The Pennsylvania State University

The Graduate School

Department of Kinesiology

THE STUDY OF SEX DIFFERENCES IN THE REGULATION OF ORTHOSTATIC BLOOD PRESSURE: THE ROLE OF THE SPLANCHNIC CIRCULATION

A Dissertation in

Kinesiology

by

Sara S. Jarvis

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2009

The dissertation of Sara S. Jarvis was reviewed and approved* by the following:

James A. Pawelczyk
Associate Professor of Physiology and Kinesiology
Dissertation Advisor
Chair of Committee

Cynthia J. Bartok
Assistant Professor of Kinesiology

Mosuk Chow
Associate Professor of Statistics

David N. Proctor
Associate Professor of Physiology and Kinesiology

Nancy I. Williams
Associate Professor of Physiology and Kinesiology

Karl M. Newell
Marie Underhill Noll Chair
Professor and Head of the Department of Kinesiology

*Signatures are on file in the Graduate School

ABSTRACT

Previous investigations have shown that women are less orthostatically tolerant than men. Several mechanisms have been proposed; however, the exact mechanism has not been defined. Blood volume distribution to high capacitance areas (i.e., the splanchnic region) influence orthostatic tolerance. Limited data suggests that the distribution of blood volume during an orthostatic stress differs between women and men, where the women tend to pool more blood in the lower abdomen/pelvic region. Accordingly, this series of experiments was designed to examine blood pressure regulation differences between the sexes as it relates to the contribution of the splanchnic circulation.

In the first study, we sought to clarify whether or not a sex difference existed in tilt table tolerance. We hypothesized that during head-up tilt (HUT) women would demonstrate less splanchnic vasoconstriction, leading to splanchnic pooling, lower blood pressure, and lower orthostatic tolerance. Mean arterial blood pressure (MAP), heart rate (HR), cardiac output ($\dot{Q}_C$, C_2H_2 rebreathing), stroke volume, splanchnic blood flow (SpBF, indocyanine green clearance), and vascular conductance (systemic, $SVC = \dot{Q}_C/MAP$; splanchnic, SpVC=SpBF/MAP; non-splanchnic, non-SpVC=SVC-SpVC) were measured during supine baseline, 70° HUT, and recovery in 14 healthy women (23±6 yrs; mean±SD) and 16 men (23±5 yrs). Neither median tilt time (15.7 vs. 21.8 min; χ^2=0.54, p=0.46), nor the proportion surviving 45 min of HUT (χ^2=2.92, p=0.09) was different between the sexes. MAP was lower in women (supine: 77±5 vs. 86±9 mmHg, p<0.01; tilt: 72±8 vs. 83±10 mmHg, p<0.01), while HR and cardiac index were not different between the sexes (supine: 66±6 vs. 64±8 bpm; tilt: 96±13 vs. 94±10 bpm; supine: 3.8±0.9 vs. 3.7±0.7 $L \cdot min^{-1} \cdot m^{-2}$; tilt: 2.7±0.8 vs. 2.3±0.5 $L \cdot min^{-1} \cdot m^{-2}$). SpBF and SpVC were lower in women at rest but not during tilt- (SpBF, supine: 1174±243 vs. 1670±391 ml/min,

$p<0.01$; SpVC, supine: 14.83±3.61 vs. 19.59±4.95 $ml \cdot min^{-1} \cdot mmHg^{-1}$, $p<0.01$; SpBF, tilt: 884±300 vs. 1094±271 ml/min; SpVC, tilt: 13.14±4.28 vs. 14.82±4.16 $ml \cdot min^{-1} \cdot mmHg^{-1}$). However, in the women SpVC was not reduced between baseline and tilt (ΔSpVC: -1.70±3.19 $ml \cdot min^{-1} \cdot mmHg^{-1}$, p=NS; -4.81±3.44 $ml \cdot min^{-1} \cdot mmHg^{-1}$, $p<0.01$), suggesting a blunted vasoconstrictor response. Thus, women tended to have lower tilt-table tolerance associated with a smaller splanchnic vasoconstrictor reserve than men.

In the second study, splanchnic hemodynamics and tilt table tolerance were assessed after an infusion of placebo or octreotide acetate, a somatostatin analog whose vascular effects are largely confined to the splanchnic circulation. We hypothesized that reductions in splanchnic blood flow (SpBF) and splanchnic vascular conductance (SpVC) would be related to improvements in tilt time. Hemodynamic variables were collected in 14 women and 16 men during baseline, 70° head-up tilt (HUT), and recovery during placebo and octreotide conditions. HUT elicited an increase in heart rate and decreases in mean arterial pressure, cardiac index, stroke index, and systemic vascular conductance (SVC). Additionally, SpVC and non-SpVC were lower during HUT. Octreotide reduced SpBF and SpVC and increased SVC and non-SpVC. A repeated measures ANOVA was used to compare changes from baseline with respect to sex and condition. Changes in SpBF and SpVC between supine and HUT were smaller in women ($p<0.05$). There was a significant improvement in tilt table tolerance in both sexes with octreotide administration. Median tilt times were increased from 15.7 and 21.8 min to 37.0 and 45.0 min for women and men, respectively. A significant relationship existed between ΔSpBF (placebo-octreotide) and Δtilt time in women (Δtilt time=2.5-0.0083 ΔSpBF, p=0.0051) but not men (Δtilt time=3.41–0.0008 ΔSpBF, p=0.59). Thus, administration of octreotide acetate improved tilt table tolerance where the principal mechanism was by decreasing splanchnic vascular conductance.

The purpose of the third study was to quantify blood volume redistribution during graded tilt. Head-up tilt (HUT) redistributes ~700 mL of blood to the dependent regions. In a gravitational field, hydrostatic pressure is balanced against vascular compliance, resulting in a hydrostatic indifferent point (HIP). Its location is independent of posture and should be coincident with a volume indifferent point (VIP). Cardiac filling is determined by the hydrostatic gradient between the HIP/VIP and right atrium. A more inferior VIP would lead to decreased preload and possibly orthostatic intolerance. We located the VIP by employing segmental impedance to examine blood volume redistribution during HUT. During HUT impedance increased above and decreased below the VIP, respectively, due to blood volume shifts. An exponential model related blood volume shifts and the hydrostatic gradient to determine the location of the VIP, which was located at 64.5±2.6% of an individual's height. This method may provide a quantitative framework to assess the effects of blood volume distribution on tilt tolerance.

The fourth and fifth studies examined the impact of splanchnic blood volume manipulation on the location of the VIP. We previously indentified that the splanchnic circulation contributes to the location of the VIP. Using segmental impedance we identified the VIP under control conditions and when blood volume within the splanchnic segment was altered. We also sought to determine the relationship between the location of the VIP and an individual's tolerance to an orthostatic stress. In Protocol 1, we found that administration of the somatostatin analog, octreotide acetate, a selective splanchnic vasoconstrictor, induced a superior shift in the location of the VIP (+1.9±3.3 cm, $p=0.03$). This finding substantiated previous reports of improvements in tilt tolerance after octreotide and suggests it might be related to relocation of the VIP. Conversely, in Protocol 2, exposure to lower body negative pressure (LBNP) induced splanchnic pooling and moved the VIP inferiorly (-6.0±7.2 cm, $p<0.01$). LBNP combined with

head-up tilt significantly decreased tilt table tolerance (median tilt times: 28.0 min vs. 4.2 min; χ^2=14.29, p<0.01); a positive relationship between ΔVIP and Δtilt time existed (Δtilt time = 3.05 + 0.12 ΔVIP, p=0.03). Thus, individuals that demonstrated the largest inferior shift in the location of the VIP also demonstrated the largest decrease in tilt table tolerance. We conclude that the splanchnic circulation plays an important role in determining the location of the VIP and its location is a determinant of tolerance to an orthostatic stress.

The results of these studies indicate that women and men regulate blood pressure differently in the upright posture. Specifically, women showed a decreased vasoconstrictor response that could be isolated to the splanchnic circulation. Administration of octreotide, a selective splanchnic vasoconstrictor, improved blood pressure regulation during head-up tilt. The mechanism by which this occured was related to alteration of the volume indifferent point to a more superior location. Conversely, decrements in tilt table tolerance were shown to be directly related to an inferior shift in the location of the volume indifferent point.

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LIST OF ABBREVIATIONS

A_D	Body surface area
Ang II	Angiotensin II
ANOVA	Analysis of variance
BMI	Body Mass Index
BP	Blood Pressure
BRS	Baroreflex sensitivity
BSA	Body Surface Area
BV	Blood Volume
CVP	Central venous pressure
E_2	Estrogen
ET-1	Endothelin-1
HCT	Hematocrit
HIP	Hydrostatic indifferent point
HR	Heart rate
HU	Hindlimb unloading
HUT	Head-up tilt
ICG	Indocyanine green
LBNP	Lower body negative pressure
MAP	Mean arterial pressure
MAST	Medical anti-shock trousers
MCFP	Mean circulatory filling pressure
MSNA	Muscle sympathetic nerve activity
NE	Norepinephrine
Non-SpVC	Non-splanchnic vascular conductance
OCT	Octreotide
OI	Orthostatic intolerance
OT	Orthostatic tolerance
PLA	Placebo
POTS	Postural orthostatic tachycardia syndrome
PV	Plasma volume
$\dot{Q}_c$	Cardiac output
$\dot{Q}_i$	Cardiac index

RAP	Right atrial pressure
SNS	Sympathetic nervous system
SpBF	Splanchnic blood flow
SpVC	Splanchnic vascular conductance
sst	Somatostatin
SV	Stroke volume
SV_i	Stroke index
SVC	Systemic vascular conductance
SVR	Systemic vascular resistance
THRIM	Tetrapolar high resolution impedance meter
TPR	Total peripheral resistance
VIP	Volume indifferent point
$\dot{V}O_{2max}$	Maximal oxygen uptake

ACKNOWLEDGEMENTS

The knowledge and experience I have gained during my time here in Noll Lab will not be easily forgotten. As my time here nears its end, I can't help but reminisce. This journey was not an easy one by any stretch of the imagination. This dissertation not only represents my work over the past 5 years, but should also be viewed as a collaborative effort of many individuals. I would be remiss if I did not acknowledge the many people who helped me throughout this process because without them the challenges may have been insurmountable.

To my advisor, Dr Jim Pawelczyk, I thank you for the many opportunities you have provided me over the years. You pushed me out of my comfort zone in many ways, but through that I learned to be a more critical thinker. You encouraged me to think limitlessly when I was a hindrance to myself. I leave Noll with an appreciation of your enthusiasm for teaching and your passion for learning.

To my committee:

Dr Bartok, thank you for your support while I transitioned into balancing my work with having a child.

Dr Chow, while you are new to my committee, I am still appreciative of the guidance you provided.

Dr Proctor, I appreciate your open door policy; I always felt you wanted nothing but the best for me.

Dr Williams, thank you for encouraging me to think more creatively about my research.

To my fellow graduate students—Beth Parker, JC Kostyak, Kelly Dougherty, and Craig Hunter—you have all mastered the fine art of knowing when to be sympathetic and when to administer tough love. I know our friendships extend beyond the walls of Noll Lab.

To the Undergraduate Army: the amount of data collected would not have been possible without your help. I hope our paths cross again in the future.

To the General Clinical Research Center staff, thank you for your flexibility and willingness to consider and come up with alternative solutions. I especially owe thanks to Laurie Aquilino. You always treated each subject with such care and respect. You never accepted less than perfection from yourself, ultimately making our research better. Thank you.

To those who provided excellent technical and equipment support—Doug Johnson, Randy McCullough, Denny Ripka, and Don Fink—thank you for working expeditiously on the many SNAFUs that occurred over the years.

To my friends and family, thank you for being interested in what I do but for also acknowledging that I am more than my research and that I have many other interests in life. I can't thank all of you enough for always being so positive and supportive. I'm honored to have made you proud.

Last, but certainly not least, to Jason and Catchr—the two most important people in my life—thank you for giving me the space to work long hours when it was needed. You two, perhaps, made the biggest sacrifices of all. Jason, thank you for making me laugh and for giving me a kick in the butt and some perspective when I needed it most. To my son, Catchr, although you won't remember these times, you have taught me things so much more valuable than anything I've learned at Penn State. I thank you for providing balance in my life. Through you I've been gently reminded that at the end of the day all that really truly matters is that I can look in the mirror and know that I "done good".

"What lies behind us and what lies before us are tiny matters compared to what lies within us."

~Ralph Waldo Emerson

Chapter 1

INTRODUCTION

"The immunity against circulatory failure in the upright posture is maintained by daily training, and is so deeply ingrained in the texture of human nature that we become aware of its existence only when it fails…."

~ Otto H. Gauer & Hans L. Thron

Background and Significance

About 500,000 Americans, particularly women of childbearing age, suffer from orthostatic intolerance (OI) (Ali *et al.*, 2000). OI is the inability to maintain blood pressure in the upright posture; it is the second most prevalent blood pressure disorder following hypertension (Ali *et al.*, 2000). Diagnosis can be difficult because the symptoms are often vague, ranging from nausea, fatigue, and light-headedness to frank syncope. Extreme cases of OI resulting from autonomic dysfunction can be debilitating, leading to a diminished quality of life. For example, some individuals suffering from severe autonomic dysfunction, are unable to tolerate an upright posture for more than 1 minute (Schroeder *et al.*, 2005; Shapiro *et al.*, 2000).

Astronauts are also at risk for developing OI upon return from spaceflight because exposure to free-fall reduces hydrostatic gradients, leading to cardiovascular deconditioning. Recently it was reported that 20% of male astronauts were able to complete a 10-min stand test upon return from space; however, 100% of women were unable to complete the orthostatic

challenge, indicating that female astronauts were disproportionately affected (Waters *et al.*, 2005).

Limited data suggest that the distribution of blood volume during an orthostatic challenge is different between the sexes, which may contribute to OI. For example, Frey and Hoffler (Frey & Hoffler, 1988) have shown that women demonstrate a greater shift of blood from the thoracic region to the lower body than do men when subjected to -50 mmHg lower body negative pressure (LBNP, an experimental approach to apply an orthostatic stress). Moreover, White and Montgomery (White & Montgomery, 1996) found that women had an 83% greater translocation of blood volume to the pelvic region during -50 mmHg of LBNP, when compared to men. The abdominal/pelvic region includes the splanchnic bed, a highly compliant region. Thus, blood pooling in this highly compliant region is sequestered from the general circulation, decreasing venous return, cardiac preload, and subsequently, cardiac output. Such differences provide clues to the disparity seen in OI between the sexes and suggestions to targeted therapeutic interventions.

Accordingly, this series of studies for this dissertation was designed to investigate sex differences relating to orthostatic tolerance, to quantify blood volume distribution during an orthostatic stress, and to determine how selectively targeting the splanchnic circulation influences these. Because the findings in the literature are equivocal in terms of whether a sex difference in tilt tolerance exists, the first study was designed to examine differences in tilt table tolerance between women and men. The second study was to determine whether a reduction in splanchnic blood flow could improve tilt table tolerance by decreasing splanchnic vascular conductance. The third study was a method validation study; it sought to quantify blood volume distribution during graded tilt, locating the volume indifferent point. The fourth study was designed to bridge the second and third studies. It sought to determine how reductions in splanchnic blood flow alter the location of the volume indifferent point. The last study, study 5, was to determine whether

decrements in orthostatic tolerance, induced by lower body negative pressure, could be quantified using the volume indifferent point model. Collectively, these studies focused on clarifying sex differences in the regulation of orthostatic blood pressure and the role in which the splanchnic circulation plays in this interaction.

Specific Aims and Hypotheses

Specific Aim 1

The purpose of the study, "Sex differences in vasoconstrictor reserve during 70° head-up tilt," was to determine whether sex differences relating to tilt table tolerance exist and whether these differences could be attributed to the splanchnic circulation.

Hypothesis 1: Women will demonstrate diminished splanchnic vasoconstriction, leading to splanchnic pooling, lower systemic resistance, and lower tilt table tolerance.

Specific Aim 2

The purpose of the study, "A somatostatin analog improves orthostatic tolerance by decreasing splanchnic vascular conductance," was to determine whether selective constriction of the splanchnic circulation would improve tilt table tolerance.

Hypothesis 2a: Administration of octreotide, which selectively constricts blood vessels in the splanchnic region, will decrease splanchnic blood flow and splanchnic vascular conductance, leading to improvements in tilt table tolerance.

Hypothesis 2b: Improvements in tilt table tolerance after administration of octreotide will be greater in women.

Specific Aim 3

The purpose of the study, "Identification of the human volume indifferent point," was to use bioelectrical impedance to quantify blood volume redistribution and locate the point where blood volume does not change during graded tilt (the volume indifferent point).

Hypothesis 3a: The volume indifferent point is analogous to the hydrostatic indifferent point, thus, it will be located roughly 2/3 of an individual's height.

Hypothesis 3b: The volume indifferent point will be located more inferiorly in women since women tend to be less orthostatically tolerant.

Specific Aim 4

The purpose of, "Experimental manipulation of the human volume indifferent point with octreotide acetate," was to determine whether octreotide improved tilt table tolerance by shifting the volume indifferent point superiorly.

Hypothesis 4: Administration of octreotide acetate will decrease splanchnic blood flow and, therefore, the volume of blood in the splanchnic region, which will shift the location of the volume indifferent point superiorly.

Specific Aim 5

The purpose of the study, "Experimental manipulation of the volume indifferent point with lower body negative pressure," was to determine whether application of -20 mmHg of lower body negative pressure could elicit an inferior shift in the volume indifferent point. Additionally, the relationship between the change in the location of the volume indifferent point with and without lower body negative pressure and the change in tilt tolerance with and without lower body negative pressure was assessed.

Hypothesis 5a: Application of -20 mmHg lower body negative pressure will increase the volume of blood pooled in the splanchnic region and will induce an inferior shift in the location of the volume indifferent point.

Hypothesis 5b: The magnitude of change in location of the volume indifferent point, due to lower body negative pressure, will be related to changes in tilt tolerance.

Chapter 2

REVIEW OF LITERATURE

This chapter will address the relevant literature relating to this dissertation. The discussion will include: 1) cardiovascular adjustments to upright posture, 2) factors influencing the regulation of orthostatic blood pressure, and 3) sex differences in orthostatic tolerance.

Cardiovascular Adjustments to Upright Posture

Upon assumption of the upright posture ~700 mL of blood translocates from the thorax to the gravitationally dependent regions, which include the lower abdomen, pelvic region, buttocks and upper thighs (Jacob & Biaggioni, 1999). Concomitant with these blood volume shifts is a rapid decrease in central venous filling pressure (CVP) from ~5 to ~0 mmHg (Smith & Ebert, 1990). Mean arterial pressure (MAP) is transiently decreased before the baroreflex elicits a compensatory response due to the drop in pressure. The baroreflex originates from the mechanoreceptors in the aortic arch and carotid arteries. When the transmural pressure within these vessels is decreased due to a loss of central blood volume, mechanoreceptor activity also decreases (Rowell, 1993). In response, the afferent mechanoreceptor signals via the vagus nerve and the glossopharyngeal nerve synapse on the nucleus tractus solitarius to initiate two pathways: 1) projections to the nucleus ambiguus in the medulla to inhibit vagal activity, which produces an increase in heart rate (HR) and cardiac output ($\dot{Q}_c$); and 2) projections from the nucleus tractus solitarius synapse on the caudal ventrolateral medulla to disinhibit the C1 area of the rostral ventrolateral medulla and lead to an increase in vasoconstriction (systemic vascular resistance, SVR) via the sympathetic chain (Boulpaep, 2003). SVR increases during an orthostatic stress due

to increases in muscle sympathetic nerve activity (MSNA) (Shoemaker *et al.*, 2001). Additionally, a hypotensive stimuli elicits increases in renal (Miki *et al.*, 1989) and splanchnic sympathetic nerve activity (Mandel & Schreihofer, 2008), which also increase SVR.

Thus, during passive head-up tilt (HUT) in healthy individuals, there is an immediate increase in HR of ~30% due to the transient drop in blood pressure (BP) (Smith & Ebert, 1990). HR then settles to ~10% above baseline in 15 sec (Smith & Ebert, 1990). In tandem, a nadir in MAP is reached within 5 seconds, followed by an increase back to baseline or slightly above baseline at about the same time HR is stabilized (Smith & Ebert, 1990). SVR increases by 30% to offset the decrease in stroke volume (SV) and $\dot{Q}_c$ (Smith & Ebert, 1990). However, when SVR cannot increase to sufficiently offset these decrements, BP cannot be maintained and syncope occurs.

Vasoconstriction (increases in SVR) contributes more to the maintenance of MAP than does the increase in HR (Hainsworth, 2000). Regions receiving the largest proportion of $\dot{Q}_c$ are the most important because they potentially have the largest volume to displace in order to support cardiac filling (Rowell, 1986). These regions include skeletal muscle and the splanchnic and renal circulations because they receive 18%, 27%, and 22% of $\dot{Q}_c$, respectively (Rowell, 1986). During HUT, reductions in flow are observed in these regions, contributing to increased systemic resistance. For example, Julu et al (Julu *et al.*, 2003) observed a 70% increase in forearm vascular resistance during HUT. Rowell and colleagues (Rowell *et al.*, 1972) posited that muscle and skin contributed to ~40% of the reduction in total conductance (the inverse of resistance) when BP maintenance was challenged. The splanchnic contribution was reported as accounting for 33% of the reduction in total conductance and the renal as 28% (Rowell *et al.*, 1972).

During prolonged orthostasis (longer than ~30 min), circulating hormones such as renin, angiotensin II (ang II), aldosterone, and antidiuretic hormone contribute to maintenance of MAP (Smith & Ebert, 1990). These hormones influence fluid balance and retention, as well as have a direct effect on the vasculature (Smith & Ebert, 1990). In terms of fluid retention and balance, renin is indirectly responsible for decreasing sodium and water excretion via increases in ang II production (Giebisch & Windhager, 2003). In terms of the direct vasoconstrictive effect, a decrease in renal perfusion pressure (as with what occurs during HUT), increases the release of renin, initiating the cascade of ang II production (Giebisch & Windhager, 2003). Ang II has been shown to be a potent vasoconstrictor that targets the splanchnic and renal beds during states when BP is low (Boulpaep, 2003).

Factors Influencing the Regulation of Orthostatic Blood Pressure

As suggested above, blood volume distribution to regions receiving large proportions of $\dot{Q}_c$ have the potential to influence BP maintenance. Of these circulations—skeletal muscle, splanchnic and renal—the splanchnic is the most compliant and, therefore, contributes greatly to the maintenance of BP (Rowell, 1986). In fact, Rowell (Rowell, 1986) purported that about 20% of the total blood volume is contained within the splanchnic veins. Skeletal muscle, however, only comprises about 600-700 mL (about 12%) of total blood volume, a small proportion for its size (Rowell, 1986). While the renal circulation receives a large percentage of $\dot{Q}_c$, it does not serve as a large volume reservoir. Accordingly, the following section will focus on regional blood volume distribution as it relates to skeletal muscle and the splanchnic circulation during HUT, how blood volume distribution can be quantified, and how pharmacological intervention might influence the maintenance of BP by redistribution of blood volume.

Regional blood volume distribution

In the supine posture blood volume is evenly distributed as gravity is exerted along the longitudinal axis of the body. During HUT or lower body negative pressure (LBNP), blood is translocated from the thoracic region to the lower abdomen, pelvic region, buttocks and legs (Jacob & Biaggioni, 1999). The amount of blood volume gain in these regions is largely dependent on the capacitance property of each circulation.

With respect to the contribution of muscle, some studies (Gaffney *et al.*, 1982; Terai *et al.*, 1991) have focused on the distribution of blood volume to the legs as it relates to cardiovascular hemodynamics. Studies of leg blood volume shifts have employed the use of medical anti-shock trousers (MAST) in attempt to quantify the contribution of the volume shift. MAST have three inflatable bladders that encapsulate each leg and the lower abdomen. Inflation of the bladders exerts pressure, which decreases the volume of blood contained in these regions and increases the effective circulating volume (stressed volume). It has been suggested, however, that increases observed in SV during MAST inflation (legs only) may be related more to the increase in SVR than any "auto-transfusion" that occurs (Gaffney *et al.*, 1981). The older studies quantifying the volume contribution of the legs have been criticized for over-estimating the volume that could theoretically be "auto-transfused" (750 mL) (McSwain, Jr., 1976). For example, Gaffney et al (Gaffney *et al.*, 1982) purported that the legs only contain 100-250 mL, of which only a small portion could be mobilized. This is not surprising since the vasculature in the legs is less compliant compared to other circulations (Gelman, 2008). Thus, while blood volume is redistributed to the legs during HUT, the volume is minimal compared to the contribution of other circulations.

A compliant circulation such as the splanchnic region contributes greatly to the maintenance of BP during orthostasis (Gelman, 2008). In fact, the venous system has been

described as a two compartment model—non-splanchnic and splanchnic—because of the important role of the splanchnic circulation (Gelman, 2008). The gut region is one of the most richly innervated by the SNS (Rowell, 1986), which means that vaso- and venoconstriction have the ability to mobilize a large volume of blood (up to 350 mL) (Rothe, 1983). A reduction in splanchnic arterial blood flow reduces flow, pressure, and volume in the splanchnic veins, leading to passive expulsion of blood volume (due to elastic recoil), resulting in an "auto-transfusion" and an increase in the mean circulatory filling pressure (Gelman, 2008).

To illustrate this point, Gelman and colleagues (Gelman *et al.*, 1994), using Tc99m labeled albumin, reported that aortic cross-clamping proximal to the celiac artery (one of the arteries feeding the splanchnic circulation) led to a reduction in splanchnic blood flow (SpBF) and a decrease in the volume contained in that circulation. An increase in gamma-emission was detected in the upper body, indicating that the reduction in splanchnic blood volume was redistributed to other regions (Gelman *et al.*, 1994).

Conversely, when a large volume of blood pools in the splanchnic region, the effective blood volume is reduced, which diminishes venous return (Gelman, 2008). The functional implication of splanchnic pooling can be illustrated through patient populations. For example, a subset of patients with postural orthostatic tachycardia syndrome (POTS) demonstrate splanchnic hypervolemia and exhibit low tolerance to an orthostatic stress (Stewart & Montgomery, 2004). Interestingly, when Stewart & Montgomery (Stewart & Montgomery, 2004) characterized these patients they noted that this subgroup had normal peripheral vasoconstrictive function, similar to individuals without POTS. This suggests that differences in tolerance to an orthostatic stress can, at least, be partially explained by the splanchnic pooling that occurs in the upright posture. Additionally, Arbeille et al (Arbeille *et al.*, 2005) examined the portal vein cross sectional area after 90 days bed rest in a group of men. They categorized subjects into "tolerant" and "non-tolerant" based on the ability to complete a tilt table tolerance test, reporting that the portal vein

cross sectional area decreased significantly between supine and 80° HUT in the tolerant individuals; this was not observed in the non-tolerant group (Arbeille *et al.*, 2005). Taken together, these studies indicate that the degree to which blood flow/blood volume can be enhanced or reduced in the splanchnic circulation during HUT influences orthostatic tolerance (OT). *Accordingly, dissertation studies 2, 4, and 5 will seek to address how manipulation of splanchnic blood flow/volume influences OT.*

The hydrostatic indifferent point

While the above studies indicate that splanchnic pooling decreases OT, they do not describe the mechanism by which this occurs. That is, how does volume gain in a compliant circulation lead to decrements in OT? The following section describes how the hydrostatic indifferent point (HIP) plays a role in determining OT.

The HIP is the point where pressure does not change in the venous system during changes in posture. Gauer & Thron (Gauer & Thron, 1965) located the HIP by measuring pressure throughout the venous system between the iliac crest and the clavicle during recumbency and 60°HUT, finding that the HIP was a few centimeters below the level of the diaphragm. The location of the HIP dictates the filling gradient for the heart; therefore, a more inferior point should translate to lower OT (Rowell, 1986). Theoretically, if the heart was located at the same level as the HIP, filling pressure would not decrease during standing and orthostatic intolerance (OI) could not be induced (Gauer & Thron, 1965).

Using the analogy of a column of blood, if the distensibility of the column was the same above and below the HIP, the HIP would not move (Gauer & Thron, 1965). However, when distensibility is not equal (as in the human body), the HIP moves toward the end that is more distensible (Gauer & Thron, 1965). Thus, the HIP is balanced by the hydrostatic gradient above

it and the mechanical properties of the compliant circulation below it (Rowell, 1986). Therefore, compliant circulations such as the splanchnic move the HIP inferiorly and, especially so, when the distending pressure in that region is reduced inadequately. Conversely, improvements in cardiac filling have been observed when the location of the HIP is moved superiorly. For example, Rowell (Rowell, 1986) asserted that water immersion up to the diaphragm in individuals with venous valvular insufficiency reduces the volume of blood in the dependent regions and supports cardiac filling.

While the HIP allows for a quantitative assessment of cardiac filling in the upright posture, its widespread use is limited because of its invasiveness. Perko et al (Perko *et al.*, 1993) examined this same concept using blood volume distribution, rather than pressure. However, this study did not precisely locate a point analogous to the HIP. Additionally, the relationship between the HIP (or an analogous point) and OT has not been established. *Accordingly, dissertation studies 3, 4, and 5 will seek to design and validate a non-invasive method analogous to the HIP, seek to experimentally manipulate the location of this point, and to determine its relationship with OT.*

Pharmacological intervention

This section of this review will focus on two drugs—midodrine and octreotide—because they have been commonly used as therapeutic interventions for individuals suffering from orthostatic hypotension. While both drugs are administered in an effort to mobilize blood back into the general circulation, these drugs target two different regions.

Midodrine

Midodrine, an α_1-adrenergic agonist, induces both vaso- and venoconstriction, leading to increases in SVR (Stewart, 2006). Midodrine has been prescribed to astronauts experiencing post-spaceflight OI (Platts *et al.*, 2004), as well as to patients with autonomic dysfunction (Hoeldtke *et al.*, 1998). For example, Hoeldtke et al (Hoeldtke *et al.*, 1998) administered 5 mg of midodrine to patients with post-prandial hypotension in order to determine whether the α_1-adrenergic agonist could ameliorate the hypotensive response. The post-prandial nadir in BP generally occurs 60-90 min after eating (Hoeldtke *et al.*, 1998). MAP was only significantly elevated for 90-105 min after the meal and standing times were not significantly increased (3.5 vs. 8.4 min) (Hoeldtke *et al.*, 1998). Additionally, the same group (Hoeldtke *et al.*, 2006) compared midodrine treatment in patients with POTS and OI, noting only improvements in standing times for patients with OI. That is, the patients with POTS—who tend to demonstrate splanchnic pooling—did not have any improvements in BP maintenance when administered an α_1-adrenergic agonist (Hoeldtke *et al.*, 2006). Taken together, these findings suggest that non-selective vasoconstriction might be less effective than other pharmacological approaches. Given the importance of the splanchnic circulation in the maintenance of orthostatic BP, a drug that selectively targets this region might be more beneficial.

Octreotide

Octreotide has also been a drug of interest for individuals with post-prandial hypotension, POTS, or OI (Hoeldtke *et al.*, 1998; Hoeldtke *et al.*, 2006). Octreotide acetate is a synthetic octapeptide mimicking somatostatin (sst) in its actions, but with a longer half-life. Five somatostatin receptor subtypes (sst_{1-5}) are expressed in the brain, stomach, liver, kidneys,

intestines, and pancreas (Patel & Srikant, 1997). Octreotide demonstrates high affinity for sst_2 and sst_5 and intermediate affinity for sst_3 (Reynaert & Geerts, 2003). Receptor subtypes sst_2 and sst_5 are preferentially located in the liver (Reynaert *et al.*, 2007), stomach, and pancreas (Patel & Srikant, 1997).

Octreotide induces an immediate (within 1-2 min) (Bosch *et al.*, 1981) reduction in SpBF of up to 35% (Eriksson & Wahren, 1989). The exact mechanism has not been clearly defined but there are three potential candidates:

1) Modulation of gut vasodilatory hormones
2) Indirect vasoconstrictive effect via potentiation of vasoconstrictors
3) Direct vasoconstrictive effect

A group of G-proteins coupled to the sst_2 receptor have been identified that include: $G_{i\alpha1}$, $G_{i\alpha3}$, and $G_{0\alpha}$ (Law *et al.*, 1995). $G_{i\alpha1}$ inhibits adenylyl cyclase activity, $G_{i\alpha3}$ increases conductance of K^+ channels, and $G_{0\alpha}$ decreases conductance of Ca^{2+} channels (Law *et al.*, 1995). These actions inhibit the secretion of peptides such as glucagon and gastric acids (Patel & Srikant, 1997), as well as vasoactive intestinal peptide (Gu *et al.*, 1992). Glucagon contributes to the resting tone of the hepatic arterioles (Benoit *et al.*, 1986). For example, in a rat model with portal hypertension, Benoit et al (Benoit *et al.*, 1986) found that administration of a glucagon antiserum significantly reduced hepatic blood flow. However, while octreotide may induce vasoconstriction through this pathway, it is unlikely that inhibition of these vasodilatory peptides can produce an immediate decrease in SpBF.

Some have purported that octreotide by itself does not have a direct vasoconstrictive effect on the vascular smooth muscle and, instead, exerts an indirect effect (Wiest *et al.*, 2001). For example, a series of studies in the prehepatic portal-hypertensive rat model illustrated that octreotide potentiated the contraction of smooth muscle vasoconstrictors such as ET-1 (Wiest *et*

al., 2001). Wiest et al (Wiest *et al.*, 2001) hypothesized that octreotide activates phospholipase A_2, which leads to the formation of cyclooxygenase-derived vasoconstrictive prostanoids. While Wiest et al (Wiest *et al.*, 2001) did not extend their hypothesis any further, it might be suggested that the formation of thromboxane A_2 is what contributed to this potentiation since Vila et al (Vila *et al.*, 2001) demonstrated that inhibition of thromboxane A_2 formation negated the vasoconstrictive effect of ET-1. It is not clear, however, if constitutive production is sufficient to cause the observed reduction in SpBF.

Findings supporting a direct effect of octreotide have been equivocal. Sieber et al (Sieber *et al.*, 1992) examined the effect of octreotide on the isolated rat superior mesenteric artery, finding that octreotide did not induce an increase in vessel resistance. In contrast, Dimech et al (Dimech *et al.*, 1995) tested the effect of octreotide on the isolated human saphenous vein. They found that octreotide induced contractions in a concentration-dependent manner that were greater than native somatostatin (Dimech *et al.*, 1995). Additionally, this group performed a series of experiments using native somatostatin in order to elucidate the vasoconstrictive mechanism. Interestingly, they ruled out interactions due to thromboxane A_2 because indomethacin (thromboxane A_2 antagonist) failed to abolish the contractile response (Dimech *et al.*, 1995). They concluded that the L-type Ca^{2+} channel was the most likely candidate since the constrictor response was attenuated when the external Ca^{2+} concentration was reduced (Dimech *et al.*, 1995). Moreover, co-administration of verapamil (L-type Ca^{2+} antagonist) or nifedipine (Ca^{2+} channel antagonist) also attenuated the contractile response (Dimech *et al.*, 1995).

Clinical trials have taken advantage of the selectivity of octreotide in terms of inducing reductions in SpBF. As previously mentioned, patients with post-prandial hypotension were administered midodrine with little improvement (Hoeldtke *et al.*, 1998). However, when patients were administered octreotide (0.5 μg/kg) an improvement in BP maintenance after a meal was observed (Hoeldtke *et al.*, 1998). Moreover, the standing times were significantly increased

(Hoeldtke *et al.*, 1998). Interestingly, an additive effect was seen when midodrine and octreotide were combined (Hoeldtke *et al.*, 1998), suggesting a synergistic effect and lends support to the idea that octreotide might induce changes in SpBF directly and indirectly.

What might be the mechanism for improved BP maintenance after the administration of octreotide? Octreotide alters blood volume distribution because it decreases blood flow to a high flow, high capacitance region. The redistribution of blood volume from the splanchnic circulation should lead to a superior shift in the location of the HIP. In fact, Wong and Sheriff (Wong & Sheriff, 2007) recently demonstrated, in the dog model, that administration of octreotide acetate induced an increase in right atrial pressure. They postulated that this increase could lead to improvements in OT. A*ccordingly, dissertation study 4 will determine the mechanism of improvement in tilt table tolerance as it relates to blood volume distribution, due to octreotide (observed in study 2).*

Sex Differences in Orthostatic Tolerance

A general consensus exists demonstrating that women are less orthostatically tolerant than men (Franke *et al.*, 2003; Fu *et al.*, 2004a; Fu *et al.*, 2005; Montgomery *et al.*, 1977; White *et al.*, 1996). Findings in the literature propose a wide range of potential mechanisms including: phase of the menstrual cycle (Cooke *et al.*, 2002; Meendering *et al.*, 2005), estrogenic influences on the vasculature (Bowyer *et al.*, 2001; Ergul *et al.*, 1998; Kneale *et al.*, 2000), baroreflex sensitivity (Cooke *et al.*, 2002; Hirshoren *et al.*, 2002; Hunt *et al.*, 2001; Minson *et al.*, 2000a), and regional blood volume distribution (Frey & Hoffler, 1988; White & Montgomery, 1996).

Orthostatic tolerance across the menstrual cycle

Several studies have examined OT across the menstrual cycle (Claydon *et al.*, 2006; Cooke *et al.*, 2002; Meendering *et al.*, 2005); however, the general conclusion is that no difference exists. For example, Meendering et al (Meendering *et al.*, 2005) examined the effect of heat stress and tilt tolerance across three phases of the cycle (early follicular, ovulation, mid-luteal), finding no difference in tolerance. Additionally, other investigative groups were unable to detect differences in tolerance across the menstrual cycle (Claydon *et al.*, 2006; Cooke *et al.*, 2002). These studies, however, did not address the effect estrogen (low, high, cyclical exposure) has on variables that may impact the differences observed between the sexes.

Estrogenic influences on the vasculature

Estrogen (E_2) has been shown to modulate nitric oxide production, which leads to a decrease in vascular tone (Orshal & Khalil, 2004). Women have demonstrated blunted responsiveness to vasoconstrictors such as norepinephrine (NE), and ET-1 (Ergul *et al.*, 1998; Kneale *et al.*, 2000). For example, Kneale and colleagues (Kneale *et al.*, 2000) reported that the degree of vasoconstriction in the forearm of women, provoked by NE infusion, was only comparable to men when propranolol, a β_2-adrenergic antagonist, was co-administered. This finding was supported by Bowyer et al's (Bowyer *et al.*, 2001) finding that women demonstrated a blunted vasoconstrictor response to NE. Furthermore, Ergul et al (Ergul *et al.*, 1998) found that ET-1 induced contractions were two times greater in men. Taken together, women demonstrate blunted responses to vasoconstrictive agents, which could have functional consequences during an orthostatic challenge.

Baroreflex sensitivity

Baroreflex sensitivity (BRS) is defined by the change in either HR or MSNA due to changes in systolic BP. It provides an index of autonomic control of BP. There are two questions that need to be addressed: 1) does BRS change across the menstrual cycle? and 2) does BRS differ between women and men? There have been inconsistent findings between investigative groups. For example, Cooke et al (Cooke *et al.*, 2002) quantified BRS (carotid-cardiac) across the naturally occurring menstrual cycle of women, noting that BRS was not different between the phases of the cycle. In contrast, others (Hirshoren *et al.*, 2002; Minson *et al.*, 2000a) reported increases in BRS (carotid-cardiac and vascular sympathetic) during the luteal phase. Furthermore, E_2 supplementation to post-menopausal women was shown to enhance BRS (vascular sympathetic) (Hunt *et al.*, 2001). Thus, the majority of studies indicate BRS increases with increasing E_2 concentration.

What might be the mechanism by which E_2 enhances BRS? Saleh & Connell (Saleh & Connell, 2000) used a rat model to answer this question. Ovarioectomized rats were given bolus injections of E_2 while vagal and renal sympathetic efferent nerve activity was measured. They reported that vagal activity was increased and renal sympathetic activity decreased immediately following the bolus injection, leading to an increase in BRS. In a subgroup of rats, they performed the opposite experiment by injecting a selective E_2 antagonist directly into the nucleus ambiguus (an important brain structure in the baroreflex arc). They noted an immediate decrease in vagal activity, increase in renal sympathetic nerve activity, and subsequent decrease in BRS (Saleh & Connell, 2000). Thus, these findings suggest that E_2 enhances resting BRS by augmenting vagal tone.

How does BRS differ between the sexes? The general consensus suggests that women have lower baroreflex sensitivity (Christou *et al.*, 2005; Convertino, 1998). Using a rat model,

Foley and colleagues (Foley *et al.*, 2005) performed hindlimb unloading (HU) studies on male and female rats. HU simulates microgravity due to the shift of blood volume toward the thoracic region. They assessed BRS by changes in renal sympathetic nerve activity due to blood pressure manipulations with phenylephrine (pressor) and sodium nitroprusside (dilator). Female rats had lower BRS compared to the males during the control condition, as well as after 12 days of HU. In fact, their data indicated that the control condition for the females was comparable to the males after HU (Foley *et al.*, 2005).

What is the functional implication of lower baroreflex responsiveness? Lower baroreflex responsiveness should decrease the ability to buffer against perturbations in BP, such as what occurs in the upright posture. Thus, this may be one explanation of the sex differences observed in OT. However, there is one caveat. It has also been reported that high resting BRS is predictive of low tolerance to an orthostatic stress (el Sayed & Hainsworth, 1995; Iacoviello *et al.*, 2008; Pitzalis *et al.*, 2003). While these findings may appear to be at odds with one another, many factors, aside from E_2, also have the potential to influence BRS. For example, low plasma volume has been associated with higher BRS and a decrease in OT (el Sayed & Hainsworth, 1995; Iacoviello *et al.*, 2008). Thus, BRS alone may not be a good indicator of sex differences relating to OT.

Regional blood volume distribution differences between the sexes

As mentioned earlier in this review, blood volume distribution influences factors such as the HIP which, in turn, influences the filling gradient for the heart. Limited data indicates that blood volume distribution is different between the sexes (Frey & Hoffler, 1988; White & Montgomery, 1996). For example, White & Montgomery (White & Montgomery, 1996) reported that women had a greater blood volume gain in the pelvic region (iliac crest to mid-thigh)

compared to men during exposure to -50 mmHg LBNP. Additionally, Frey & Hoffler (Frey & Hoffler, 1988) found that women had greater thoracic impedance during -50 mmHg LBNP, without a concomitantly greater increase in calf circumference. (Impedance is inversely related to blood volume.) These findings suggest that women tend to pool more blood in the pelvic region during LBNP. The pelvic region, defined by White & Montgomery (White & Montgomery, 1996), includes part of the splanchnic circulation. Thus, it is possible that more splanchnic pooling occurs in women during an orthostatic stress which leads to decrements in the maintenance of BP. *Accordingly, dissertation studies 1, 2, 4, and 5 will address how selective constriction of the splanchnic circulation and splanchnic pooling affect tilt table tolerance. Additionally, these studies will quantify blood volume distribution, locating a point analogous to the HIP, and will seek to experimentally manipulate the location of this point to determine its relationship with OT.*

Summary

A review of the relevant literature regarding the regulation of BP in the upright posture and potential sex differences reveals these major findings: 1) regional blood volume distribution during an orthostatic stress impacts OT; 2) the HIP is one way to quantify blood volume distribution as it relates to venous return; 3) pharmacological interventions selectively targeting the splanchnic circulation produce improvements in OT; 4) vascular responsiveness to various vasoconstrictors, such as NE and ET-1, is attenuated in women; and 5) women tend to redistribute blood volume differently than do men during LBNP. Taken together, these findings reveal a gap in our current understanding of the relationship between blood volume distribution and OT, especially in the context of sex differences.

Chapter 3

SEX DIFFERENCES IN VASOCONTRICTOR RESERVE DURING 70° HEAD-UP TILT

Introduction

About 500,000 Americans suffer from orthostatic intolerance (Robertson, 1999), the inability to maintain blood pressure in an upright posture. Orthostasis requires a combination of integrated compensatory mechanisms in order for blood pressure to be maintained. Upon quiet standing, about 700 ml of blood translocates from the chest to the gravitationally dependent regions (Jacob & Biaggioni, 1999). Blood pooled in compliant regions, such as the splanchnic region, is sequestered from the general circulation leading to decreased venous return, cardiac preload, and, subsequently, cardiac output (Rowell, 1993). A compensatory response is mounted via sympathetic disinhibition upon unloading of arterial baroreceptors (Rowell, 1993), where the autonomic nervous system defends blood pressure with increases in heart rate and α_1-adrenergic stimulated vasoconstriction (Boron & Boulpaep, 2003). During prolonged orthostasis, circulating hormones such as renin, angiotensin II, aldosterone, and antidiuretic hormone contribute to the maintenance of blood pressure via changes in fluid balance/retention (Rowell, 1993). When these compensatory mechanisms are insufficient blood pressure cannot be maintained and syncope may occur (van Lieshout *et al.*, 1991).

Orthostatic intolerance affects more women than men (Convertino, 1998; Montgomery *et al.*, 1977; Waters *et al.*, 2002; White *et al.*, 1996); however, it is unclear why this disparity exists. Past investigations examining sex differences have reported on a variety of influential factors to include: hormone status (Meendering *et al.*, 2005), norepinephrine transporter function

(Schroeder *et al.*, 2004), cardiac mechanics (Fu *et al.*, 2004a), the vestibulosympathetic reflex (Ray, 2000), and peripheral vasoconstrictor reserve (Fu *et al.*, 2004b).

Many factors, to include those listed above, potentially contribute to an individual's tolerance to an orthostatic stress; however, the degree to which each variable contributes is controversial or remains unanswered. For example, hormone status (i.e., estrogen) has the potential to affect blood pressure maintenance via changes in baroreflex sensitivity (BRS) and/or changes to the peripheral vasculature. However, findings in this area have been inconsistent (Cooke *et al.*, 2002; Hunt *et al.*, 2001; Minson *et al.*, 2000b) making them difficult to interpret.

Limited data point to a role for abdominal/pelvic pooling (Frey & Hoffler, 1988; White & Montgomery, 1996) in the lower orthostatic tolerance of women. For example, White and Montgomery reported that during -50 mmHg of lower body negative pressure (LBNP) women demonstrated an 83% greater increase in blood volume to the pelvic region (White & Montgomery, 1996). Additionally, Frey and Hoffler showed during -50 mmHg of LBNP that women had a greater change in thoracic impedance without a concomitantly large increase in calf circumference, when compared to men (Frey & Hoffler, 1988). These studies (Frey & Hoffler, 1988; White & Montgomery, 1996) demonstrate that blood volume distribution during an orthostatic stress differs between women and men, suggesting that the splanchnic circulation may play a key role in the observed differences in orthostatic tolerance between the sexes.

Thus, the purpose of the present investigation was to examine: 1) tilt table tolerance in women and men; and 2) determine the splanchnic hemodynamic differences between women and men during supine baseline and 70° head-up tilt. We hypothesized that women would have less splanchnic vasoconstriction, leading to splanchnic pooling, lower systemic vascular resistance, and, subsequently, lower orthostatic tolerance.

Methods

Subjects

Thirty subjects (14 women, 16 men) gave written informed consent to participate in the study, which was approved by the Institutional Review Board at the Pennsylvania State University. Descriptive characteristics of the subjects are outlined in Table 1. Exclusionary criteria included: smoking, BMI <20 kg/m^2 or ≥30 kg/m^2, hormonal contraceptive use, autonomic dysfunction or clinically diagnosed orthostatic intolerance, peripheral vascular disease, and allergies to local anesthetics, shellfish, iodides, penicillin, or sulfa drugs.

Before participating in any part of the experiment, subjects underwent a screening protocol which consisted of a 12-lead resting electrocardiogram and blood pressure measurements, a blood panel (complete blood count, CHEM 24, and coronary risk profile), a physical exam, evaluation of medical history, and a graded treadmill exercise test.

Subjects were admitted to the General Clinical Research Center (GCRC) at the Pennsylvania State University for study visits and were instructed to fast for at least 8 hrs; they also refrained from alcohol intake for 24-hrs, and caffeine intake for 48-hrs prior to each study visit. Female participants were tested during the early follicular phase of the menstrual cycle (days 2-6) and submitted urine samples for pregnancy testing during screening, as well as on the day of the study.

Extraction ratio of ICG and plasma volume determination

On a separate visit from screening, we determined the hepatic extraction ratio of indocyanine green (ICG; Akorn Inc.), based on a two-compartment model (Grainger *et al.*, 1983). The subject had one antecubital intravenous (18-20 gauge) catheter placed into each arm. The

left arm was used for bolus injection and infusion, while the right arm was used for blood sample withdrawal. Subjects laid supine for a minimum of 30 minutes prior to withdrawal of an aliquot of blood to serve as a spectrophotometer blank, followed by the bolus injection of 0.5 mg/kg ICG. After the intravenous bolus injection of ICG, a 3-ml blood sample was withdrawn every five minutes, followed by a 3-ml saline flush. Samples were centrifuged at 3,000 rpm for 20 minutes, and the plasma concentration of ICG was measured by spectrophotometry (805 nm for absorbance and 910 nm for turbidity). A separate extraction ratio was calculated for each subject from the exponential decay of the plasma disappearance curve of ICG (Sigma Plot, San Rafael, CA) utilizing the Marquardt-Levenberg algorithm. In addition to the extraction ratio, plasma volume was estimated for each subject from the extrapolated time-zero plasma concentration of dye.

70° head-up tilt

For this study visit, subjects received a brief physical exam by a GCRC clinician, followed by catheter placement (one antecubital in each arm, the right arm for bolus injection and infusion of ICG and the left arm for blood sample withdrawal). Subjects were then instrumented in our laboratory for electrocardiogram (3-lead ECG; Hewlett-Packard 78534A) and blood pressure (arterial applanation tonometry for beat-to-beat measurements and auscultatory for calculation of conductance measurements, Colin 7000; Colin Medical Instruments). Subjects were instructed to lay supine on the modified tilt table (Model OT-9003, Omni Technologies) with arms outstretched and supported at the level of the heart.

Ninety min after instrumentation and baseline measurements, the subject was tilted 70º head-up. Subjects were instructed to stand quietly with feet shoulder width apart, keeping as still as possible to avoid muscle pump activity. Subjects remained in the 70° head-up tilt position for

45 min, until presyncope, or at the subject's request to stop. Presyncope was defined as loss of hemodynamic stability (decrease in blood pressure >20/10 mmHg and/or rapid decline in heart rate >25 bpm), diaphoresis, nausea, light-headedness, and/or hyperventilation. At the onset of presyncope the subject was placed in the Trendelenburg position (-10°) until blood pressure and heart rate stabilized. Once blood pressure and heart rate were stabilized the subject was moved to supine. Twenty min of recovery data were collected in the supine position.

Heart rate (HR) and blood pressure (BP)

HR and BP were sampled continuously at 1000- and 100-Hz, respectively, and stored in beat-to-beat format using a customized data collection program. BP was also obtained via auscultation just prior to the cardiac output measurements for determinations of systemic and splanchnic vascular conductance.

Cardiac output ($\dot{Q}_c$) and stroke volume (SV)

Before data recording began the subject was instructed on how to perform the $\dot{Q}_c$ rebreathing maneuver and was allowed to perform two practice maneuvers. We used the acetylene (C_2H_2) rebreathing technique as described by Triebwasser et al (Triebwasser *et al.*, 1977), which has been previously validated against direct Fick and thermodilution (Jarvis *et al.*, 2007). A mass spectrometer (Perkin-Elmer MGA 1000) was used to analyze the gas concentrations during the 20 sec rebreathing period. $\dot{Q}_c$ was measured every 10-min during baseline and recovery. A $\dot{Q}_c$ measurement was obtained upon HUT and then every 10-min during tilt. To correct for size differences $\dot{Q}_c$ was normalized to cardiac index, $\dot{Q}_i$

($\dot{Q}_i = \dot{Q}_c$ /body surface area), where body surface area (BSA) =$0.202 \cdot kg^{0.425} \cdot m^{0.725}$ (Du Bois & Du Bois, 1916). SV was calculated by $\dot{Q}_c$/HR and normalized to stroke volume index (SV_i=SV/BSA).

Splanchnic blood flow (SpBF)

SpBF was determined from continuous infusion of ICG (Grainger *et al.*, 1983). This method has been previously validated against hepatic vein catheterization (Grainger *et al.*, 1983). Blank samples for spectrophotometry were drawn prior to ICG bolus and infusion. Time zero began upon the priming bolus injection, followed immediately by continuous infusion of ICG (0.5 mg/min) until the experiment was completed. Every 5-min throughout baseline, tilt, and recovery a 3-ml blood sample was drawn, followed by a 3-ml saline flush. Plasma ICG concentration was determined in the same manner as the extraction ratio procedure. To determine SpBF we first determined splanchnic plasma flow (SpPF) by the following equation: $SpPF=\{I-[(C_{a2}-C_{a1})/dt]\cdot PV\}/(ER\cdot C_a)$, where I=infusion rate ($mg \cdot min^{-1}$); C_{a2} and C_{a1}=ICG concentrations at times 2 and 1; d*t*=time between samples; PV=plasma volume; ER=extraction ratio of ICG; and C_a=ICG concentration. SpBF was then calculated as SpPF/(1-hematocrit).

Vascular conductance

Systemic vascular conductance (SVC= $\dot{Q}_c$ /MAP) was calculated using blood pressure obtained via auscultation. Splanchnic vascular conductance (SpVC) was calculated as SpVC=SpBF/MAP. Non-splanchnic vascular conductance (non-SpVC) was calculated from the difference between SVC and SpVC (non-SpVC=SVC–SpVC).

Baroreflex sensitivity (BRS)

Spontaneous BRS was assessed during the three stages of the protocol using WinCPRS (Absolute Aliens, Öy, Finland). Briefly, spontaneous sequences were identified as SBP ramps (up or down) followed by the immediate (next beat) lengthening or shortening of the R-R interval, respectively. SBP changes of at least 1 mmHg with a corresponding change in R-R interval of at least 5 ms were necessary for a sequence to be identified. The slope of the linear regression line (R-R interval vs. SBP) was computed for each sequence. *A priori*, a correlation coefficient of >0.80 was selected in order to consider the sequence to be baroreflex-mediated. BRS for each subject was computed as the average of all slopes (up and down) for each stage.

Data summary and statistical analyses

Data were summarized as 5-min averages. Baseline included data collected in the supine position from min 80 to min 85, or 10 min prior to tilt. Tilt included a 3-min average of data during the last 5-min of the tilt with the 2-min immediately preceding tilt termination excluded from analyses. The tilt time point was chosen to capture a period of hemodynamic stability prior to presyncope. Recovery included data collected 10-min after the subject was returned to the supine position.

Kaplan-Meier survival analysis and Mood's median test were used to assess differences in tilt tolerance between the sexes. A repeated measures analysis of variance (sex × stage) was used for comparisons with Tukey post-hoc analysis used when significance was found. Analyses were performed using SAS 9.1. All data are presented as mean±SD. In all cases, differences with $p<0.05$ were considered significant.

Results

Table **3-1** summarized the subject characteristic. The groups were different in height, weight, BSA, $\dot{V}O_{2max}$, hematocrit, and blood volume (corrected for body weight) but age, BMI, and plasma volume (corrected for body weight) were not different between the groups.

Tilt tolerance. Figure **3-1** illustrates there was no difference in the number of women (0/14) completing 45 min of tilt compared to men (3/16) (χ^2=2.92, p=0.09) or in the median times to presyncope (15.7 vs. 21.8 min; χ^2=0.54, p=0.46).

Blood pressure, heart rate, cardiac index, stroke index, and systemic vascular conductance. MAP, HR, $\dot{Q}_i$, SV_i, and SVC are shown in Figure **3-2**. MAP was lower in women during supine baseline (77±5 vs. 86±9 mmHg, p<0.01), as well as immediately preceding presyncope (72±8 vs. 83±10 mmHg, p<0.01). There was a main effect of sex (p=0.02) with systolic blood pressure with women having lower values throughout the experiment (baseline: 114±7 vs. 130±12 mmHg; tilt: 91±17 vs. 110±15 mmHg; recovery: 119±11 vs. 131±12 mmHg). Diastolic blood pressure was lower in the women during rest and neared a significant difference during tilt (baseline: 58±5 vs. 66±8 mmHg, p<0.01; tilt: 52±9 vs. 60±13 mmHg, p=0.047; recovery: 61±7 vs. 65±10 mmHg). Systolic and diastolic blood pressures were lower during tilt than during supine rest (p<0.01). HR was not different between the sexes (baseline: 66±6 vs. 64±8 bpm; tilt: 96±13 vs. 94±10 bpm) and was significantly higher (p<0.01) during HUT. $\dot{Q}_c$ nor $\dot{Q}_i$ were different between the sexes ($\dot{Q}_c$,baseline: 6.4±1.5 vs. 7.5±1.4 L/min; $\dot{Q}_c$, tilt: 4.5±1.4 vs. 4.6±1.1 L/min; $\dot{Q}_c$, recovery: 6.4±1.0 vs. 6.7±1.2 L/min; $\dot{Q}_i$, baseline: 3.8±0.9 vs. 3.7±0.7 $L \cdot min^{-1} \cdot m^{-2}$; $\dot{Q}_i$, tilt: 2.7±0.8 vs. 2.3±0.5 $L \cdot min^{-1} \cdot m^{-2}$; $\dot{Q}_i$, recovery: 3.8±0.6 vs. 3.3±0.6 $L \cdot min^{-1} \cdot m^{-2}$) but were lower

during HUT in both sexes ($p<0.01$). Like $\dot{Q}_c$, SV was not different between the sexes during any stage (baseline: 94.6±23.4 vs. 101.6±20.3 ml/beat; tilt: 48.8±15.7 vs. 47.4±8.2 ml/beat; recovery: 98.2±21.0 vs. 94.6±20.9 ml/beat). SV_i was significantly lower during HUT in both sexes ($p<0.01$) and a main effect of sex ($p<0.001$) was present where the women had higher values throughout the experiment (baseline: 56.6±12.4 vs. 50.4±10.3 ml/m^2; tilt: 28.8±8.4 vs. 23.6±4.1 ml/m^2; recovery: 58.8±12.5 vs. 46.6±9.4 ml/m^2). SVC was not different between the sexes during any stage (baseline: 83.86±21.50 vs. 90.46±15.97 ml·min^{-1}·mmHg^{-1}; tilt: 61.05±15.53 vs. 57.51±16.10 ml·min^{-1}·mmHg^{-1}; recovery: 79.73±18.29 vs. 76.43±13.12 ml·min^{-1}·mmHg^{-1}) and was lower during tilt ($p<0.01$).

Splanchnic blood flow. SpBF (Figure **3-3**) was lower in women during rest (baseline: 1174±243 vs. 1670±391 ml/min, $p<0.01$; recovery: 1087±217 vs. 1583±406 ml/min, $p<0.01$; with a sex × stage interaction, $p<0.01$). Head-up tilt elicited a significant decrease in SpBF from baseline in both women and men ($p<0.01$), where women and men exhibited significant differences (884±300 vs. 1094±271 ml/min, $p<0.01$). Normalizing SpBF with BSA did not remove this difference during baseline or recovery (not shown), suggesting that the variation in size between the sexes does not account for the observed difference.

Splanchnic and non-splanchnic vascular conductance. Figure **3-4** portrays SpVC and non-SpVC. Using a two compartment model, we divided SVC into the SpVC and the non-SpVC circulations. We originally proposed women would have greater pooling in the splanchnic region (i.e., have greater SpVC). However, we found women were more constricted in the splanchnic region during rest since SpVC was lower in women during baseline (14.83±3.61 vs. 19.59±4.95 ml·min^{-1}·mmHg^{-1}, $p<0.01$) and recovery (12.99±3.71 vs. 17.96±4.39 ml·min^{-1}·mmHg^{-1}, $p<0.01$), where a significant sex × stage interaction existed ($p=0.02$). However, women did not

demonstrate a decrease in SpVC between baseline and HUT (ΔSpVC: -1.70±3.20 vs. -4.81±3.44 $ml \cdot min^{-1} \cdot mmHg^{-1}$), whereas the men showed a significant decrease ($p<0.01$), indicating women were less able to further constrict the splanchnic circulation during the orthostatic challenge. During HUT SpVC was not different between women and men (13.14±4.28 vs. 14.82±4.16 $ml \cdot min^{-1} \cdot mmHg^{-1}$). Non-SpVC was not different between the sexes during any stage (baseline: 69.04±21.56 vs. 70.89±14.19 $ml \cdot min^{-1} \cdot mmHg^{-1}$; tilt: 47.91±15.34 vs. 41.93±14.62 $ml \cdot min^{-1} \cdot mmHg^{-1}$; and recovery: 66.31±16.77 vs. 57.75±13.59 $ml \cdot min^{-1} \cdot mmHg^{-1}$), nor was the change between baseline and HUT; however, during HUT non-SpVC was significantly lower in both sexes ($p<0.01$).

Baroreflex sensitivity. BRS (Figure **3-5**) was higher in women ($p=0.01$). HUT elicited a significant decrease ($p<0.0001$) in BRS compared to baseline (baseline: 21.95±10.76 vs. 15.63±7.20 ms/mmHg; tilt: 5.26±2.68 vs. 4.59±2.52 ms/mmHg; recovery: 19.50±8.35 vs. 14.77±6.68 ms/mmHg).

Discussion

Based on previous studies (Frey & Hoffler, 1988; White & Montgomery, 1996), we hypothesized that women would have lower orthostatic tolerance and demonstrate greater blood flow to the splanchnic region during HUT, leading to splanchnic pooling and lower orthostatic tolerance. Despite a general consensus in the literature that women are less orthostatically tolerant than men (Convertino, 1998; Montgomery *et al.*, 1977; Waters *et al.*, 2002; White *et al.*, 1996), we did not find a statistical difference between women and men in the median tilt time to lipothymia or the proportion of survivors at 45 min of 70° HUT. We, however, found that women were 28% less tolerant than the men, which is consistent with past investigations

examining lower body negative pressure tolerance differences between the sexes (Convertino, 1998; Franke *et al.*, 2003). The lack of a statistically significant difference can be interpreted in several ways. First, others have also found no difference in orthostatic tolerance between women and men (Geelen *et al.*, 2002; Shvartz & Meyerstein, 1970). For example, although tilt time was not an endpoint in their study, Geelen and colleagues reported that 46 out of 109 subjects were unable to complete 13 min of 70° HUT and that those individuals were divided about evenly with the sexes (Geelen *et al.*, 2002). Furthermore, Shvartz and Meyerstein did not find a statistical difference between the number of women who fainted or showed "orthostatic weakness" when compared to the men during 20 min of 70° HUT (Shvartz & Meyerstein, 1970). Second, our study may have been underpowered to detect a difference. The tilt tolerance of the 30 subjects was not the primary outcome variable on which the original power calculations were based. Thus, the possibility of a Type II statistical error cannot be ignored.

Despite the similarity with median tilt times and the number of survivors at 45 min of tilt in our study, we found that blood pressure and regulation of blood pressure differed between the sexes. MAP was lower in women during supine rest, as well as immediately preceding presyncope, which is consistent with previous investigations (Franke *et al.*, 2003; Frey & Hoffler, 1988; Montgomery *et al.*, 1977). $\dot{Q}_i$ was comparable between the sexes, which has also been reported by others (Fu *et al.*, 2005; Shoemaker *et al.*, 2001). Additionally, we found that HR was not different between the sexes during baseline or immediately preceding presyncope. Absolute SV was not different between the sexes; however, SV_i was higher in women throughout the protocol. Our findings are consistent with Gotshall et al who reported no difference in HR and SV immediately preceding presyncope (Gotshall, 2000). However, our HR findings contrast with other studies reporting that women have a higher HR to compensate for the concomitantly lower SV or SV_i (Franke *et al.*, 2003; Shoemaker *et al.*, 2001).

Since $\dot{Q}_i$ was similar between the sexes, despite a higher SV_i in women, we further investigated other components of MAP to determine what might account for differences in orthostatic blood pressure between women and men. Our findings were similar to Shoemaker et al (Shoemaker *et al.*, 2001) who reported no difference in peripheral vascular resistance responses between women and men during HUT and Gotshall et al (Gotshall, 2000) who found no difference during exposure to stepwise lower body negative pressure. However, when the components of SVC were dissected into SpVC and non-SpVC, we found that sex differences existed in the splanchnic vasoconstrictor response.

Lastly, women demonstrated greater BRS than did the men in the supine position. Several studies indicated women had lower BRS when compared to men (Beske *et al.*, 2001; Convertino, 1998). This finding is consistent with other investigators (el Sayed & Hainsworth, 1995; Iacoviello *et al.*, 2008; Pitzalis *et al.*, 2003) who found that higher BRS was associated with lower orthostatic tolerance.

Thus, women exhibited three characteristics different from the men: 1) lower SpVC at rest; 2) an attenuated splanchnic vasoconstrictor response to HUT; and 3) enhanced BRS at rest. This section associates these principal findings with other features of blood pressure regulation.

Role of the splanchnic circulation in orthostatic blood pressure regulation

Rowell posited that in a closed circuit with a fixed volume, varying degrees of vasoconstriction occur in different regions to maintain central venous pressure and/or cardiac output (Rowell, 1986; Zidon & Sheriff, 2006). Compliant regions have the greatest volume to redistribute in order to maintain or increase central venous pressure/right atrial pressure. For example, Zidon and Sheriff, using a dog model, determined that diversion of blood from a non-compliant region (skeletal muscle) to a compliant circulation (splanchnic) could reduce right

atrial pressure by 0.75 mmHg (Zidon & Sheriff, 2006). Thus, regulation of compliant circulations plays a critical role in closed-loop cardiovascular control.

The significance of this blood volume redistribution can also be illustrated by Arbeille et al (Arbeille *et al.*, 2005). Recent evidence from their 90 day 6° head-down bed rest study suggested orthostatic intolerance was related to the degree of reduction in the cross sectional area of the portal vein during standing (Arbeille *et al.*, 2005). Although Arbeille and colleagues did not include women in their investigation, they noted a significant decrease in the portal vein cross sectional area between supine and standing in the orthostatically tolerant group, which was not observed in the intolerant group (Arbeille *et al.*, 2005). Consistent with this thought, the women in our study tended to be less tolerant than the men and exhibited a lesser degree of splanchnic vasoconstriction during HUT.

Sex differences in splanchnic blood flow

We hypothesized that women would have higher SpBF (i.e., less splanchnic vasoconstriction) when compared to the men. However, contrary to our hypothesis, SpBF was lower in the women during all stages. A direct comparison between our study and others is difficult since: 1) few studies have examined sex differences in SpBF; and 2) those available use Doppler ultrasound, which may not yield quantitatively similar results.

We were able to make a direct comparison with only two studies in the literature. Jensen et al used ICG to determine SpBF during feeding to determine regional substrate utilization (Jensen *et al.*, 1995; Jensen *et al.*, 1998). Our baseline data are consistent with the resting data Jensen and colleagues reported in their studies (Jensen *et al.*, 1995; Jensen *et al.*, 1998). Thus, there is support in the literature for the concept that SpBF is lower in women at rest.

Other studies examining SpBF used Doppler ultrasound to examine the superior mesenteric artery and/or the portal vein. For example, Szinnai et al were interested in sex differences relating to basal and post-prandial splanchnic hemodynamics (Szinnai *et al.*, 2001). They reported lower blood pressure and higher mean superior mesenteric artery velocity in women, with the vessel diameter comparable between the sexes (Szinnai *et al.*, 2001). These results would indicate that men showed a greater degree of vasoconstriction at rest compared to the women (Szinnai *et al.*, 2001). By contrast, a study by Qamar et al did not show differences with superior mesenteric artery blood flow between women and men during resting conditions (Qamar *et al.*, 1986). It should be noted, however, that Qamar et al did not separately report blood pressure, vessel cross-sectional area or diameter, or velocity. Thus, one cannot determine whether any sex differences existed in terms of superior mesenteric artery resistance or conductance (Qamar *et al.*, 1986).

The portal vein diameter is roughly twice that of the superior mesenteric artery with a corresponding flow of almost double (Maconi *et al.*, 1998). It could be argued that portal vein flow is more representative for determining changes in SpBF since the portal vein flow comprises 75% of hepatic blood flow (Segal, 2003). With regard to sex differences, Maconi et al measured portal vein flow in both women and men with Crohn's disease, reporting a trend toward lower portal vein flow in women (Maconi *et al.*, 1998). However, just as with the study by Qamar et al, these investigators did not report blood pressure, vessel size, or velocity so comparisons of conductance or resistance are not possible (Maconi *et al.*, 1998). To summarize, Doppler studies of the conduit vessels comprising the splanchnic circulation yield equivocal findings in terms of sex differences.

If resting SpBF is lower in women than men, what might be the reason(s) for this difference? Since cardiovascular hemodynamic variables such as $\dot{Q}_c$ and SV are normalized by taking into consideration body surface area we also normalized SpBF (Sp_i) as part of our analysis.

However, the disparity we observed in SpBF remained present despite normalization, indicating that differences in flow were not due to body size. One potential explanation for the lower resting SpBF in women is that they were relatively hypovolemic compared to the men. Specifically, even after normalizing for body weight, women had significantly lower blood volume than the men (Table 1). Past investigations have shown that hypovolemia can elicit increases in sympathetic activity (Fu *et al.*, 2005), which may lead to greater vasoconstriction in the splanchnic circulation. Therefore, it is conceivable that the women compensated for a lower blood volume with increased splanchnic vasoconstriction.

Are sex differences in orthostatic blood pressure regulation related to attenuated adrenergic responsiveness/vasoconstrictor reserve?

Our data may be the first to 1) document that the vasoconstrictor response in the splanchnic circulation; and 2) show that this response is blunted in women. Some, but not all investigations (Convertino, 1998; Franke *et al.*, 2003) report that other circulations in women demonstrate a blunted vasoconstrictor response when compared to men (Bowyer *et al.*, 2001; Ergul *et al.*, 1998; Kneale *et al.*, 2000). For example, an infusion of norepinephrine elicited smaller vasoconstrictor responses in the forearm of women (Kneale *et al.*, 2000). Kneale et al posited that women had greater β_2-adrenergic sensitivity because a significant increase in vasoconstriction was observed in women only when norepinephrine was co-infused with propranolol, a β-adrenergic blockade (Kneale *et al.*, 2000).

Additionally, an animal model suggests that differences between the sexes exist in intestinal blood flow during stress. Using a hemorrhagic shock model, Deitch et al reported female rats in the proestrous phase better preserved blood flow, including blood flow to the splanchnic circulation, even when corrected for tissue mass (Deitch *et al.*, 2008). Moreover,

when male rats were administered either a testosterone receptor blockade (Ba *et al.*, 2001) or estrogen (Kuebler *et al.*, 2002), improvements in organ blood flow during hemorrhagic shock were observed. However, it is also not clear how the vasculature adapts to long term, cyclical exposure to estrogen as in the normally cycling female. Taken together, previous findings suggest females demonstrate preserved intestinal blood flow compared to males, which may be due to lower testosterone and higher estrogen in women.

What might be the functional implication of these vascular responsiveness differences? Fu et al hypothesized the difference in an individual's tolerance to an orthostatic stress was dependent upon the degree of available vasoconstrictor reserve (Fu *et al.*, 2004b). They superimposed a cold pressor test to progressive LBNP, showing increases in MAP, muscle sympathetic nerve activity, and total peripheral resistance above that seen with LBNP alone (Fu *et al.*, 2004b). Furthermore, they found a significant positive correlation between increases in muscle sympathetic nerve activity with increases in tolerance to LBNP, with those individuals having the largest increase in muscle sympathetic nerve activity having the greatest increase in tolerance (Fu *et al.*, 2004b). Our findings support those of Fu and colleagues (Fu *et al.*, 2004b) since the women in our study demonstrated a lesser degree of splanchnic vasoconstriction during HUT and tended to be less tolerant.

What role does BRS play?

A general consensus in the literature, with respect to gender differences in BRS, does not exist. Several studies (el Sayed & Hainsworth, 1995; Iacoviello *et al.*, 2008; Pitzalis *et al.*, 2003) support our findings that individuals demonstrating lower tolerance also demonstrate enhanced BRS. This finding is difficult to interpret as it appears counter intuitive, but can probably be best explained by the relationship between plasma volume and BRS. Hypovolemia reportedly

contributes to OI because the circulating blood volume is reduced (el Sayed & Hainsworth, 1995). However, it has been also suggested that BRS is enhanced during hypovolemic conditions (Billman *et al.*, 1981; el Sayed & Hainsworth, 1995; Pawelczyk & Raven, 1989). The women in our study were relatively hypovolemic when compared to the men. It should also be noted, however, that baroreflex control of HR does not address control of the vasculature (systemic vascular conductance); thus, using an index of baroreflex control of the heart to baroreflex control of the vasculature is speculative.

Experimental considerations

Menstrual cycle status was a consideration with female subjects. We controlled for hormone status by having our female subjects complete all visits (blood volume determination/extraction ratio and tilt tolerance) during the early follicular phase of the menstrual cycle (days 2-6). Additionally, female subjects were queried before entrance into the study about the regularity of their cycle and none of the subjects were taking hormonal contraceptives during the course of the study, or for the previous 6 months.

A second consideration focuses on the $\dot{Q}_c$ determinations we reported. We used a rebreathing method that has been validated against invasive determinations such as direct Fick and thermodilution (Jarvis *et al.*, 2007). Unfortunately, due to the experimental constraints of a rebreathing method, it is not possible to obtain repeat measurements because a sufficient period of time must pass in order for the C_2H_2 from the gas mixture to clear the lungs (typically 5 minutes). However, this consideration does not change our overall finding that SpVC (i.e., vasoconstrictor reserve) was different between the sexes.

A third consideration is that we could not measure regional blood flow in other areas, such as the renal circulation. We acknowledge that a two compartment model is a simplistic

approach to decompose SVC. The non-SpVC portion of the model accounts for ~75% of SVC, assuming the splanchnic circulation receives ~25% of $\dot{Q}_c$. We cannot determine the contribution of other highly perfused beds (e.g., the kidneys) which would allow us to better assess SVC as a whole. One might hypothesize that flow in renal, or other vascular beds, may have offset the sex differences in SpVC such that SVC remained similar between the sexes. For example, Miller et al (Miller *et al.*, 1999) examined differences in renal hemodynamics between the sexes. In their analysis they reported no sex difference with respect to renal vascular resistance, however, they did not control for menstrual status. Further analysis of their data, dividing the women into "high" and "low" estrogen groups, revealed that a significant difference between estrogen statuses existed in renal vascular resistance with resistance being markedly lower in the low estrogen group. This would support our findings as the women in our investigation were in the low hormone phase of the menstrual cycle.

The last consideration is how to normalize for body size differences between the two groups. When appropriate, we corrected for body size by normalizing to BSA (cardiac output, stroke volume) or body weight (blood volume, plasma volume). We found that women had 13% lower blood volume, even after adjusting for body weight. It is possible that differences in body composition between the sexes could account for this difference since adipose tissue is poorly vascularized, compared to other tissues, and women tend to have a higher body fat percentage (American College of Sports Medicine, 2000). We did not perform measurements of body composition in our subjects. However, based on normative data, there exists about a 6% difference in body fat percentage between women and men at the 50^{th} percentile (15.9% for men and 22.1% for women) (American College of Sports Medicine, 2000). Based on these values the women and men in our study, on the average, both have about 13 kg of body fat. Assuming blood flow/volume to adipose tissue does not vary between the sexes, it is unlikely that body composition accounts for the difference in blood volume.

Conclusions

In conclusion, women demonstrated lower blood pressure during baseline and HUT and tended toward lower tilt-table tolerance. Women exhibited reduced SpBF and had lower SpVC during rest, suggesting higher resting tone, possibly due to increased baroreflex sensitivity resulting from decreased blood volume. Upon HUT both sexes decreased blood flow to the splanchnic region; however, women demonstrated a smaller decrease compared to the men. The principal reason for this difference appears to be an attenuated vasoconstrictor response in the splanchnic circulation.

Acknowledgements

We would like to thank Sandy Smithmyer, Christopher Sirolli, Dave Leone, Kyle Preston, Matt Hinkley, the General Clinical Research Center, and Critical Care Systems for their assistance with this study. This research was supported by NASA NNJ04HF45G (to J.A. Pawelczyk) and NIH MO1RR10732 (to General Clinical Research Center)

Table **3-1**: Subject characteristics. Values are means ± SD. BMI, body mass index; A_D, body surface area; $\dot{V}O_{2max}$, maximal oxygen uptake. *Significantly different from men, $p<0.05$.

	Women	**Men**
Age, yr	23 ± 6	23 ± 5
Height, cm	164.6 ± 6.6*	180.0 ± 6.3
Weight, kg	62.6 ± 12.5*	82.2 ± 7.4
BMI, kg/m^2	23 ± 3	25 ± 2
A_D, m^2	1.68 ± 0.18*	2.01 ± 0.11
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	39.3 ± 6.3*	47.1 ± 6.6
Hematocrit, %	38 ± 3*	44 ± 2
Plasma volume, ml/kg	29.4 ± 10.1	32.3 ± 4.0
Blood volume, ml/kg	50.1 ± 11.2*	57.8 ± 7.4

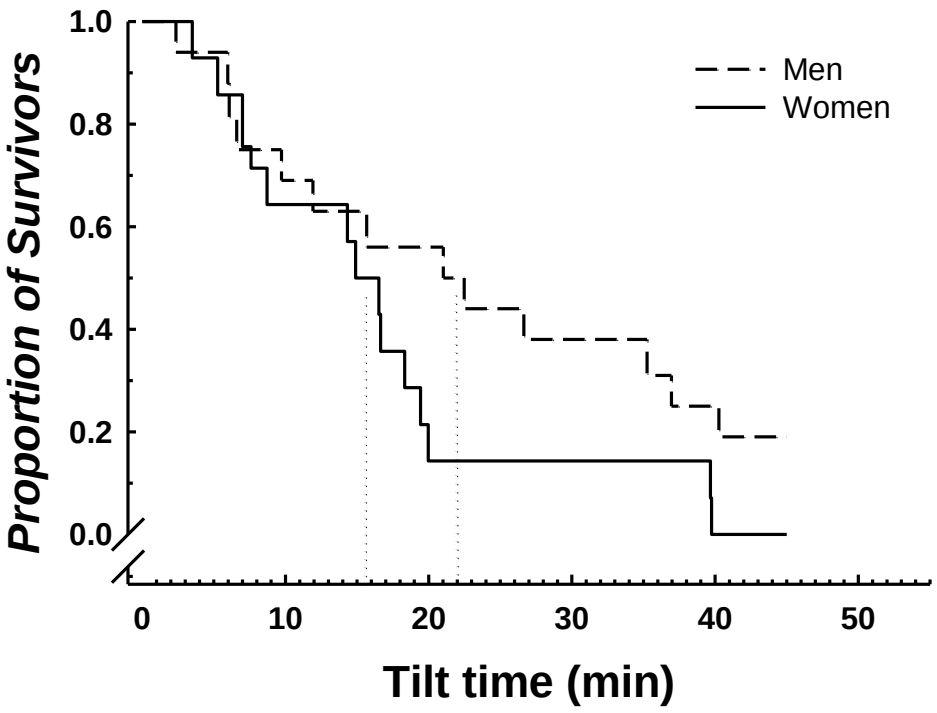

Figure **3-1**: Median tilt times and proportion of women survivors versus men. Median tilt times to presyncope (indicated by the vertical dashed lines) tended to be lower in women when compared to men (15.7 vs. 21.8 min) but were not statistically different (χ^2=0.54, p=0.46). There was no significant difference between the proportion of women surviving 45 min of tilt compared to the proportion of men (χ^2=2.92, p=0.09).

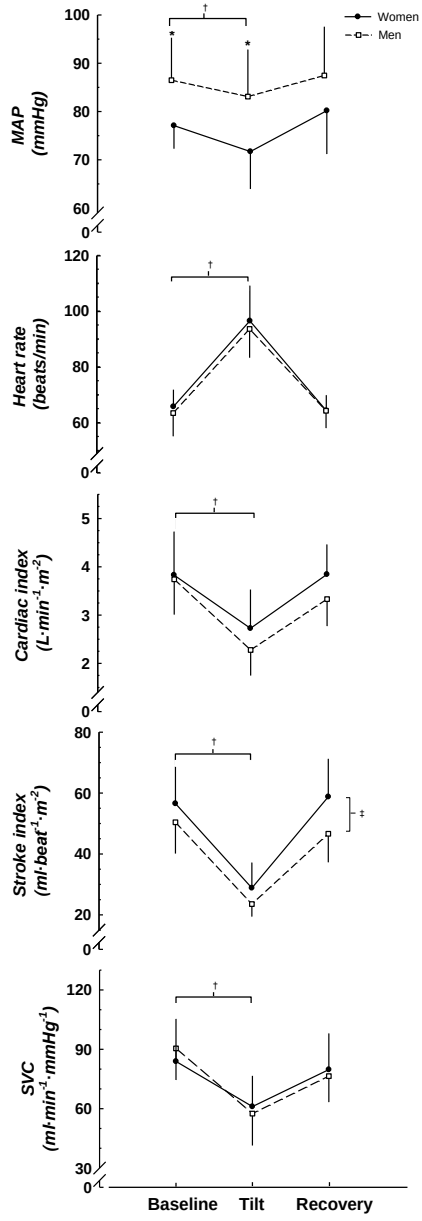

Figure **3-2**: Hemodynamic variables. Beat-to-beat MAP was lower immediately preceding presyncope and was lower in women at baseline and immediately preceding presyncope, but was not different during recovery. HR was higher during HUT compared to baseline and was not different in women compared to men during baseline, tilt, or recovery. $\dot{Q}_i$ was lower during HUT compared to baseline and was not different between the sexes at any time during the experiment. SV_i was lower during HUT compared to baseline. A main effect of sex was present with SV_i. SVC was not different between the sexes but was lower in both sexes during HUT. *Sex difference, $p<0.01$. †Difference in both sexes between baseline and tilt, $p<0.01$. ‡Main effect of sex, $p=0.02$.

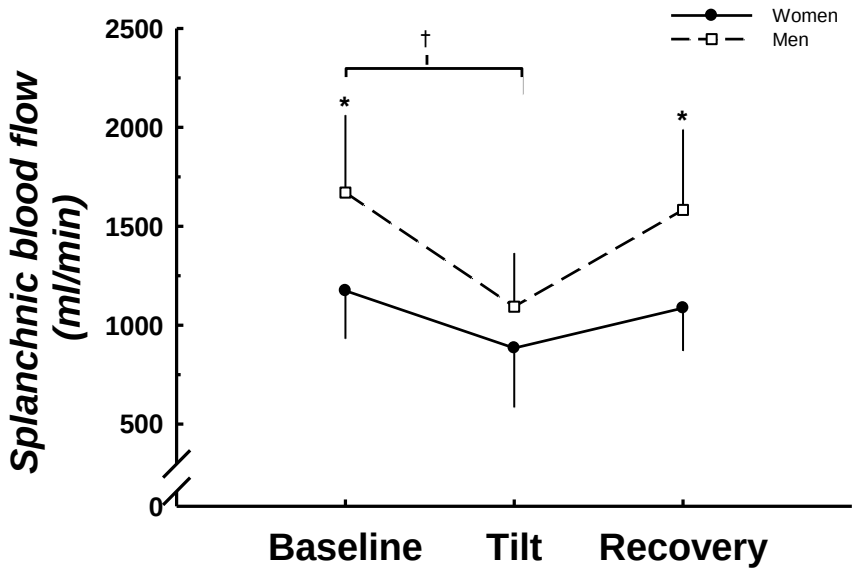

Figure **3-3**: Splanchnic blood flow. SpBF was lower in women during rest and recovery. *Sex difference, $p<0.01$. †Difference in both sexes between baseline and tilt, $p<0.01$.

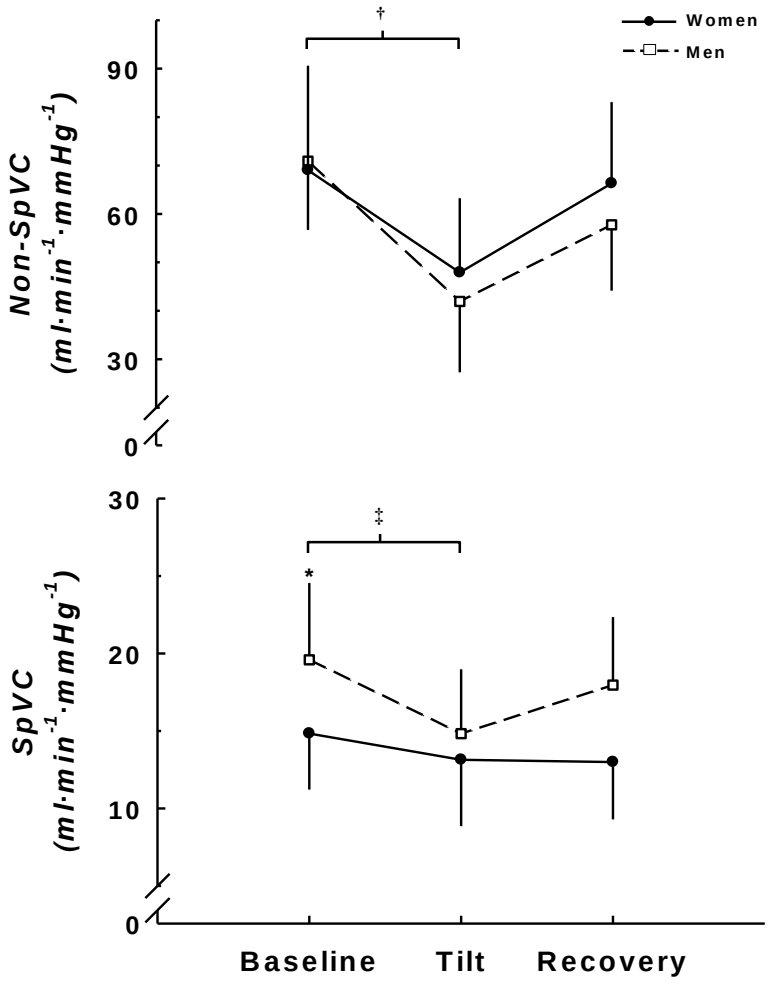

Figure **3-4**: Splanchnic and non-splanchnic vascular conductance. SpVC was lower in women during baseline but was not from the men during tilt. The change in SpVC from baseline to tilt (ΔSpVC) in women was half of that seen in men, where SpVC was significantly lower during HUT compared to baseline in men. Non-SpVC was significantly lower during HUT in both sexes and was not different between the sexes during any stage. *Sex difference, $p<0.01$. †Difference in both sexes between baseline and tilt, $p<0.01$. ‡ Difference in males between baseline and tilt, $p<0.05$.

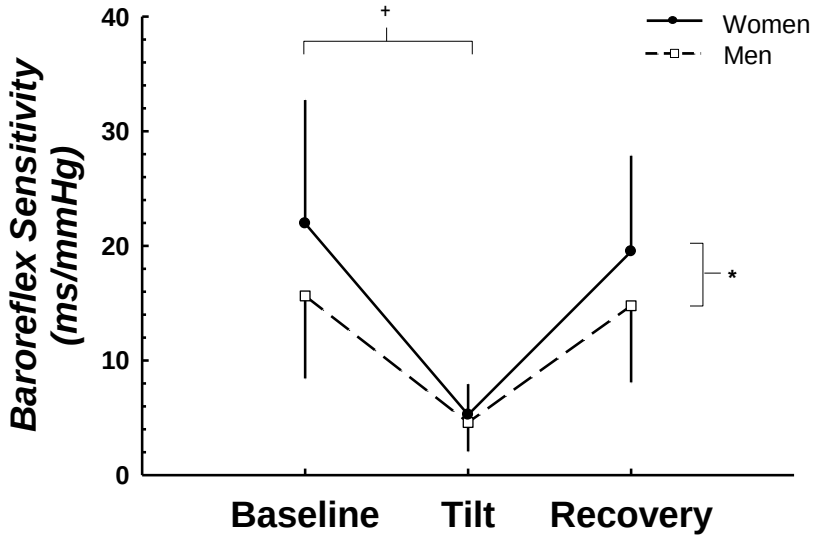

Figure **3-5**: Baroreflex sensitivity. BRS was higher in women in the supine position. HUT elicited a significant reduction in BRS. *Main effect of sex, p=0.01. †Difference between baseline and tilt, p<0.01.

Chapter 4

A SOMATOSTATIN ANALOG IMPROVES TILT TABLE TOLERANCE BY DECREASING SPLANCHNIC VASCULAR CONDUCTANCE

Introduction

Orthostatic intolerance (OI), the inability to maintain blood pressure in the upright posture, can affect a wide range of individuals. For example, OI can manifest itself in individuals that have undergone deconditioning due to bed rest (Levine *et al.*, 1997; Watenpaugh *et al.*, 2007), in patients with autonomic dysfunction (Hoeldtke *et al.*, 2006), or in astronauts upon return from spaceflight (Meck *et al.*, 2004; Platts *et al.*, 2004). Additionally, some (Convertino, 1998; Montgomery *et al.*, 1977; Waters *et al.*, 2002; White & Montgomery, 1996), but not all (Geelen *et al.*, 2002; Shvartz & Meyerstein, 1970), studies suggest that women are less orthostatically tolerant than men. The mechanism(s) behind OI are multi-factorial; however, since such varied populations can be affected it is plausible that a common characteristic between these groups might exist making these individuals more susceptible.

Past investigations have suggested that an attenuated vasoconstrictor reserve contributes to OI (Arbeille *et al.*, 2005; Fu *et al.*, 2004b). For example, Fu et al were the first to demonstrate that an increase in muscle sympathetic nerve activity (MSNA), provoked by a cold pressor stimulus, was related to improvements in the time to presyncope. That is, Individuals showing the greatest increase in MSNA also had the largest increase in total peripheral resistance during the cold pressor test, which resulted in greater tolerance to lower body negative pressure (Fu *et al.*, 2004b).

Along these lines, Arbeille et al examined the constrictor response in the portal vein during 80° head-up tilt. They identified "tolerant" and "non-tolerant" individuals, noting that the portal vein cross-sectional area decreased from supine to head-up tilt in the tolerant individuals; however, this was not observed in the non-tolerant group (Arbeille *et al.*, 2005). We have previously reported similar findings (manuscript in review). During 70° head-up tilt, women tended to be less orthostatically tolerant and also did not demonstrate a decrease in splanchnic vascular conductance, which was observed in the men (manuscript in review). The findings of Arbeille and colleagues (Arbeille *et al.*, 2005), as well as findings from our own laboratory, suggest that the inability to adequately vasoconstrict the splanchnic circulation influences an individual's orthostatic tolerance. Taken together, these studies suggest that therapies targeting splanchnic vasoconstriction/vascular conductance during an orthostatic stress should improve tolerance.

While several approaches may be used to elicit splanchnic vasoconstriction, administration of a somatostatin (sst) analog offers a somewhat selective intervention because of the preferential distribution of sst_2 and sst_5 receptors in the gut. For example, Hoeldtke et al (Hoeldtke *et al.*, 1998) administered octreotide acetate, a sst analog with a biological half-life considerably longer than native sst, to patients with post-prandial hypotension. They noted improvements in blood pressure maintenance that were greater than those observed with midodrine, an α_1-adrenergic agonist (Hoeldtke *et al.*, 1998). In a separate study, Hoeldtke et al (Hoeldtke *et al.*, 2006) reported improvements with standing times in patients with OI after octreotide. These findings support the idea that selective constriction of the splanchnic circulation improves blood pressure maintenance. However, to the best of our knowledge, no group has fully characterized splanchnic and systemic hemodynamics after the administration of the octreotide with respect to orthostatic tolerance in otherwise healthy groups.

Thus, the purpose of this randomized, double-blinded study was: 1) to characterize splanchnic and systemic hemodynamics in response to octreotide acetate; and 2) to determine the effect of selective splanchnic vasoconstriction on tilt table tolerance. We hypothesized that octreotide acetate would reduce SpBF and SpVC and that these reductions would lead to improvements in tilt table tolerance. Additionally, we hypothesized that the improvement in tilt tolerance would be greater in the women.

Methods

Subjects

Thirty subjects (14 women, 16 men) gave written informed consent to participate in the study, which was approved by the Institutional Review Board at the Pennsylvania State University. Descriptive characteristics of the subjects are outlined in Table 1. Exclusionary criteria included: smoking, BMI <20 kg/m^2 or ≥30 kg/m^2, hormonal contraceptive use, autonomic dysfunction or clinically diagnosed OI, peripheral vascular disease, and allergies to local anesthetics, shellfish, iodides, penicillin, or sulfa drugs.

Subjects were admitted to the General Clinical Research Center (GCRC) at the Pennsylvania State University for study visits and were instructed to fast for at least 8 hrs; they also refrained from alcohol intake for 24-hrs, and caffeine intake for 48-hrs prior to each study visit. Female participants were tested during the early follicular phase of the menstrual cycle (days 2-7). Minson et al (Minson *et al.*, 2000a) previously reported a direct relationship between MSNA and estrogen. Thus, the early follicular phase was chosen because estrogen concentration would be the lowest. This should have maximized our ability to detect any sex differences because sympathetic activity differences between the sexes should have been the greatest.

Female subjects submitted urine samples for pregnancy testing during screening, as well as on the day of the study.

Experimental Design

Subjects completed a total of five visits. On the first visit, subjects had a 12-lead resting electrocardiogram and blood pressure measurements, and blood drawn for a complete blood count, CHEM 24, and coronary risk profile. Subjects returned within a week for a physical exam, evaluation of medical history, and a maximal graded treadmill exercise test. The third visit was comprised of the extraction ratio and plasma volume determination. During the fourth and fifth visits subjects underwent a tilt tolerance test, with the order of treatment (placebo or octreotide) randomly assigned.

Extraction ratio of ICG and plasma volume determination

We determined the hepatic extraction ratio of indocyanine green (ICG; Akorn Inc.), based on a two-compartment model (Grainger *et al.*, 1983). The subject had one antecubital intravenous (18-20 gauge) catheter placed into each arm. The left arm was used for ICG injection, while the right arm was used for blood sample withdrawal. Subjects were supine for a minimum of 30 minutes prior to withdrawal of an aliquot of blood to serve as a spectrophotometer blank, followed by the bolus injection of 0.5 mg/kg ICG. After the bolus injection, a 3-ml blood sample was withdrawn from the other arm every five minutes, followed by a 3-ml saline flush. Samples were centrifuged at 3,000 rpm for 20 minutes, and the plasma concentration of ICG was measured by spectrophotometry (805 nm for absorbance and 910 nm for turbidity). A separate extraction ratio was calculated for each subject from the exponential

decay of the plasma disappearance curve of ICG (SigmaPlot 9.0, San Rafael, CA) fit utilizing the Marquardt-Levenberg algorithm. In addition to the extraction ratio, plasma volume was estimated by indicator-dilution by back extrapolation of the plasma concentration of dye to the moment of injection.

70° head-up tilt (HUT)

The placebo and octreotide conditions were randomly assigned during the visits when tilt tolerance was assessed. On these visit days, the subjects received a brief physical exam by a GCRC clinician, followed by catheter placement (one antecubital in each arm, the right arm for ICG and placebo/octreotide and the left arm for blood sample withdrawal). Subjects were then moved to the laboratory and instrumented in our laboratory for electrocardiogram (3-lead ECG; Hewlett-Packard 78534A) and blood pressure (arterial applanation tonometry, Colin 7000; Colin Medical Instruments). Subjects were instructed to lay supine on a modified tilt table (Model OT-9003, Omni Technologies). The arms outstretched and supported at the level of the heart.

Fifty min after instrumentation subjects received an infusion of either placebo (50 mL saline) or octreotide acetate (100 μg or 125 μg octreotide acetate mixed with 50 mL of saline for the women and men, respectively) over 15 min. The doses were chosen based on the average weight of each sex. Ninety min after instrumentation and baseline measurements, the subject was tilted 70º head-up. Subjects were instructed to stand quietly with feet shoulder width apart. Subjects remained this position for 45 min, until presyncope, or at the subject's request to stop. Presyncope was defined as loss of hemodynamic stability (decrease in blood pressure >20/10 mmHg and/or rapid decline in heart rate >25 bpm), diaphoresis, nausea, light-headedness, and/or hyperventilation. At the onset of presyncope the subject was placed in Tredelenburg position

(-10°) until blood pressure and heart rate stabilized. Twenty min of recovery data were collected in the supine position.

Heart rate (HR) and blood pressure (BP)

HR and BP were sampled continuously at 1000- and 100-Hz, respectively, and stored in beat-to-beat format using a customized data collection program. BP was also obtained via auscultation for determinations of systemic and splanchnic vascular conductance.

Cardiac output ($\dot{Q}_c$) and stroke volume (SV)

We used the acetylene (C_2H_2) rebreathing technique as described by Triebwasser et al (Triebwasser *et al.*, 1977), which has been previously validated against direct Fick and thermodilution (Jarvis *et al.*, 2007). Before data recording began the subject was instructed on how to perform the $\dot{Q}_c$ rebreathing maneuver and was allowed to perform two practice maneuvers. A mass spectrometer (Perkin-Elmer MGA 1000) was used to analyze the gas concentrations during the 20 sec rebreathing period. $\dot{Q}_c$ was measured every 10-min during baseline and recovery, except during the infusion of placebo or octreotide. A $\dot{Q}_c$ measurement was obtained upon HUT and then every 10-min during tilt. To correct for size differences $\dot{Q}_c$ was normalized to cardiac index, $\dot{Q}_i$ ($\dot{Q}_i = \dot{Q}_c$ /body surface area), where body surface area (BSA) $=0.202 \cdot kg^{0.425} \cdot m^{0.725}$ (Du Bois & Du Bois, 1916). SV was calculated as $\dot{Q}_c$ /HR and normalized to stroke volume index (SV_i=SV/BSA).

Splanchnic blood flow (SpBF)

SpBF was determined from continuous infusion of ICG (Grainger *et al.*, 1983). Blank samples for spectrophotometry were drawn prior to ICG bolus and infusion. Time zero began upon the priming bolus injection, followed immediately by continuous infusion of ICG (0.5 mg/min) until the experiment was completed. Every 5-min throughout baseline, tilt, and recovery a 3-ml blood sample was drawn, followed by a 3-ml saline flush. Plasma ICG concentration was determined in the same manner as the extraction ratio procedure. To determine SpBF we first determined splanchnic plasma flow (SpPF) by the following equation: $SpPF=\{I-[(C_{a2}-C_{a1})/dt]\cdot PV\}/(ER\cdot C_a)$, where I=infusion rate ($mg\cdot min^{-1}$); C_{a2} and C_{a1}=ICG concentrations at times 2 and 1; d*t*=time between samples; PV=plasma volume; ER=extraction ratio of ICG; and C_a=ICG concentration. SpBF was then calculated as SpPF/(1-hematocrit).

Vascular conductance

Systemic vascular conductance ($SVC=\dot{Q}_c/MAP$) was calculated using blood pressure obtained via auscultation. Splanchnic vascular conductance (SpVC) was calculated as SpVC=SpBF/MAP. Non-splanchnic vascular conductance (non-SpVC) was calculated from the difference between SVC and SpVC (non-SpVC=SVC–SpVC). Since the numerator and the denominator scale to body size, the quotients (i.e., conductance) were not normalized to body size.

Data summary and statistical analyses

Data were summarized as 5-min averages. The infusion of octreotide or placebo started 50 min after the subject was placed in the supine position and was complete 15 min before tilting began. Baseline was designated as min 80 to min 85, or 10 min prior to tilt. Tilt included a 3-min average of data during the last 5-min of the tilt with the 2-min immediately preceding tilt termination excluded from analyses. This tilt time point was chosen to capture a period of hemodynamic stability prior to presyncope. Recovery included data collected 10-min after the subject was returned to the supine position.

Kaplan-Meier survival analysis and Mood's median test were used to assess differences in tilt tolerance between the sexes and conditions. Sex differences were analyzed using repeated measures analysis of variance ANOVA (sex × stage × treatment). Data were expressed as changes from baseline (after the infusion) to HUT (Δbaseline). A repeated measures ANOVA was also used to assess the time course of treatment (pre and post) and HUT using a baseline prior to infusion of octreotide or placebo. Tukey post-hoc analysis was used when significance was found. The LIFEREG procedure in SAS was used to assess the relationship between the change in SpBF and the change in time between the two conditions. LIFEREG allows for regression analysis to be performed on right censored data since we ended tilt tests at 45 min if individuals did not become presyncopal. Analyses were performed using SAS 9.1 (Cary, NC). Data are presented as mean ± SD. In all cases, differences with $p<0.05$ were considered significant.

Results

Table **4-1** summarizes the subject characteristics. The groups were different in height, weight, BSA, $\dot{V}O_{2max}$, hematocrit, and blood volume (ml/kg) but age, BMI, and plasma volume (ml/kg) were not different between the groups.

Tilt tolerance. Figure **4-1** illustrates the median tilt times and proportion of individuals surviving 70° HUT after administration of placebo or octreotide. Both women and men demonstrated improvements in tilt time after administration of octreotide where 7/14 (χ^2= 9.83, $p<0.01$) women and 10/16 (χ^2=7.55, $p<0.01$) men completed 45 min of tilt. Median tilt times were improved from 15.7 min to 37.0 min for the women and 21.8 to 45.0 min for the men (χ^2=9.60, $p<0.01$). There was no difference between the sexes in the median tilt time after the administration of octreotide.

Blood pressure, heart rate, cardiac index, stroke index, and systemic vascular conductance. MAP, HR, $\dot{Q}_i$, SV_i, and SVC are shown in Table **4-2** and Figure **4-2**. HUT induced significant changes in all hemodynamic variables ($p<0.01$). Women demonstrated higher $\dot{Q}_i$ ($p<0.05$), higher SV_i ($p<0.05$), and lower SVC ($p<0.05$) compared to the men. Changes in MAP, HR, $\dot{Q}_i$, and SV_i, elicited by tilt were not different between the sexes. Octreotide induced an increase in SVC ($p<0.05$).

Splanchnic blood flow. We saw an immediate reduction (20±19%) in SpBF after the infusion of octreotide was initiated where its nadir was achieved, in most individuals, 10 min after the onset of infusion. This reduction was not significant for the time points we chose for analysis. However, a separate paired t-test indicated a significant reduction ($p<0.01$) was present 5 min

after the infusion was complete. HUT elicited a decrease in SpBF ($p<0.01$). Analysis of variance of SpBF (Table **4-2** and Figure **4-3**) indicated that a significant sex × stage interaction was present ($p<0.01$), where women demonstrated a smaller reduction in SpBF between baseline and tilt (Δbaseline) when compared to the men.

Splanchnic and non-splanchnic vascular conductance. SpVC and non-SpVC are outlined in Table **4-2** and portrayed in Figure **4-4**. SpVC was reduced after administration of octreotide ($p<0.05$). SpVC and non-SpVC were lower during tilt ($p<0.01$) compared to supine. A significant difference in the SpVC response to tilt (Δbaseline) existed between the women and men where the women showed little change ($p<0.05$). Women demonstrated higher non-SpVC compared to the men ($p<0.01$). Octreotide elicited a treatment effect, raising non-SpVC ($p<0.05$).

Relationship between SpBF and tilt table tolerance. To explore the impact of the reduction in SpBF induced by octreotide on tilt tolerance we examined the differences in average SpBF during tilt for both conditions and assessed its relationship with changes in tilt tolerance (Figure **4-5**). The LIFEREG procedure for right censored data indicated a significant relationship existed between the change in SpBF induced by octreotide acetate and the improvements in tilt table tolerance in women (Δtilt time = 2.5 - 0.0083 ΔSpBF, $p=0.0051$) but not men (Δtilt time = 3.41 - 0.0008 ΔSpBF, $p=0.59$).

Discussion

What we present that differs from past investigations is an assessment of splanchnic and systemic hemodynamics in humans after octreotide administration and its corresponding effect on

tilt table tolerance. Based on the findings of previous investigations (Arbeille *et al.*, 2005; Fu *et al.*, 2004b), we hypothesized that selective constriction of the splanchnic circulation with a sst analog would lead to improvements in tilt table tolerance and that this improvement would be greater in women. The principal findings from this investigation are: 1) administration of the 100-125 μg octreotide acetate significantly reduced SpBF and SpVC and improved tilt table tolerance; this improvement was comparable between the sexes, contrary to our hypothesis; 2) women demonstrated a blunted response in splanchnic hemodynamics when compared to men; and 3) the systemic effects of octreotide acetate differed in the women. Thus, the following section addresses the vascular effects of octreotide acetate (a somatostatin analog), the functional implications of decreasing SpBF and SpVC, and the potential reasons for the different responses observed in women after octreotide administration.

The vascular effects of somatostatin

The pharmacology and physiology of octreotide have been reviewed extensively (Katz & Erstad, 1989; Reichlin, 1983; Reynaert & Geerts, 2003). Briefly, five somatostatin receptor subtypes (sst_{1-5}) are expressed in the brain, stomach, liver, kidneys, intestines, and pancreas (Patel & Srikant, 1997). Receptor subtypes sst_2 and sst_5 are preferentially located in the stomach and pancreas (Patel & Srikant, 1997). Octreotide acetate is a synthetic octapeptide and is a somatostatin analog that demonstrates high affinity for sst_2 and sst_5 and intermediate affinity for sst_3 (Reynaert & Geerts, 2003). The relatively long half-life of octreotide, ~100 min vs. 1-3 min (Sheppard *et al.*, 1979), make it attractive for experimental and therapeutic manipulation.

The exact mechanism of octreotide, in terms of reducing SpBF, has not been clearly defined but there are three potential candidates: modulation of gut vasodilatory hormones (Law *et al.*, 1995), an indirect vasoconstrictive effect via potentiation of vasoconstrictors such as

endothelin-1 (Wiest *et al.*, 2001), and a direct vasoconstrictive effect (Dimech *et al.*, 1995). During pilot testing for the current study we initially administered octreotide as a bolus injection but noted such an immediate pressor response (similar to phenylephrine) that we modified our protocol to an i.v. infusion. Others (Bosch *et al.*, 1981; Panes *et al.*, 1994) have also reported near immediate reductions in SpBF. Thus, the rapidity in which octreotide induces a reduction in SpBF suggests a direct vascular effect. Dimech et al (Dimech *et al.*, 1995) reported that octreotide induced contractions in the isolated human saphenous vein in a concentration-dependent manner that were greater than native somatostatin. They ruled out octreotide interactions with endothelium-dependent release of thromboxane A_2 and norepinephrine induced contractions because both indomethacin (thromboxane A_2 antagonist) and phentolamine (norepinephrine antagonist) failed to reduce the contractile response (Dimech *et al.*, 1995). Dimech and colleagues (Dimech *et al.*, 1995) concluded that octreotide increases Ca^{2+} entry through the L-type Ca^{2+} channel because co-administration of verapamil and nifedipine (L-type Ca^{2+} antagonists) attenuated the contractile response.

The functional implication of reducing splanchnic blood flow and splanchnic vascular conductance

We previously reported that women were less able to vasoconstrict the splanchnic circulation because they showed no significant reduction in SpVC between supine and HUT (manuscript in review). Because the splanchnic circulation is highly compliant and receives a large proportion of cardiac output during resting conditions, a gravity induced shift in blood volume promotes pooling in compliant regions such as the gut (Rowell, 1993). Individuals lacking the ability to adequately constrict this circulation reduce the effective circulating blood volume, decreasing venous return, cardiac preload, and, therefore, cardiac output; these

reductions challenge the maintenance of blood pressure (Rowell, 1993). We previously outlined the candidate reasons for the blunted response observed in the splanchnic circulation of women (manuscript in review).

When these same women were exposed to octreotide acetate in the current investigation there was a significant improvement in tilt table tolerance. However, we also noted a non-responsiveness similar to the placebo conditions with both SpVC and SpBF. This finding is generally consistent with studies of other circulations in women (Bowyer *et al.*, 2001; Ergul *et al.*, 1998; Kneale *et al.*, 2000).

Octreotide did not induce larger changes in SpBF or SpVC during HUT; however, it produced a lower flow and conductance compared to placebo conditions. Diversion of blood from compliant to non-compliant circulations may increase the effective circulating blood volume (Rowell, 1986; Zidon & Sheriff, 2006). Wong and colleagues recently reported modest but significant increases in right atrial pressure after administration of 1.5 µg/kg octreotide acetate in the conscious dog (Wong & Sheriff, 2007). Thus, we speculate that right atrial filling pressure was supported by a reduction in blood flow to the gut. This was suggested by higher, although not statistically significant, $\dot{Q}_i$ and SV_i during HUT in the octreotide trial.

It is not clear why the relationship between the change in SpBF and the change in tilt table tolerance was only significant in women. However, there are two plausible explanations for this finding. First, a greater percentage of the men's data were right censored because three men completed 45 min of tilt not only during the octreotide trial but during the placebo trial as well. Thus, we do not have an assessment of the true improvement due to the octreotide intervention. This concern holds true for any subject's data that was censored during tilt. Alternatively, this finding might represent, consistent with past findings, true physiological differences exist between women and men in terms of the vasoconstrictor response. Thus, selective constriction with a somatostatin analog could translate to larger improvements for this group.

Potential explanations for the divergent response in systemic vascular conductance and non-splanchnic vascular conductance in women after octreotide administration

Unexpectedly, we found a treatment effect of octreotide in SVC and non-SpVC. Even more unexpectedly, we found differences between the sexes with this response. The women responded differently after an infusion of octreotide acetate compared to their control trial, as well as to the men during both trials. An increase in SVC and non-SpVC suggests that another circulation is vasodilating in response to octreotide. The most logical choice would be the renal circulation because 1) it also expresses sst receptors (Bhandari *et al.*, 2008; Patel & Srikant, 1997); 2) receives a large proportion of $\dot{Q}_c$ (Rowell, 1986); and 3) contributes to 30% of SVC (Pricher *et al.*, 2004). Therefore, this section will outline the effect of octreotide on renal blood flow and then will explore potential explanations for a divergent sex response we observed.

The effect of octreotide on renal blood flow is not well defined because there have been reports of increases (Kalambokis *et al.*, 2005) or no change (Ottesen *et al.*, 2001) in flow. One consistent finding, however, indicates that renin is significantly reduced after octreotide is administered (Kalambokis *et al.*, 2005; Sabat *et al.*, 1998; Sieber *et al.*, 1988). This may be of functional importance since during states of reduced renal perfusion pressure, such as during HUT, angiotensin II induces constriction in both the splanchnic and renal beds (Boulpaep, 2003). Therefore, removal or blunting of renin's influence on angiotensin II production could translate into increases in SVC and non-SpVC.

The differential effect of octreotide in women could be explained by the interaction of estrogen and renin since estrogen has been shown to modulate plasma renin activity (Hirshoren *et al.*, 2002). For example, Hirshoren et al (Hirshoren *et al.*, 2002) examined several cardiovascular regulatory hormones throughout the menstrual cycle, reporting that the low estrogen phase (consistent with our study population) was associated with the lowest renin concentration (Hirshoren *et al.*, 2002). Taken together, these findings suggest that women might be more

sensitive to the effect of octreotide and its influence on the renal circulation, especially when cardiovascular regulatory hormones, such as renin, might already be reduced.

Conclusions

In conclusion, populations unable to adequately vasoconstrict the splanchnic region might be susceptible to OI. We pharmacologically induced splanchnic vasoconstriction with a somatostatin analog, which decreased SpBF and SpVC. These reductions led to improvements in tilt table tolerance in both women and men. Similar to previous reports that women demonstrate an attenuated constrictor response, the women in our study showed a blunted response in splanchnic hemodynamics to HUT. However, only in the women, was the improvement in tilt time significantly related to the reduction in SpBF induced by octreotide.

Acknowledgements

We would like to thank Sandy Smithmyer, Christopher Sirolli, Matt Hinkley, the General Clinical Research Center, and Critical Care Systems for their assistance with this study. This research was supported by NASA NNJ04HF45G (to J.A. Pawelczyk), NIH MO1RR10732 (to General Clinical Research Center), and the NASA Harriet G. Jenkins Pre-doctoral fellowship (to S.S. Jarvis).

Table **4-1**: Subject characteristics. Values are means±SD. BMI, body mass index; A_D, body surface area; $\dot{V}O_{2max}$, maximal oxygen uptake. *Significantly different from men, $p < 0.05$.

	Women	**Men**
Age, yr	23 ± 6	23 ± 5
Height, cm	164.6 ± 6.6*	180.0 ± 6.3
Weight, kg	62.6 ± 12.5*	82.2 ± 7.4
BMI, kg/m^2	23 ± 3	25 ± 2
A_D, m^2	1.68 ± 0.18*	2.01 ± 0.11
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	39.3 ± 6.3*	47.1 ± 6.6
Hematocrit, %	38 ± 3*	44 ± 2
Plasma volume, ml/kg	29.4 ± 10.1	32.3 ± 4.0
Blood volume, ml/kg	50.1 ± 11.2*	57.8 ± 7.4

Table **4-2**: Hemodynamic variables. Values are means±SD. MAP, mean arterial pressure (mmHg); HR, heart rate (bpm); $\dot{Q}_i$, cardiac index ($L \cdot min^{-1} \cdot m^{-2}$); SV_i, stroke index ($ml \cdot beat^{-1} \cdot m^{-2}$); SVC, systemic vascular conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$); SpVC, splanchnic vascular conductance $ml \cdot min^{-1} \cdot mmHg^{-1}$); non-SpVC, non-splanchnic vascular conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$); SpBF, splanchnic blood flow (ml/min). *Difference between sexes, main effect, $p<0.05$. **Δbaseline sex × stage interaction, $p<0.05$. †Δbaseline difference between treatments, $p<0.05$. ‡Difference induced by HUT, $p<0.05$. §Difference pre- and post-infusion, $p<0.05$.

Placebo

	Women			**Men**			**Significance**
	Baseline	**Tilt ‡**	**Recovery**	**Baseline**	**Tilt**	**Recovery**	
MAP	77 ± 5	72 ± 8	80 ± 9	86 ± 9	83 ± 10	87 ± 10	
HR	66 ± 6	96 ± 13	64 ± 6	64 ± 8	94 ± 10	64 ± 6	
$\dot{Q}_i$	3.83 ± 0.90	2.67 ± 0.79	3.84 ± 0.62	3.66 ± 0.67	2.28 ± 0.53	3.33 ± 0.56	*
SV_i	56.54 ±	28.81 ± 8.35	58.78 ± 12.46	48.19 ± 8.92	23.55 ± 4.11	45.74 ± 9.09	*
SVC	83.87 ±	61.05 ± 15.53	79.73 ± 18.29	90.47 ± 15.95	57.51 ± 16.10	76.43 ± 13.11	* †
SpBF	1174 ± 243	884 ± 300	1087 ± 217	1671 ± 391	1124 ± 273	1637 ± 447	**
SpVC	14.83 ± 3.60	13.14 ± 4.28	12.99 ± 3.70	19.59 ± 4.94	15.59 ± 5.05	18.67 ± 5.05	**
Non-SpVC	69.04 ±	47.91 ± 15.34	66.30 ± 16.77	70.87 ± 14.19	41.92 ± 14.62	57.76 ± 13.60	* †

Octreotide

	Women			**Men**			**Significance**
	Baseline	**Tilt ‡**	**Recovery**	**Baseline**	**Tilt**	**Recovery**	
MAP	80 ± 9	74 ± 6	75 ± 11	88 ± 12	82 ± 9	85 ± 7	
HR	66 ± 4	96 ± 12	69 ± 6	67 ± 7	94 ± 15	66 ± 8	
$\dot{Q}_i$	3.63 ± 0.84	2.91 ± 0.78	3.87 ± 0.79	3.81 ± 0.75	2.42 ± 0.59	3.49 ± 0.67	*
SV_i	53.82 ± 14.24	32.29 ± 12.10	57.40 ± 11.09	51.23 ± 9.94	24.94 ± 6.09	46.43 ± 8.29	*
SVC	73.06 ± 17.93	71.54 ± 26.97	80.50 ± 19.33	90.91 ± 15.31	60.97 ± 23.44	83.19 ± 13.23	*
SpBF	1004 ± 201	697 ± 180	920 ± 164	1496 ± 426	1023 ± 291	1644 ± 418	**
SpVC	11.82 ± 2.85	10.40 ± 2.40	9.78 ± 4.03	16.76 ± 4.53	12.29 ± 3.68	19.27 ± 7.31	** §
Non-SpVC	61.24 ± 16.61	61.14 ± 25.44	70.72 ± 18.30	74.15 ± 16.32	48.68 ± 21.84	63.91 ± 17.38	*

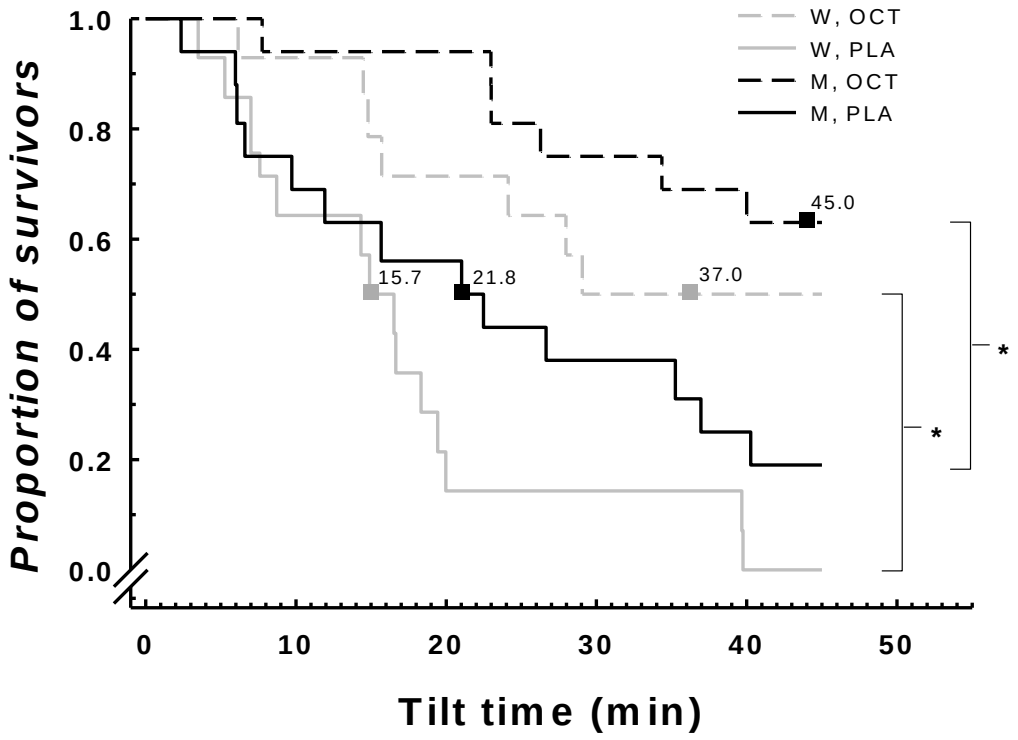

Figure **4-1**: Median tilt times and proportion of individuals surviving 70° HUT after administration of placebo or octreotide. Both women and men demonstrated improvements in tilt time after administration of octreotide where 7/14 (χ^2= 9.83, p<0.01) women and 10/16 (χ^2=7.55, p<0.01) men completed 45 min of tilt. Median tilt times were improved from 15.7 min to 37.0 min for the women and 21.8 to 45.0 min for the men (χ^2=9.60, p<0.01). There was no difference between the sexes in the median tilt time after the administration of octreotide. PLA=placebo; OCT=octreotide. *Difference between conditions for survival analyses, p<0.01.

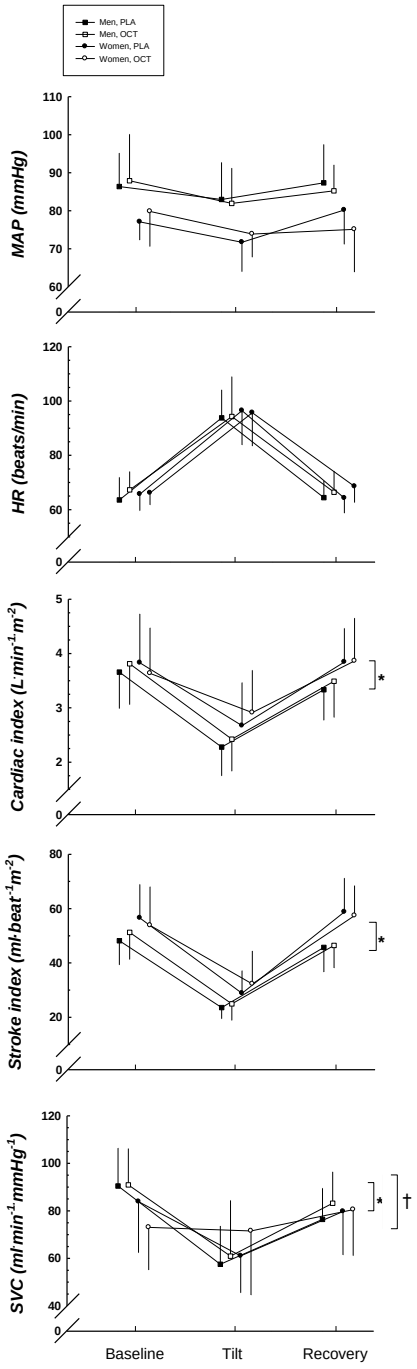

Figure **4-2:** Hemodynamic variables. The changes from baseline to HUT (Δbaseline) were comparable between the sexes with respect to MAP, HR, $\dot{Q}_i$, and SV_i. Women demonstrated higher $\dot{Q}_i$, higher SV_i, and lower SVC compared to the men. Octreotide induced an increase in SVC. PLA=placebo; OCT=octreotide. *Difference between sexes, $p<0.05$. †Difference between treatments, $p<0.05$.

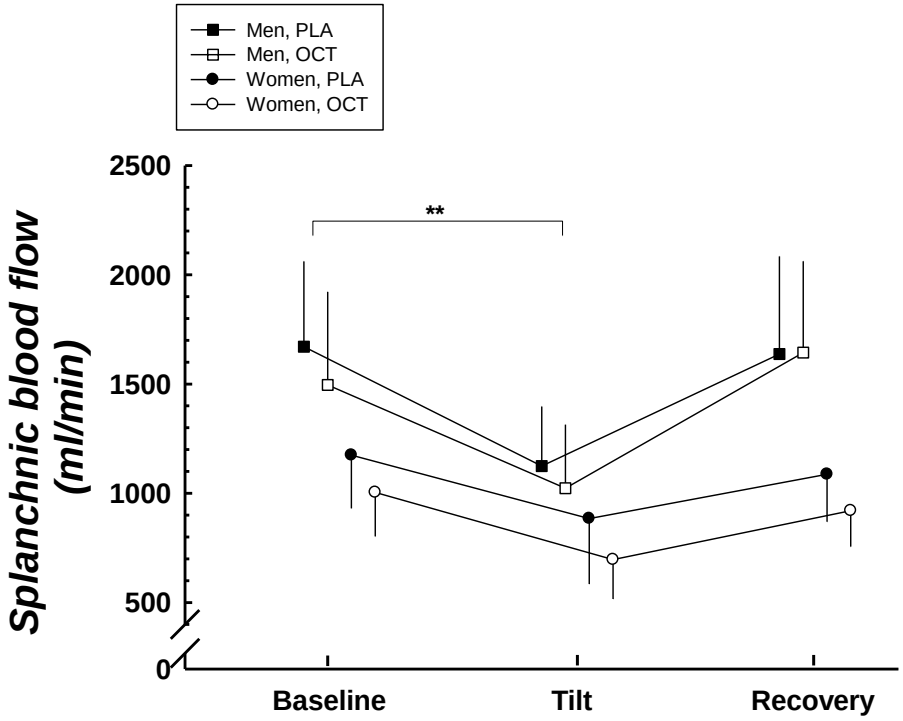

Figure **4-3**: Splanchnic blood flow. The women demonstrated a smaller reduction in SpBF between baseline and tilt (Δbaseline) when compared to the men. PLA=placebo; OCT=octreotide. **Difference between sexes in the change between supine and HUT, $p<0.01$.

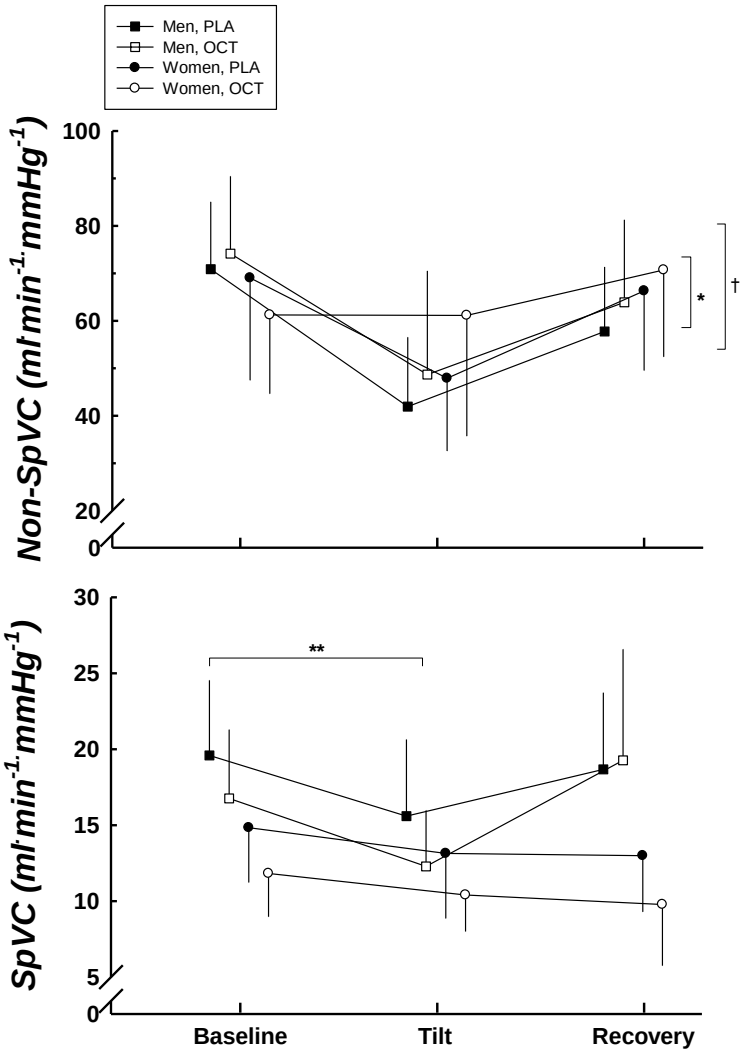

Figure **4-4**: Splanchnic and non-splanchnic vascular conductance. A significant difference in SpVC in response to tilt (Δbaseline) existed between the women and men where the women showed little change from baseline. Women demonstrated higher non-SpVC compared to the men. Octreotide elicited a treatment effect, raising non-SpVC. PLA=placebo; OCT=octreotide. *Difference between sexes, $p<0.01$. **Difference between sexes in the change between supine and HUT, $p<0.05$. †Difference between treatments, $p<0.05$.

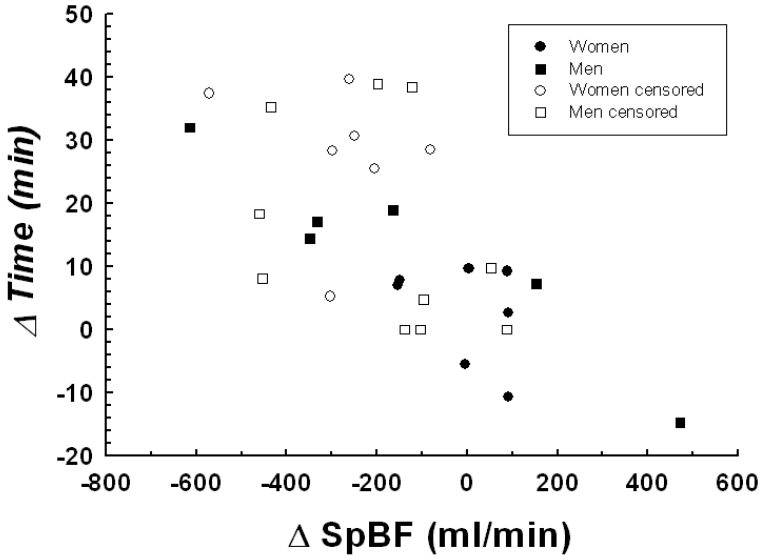

Figure **4-5**: The LIFEREG procedure revealed that a significant relationship existed between the change in SpBF induced by octreotide acetate and the improvements in tilt table tolerance in women (Δtilt time = 2.5 - 0.0083 ΔSpBF, p=0.0051) but not men (Δtilt time = 3.41 – 0.0008 ΔSpBF, p=0.59). The open symbols correspond to those points that were right censored with respect to time. Thus, the change in tilt tolerance may be greater than what is illustrated.

Chapter 5

IDENTIFICATION OF THE HUMAN VOLUME INDIFFERENT POINT

Introduction

Blood volume redistribution during standing or head-up tilt (HUT) is one factor that contributes to an individual's tolerance to an orthostatic stress. Upon assumption of the upright posture about 700 ml of blood is redistributed from the thorax to the gravitationally dependent regions (pelvis, abdomen, buttocks, and legs). In particular, blood pooling in compliant regions, such as the splanchnic circulation, reduces the circulating volume in the system and challenges cardiac filling.

How might one quantify the influence of blood volume redistribution during HUT? Gauer and Thron (Gauer & Thron, 1965) were the first to postulate a hydrostatic indifferent point (HIP) in humans. In a hydrostatic gradient, a compliant circulation will possess an HIP, a point where pressure remains unchanged, independent of posture (Gauer & Thron, 1965). This point is similar to the center of mass of a solid object and should be coincident with a volume indifferent point (VIP). Its location is influenced by regional compliances, as it is determined by the balance between the hydrostatic pressure and the mechanical properties of the compliant circulation below it (Rowell, 1986). The location of the HIP should determine an individual's tolerance to an orthostatic stress because the location (or the vertical distance between the HIP and the right atrium) dictates the filling pressure for the heart. For example, a more inferior HIP would lead to diminished cardiac filling pressure (Frey & Hoffler, 1988) and, therefore, increased incidence of orthostatic intolerance. Conversely, an improvement in blood pressure maintenance can be observed during water immersion up to the diaphragm in individuals with venous valvular

insufficiency because the counter pressure exerted by the water decreases the volume of blood pooled in the dependent regions, raising the HIP to support cardiac filling (Rowell, 1986).

Although determination of the HIP allows for a quantitative analysis of the filling gradient to the heart, the methodology is highly invasive as it requires measuring pressure throughout the venous system in the supine and upright postures (Gauer & Thron, 1965). This limitation makes this manometric method less desirable for general use.

Can an alternative approach be used? Since blood volume distribution influences the location of the HIP an alternate approach might focus on blood volume rather than pressure during HUT. Perko and colleagues used segmental bioelectrical impedance to conclude that an "electrical impedance indifferent point" existed somewhere between the umbilicus and the iliac crest (Perko *et al.*, 1993). In other words, this compartment might include the HIP described by Gauer and Thron (Gauer & Thron, 1965). However, neither its precise location nor its inter- and intra-subject variability have been identified in humans.

Thus, the purpose of this study was to develop an experimental paradigm to determine the location of the VIP. Accordingly, we sought to: 1) determine the exact location in the transverse plane of the VIP in humans, and 2) determine its repeatability.

Methods

Subjects

Eighteen subjects (10 men, 8 women) gave written informed consent to participate in the study, which was approved by the Institutional Review Board at The Pennsylvania State University. Descriptive characteristics of the subjects are outlined in Table 1. Exclusionary criteria included: smoking, body mass index <18.5 kg/m^2 or ≥ 30.0 kg/m^2, waist-to-hip ratio

>0.97, current pregnancy or breastfeeding, hypertension or other form of cardiovascular disease, hormonal contraceptive use, autonomic dysfunction or clinically diagnosed orthostatic intolerance, peripheral vascular disease, irritable bowel syndrome, biliary cholecystatic disease, or pacemaker.

Before participating in any part of the experiment, subjects underwent a screening protocol, which consisted of a 12-lead resting electrocardiogram and blood pressure measurements, a blood panel (complete blood count, CHEM 24, and coronary risk profile), a physical exam, evaluation of family and medical history, and a graded treadmill exercise test.

Subjects were admitted to the General Clinical Research Center (GCRC) at The Pennsylvania State University for study visits and were instructed to fast for at least 8 hrs, refrained from alcohol and caffeine intake for 24-hrs, and avoid strenuous exercise for 12-hrs prior to each visit. Subjects were also instructed to maintain their normal hydration pattern (up to 8 glasses of juice, water, or sports drink per day) for the 48-hrs preceding the experimental visits. Female participants were tested during the early follicular phase of the menstrual cycle (days 2-7) and submitted urine samples for pregnancy testing during screening, as well as on the days of the study.

Heart rate and blood pressure

HR was obtained via electrocardiogram (3-lead ECG, Hewlett-Packard 78534A) and BP via beat-to-beat arterial applanation tonometry (Colin 7000; Colin Medical Instruments). HR and BP were sampled continuously at 1000- and 100-Hz, respectively, and stored in beat-to-beat format using a custom data collection program.

Segmental bioelectrical impedance

Changes in biolelectrical impedance in different segments within the abdominal region and legs were determined using an electrical impedance technique (tetrapolar high resolution impedance meter, THRIM, UFI). ECG electrodes (Red dot electrodes, 3M) were used to bracket 8 segments along the torso and legs (Figure **5-1**). These segments were chosen to: 1) be consistent with the existing literature; and 2) to bracket regions above, below, and containing the region we expected to locate the VIP. Electrodes for each segment were aligned on the left side between the midline and mid-axillary line of the body at the following anatomical sites:

1 – jugular notch & inguinal crease (as determined by hip flexion)

2 – 6^{th} rib & iliac crest (superior portion as determined by palpation)

3 – umbilicus & iliac crest

4 – iliac crest & mid thigh (halfway point between inguinal crease and superior portion of the patella during hip and knee flexion)

5 – jugular notch & 6^{th} rib

6 – 6^{th} rib & inguinal crease

7 – iliac crest & inguinal crease

8 – mid thigh & ankle (distal tibia during dorsiflexion)

In some cases, the level of the umbilicus and iliac crest were coincident. Accordingly, the electrode at the level of the umbilicus was moved superior (midway between the xyphoid process and iliac crest) to accommodate all of the segments and was kept consistent within the same subject during all measurements. The height of each electrode from the floor was carefully measured to the nearest millimeter using a stadiometer (GPM).

The THRIM introduces a low frequency (51.2 kHz) constant alternating current (1 mA) between the arm and foot electrodes. The drive, or current injecting, electrodes were placed on the left forearm and left foot. The electrodes on the abdominal region and legs detected the voltage change in each segment as the current passed through.

Segmental blood volume

Impedance was continuously measured throughout the experiment but only the average of min 4 for each stage was used for analysis since 2-3 min is required to achieve equilibration in blood volume shifts after changing tilt angles. The impedance values obtained were used to calculate changes in segmental blood volume, where the following equation was used:

$$\Delta \text{ segmental blood volume (mL)} = \rho \cdot (L^2 / R_0 R_1) \cdot \Delta R$$

where, ρ=the electrical conductivity of blood estimated as $53.2 \cdot \log e^{(\text{hematocrit} \cdot 0.022)}$, L=length of the segment (cm), R_0=initial impedance (Ω), R_1=final impedance (Ω), and $\Delta R = R_1 - R_0$ (Geddes & Baker, 1989).

The change in segmental blood volume was defined as the change from baseline or 0° for each tilt angle (e.g., 15°-0°, 30°-0°, 50°-0°).

Graded head-up tilt

On two separate visits subjects arrived to the GCRC for a brief check of their health status, followed by catheter placement (one antecubital in the left arm for hematocrit determination). Subjects were then moved to the laboratory and instrumented for electrocardiogram and blood pressure. Subjects were instructed to lay supine, relaxed and motionless, on the modified tilt table (Model OT-9003) with arms outstretched and supported at

the level of the heart. Subjects were passively tilted from 0° (baseline) to 15°, 30°, and 50° head-up, each lasting 6 min for continuous measurements in segments 1-4. These tilt angles correspond to hydrostatic pressure gradients of 0%, 26%, 50%, and 77% of standing ($\sin\theta=\rho gh$). The length of time was chosen to allow for hemodynamic stability for impedance measurements and adequate time to obtain a blood sample and cardiac output measurement. A second tilt series was used to obtain measurements in segments 5-8. A graded protocol was chosen so that it could be determined if the degree of tilt angle influenced the location of the VIP.

A blood sample for hematocrit determination was withdrawn 3 ½ min into each stage, followed by a blood pressure determination via auscultation 4 ½ min into the stage. The protocol was terminated if the subject began to show signs of presyncope (decline in systolic BP <80 mmHg, sustained systolic BP <90 mmHg with other symptoms or presyncope, or a rapid decline in HR of >25 bpm) or at the subject's request.

Determination of the volume indifferent point

Figure **5-2** illustrates how the VIP was determined. Changes in segmental blood volume for all tilt angles (15°-0°, 30°-0°, 50°-0°) were plotted against the distance from the floor to the middle of each segment using an exponential model ($y=a+ae^{bx}+c$), where a = the amplitude of the exponential or the largest expected change in blood volume, b = rate constant or volume change, constrained as $b>0$, c = the y-intercept or the average expected volume change for any given segment. A non-linear model was used since the volume-pressure relationship in most vascular beds is non-linear (Rowell, 1986; Rowell, 1993). Segmental blood volume changes from segments 2, 3, 4, and 5 were used to calculate the VIP as the distance from the floor at which the change in impedance would be zero. During data analysis we determined these four segments would be sufficient for the determination of the VIP. Accordingly, segments above, below, and

containing the region we would expect to find the VIP were chosen for analysis. We normalized the VIP by reporting its location as a percent of the individual's height.

Statistical analyses

A paired t-test and Bland-Altman analysis were used to determine the repeatability of the VIP within the same person when measurements were obtained on different days. Further, the Bland-Altman analysis was used to assess whether systematic differences (bias) existed between the two determinations. A repeated measures ANOVA (VIP location × tilt angle) was used to explore whether the location of the VIP differed depending on the angle used to make the determination (e.g., 15°-0° vs. 30°-0° vs. 50°-0°). Stepwise regression was used to determine what variables contributed most to the location of the VIP. All data are presented as mean±SD. Differences with $p<0.05$ were considered significant.

Results

Table **5-1** summarizes the subject characteristics. Initially we separated the women from the men to assess whether a sex difference existed in the location of the VIP. The groups were different in height, weight, body mass index, and body surface area but age, waist-to-hip ratio, and maximal oxygen uptake were not different between the groups. Since we did not find a difference in the location of the VIP, the data were subsequently pooled. One subject's data were not suitable for analysis because there was an insufficient number of data points. Accordingly, their data were excluded from the results.

As expected HR (Figure **5-3**) increased significantly from 56±7 bpm during supine baseline (0°) to 63±10 bpm ($p=0.03$) during 30° HUT and 72±12 bpm ($p<0.0001$) during 50° HUT. HR (58±10 bpm) during 15° HUT was not different from supine baseline. This relationship held true for all series of tilts, even on different days. A paired t-test indicated that HR responses were not different ($p=0.75$) between the two visits (Figure **5-3**).

Mean arterial pressure (MAP) was not different ($p=0.31$) throughout the different tilt angles (Figure **5-3**). For example, MAP during the first tilt series during the first visit was: baseline, 0°: 76±6 mmHg; 15°: 75±6 mmHg; 30°: 73±6 mmHg; 50°: 73±5 mmHg. A paired t-test, however, indicated that the MAP was higher ($p=0.01$) during the first visit (Figure **5-3**).

As anticipated, when the subjects were tilted from the supine to head-up position, impedance in the chest (above the VIP) increased and impedance in the lower abdomen (below the VIP) decreased (Figure **5-4**). Since an inverse relationship exists between impedance and blood volume, the increase in impedance above the VIP occured as a result of a decrease in blood volume in the thorax and the decrease in impedance below the VIP occured as a result of an increase in blood volume in the gravitationally dependent regions (Figure **5-4**). A paired t-test indicated that segmental blood volume changes within the same individual were not different ($p=0.94$) between the two determinations. However, the blood volume changes differed considerably between subjects (Figure **5-4**).

A non-linear regression model was used to estimate the location of the VIP. As previously mentioned, this model was chosen because the volume-pressure relationship in most vascular beds is non-linear (Rowell, 1986; Rowell, 1993). At higher tilt angles a larger shift of blood volume was observed from regions above the VIP to regions below the VIP. However, despite the differences in the volume of blood shifted, the VIP remained unchanged. A repeated measures ANOVA indicated there was no difference ($p=0.72$) in the location of the VIP when only one of the tilt angles was used to determine the VIP (e.g., 15°-0° vs. 30°-0° vs. 50°-0°),

suggesting that the magnitude of the tilt angle did not influence the location (see Table **5-2**). It should be noted, however, that for three of the subjects an insufficient number of data points existed, thus, using only one tilt angle to determine the VIP was not possible.

Representative data from one subject is shown in Figure **5-5**. The average r^2 for all subjects was 83±14% and 87±8% for the two visits. We found that the VIP was located between the xyphoid process and iliac crest, at 64.5±2.6% of an individual's height. The location of the VIP was repeatable as we did not find a statistical difference ($p=0.95$) in its location, within the same person, when measurements were obtained on different days (Figure **5-6**). Additionally, we did not find a difference in the location of the VIP between using only segments 2, 3, 4, and 5 vs. all 8 segments in a subset of subjects. Bland-Altman analysis (Figure **5-7**) indicated that 15 of the 17 subjects were within the limits of agreement (± 2 SD) and that no bias was present. Further inspection of the data suggested that the two subjects' measurements that fell outside of the limits of agreement likely occurred due to subject movement/talking.

Stepwise regression (backward elimination) using segmental blood volume changes (50°-0°) for segments 2, 3, 4, and 5, height, weight, body surface area, waist:hip, and body mass index indicated that only blood volume changes in segment 2 contributed to predicting the location of the VIP.

Discussion

The focus of this study was to develop and validate a method to quantitatively assess blood volume redistribution during HUT. Accordingly, we sought to determine the location of the VIP in humans and to determine its repeatability. As expected, during HUT, impedance increased in the upper body and decreased in the lower body, indicating redistribution of blood volume. Our findings are generally consistent with the works of others (Perko *et al.*, 1993). For

example, Gauer and Thron reported the HIP at the level of the diaphragm (Gauer & Thron, 1965) and Perko and colleagues reported that the electrical impedance indifferent point was likely to be located between the umbilicus and the iliac crest (Perko *et al.*, 1993). Our study provides greater accuracy and precision, allowing for comparisons within, as well as between subjects. In our hands, the exact location (in the transverse plane) of the VIP was 64.5±2.6% of an individual's height. This percentage was independent of the subject's height.

What influences the location of the VIP?

In humans, during quiet standing or HUT, about 70% of the total blood volume accumulates in the compliant venous system, below the level of the heart (Rowell, 1993). It has been previously shown that water immersion (Rowell, 1986) and compression stockings (Melchior *et al.*, 1996) improve blood pressure maintenance during an orthostatic stress. During these conditions a portion of the volume of blood in the compliant venous system is expelled, increasing circulating blood volume and supporting cardiac filling. We found the largest shifts in blood volume occurred in the thoracic region (decrease) and in the upper leg (increase)—sites located above and below the VIP, respectively. The segment most likely to contain the splanchnic circulation (segment 2) had the smallest change in blood volume; not surprising since it is so close to the VIP. These findings might suggest that improvements in blood pressure regulation in the upright posture should target the iliac crest to mid-thigh region because this segment received the largest increase in blood volume during HUT.

However, since the veins in the extremities are less compliant than those in the splanchnic circulation (Gelman, 2008) they have less volume to redistribute. Our findings, as well as those by Stewart et al (Stewart *et al.*, 2006), suggest that the splanchnic region plays a larger role than do the upper legs in the maintenance of cardiac filling during an orthostatic stress.

Stepwise regression indicated that the upper legs (iliac crest to mid-thigh) did not significantly contribute to the location of the VIP. This is not surprising since those veins are less compliant (Gelman, 2008). The splanchnic circulation can hold about 1200 mL of blood during rest (~25% of cardiac output, 20% of blood volume). Therefore, it is has a large effect on the unstressed volume of the circulation and mean circulatory filling pressure (Gelman, 2008). During an orthostatic stress, blood pressure is supported by a decrease in blood flow to the splanchnic circulation, which consequently decreases its volume ("passive constriction") (Rowell, 1993). When this mechanism fails or is insufficient syncope may occur. For example, patients with postural orthostatic tachycardia syndrome (POTS) exhibit low tolerance to an orthostatic stress (Stewart *et al.*, 2006) and a subset of POTS patients demonstrate several unique characteristics that suggest the VIP might be located more inferiorly in these patients. They demonstrate excessive thoracic hypovolemia and exhibit excessive splanchnic pooling during modest levels (35°) of HUT, but have normal peripheral vasoconstrictive function (Stewart *et al.*, 2006). This suggests that blood volume distribution to the legs in this subset of patients is no different from an individual without POTS, yet these patients demonstrate very low tolerance to an orthostatic stress. The difference in tolerance, therefore, can at least be partially explained by the accumulation of blood volume in the splanchnic circulation.

The utility of determining the VIP

The location of the VIP is the result of two factors: the sum of regional compliances and the pressure exerted at each compliant vascular bed. Thus, the VIP can be used theoretically to quantify the effects of blood volume distribution and vascular volume on cardiac filling in an organism exposed to a hydrostatic pressure gradient. This parameter provides a means to understand inter- and intra-subject variability in tilt tolerance and to gauge the effects of both

pathophysiological and therapeutic perturbations that affect tilt tolerance. For example, one would expect that POTS patients would have a more inferiorly located VIP when compared to a healthy population.

These data further highlight the importance of the anatomical location of the most compliant circulations with respect to the VIP. In this regard, the significance of the splanchnic circulation and its ability to accumulate or expel blood passively cannot be overlooked (Gelman, 2008). This physical property of the circulation, prominent in human cardiovascular regulation (Rowell, 1993), would be diminished considerably if the splanchnic bed were located distant from the VIP. Future work should define the relationship between tilt tolerance and the location of the VIP. We suspect that an inverse relationship exists; if so, decrements or improvements in tilt tolerance can be understood in terms of the filling gradient to the heart that depends on the location of the VIP.

Experimental considerations

There are several assumptions taken into consideration with this analysis. One, bioelectrical impedance analysis is sensitive to proper subject preparation (hydration, skin wettedness, distribution of body water). This consideration was minimized through pre-study instructions (exercise, food/liquid intake) such that the hydration state of the subject was maintained between trials; analysis of baseline hematocrit values for the two visits indicated no difference between the trials. Second, menstrual cycle status is a consideration with female subjects. We controlled for hormone status by having our female subjects complete all visits during the early follicular phase (days 2-7) of the menstrual cycle. None of the subjects were taking hormonal contraceptives during the course of the study or had during the previous 6 months. The third consideration relates to the repeatability of the VIP determinations. A paired

t-test indicated no that statistical difference between VIP determinations existed; however, the Bland-Altman analysis indicated that five individuals had differences between the two days that may be of biological importance. We are confident that two of these subject differences can be accounted for as subject movement/talking during the protocol. However, the other three subjects showed about a 5 cm difference between the two days. We leave the importance of these differences to the interpretation of the reader. Lastly, performing multiple tilts on the same person within the same visit may affect impedance. Pilot studies demonstrated that impedance was not different when data were collected continuously (graded tilt) when compared to discontinuously (baseline to 50°), suggesting tilts performed more than once or in a different sequence did not affect the values obtained.

Conclusions

In conclusion, we developed and validated an experimental paradigm to represent blood volume distribution during HUT in a single parameter. During HUT, blood volume redistributed to the dependent regions of the body, where the largest volume increase occurred in the upper legs and the smallest change occurred in/near the splanchnic circulation. This is not surprising as the splanchnic circulation is located so close to the VIP. We precisely and reproducibly located a point unaffected by hydrostatic gradients at 64.5±2.6% of an individual's height. This method may provide a quantitative framework to assess the effects of blood volume distribution and gravitational fields on tilt tolerance in order to investigate differences between groups or in response to interventions.

Acknowledgements

We would like to thank Jeff Banas, Bill Hennon, Katie Homan, Kylie Kaminski, Dave Leone, Joy Lithgow, Kevin Mao, Heidi Nadeau, Adam Spinner, Kylie Weaver, and the General Clinical Research Center for their assistance with this study. We would also like to thank Don Sheriff for his advice throughout this project. This project was supported by the National Aeronautics and Space Administration NNJ04HF45G (to J.A. Pawelczyk), the National Institutes of Health MO1RR10732 (to General Clinical Research Center), and the National Aeronautics and Space Administration Harriet G. Jenkins Pre-doctoral fellowship (to S.S. Jarvis).

Table **5-1**: Subject characteristics. Values are means±SD. BMI, body mass index; A_D, body surface area; $\dot{V}O_{2max}$, maximal oxygen uptake. *Significant group difference, $p<0.05$.

	Women	**Men**
	(N=8)	(N=10)
Age, yr	20±1	22±5
Height, cm	164.7±5.5*	175.9±7.2
Weight, kg	61.5±6.9*	78.5±10.5
BMI, kg/m^2	22.8±1.5*	25.4±2.5
Waist-to-hip ratio	0.82±0.03	0.84±0.04
A_D, m^2	1.67±0.11*	1.94±0.15
$\dot{V}O_{2max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	44.2±3.7	47.4±2.7

Table **5-2:** The location of the VIP (cm) with respect to tilt angle. Values are means±SD. Women, N = 7; Men, N=10. *Denotes cases where an insufficient number of data points were available to calculate the VIP for that tilt angle alone. No differences in the location of the VIP existed between the different determinations.

Subject	**All**	**15°- 0°**	**30°- 0°**	**50°- 0°**
1	104.8	*	*	*
2	101.0	100.7	102.4	*
3	98.7	95.7	96.5	*
4	103.0	102.5	104.2	102.1
5	110.6	111.6	110.4	110.7
6	106.5	106.6	106.9	105.7
7	115.5	113.9	116.1	114.7
8	122.2	*	*	*
9	124.1	125.8	120.2	126.4
10	116.9	116.9	118.3	117.6
11	101.9	113.2	*	*
12	112.5	115.0	113.3	*
13	116.9	*	116.3	116.6
14	108.9	*	*	*
15	114.8	119.0	113.3	*
16	107.2	106.1	105.9	*
17	112.5	114.7	113.2	*
Mean	110.5±7.4	110.9±8.2	110.5±6.9	113.4±8.1

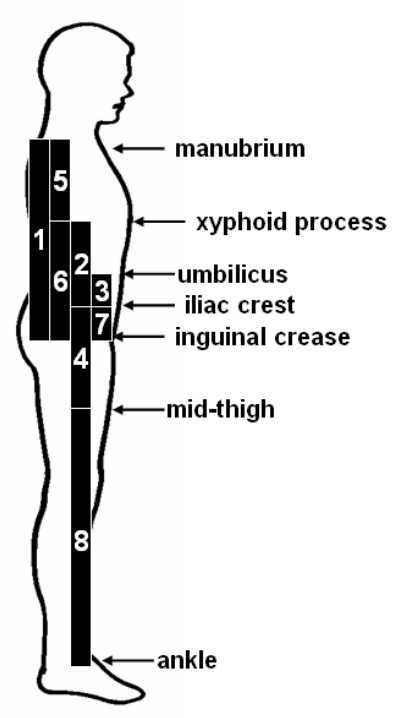

Figure **5-1**: Electrode placement for segmental impedance. Eight segments were bracketed along the legs and torso. These segments were chosen to bracket regions above, below, and containing the region where we expected to locate the VIP.

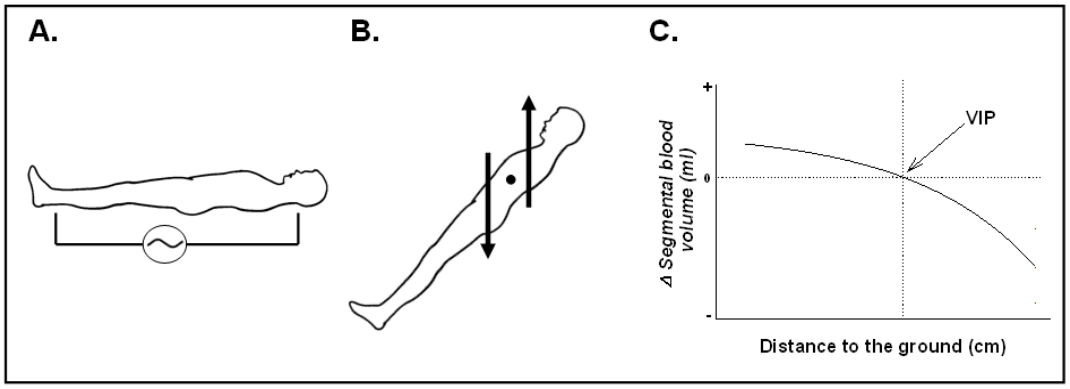

Figure **5-2**: Theoretical determination of the human volume indifferent point. We measured impedance to a high frequency, alternating current injected into the forearm and foot (Panel A). Impedance increased above the HIP/VIP and decreased below the HIP/VIP during HUT since blood volume shifted to the gravitationally dependent regions (Panel B). Impedance changes were used to calculate blood volume changes. The distance from the middle of each segment to the floor was plotted against Δ segmental blood volume (Panel C). The point where segmental blood volume does not change is the VIP.

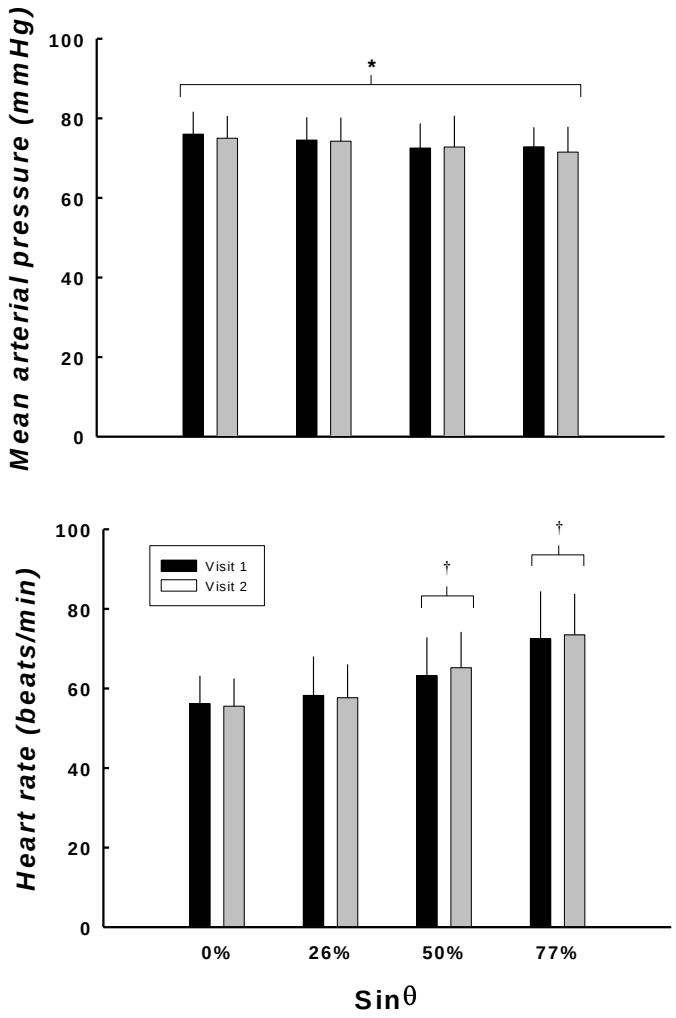

Figure **5-3:** Hemodynamic responses to graded tilt. MAP was higher during the first visit but was well maintained throughout the protocol. HR was not different between the two visits but rose significantly from baseline at 30° and 50°, 50% and 77%, respectively, of standing. *Difference between the first and second visit (paired t-test, $p=0.01$). †Difference from baseline ($p<0.05$).

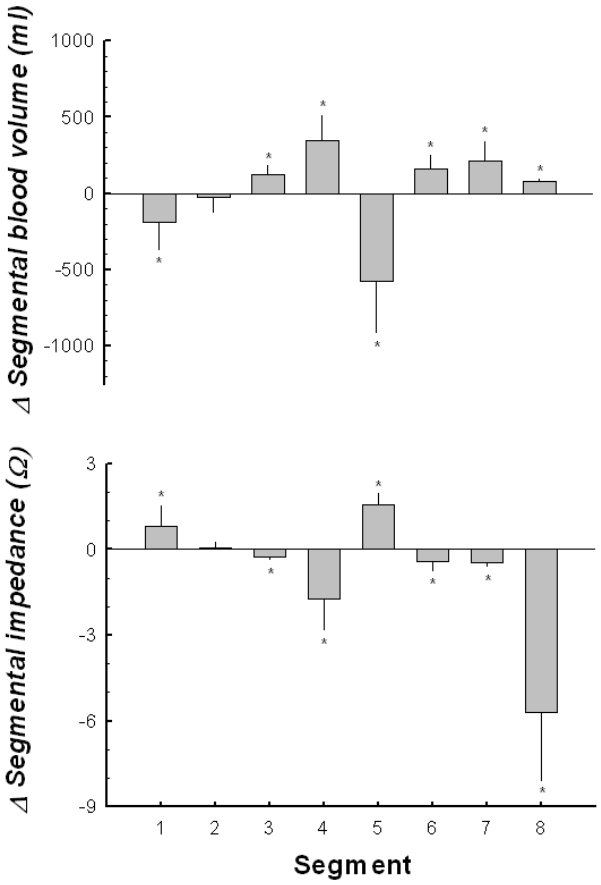

Figure **5-4**: Changes in segmental impedance and blood volume (50°-0°). Segments are defined in Figure 1. An inverse relationship exists between impedance to an applied electrical current and blood volume. Segments above the VIP demonstrated a decrease in blood volume with a concomitant increase in impedance. Segments below the VIP demonstrated an increase in blood volume with a decrease in impedance. *Significantly different from zero. N=17.

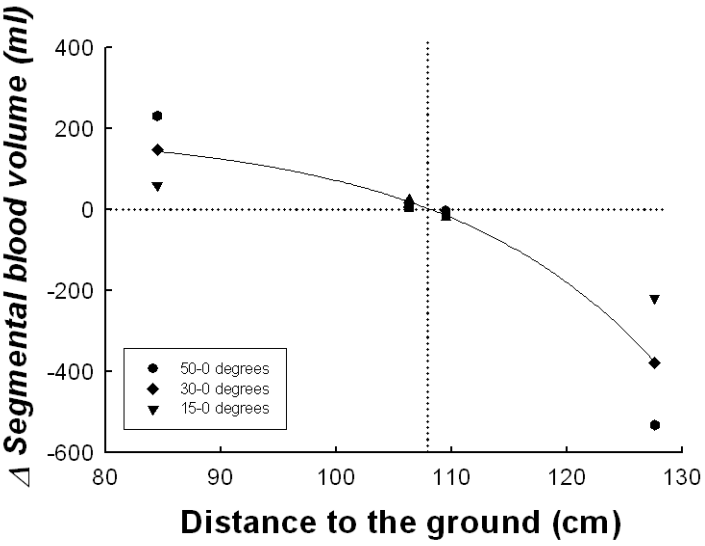

Figure **5-5**: Representative data from one subject. An exponential curve was used to determine the location (distance from the ground) where blood volume did not change during tilt.

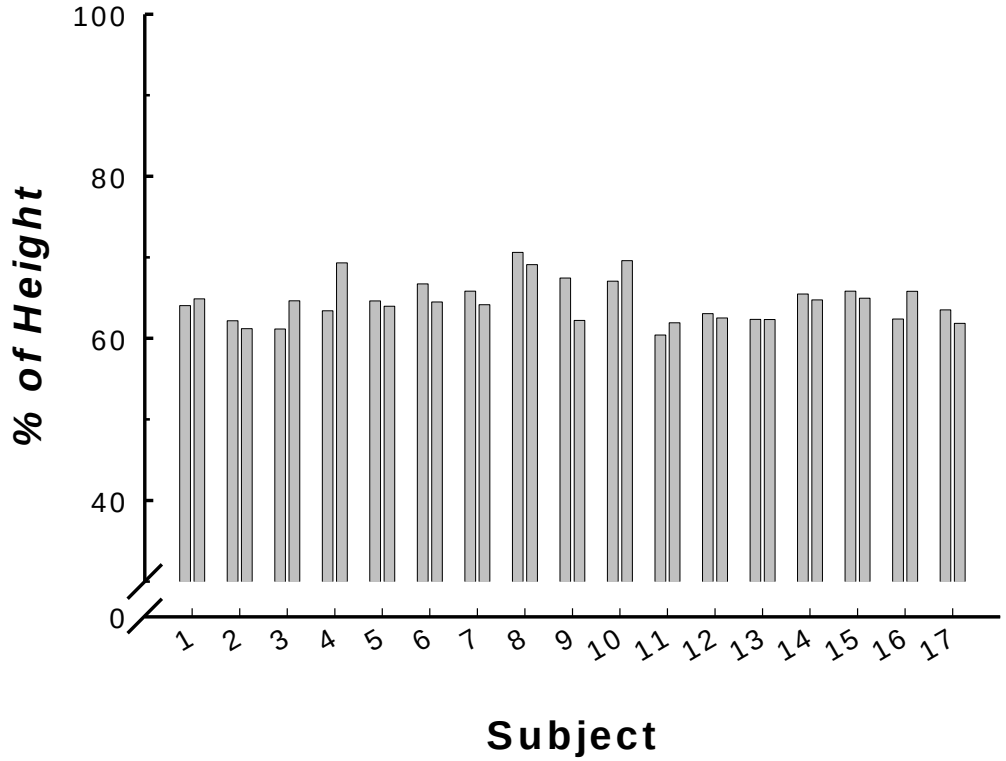

Figure **5-6**: Repeatability of the VIP determination. Two determinations were made for each subject, separated by at least 2 weeks. There was no significant difference (p=0.95) in the location of the VIP in the same subject on different days, indicating the VIP is reproducible. Further inspection of the data suggested that two of the subjects' measurements (subject 4 and 9) may have been altered due to subject movement during the protocol.

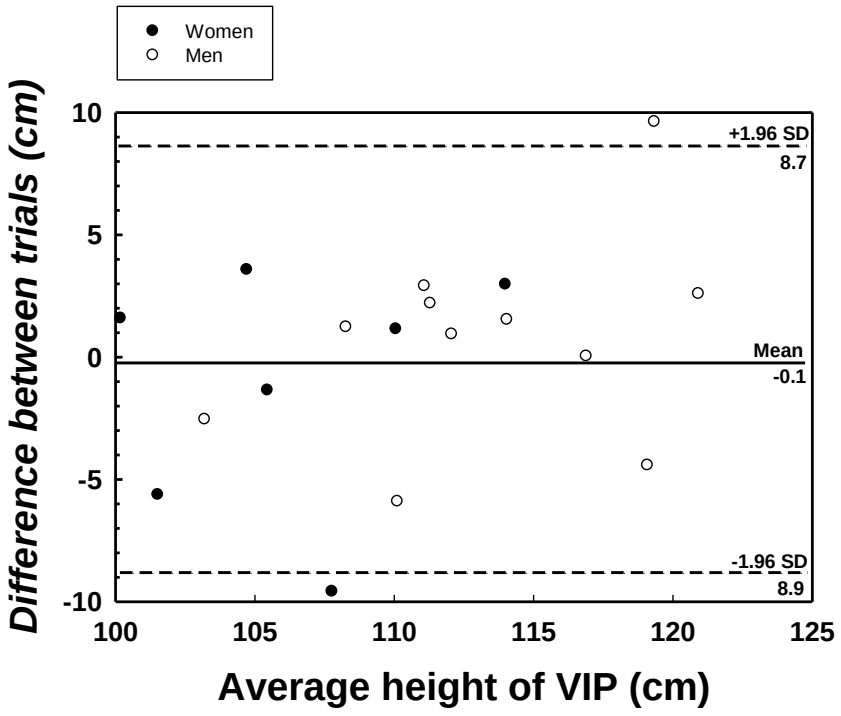

Figure **5-7**: Repeatability of the VIP on successive days. Bland-Altman analysis indicated that no bias was present with VIP determinations made on different days. Further inspection of the data suggested that the two subjects' measurements that fell outside of the limits of agreement may have occurred due to subject movement during the protocol. N=17.

Chapter 6

THE HUMAN VOLUME INDIFFERENT POINT PREDICTS ORTHOSTATIC TOLERANCE

Introduction

An individual's tolerance to an orthostatic stress depends on the maintenance of cardiac preload. In humans, about 70% of the total blood volume is contained in the compliant venous system below the level of the heart (Rowell, 1993). The distribution of blood volume, therefore, plays an integral role in the maintenance of blood pressure. For example, patients with postural orthostatic tachycardia syndrome (POTS) demonstrate splanchnic hypervolemia during low levels of head-up tilt and exhibit low tolerance to an orthostatic stress (Stewart *et al.*, 2006). Successful strategies focused on improving orthostatic tolerance have concentrated on reducing the volume of blood in these compliant regions (Hoeldtke *et al.*, 2006; Hoeldtke *et al.*, 2007; Melchior *et al.*, 1996; Rowell, 1986).

Previous investigations (Hoeldtke *et al.*, 2006; Hoeldtke *et al.*, 2007) have focused on the splanchnic region as it relates to blood pressure maintenance because of its large capacitance. We previously reported that administration of octreotide acetate, a somatostatin analog whose vascular effects are largely confined to the splanchnic circulation, improved tilt table tolerance in healthy volunteers (manuscript in review). However, the mechanism by which this improvement occured has not been fully addressed. Wong and Sheriff (Wong & Sheriff, 2007) measured right atrial pressure in the conscious dog after administration of octreotide acetate, noting a small but significant increase. They concluded that the reduction in vascular capacity in the splanchnic circulation could lead to improvements in orthstatic tolerance (Wong & Sheriff, 2007).

Recently our laboratory developed and validated an experimental paradigm that examines blood volume distribution during head-up tilt (HUT) (manuscript in review). The premise of this experimental paradigm parallels the concept of the hydrostatic indifferent point (HIP) introduced by Gauer and Thron (Gauer & Thron, 1965). The HIP was located at the level of the diaphragm (Gauer & Thron, 1965); consistent with this report, we located the VIP at 64.5±2.6% of an individual's height (manuscript in review). We noted that the splanchnic circulation, probably because of its close proximity, contributed the most to the location of the VIP (manuscript in review).

In the current investigation we sought to manipulate the volume contained in the splanchnic circulation to determine how it alters the location of the VIP. Secondly, we sought to related changes in the location of the VIP with an individual's orthostatic tolerance. We hypothesized that octreotide acetate would induce a superior shift in the location of the VIP. We also postulated that an inferior shift in the location of the VIP, provoked by -20 mmHg lower body negative pressure, would be related to decrements in tilt table tolerance.

Methods

Subjects

Thirty-two subjects (14 women, 18 men) gave written informed consent to participate in these two studies (Protocol 1 and Protocol 2), which were approved by the Institutional Review Board at The Pennsylvania State University. Descriptive characteristics of the subjects are outlined in Table **6-1**. Exclusionary criteria included: smoking, body mass index <18.5 kg/m^2 or ≥30.0 kg/m^2, waist-to-hip ratio >0.97, current pregnancy or breastfeeding, hypertension or other form of cardiovascular disease, hormonal contraceptive use, autonomic dysfunction or clinically

diagnosed orthostatic intolerance, peripheral vascular disease, irritable bowel syndrome, biliary cholecystatic disease, or pacemaker.

Before participating in any part of the experiment, subjects underwent a screening protocol, which consisted of a 12-lead resting electrocardiogram and blood pressure measurements, a blood panel (complete blood count, CHEM 24, and coronary risk profile), a physical exam, and an evaluation of family and medical history.

On study visits subjects were admitted to the General Clinical Research Center (GCRC) at The Pennsylvania State University. Subjects were instructed to fast for at least 8 hrs, refrained from alcohol and caffeine intake for 24-hrs, and avoid strenuous exercise for 12-hrs prior to each visit. Subjects were also instructed to maintain their normal hydration pattern (up to 8 glasses of juice, water, or sports drink per day) for the 48-hrs preceding the experimental visits. After a brief check of their health status, one antecubital catheter was placed in the left arm (hematocrit determination and octreotide infusion). Female participants were tested during the early follicular phase of the menstrual cycle (days 2-7) and submitted urine samples for pregnancy testing during screening, as well as on the days of the study.

Experimental design

Protocol 1: Pharmacological manipulation of the VIP with octreotide acetate

Eight women and ten men participated in Protocol 1. The VIP was determined twice (described below): once under control conditions and once after administration of octreotide acetate. Subjects received an infusion of 1.7 μg/kg octreotide acetate (mixed with 50 mL saline) over 15 min. Forty min after the beginning of the infusion a second set of impedance measurements were obtained.

Protocol 2: Physiological manipulation of the VIP with lower body negative pressure

Six women and 8 men participated in Protocol 2. As in Protocol 1, the VIP was determined twice once under control conditions and, after 40 min of rest in the supine position, again during exposure to -20 mmHg lower body negative pressure (LBNP). The LBNP skirt that creates the seal between the subject and the LBNP chamber was modified so that it did not exert any pressure on the abdomen. This is of particular importance because it had the potential to influence measurements of impedance and, therefore, blood volume distribution. Additionally, these subjects underwent two tilt tolerance tests, once under control conditions and once with concomitant -20 mmHg LBNP.

Plasma volume determination (Protocol 2 only)

On a separate visit from screening, we determined the plasma volume of each subject using indocyanine green (ICG; Akorn Inc.). The subject had one antecubital intravenous (18-20 gauge) catheter placed into each arm. The left arm was used for bolus injection, while the right arm was used for blood sample withdrawal. Subjects laid supine for a minimum of 30 minutes prior to withdrawal of an aliquot of blood to serve as a spectrophotometer blank, followed by the bolus injection of 0.5 mg/kg ICG. Two min after the intravenous bolus injection of ICG, a 3-ml blood sample was withdrawn, which was then followed by withdrawal of a 3-ml blood sample every 3 min until min 32. Each sample withdrawal was followed by a 3-ml saline flush. Samples were centrifuged at 3,000 rpm for 20 minutes, and the plasma concentration of ICG was measured by spectrophotometry (805 nm for absorbance and 910 nm for turbidity). The plasma volume was estimated for each subject by the moment of injection by indicator-dilution by back extrapolation of the plasma concentration of the dye.

Heart rate (HR) and blood pressure (BP)

HR was obtained via electrocardiogram (3-lead ECG; Hewlett-Packard 78534A) and BP via beat-to-beat arterial applanation tonometry (Colin 7000; Colin Medical Instruments). HR and BP were sampled continuously at 1000- and 100-Hz, respectively, and stored in beat-to-beat format using a custom data collection program.

Cardiac output ($\dot{Q}_c$) and stroke volume (SV)

We used the acetylene (C_2H_2) rebreathing technique as described by Triebwasser et al (Triebwasser *et al.*, 1977), which has been previously validated against direct Fick and thermodilution (Jarvis *et al.*, 2007). Before data recording began the subject was instructed on how to perform the $\dot{Q}_c$ rebreathing maneuver and performed two practice maneuvers. A mass spectrometer (Perkin-Elmer MGA 1000) was used to analyze the gas concentrations during the 20 sec rebreathing period. During the VIP determination, $\dot{Q}_c$ was measured once during each stage. During the tilt tolerance test, $\dot{Q}_c$ was measured upon HUT and the every 10-min during tilt and recovery. To normalize for size differences $\dot{Q}_c$ was expressed as cardiac index, $\dot{Q}_i$ ($\dot{Q}_i = \dot{Q}_c$ /body surface area), where body surface area (BSA) $=0.202 \cdot kg^{0.425} \cdot m^{0.725}$ (Du Bois & Du Bois, 1916). SV was calculated by $\dot{Q}_c$/HR and normalized as stroke volume index (SV_i=SV/BSA).

Determination of the VIP

We have previously reported the methodology for the determination of the VIP (manuscript in review). Briefly, changes in biolelectrical impedance in four segments (Figure **6-1**) were determined (tetrapolar high resolution impedance meter, THRIM, UFI):

A (splanchnic): 6^{th} rib & iliac crest (superior portion as determined by palpation)

B (pelvic): umbilicus & iliac crest

C (upper thigh): iliac crest & mid thigh (halfway point between inguinal crease and superior portion of the patella during hip and knee flexion

D (thoracic): jugular notch & 6^{th} rib

Subjects were passively tilted on a tilt table (Model OT-9003, Omni Technologies) from 0° (baseline) to 15°, 30°, and 50° head-up, each lasting 6 min for continuous measurements. These tilt angles correspond to hydrostatic pressure gradients of 0%, 26%, 50%, and 77% of standing ($\sin\theta=\rho gh$). The change in segmental blood volume was defined as the change from baseline or 0° for each tilt angle (e.g., 0°-15°, 0°-30°, 0°-50°). Non-linear regression was used to determine the distance from the ground where blood volume did not change (VIP) during graded tilt.

Tilt tolerance test (Protocol 2 only)

70° head-up tilt (Control). The order of the tilt tolerance tests was randomly assigned. For these study visits, subjects received a brief physical exam by a GCRC clinician, followed by catheter placement (one antecubital). Subjects were then instrumented in our laboratory for electrocardiogram and blood pressure and instructed to lay supine on the modified tilt table. The subjects' arms were outstretched and supported at the level of the heart.

Ninety min after instrumentation and baseline measurements, the subject was tilted 70º head-up. The subject was instructed to stand quietly with feet shoulder width apart. Subjects remained in the 70° head-up tilt (HUT) position for 45 min, until presyncope, or at the subject's request to stop. Presyncope was defined as loss of hemodynamic stability (decrease in blood pressure >20/10 mmHg and/or rapid decline in heart rate >25 bpm), diaphoresis, nausea, light-headedness, and/or hyperventilation. At the onset of presyncope the subject was placed in the Trendelenburg position (-10°). Once blood pressure and heart rate were stabilized the subject was moved to supine. Twenty min of recovery data were collected in the supine position.

70° head-up tilt + LBNP. Instrumentation was similar to the control tilt except that the subject donned a modified LBNP skirt for the LBNP protocol. The LBNP skirt was modified so that it did not exert any pressure on the abdomen. Ninety min after instrumentation and baseline measurements, the subject was exposed to -20 mmHg LBNP and then tilted to 70º head-up for 45 min or until presyncope.

Statistical analyses

For Protocol 1, a paired t-test was used to assess changes in the location of the VIP, within the same individual, between treatment conditions (control vs. octreotide). A repeated measures analysis of variance (stage × condition) was used to examine hemodynamic responses during the VIP determination. For Protocol 2, a paired t-test was used to assess changes in the location of the VIP, within the same individual, between treatment conditions (control vs. LBNP). Kaplan-Meier survival analysis and Mood's median test were used to assess differences in tilt tolerance between the conditions for Protocol 2. A repeated measures analysis of variance (stage × condition) was used to examine hemodynamic responses to the tilt tolerance tests. The

LIFEREG procedure in SAS was used to assess the relationship between the change in the location of the VIP and the change in tilt time between the two conditions (control vs. LBNP). LIFEREG performs regression analysis on right censored data because in some instances the subject did not become presyncopal before 45 min. Analyses were performed using SAS 9.1 (Cary, NC). Data are presented as mean±SD. In all cases, differences with $p<0.05$ were considered significant.

Results

Table **6-1** summarizes the subject characteristics. Initially we separated the women from the men to assess whether a sex difference existed in the location of the VIP. Since we did not find a difference in the location of the VIP between the sexes in either protocol, the data were subsequently pooled. One subject's data (Protocol 1) were not suitable for analysis because there was an insufficient number of data points. Accordingly, their data were excluded from the results.

Protocol 1

Figure **6-2** (panel A) represents the hemodynamic variables during the graded tilt procedure that was used to determine the location of the VIP. As expected, HR increased significantly from during supine baseline (57±7 and 58±7 bpm, control and octreotide, respectively), during 30° HUT (63±8 and 62±9 bpm, $p=0.01$), and 50° HUT (70±10 and 69±12 bpm, $p<0.01$). HR during 15° HUT (58±7 and 58±9 bpm) was not different from supine baseline. MAP was lower during 30° HUT (72±8 and 74±6 mmHg) compared to baseline (75±6 and 78±7 mmHg, $p=0.04$) but values obtained at 15° HUT (74±6 and 76±7 mmHg) and 50° HUT

(73±7 and 74±6 mmHg) were not different from baseline. MAP was higher during the octreotide trial ($p<0.01$).

The VIP was located in Segment A (splanchnic) for a majority of subjects (11/17). As shown in Figure **6-3** (panel A), octreotide induced a superior shift (+1.9±3.3 cm, $p=0.03$) in the location of the VIP. The changes in segmental blood volume (0°-50°) are depicted in Figure **6-4** (panel A). The splanchnic region was the only segment that showed a significant difference ($p<0.01$) between the conditions.

Protocol 2

During the VIP determination in Protocol 2, HR (Figure **6-2**, panel B) progressively increased from baseline (57±8 and 64±10 bpm, control and LBNP, respectively) during 15° HUT (60±8 and 73±11 bpm, $p=0.02$), 30° HUT (68±9 and 82±12 bpm, $p=<0.01$) and 50° HUT (75±8 for the control trial, $p<0.01$). HR during LBNP was higher than the control trial ($p<0.01$). MAP was not different ($p=0.57$) throughout the different stages nor between the conditions ($p=0.90$) (Figure **6-2**). An inadequate sample size was available at 50° HUT during the LBNP protocol, thus, these data were removed from further analysis.

The VIP was located in Segment A (splanchnic) for most of the subjects (10/14) during the control measurments, consistent with Protocol 1. LBNP induced a significant inferior shift (-6.0±7.2 cm, $p<0.01$) (Figure **6-3**, panel B). Segmental blood volume changes (0°-15°) (Figure **6-4**, panel B) were different ($p<0.05$) between the conditions.

LBNP during 70° HUT reduced tilt table tolerance in all individuals. The median tilt time during the control tilt was 28.0 min and 4.2 min during the LBNP protocol ($\chi^2=14.29$, $p<0.01$). Survival anlysis (Figure **6-5**) indicated a significant difference existed between the

proportion surviving 45 min of tilt (6/14 vs. 0/14) between the conditions (χ^2=26.03, p<0.01). No sex differences in tilt tolerance existed in either the control or LBNP trials.

Figure **6-6** illustrates the hemodynamic responses before, during, and after the tilt tolerance tests. MAP was not significantly altered by HUT and was similar between conditions (baseline: 73±7 and 75±7 mmHg; tilt: 72±11 and 71±9 mmHg; control and LBNP, respectively). A significant stage × condition interaction existed during tilt (p=0.02) where HR was higher during the LBNP trial (tilt: 94±14 vs. 108±24 bpm). HUT reduced $\dot{Q}_i$, SV_i, and SVC with no statistical difference in these variables between the control and LBNP conditions. $\dot{Q}_i$ decreased (p<0.01) from 3.00±0.58 and 2.95±0.83 $L \cdot min^{-1} \cdot m^{-2}$ to 2.48±0.63 and 2.21±0.76 $L \cdot min^{-1} \cdot m^{-2}$ during tilt. SV_i was reduced (p<0.01) from 44.52±8.51 and 42.14±14.89 $ml \cdot beat^{-1} \cdot m^{-2}$ to 25.51±7.42 and 20.44±9.23 $ml \cdot beat^{-1} \cdot m^{-2}$. A reduction (p<0.01) was observed in SVC, baseline decreased from 73.09±20.52 and 78.20±20.43 $ml \cdot min^{-1} \cdot mmHg^{-1}$ to 59.88±16.60 and 52.98±19.55 $ml \cdot min^{-1} \cdot mmHg^{-1}$ during tilt. It should be noted that LBNP was only applied during HUT and not during the supine stages (i.e., baseline and recovery).

Lastly, a significant relationship between ΔVIP and Δtilt time existed (Δtilt time = 3.05 + 0.12 ΔVIP, p=0.03), indicating that those individuals with the largest inferior shift in the location of the VIP also demonstrated the largest decrease in tilt table tolerance (Figure **6-7**).

Discussion

The current investigation sought to determine the relationship between the VIP and an individual's tolerance to an orthostatic stress. We hypothesized that the improvements in tilt table tolerance, previously reported by our group, were related to a superior shift in the location of the VIP. Additionally, we postulated that decrements in tilt tolerance could be induced by

splanchnic pooling with low level LBNP and that these decrements would be associated with an inferior shift in the location of the VIP.

A superior shift in the location of the VIP

Selective vasoconstriction of the splanchnic circulation with octreotide acetate has been shown to increase an individual's tolerance to an orthostatic stress (Hoeldtke *et al.*, 1998; Hoeldtke *et al.*, 2007). However, this mechanism has not been fully described. In Protocol 1 of the current study, we found that an infusion of 1.7 μg/kg octreotide acetate induced a superior shift in the location of the VIP compared to the control trial. Recently, Wong and Sheriff (Wong & Sheriff, 2007) reported that octreotide increased right atrial pressure by 0.6 mmHg. Thus, consistent with their findings, the +1.9 cm shift in the VIP we observed can be theoretically translated into a 1.4 mmHg increase in filling pressure(Wong & Sheriff, 2007).

Blood volume changes in the splanchnic region contributed to the relocation of the VIP due to octreotide. During the control VIP determination, the splanchnic segment demonstrated a loss of blood volume but during the octreotide determination a gain in blood volume. This may appear counter intuitive since octreotide constricts the splanchnic circulation, which should cause passive and active reduction in splanchnic blood volume (Rowell, 1986). The VIP is the point where blood volume does not change, which means, by definition, that a loss of blood volume that occurs above the VIP must be balanced by a gain below it. Segmental impedance, however, only allows one to estimate the net blood volume change in a given segment. Thus, the most plausible interpretation of these findings is that a superior shift in the VIP exposes a greater portion of that segment to volume gain (Figure **6-8**, panel C).

Figure **6-8** portrays this idea conceptually. If the body is treated as a column of blood (or a fluid filled cylinder), in the supine position blood volume is evenly distributed because gravity

is exerted along the longitudinal axis. In the upright position the change in segmental blood volume (from supine) along this column of blood varies and is depedent on regional compliances. The sum of these regional compliances will affect the location of the VIP. Vasoconstriction and venocontriction of a compliant circulation contracts the distensible compartment (dotted line) shown in panel A. Since blood volume is fixed and the circulation is closed, this would lead to a superior shift in the location of the VIP, which would support cardiac filling in a gravitational field.

While Protocol 1 did not include tilt tolerance testing, the hemodynamic variables during the graded HUT for the VIP determination indicated that blood pressure was higher after administration of octreotide. These data are consistent with reports that the splanchnic circulation plays an integral role in the maintenance of blood pressure during an orthostatic challenge (Rowell, 1986) and that cardiac filling is maintained via relocation of the VIP.

An inferior shift in the location of the VIP

If the location of the VIP affects tilt table tolerance then an inferior shift in the VIP should translate into decrements in tilt table tolerance. Changes in segmental blood volume were different during tilt with LBNP compared to tilt alone. We noted that the difference in the splanchnic segment between the two conditions (control vs. LBNP) was directionally opposite to Protocol 1. That is, during LBNP, the splanchnic region demonstrated a larger loss of blood volume, which has also been reported by Taneja et al (Taneja *et al.*, 2007). Again, this appears counter intuitive since lower body suction produces splanchnic pooling (Montgomery *et al.*, 1977; White & Montgomery, 1996). However, this is explained by the relocation of the VIP. An inferior shift positions a larger portion of the splanchnic segment above the VIP where one would expect to see a loss of blood volume.

Again, Figure **6-8** (panel A and C) depicts this idea conceptually. Since LBNP induces splanchnic pooling (dashed line), blood volume is increased in one of the most compliant regions below the VIP, leading to an expansion of this distensible compartment and an inferior shift in the VIP. The -6.0 cm shift in the VIP, theoretically, translates into a 4.4 mmHg decrease in filling pressure, slightly less than what has been reported by Fu and colleagues (Fu *et al.*, 2004a). Fu et al measured right atrial pressure during -15 and -30 mmHg LBNP; therefore, by extrapolating from the data they present ~5.0 mmHg decrease in right atrial pressure would occur during -20 mmHg LBNP.

We were interested in determining the relationship between alterations in the VIP and tilt tolerance. A significant relationship exists between these variables, indicating that those individuals with the largest inferior shift in the VIP had the largest decrement in tolerance. This underscores the important role the splanchnic circulation plays in determining the location of the VIP and that the location of the VIP is a determinant of tolerance to an orthostatic stress. It might suggest that populations that demonstrate low orthostatic tolerance have more inferiorly located VIPs. For example, patients with POTS exhibit both splanchnic hypervolemia and low orthostatic tolerance (Stewart *et al.*, 2006).

Conclusions

In summary, we manipulated the location of the VIP and related its location to orthostatic tolerance. Selective constriction of the splanchnic circulation moved the VIP closer to the heart and exposure to LBNP moved the VIP closer to the feet. These changes were related to improvements and decrements, respectively, in a controlled study of tilt table tolerance. Lastly, we found that changes in VIP location can predict an individual's tolerance to HUT, which

indicates that individuals susceptible to experiencing intolerance may be indentified using this methodology.

Acknowledgements

We would like to thank Sandy Smithmyer, Lauren Gianoni, Matt Hinkley, Dave Leone, Xenophon Mucciarone, Kyle Preston, Kylie Weaver, and the General Clinical Research Center for their assistance with this study. This project was supported by the National Aeronautics and Space Administration NNJ04HF45G (to J.A. Pawelczyk), the National Institutes of Health MO1RR10732 (to General Clinical Research Center), and the National Aeronautics and Space Administration Harriet G. Jenkins Pre-doctoral fellowship (to S.S. Jarvis).

Table **6-1**: Subject characteristics. Values are means±SD. BMI, body mass index; A_D, body surface area. *Significant group difference within Protocol, $p<0.05$. No significant differences between Protocol A and Protocol B existed.

	Protocol 1		**Protocol 2**	
	Women (N=8)	**Men** (N=10)	**Women** (N=6)	**Men** (N=8)
Age, yr	20±1	22±5	25±4	24±4
Height, cm	164.7±5.5*	175.9±7.2	160.2±6.0*	176.1±10.6
Weight, kg	61.5±6.9*	78.5±10.5	62.1±13.6	74.4±9.0
BMI, kg/m^2	22.8±1.5*	25.4±2.5	24.1±4.8	23.9±1.8
Waist-to-hip ratio	0.82±0.03	0.84±0.04	0.76±0.02*	0.79±0.04
A_D, m^2	1.67±0.11*	1.94±0.15	1.64±0.18*	1.90±0.17
Plasma volume	—	—	2453±557	2570±260
Plasma volume (ml/kg)	—	—	40.9±11.2	34.9±4.7
Blood volume	—	—	3852±793	4548±552
Blood volume (ml/kg)	—	—	64.4±18.5	61.9±9.5

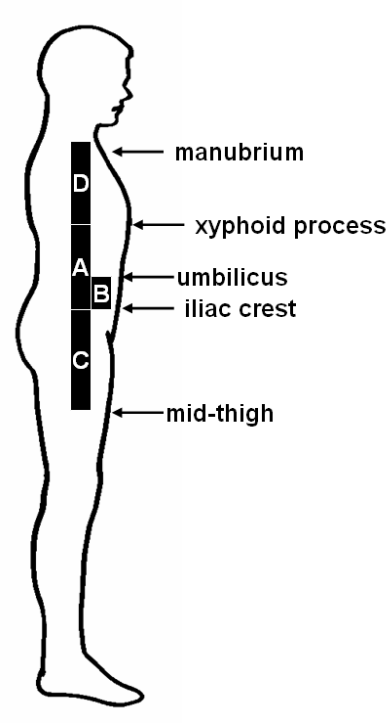

Figure **6-1**: Electrode placement for segmental impedance. Four segments were bracketed along the legs and torso. A: splanchnic; B: pelvic; C: upper thigh; and D: thoracic.

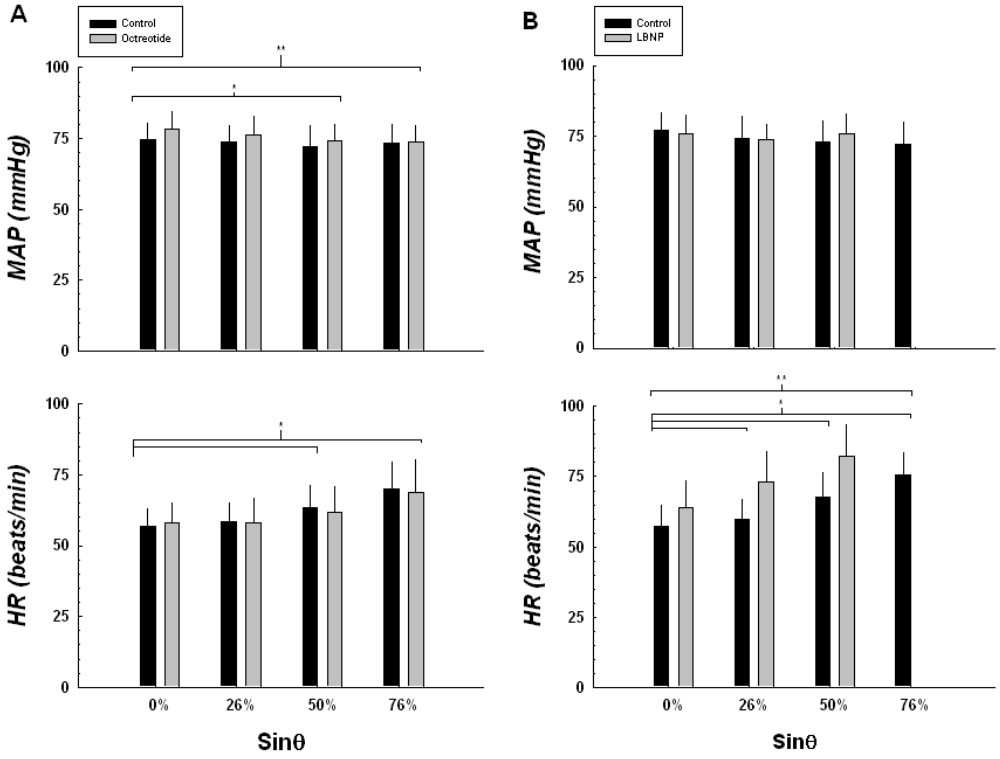

Figure **6-2**: Hemodynamic responses during graded tilt for the VIP determinations. Panel A illustrates the HR and MAP responses during graded tilt for Protocol 1. HR was higher than baseline (0°) during 30° and 50° HUT, corresponding to 50% and 76% of standing, respectively. MAP was lower during 30° HUT but not different from baseline during 15° HUT or 50° HUT. Panel B represents HR and MAP responses for Protocol 2. HR progressively increased from baseline in each subsequent stage and was higher during the LBNP protocol. MAP was well maintained throughout the graded tilt and was not different between conditions. An adequate sample size was not available at 50° HUT during the LBNP protocol, thus, these data were omitted. *Difference from 0°, $p<0.05$. **Difference between conditions, $p<0.01$.

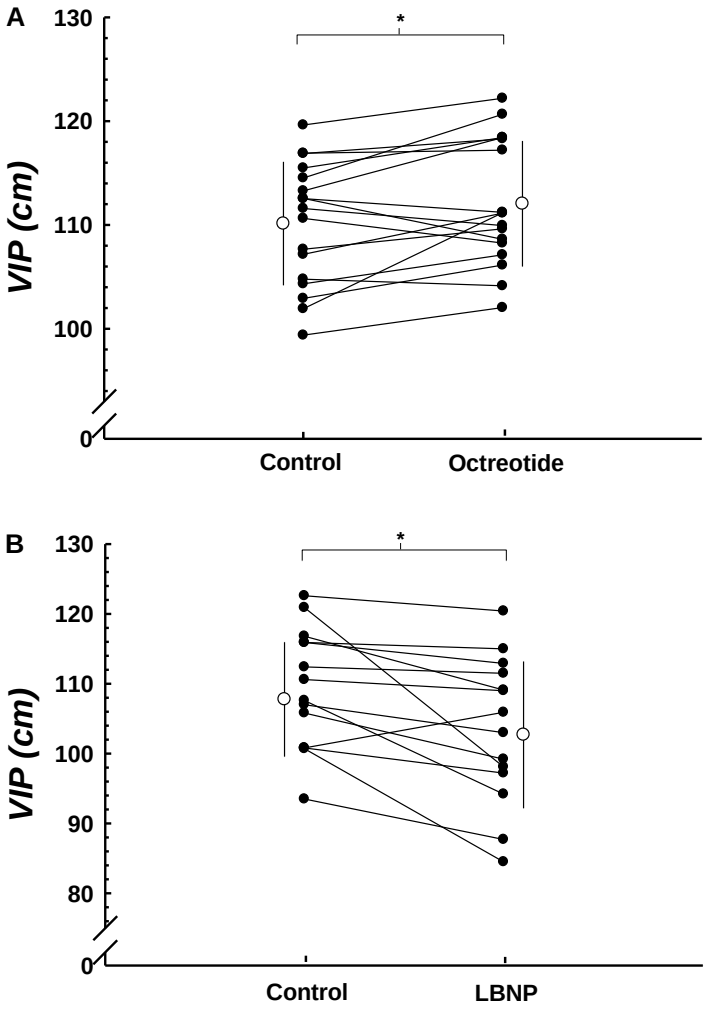

Figure **6-3**: The location of the VIP during control and experimental conditions. Panel A represents the octreotide induced shift of +1.9±3.3 cm in the location of the VIP in Protocol 1. Panel B illustrates the LBNP induced inferior shift of 6.0±7.2 cm compared to the control trial in Protocol 2. *Difference between conditions, $p<0.05$.

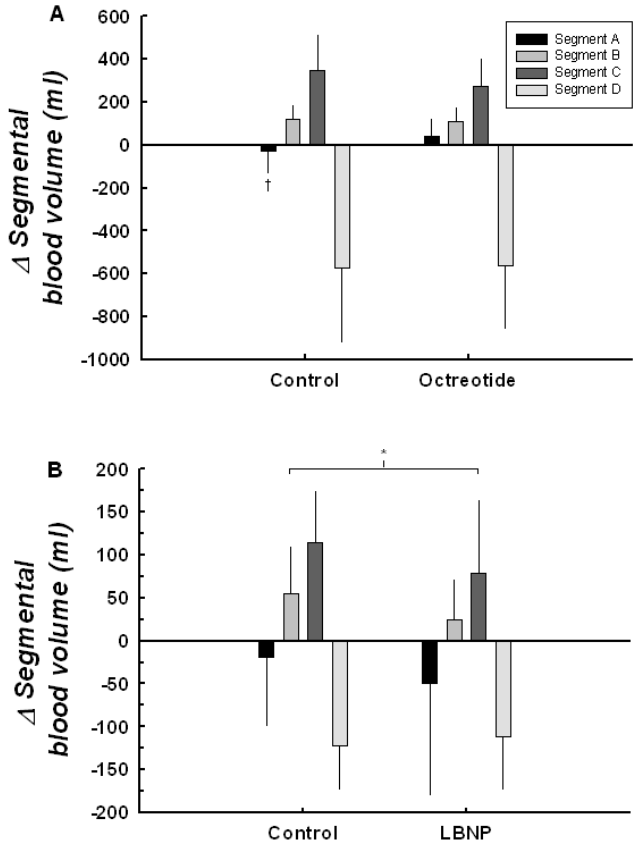

Figure **6-4**: Segmental blood volume changes during the VIP determination. Panel A represents the change in blood volume (0°-50°) during Protocol A. Changes in Segment A (splanchnic) were different between the conditions. Panel B represents the changes in blood volume (0°-15°) during Protocol 2. The magnitude of change is smaller in Procotol 2 because comparisons between 0°-15° were used vs. 0°-50° during Protocol 1. An insufficient number of data points existed at the higher tilt angles during Protocol 2. *Difference between conditions, $p<0.05$. †Difference between conditions, $p<0.01$.

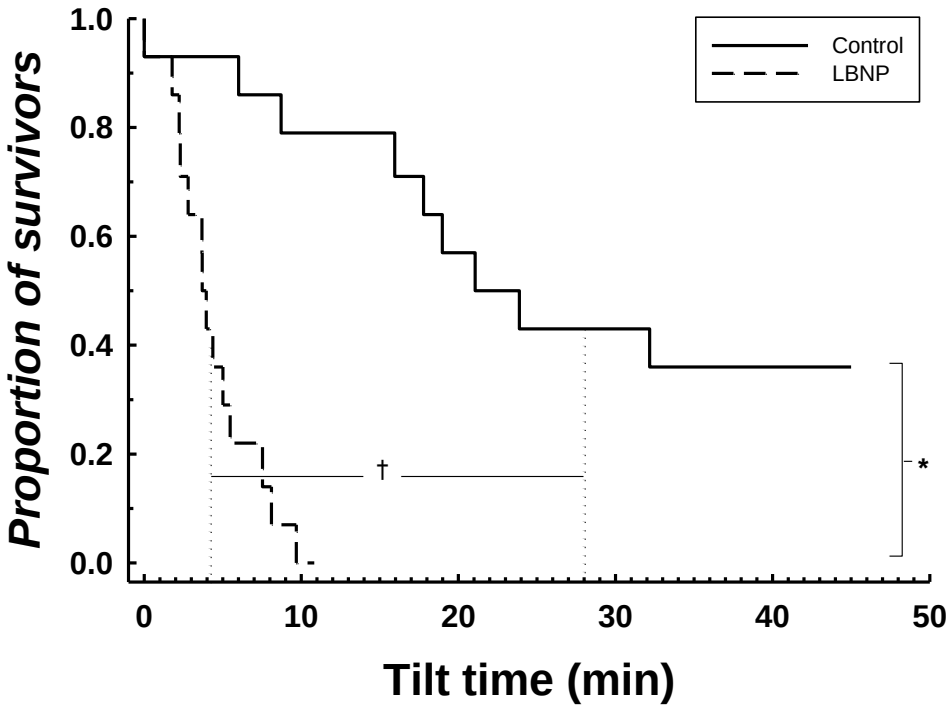

Figure **6-5**: Median tilt times and proportion of survivors during 70° HUT with and without -20 mmHg LBNP. The median tilt time (dotted lines) during the control tilt was 28.0 min and 4.2 min during the LBNP protocol. Survival anlysis indicated a difference existed between the proportion surviving 45 min of tilt (6/14 vs. 0/14) between the conditions. *Difference between conditions, $p<0.01$. †Difference in median tilt times, $p<0.01$.

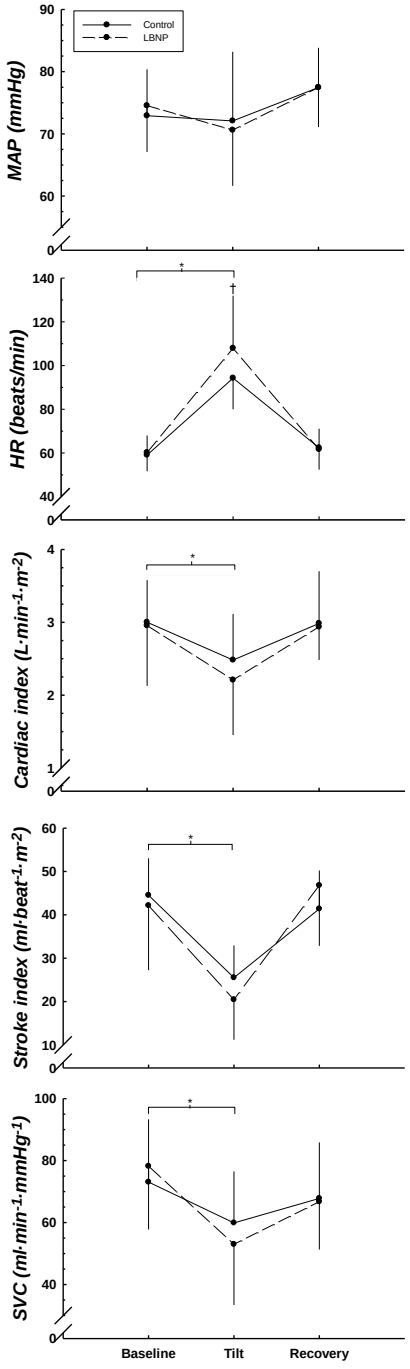

Figure **6-6**: Hemodynamic responses to tilt tolerance tests. MAP was not significantly altered by HUT and was similar between conditions. HR was higher during tilt than during supine and was higher during tilt combined with LBNP. HUT reduced $\dot{Q}_i$, SV_i, and SVC with no statistical difference in these variables between conditions. N.B.: LBNP was only applied during the HUT portion of data collection. *Difference between baseline and HUT, $p<0.01$. †Difference between conditions, $p<0.05$.

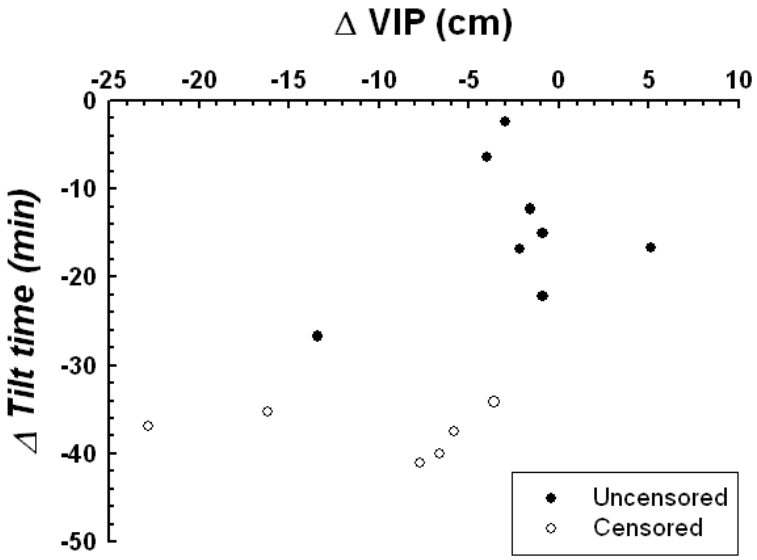

Figure **6-7**: The change in the location of the VIP predicts tilt time. A significant relationship between ΔVIP and Δtilt time existed (Δtilt time = 3.05 + 0.12 ΔVIP, p=0.03) indicating that those individuals that demonstrated the largest inferior shift in the location of the VIP. The open circles represent those individuals that were right censored (completed 45 min of tilt during the control condition). Thus, the true difference in tilt time for these individuals may be larger than was is illustrated.

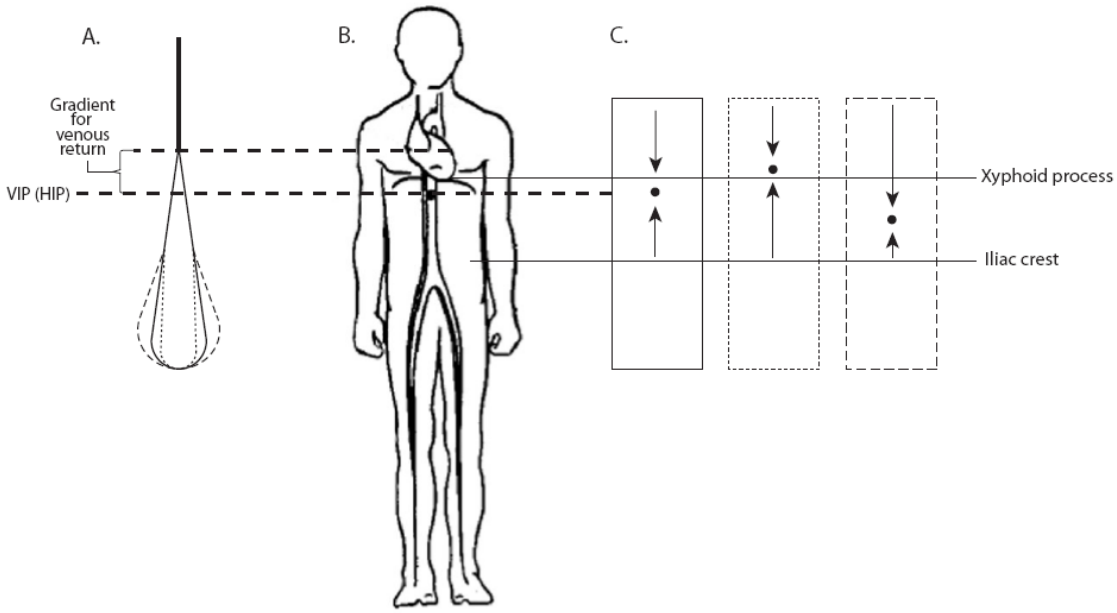

Figure **6-8**: Theoretical conceptualization of the VIP during control and experimental manipulations. Panel A represents the sum of regional compliances that contribute to the location of the VIP. The solid line (—) depicts a homeostatic condition. The dotted line (····) illustrates that selective splanchnic vasoconstriction with octreotide contracts a circulation that contributes greatly to the VIP and, therefore, induces a superior shift. The dashed line (---) shows that -20 mmHg LBNP results in splanchnic pooling, which moves the VIP inferiorly. Panel B references the human body to the VIP and to the net change in blood volume in the splanchnic segment. Panel C illustrates how manipulation of the location of the VIP effects the net blood volume change in the splanchnic segment. The dot (•) represents the VIP and the arrows a loss (↓) or gain (↑) of blood volume. The size of the arrow is related to the magnitude of loss or gain. The first box represents a homeostatic condition. The middle box illustrates how octreotide moves the VIP superior, thereby exposing the splanchnic segment to blood volume gain. The last box shows how LBNP moves the VIP inferiorly, which exposes a large portion of the splanchnic segment to volume loss. Figure adapted from Rowell (Rowell, 1986).

Chapter 7

CONCLUSIONS

The five studies comprising this dissertation were designed to explore sex differences relating to blood pressure regulation in the upright posture. Additionally, these studies sought to quantify the impact of blood volume manipulation of the splanchnic region as it relates to OT. This chapter is intended to summarize the results from these studies. The future direction of this research will also be addressed.

Vasoconstrictor Reserve during Head-up Tilt

The principal findings of this study (Chapter 3) were that women had lower SpBF and SpVC at rest and demonstrated a smaller decrease in SpBF and SpVC between baseline and HUT. Women tended to be less orthostatically tolerant than the men where the primary reason appeared to be an attenuated vasoconstrictor response in the splanchnic circulation.

A Somatostatin Analog Improves Tilt Table Tolerance

The primary finding from this study (Chapter 4) was that administration of octreotide acetate, a sst analog, improved tilt table tolerance in women and men. Administration of 100-125 μg octreotide decreased SpVC and improved median tilt times significantly. A relationship between the change in SpBF (placebo and octreotide conditions) and improvements in tilt time was observed for the women. Thus, in women, those who demonstrated the greatest decrease in SpBF between the conditions also demonstrated the largest improvements in tilt table tolerance.

Identification of the Volume Indifferent Point

The goal of this study (Chapter 5) was to develop an experimental paradigm to represent blood volume distribution during HUT in a single parameter (the VIP). During HUT, blood volume redistributes from the upper body to the lower body where the smallest change occurred in/near the splanchnic circulation. The VIP was repeatably located at 64.5±2.6% of an individual's height. This method may provide a quantitative framework to assess the effects of blood volume distribution and gravitational fields on tilt tolerance in order to examine differences between groups or in response to interventions.

The Location of the Volume Indifferent Point Predicts Orthostatic Tolerance

The primary finding from this study (Chapter 6) was that the location of the VIP predicts tolerance to an orthostatic stress. Administration of 1.7 μg/kg octreotide, a selective splanchnic vasoconstrictor, induced a superior shift (+1.9±3.3 cm) in the location of the VIP, which may explain the improvements in tilt tolerance observed in study 2. Conversely, application of -20 mmHg LBNP moved the VIP inferiorly (-6.0±7.2 cm). The displacement was directly associated with decrements in tilt table tolerance, where individuals that had the largest shift in the VIP also had the largest decrease in tilt tolerance.

Summary

This section summarizes the original hypotheses and the observations that support or refute these statements.

Hypothesis 1: Women will demonstrate diminished splanchnic vasoconstriction, leading to splanchnic pooling, lower systemic resistance, and lower tilt table tolerance.

Observation 1: Women demonstrated an attenuated vasoconstrictor response during HUT and tended to have lower tilt table tolerance. Women did not have lower systemic resistance. Unexpectedly, when the components of systemic resistance were dissected into splanchnic and non-splanchnic, women demonstrated greater resting tone in the splanchnic circulation.

Hypothesis 2a: Administration of octreotide, which selectively constricts blood vessels in the splanchnic region, will decrease splanchnic blood flow and splanchnic vascular conductance, leading to improvements in tilt table tolerance.

Observation 2a: Administration of octreotide decreased splanchnic vascular conductance and improved tilt table tolerance in both women and men.

Hypothesis 2b: Improvements in tilt table tolerance after administration of octreotide will be greater in women.

Observation 2b: Tilt tolerance was comparable between the sexes after octreotide was given. However, reductions in splanchnic blood flow between the placebo and drug interventions were directly related to improvements in tilt table tolerance in the women but not the men.

Hypothesis 3a: The volume indifferent point is analogous to the hydrostatic indifferent point, thus, it will be located ~2/3 of an individual's height.

Observation 3a: The volume indifferent point was repeatably located at 64.5±2.6% of an individual's height. The splanchnic segment contributed most to the location of the VIP.

Hypothesis 3b: The volume indifferent point will be located more inferiorly in women since women have been reported to redistribute a greater proportion of the blood volume to the lower abdominal/pelvic region *and* tend to be less orthostatically tolerant.

Observation 3b: There was no difference in the location of the VIP between the sexes.

Hypothesis 4: Administration of octreotide will decrease splanchnic blood flow and, therefore, the volume of blood in the splanchnic region, which will shift the location of the volume indifferent point superiorly.

Observation 4: Administration of octreotide moved the VIP to a more superior location (+1.9±3.3 cm).

Hypothesis 5a: Application of -20 mmHg lower body negative pressure will increase the volume of blood pooled in the splanchnic region and will induce an inferior shift in the location of the volume indifferent point.

Observation 5a: Application of -20 mmHg lower body negative pressure induced an inferior shift in the location of the VIP (-6.0±7.2 cm).

Hypothesis 5b: The magnitude of change in location of the volume indifferent point, due to lower body negative pressure, will be related to changes in tilt tolerance.

Observation 5b: Decrements in tilt table tolerance (with and without lower body negative pressure) were associated with the magnitude of the change in terms of the location of the VIP.

Conclusions

Women and men regulate blood pressure differently in the upright posture. Consistent with studies examining other circulations in women (Bowyer *et al.*, 2001; Ergul *et al.*, 1998; Kneale *et al.*, 2000), the women in our studies demonstrated an attenuated vasoconstrictor response that was isolated to the splanchnic circulation. Rowell (Rowell, 1986) and others (Gelman, 2008) have reported that the splanchnic circulation plays a large role in the maintenance of blood pressure because it is a high flow, high capacitance region. We found that individuals unable to adequately constrict this circulation during an orthostatic challenge tended to be less

tolerant. This has also been shown in two other populations: bed rest subjects and patients with POTS. Arbeille et al (Arbeille *et al.*, 2005) demonstrated that individuals who were orthostatically intolerant after 90 days of bed rest also exhibited a significantly smaller decrease in the portal vein cross sectional area during HUT. Additionally, patients with POTS pool a greater volume of blood in the gut region during standing or HUT (Stewart & Montgomery, 2004). Thus, blood volume distribution in the upright posture influences an individual's tolerance to an orthostatic stress.

Clinical studies have utilized a wide range of pharmacological agents for the treatment of OI. For example, Hoeldtke et al (Hoeldtke *et al.*, 1998) administered midodrine (an α_1-adrenergic agonist) to a group of POTS patients, finding that OI was not ameliorated, indicating that non-selective vasoconstriction was not effective for this group. When we administered a selective splanchnic vasoconstrictor (octreotide) to our healthy volunteers we found a significant improvement in tilt table tolerance; these improvements were comparable between the sexes. Others have also found that selective vasoconstriction of the splanchnic circulation improves standing times in POTS patients (Hoeldtke *et al.*, 1998). Thus, manipulation of blood flow to the splanchnic circulation affects tilt tolerance. However, studies of patient populations have done little to determine the mechanism of the improvement in blood pressure regulation after the administration of octreotide.

Accordingly, we developed and validated an experimental paradigm that allowed for a quantitative assessment of blood volume distribution during HUT that could be defined in a single parameter (the VIP). The location of the VIP should determine the distance needed to overcome in order to fill the right atrium. Its location is determined by the balance between the hydrostatic pressure and the mechanical properties of the compliant circulation below it (Rowell, 1986). A more superiorly located point should translate into improvements in tilt tolerance and a more inferiorly located point should lead to decrements in tilt tolerance. Others (Perko *et al.*,

1993) have suggested that a VIP exists but past investigations have not identified its exact location (in the transverse plane), nor have past investigations tried to experimentally manipulation the location of this point.

We found that the location of the VIP was most influenced by blood volume in the splanchnic circulation. When blood flow and blood volume to the gut region was reduced with administration of octreotide, there was a superior shift in the location of the VIP and an associated improvement in tilt table tolerance. Conversely, when LBNP was used to mimic a POTS-like patient population, by inducing splanchnic pooling, there was an inferior shift in the location of the VIP and a worsening of tilt table tolerance. Moreover, there was a direct relationship between decrements in tilt table tolerance and an inferior positioning of the VIP, indicating that those individuals that had the largest inferior shift in the VIP during LBNP also demonstrated the largest decrement in tilt table tolerance.

It is not clear why a sex difference was not present in all of the dissertation studies, particularly studies 3, 4, and 5. While more women present with OI in a clinical setting, it may be that the samples under investigation for the dissertation studies was not representative of the clinical population. Our subjects were normal, healthy college-aged individuals without prior history of OI. Access to an adequate sample size of a patient population in this area is nearly impossible to achieve. With this limitation in mind, study 5 was designed to specifically address this issue and to simulate a POTS-like patient through the application of LBNP. Future studies, as outlined below, should utilize the theoretical groundwork developed in these studies and should seek to further our understanding of whether the location of the VIP differs in a patient population.

Future Research Directions

The studies comprising this dissertation provide insight into differences between women and men in terms of blood pressure regulation during orthostasis. These studies also emphasize the important role of the splanchnic circulation and extend the current view by providing a quantitative assessment of blood volume distribution in the upright posture. These studies raise several questions that should be addressed in future investigations:

1. Further explore and indentify the mechanism(s) for the reduced splanchnic blood flow observed in women during resting conditions.
2. Because the women demonstrated a different response to octreotide (non-splanchnic and systemic vascular conductance), simultaneously address the renal and splanchnic contributions to systemic conductance during HUT, with and without octreotide.
3. Based on the response (non-splanchnic and systemic vascular conductance) of the women during the octreotide trial, characterize the effect of octreotide on the renal circulation.
4. Characterize the distribution of sst receptors and whether differences between women and men exist in terms of distribution, density, and/or sensitivity.
5. Better characterize the mechanism(s) by which octreotide induces splanchnic vasoconstriction.
6. Validate the VIP against the HIP.
7. Determine whether the location of the VIP differs in the patient population (e.g., patients with POTS).
8. Determine whether individuals susceptible to decrements in tilt tolerance after a bed rest study can be identified *a priori*.
9. Determine whether combined octreotide and midodrine also have an additive effect on raising the location of the VIP.

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Appendix

INFORMED CONSENT FORMS

Informed consent for study 1 and 2

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY
The Pennsylvania State University
Protocol 2

Title of Project: Improving Orthostatic Tolerance in Women: Control of Splanchnic and Cutaneous Vascular Capacitance

Principal Investigator: James A. Pawelczyk, Ph.D.

Other Investigators: W. Larry Kenney, Ph.D.
Urs Leuenberger, M.D.
Nancy Williams, Sc.D.

Graduate Students: John Florian
Sara Jarvis

Research Assistant: Sandra Smithmyer

This is to certify that I,_____, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. James A. Pawelczyk.

1. Purpose of the study:
The purpose of this investigation is to test your ability to control blood pressure with and without an injection of a drug called octreotide, which causes blood flow in your splanchnic (gut) region to decrease and may improve your body's ability to control its blood pressure. These measurements will be done by tilting subjects at different angles on a tilt table. A total of 16 male and 16 female volunteers will be included in this investigation. To complete this study you will be asked to visit the Noll Physiological Research Center and/or the General Clinical Research Center (GCRC), on five separate occasions.

2. Procedures to be followed:
General information: A total of 5 days are required to complete this study. These include:

Pre-study Screening Days:
- Blood draw and lab tour (45 minutes)
- Physical and exercise test (2 hours)

Preparation Day: Splanchnic (gut) extraction before and after octreotide injection (2 hours)

Study Day 1: Tilt test with octreotide injection (4 hours)
Study Day 2: Tilt test without octreotide injection (4 hours)

Pre-study Screening Day: During your first visit we will give you a tour of Noll laboratory. You will complete a detailed medical history and a GCRC clinician will draw a small amount of blood from your arm (less than a tablespoon or 15 milliliters) with a needle in order to perform routine blood checks for anemia, kidney function, and liver function. On a second day, about 3 days later, you will receive a physical examination by a designated GCRC clinician. We will record the electrical activity of your heart (electrocardiogram). In addition, you will undergo a graded exercise test (GXT). This test is detailed on the separate GXT informed consent sheet.

Menstrual History (female participants): You will complete a menstrual history questionnaire to document your gynecological age, prior history of menstrual cycle lengths, and menstrual cycle characteristics. The study days will take place on the day of your menstrual cycle when estrogen levels are lowest (approximately 3-5 days after your period begins).

Pregnancy Test (female participants): An over the counter urine pregnancy test will be performed before each experimental day. If the test result is positive you will be excused from participating in the study.

Preparation Day- Splanchnic (gut) removal of dye: The ability of your liver to remove the dye used later in the study will be measured before and after an injection of octreotide. Following the application of a numbing cream, a catheter (small tube) will be inserted into each hand or forearm. After 30 minutes of rest, this dye, indocyanine green (ICG), will be injected into one of the tubes inserted into a vein. Small samples of your blood will be taken from the second catheter every three minutes for one-half hour. The total amount of blood removed will be about 2 tablespoons (30 mls). This will be repeated after you are given an injection of octreotide. Right before and then following the octreotide injection, we will take small amounts of blood (about 1 teaspoon total) to measure your blood sugar levels. This is done to make sure that your blood sugar doesn't become too low following the injection of octreotide.

ICG is a safe non-toxic substance that is easily cleared by the liver and can be safely injected with little risk when subjects are properly screened. **People who are sensitive or allergic to penicillin, iodides, shellfish, or sulfa drugs should not participate because they have a greater chance of being allergic to the dye**. Therefore, you will be asked if you have had any reactions to these types of medicines in the past, and if you have, you will not be allowed to participate in the project. This is a necessary requirement to reduce the slight possibility of an allergic reaction to ICG.

Study Day 1 and 2: Following the application of a numbing cream, a catheter (small tube) will be inserted into each hand or forearm. You will then be placed on a tilt table and again given an injection of either octreotide or saline. After twenty minutes, you will be slowly raised to a tilt angle of 70˚ (almost standing). While you are lying flat and during the tilting, we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- blood flow from cuffs attached to your wrist and upper arm,
- your heart's pumping ability by breathing in and out of a bag containing a small amount of a gas called acetylene,

- blood sugar levels by drawing small amounts (about 1 teaspoon total) of blood from your arm,
- Fluid volume changes in your abdomen,
- splanchnic (gut) blood flow by similar infusion of ICG described above.

You will remain tilted for no more than 45 minutes. During that time you should stay as relaxed and motionless as possible. If your blood pressure begins to fall or you begin to feel like you may pass out, we will return you to a flat position and you will feel better within minutes. After the tilt test, you will be lowered to a flat position.

After you are finished with day 1 of the study, you will be asked to return to the lab one more time to repeat the tilt test during the same phase of your menstrual cycle if you are a female. If you received an injection of octreotide before the first tilt test, you will receive an injection of saline before the second tilt test and vice versa.

On rare occurrences we may need to infuse more ICG during the study. This would add an additional 30-40 minutes to the length of the study.

If we discover that you are pregnant at any time during your involvement in the study, you will be notified immediately and eliminated from the study.

Researchers of either sex will be available to administer tests if you so desire.

A more detailed description of these days, and the procedures to be conducted, is provided below. All procedures will take place at the Noll Physiological Research Center or the GCRC. For two days before each day of the study you should be sure to drink normal amounts of fluid (8 glasses water, juice, or sports drinks per day). You should avoid using any type of stimulant (including cold medications and chocolate), drinking caffeine (coffee, tea, cola), and drinking alcohol after 9 PM the night before any procedure.

Detailed description of the procedures to be used:
Please read the detailed description of each procedure below and initial each one to indicate that you have read and understand them.

____ Heart Rate. Electrodes (sticky patches) will be applied to the skin of your chest to measure your heart's electrical activity. Other than skin irritation from the sticky electrodes, there is no known risk involved with this procedure.

___ Blood Pressure. A wrist brace and sensor will be placed over your wrist and a cuff will be placed on your upper arm. The sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed. There is no known risk involved with this procedure.

___ Blood Flow. Periodically we will measure blood flow to your forearm. Velcro blood pressure cuffs will be wrapped around your upper arm and around your wrist, and thin rubber tubes filled with mercury will be wrapped around the middle of your arm. The wrist cuff will be inflated to a pressure high enough to stop blood flow to your hand. This causes no problems for the 2-5 minute period the cuff will be inflated. While this cuff is inflated, the other cuff will be inflated to a much lower pressure for 10-15 seconds, about 3 times a minute. While this cuff inflates you may feel that your arm is swelling. This sensation will cease when the cuffs are released.

___ Abdominal fluid measurement (bioimpedance). We will place sticky tape around your chest, waist, legs, and lower neck. A cable will be connected to the tape around your legs and around your neck. A very small current of electricity will flow through the tape and through your body. You will not be able to detect the current as it passes through your body.

___ Cardiac Output (acetylene rebreathing). We will measure your heart's pumping rate by analyzing the air you rebreathe in and out of a bag using fairly deep breaths for 15-20 seconds. The bag will contain a small concentration of two gases, acetylene and helium. The concentrations of these gasses are so small that there is no risk of them catching fire. Some people report a slightly "tangy" taste from the rebreathing gas. Although you may become light-headed for a few seconds during rebreathing or develop a slight headache from repeated rebreathing, there are no other known risks to performing this procedure. The gas disappears from your lungs and blood in less than 5 minutes.

___ Splanchnic (gut) blood flow. The greenish dye called ICG will be pumped into a tube inserted into a vein in your forearm throughout the test. A little more than 1 tablespoon (15 mls) of ICG will be injected just prior to starting the pump. Small samples of your blood will then be taken from a second catheter inserted into a vein near your hand every 5 minutes during tilting. ICG is a safe non-toxic substance that is easily cleared by the liver and can be safely injected with little risk in subjects who show no allergic reactions. An allergic reaction to ICG, though very rare, could be life-threatening.

___ Head-up Tilt. You will lie on a table for 20 minutes and then you will be tilted at an angle of 70˚ on a tilt table for up to 45 minutes. During that time we ask that you relax and stay as motionless as possible. We will stop tilting if your blood pressure becomes too low, you experience an abnormal heart beat, or you feel light-headed or dizzy.

3. Discomforts and risks:

All procedures carry risk. Risk has two aspects: severity and frequency. Severe risk might threaten the loss of life or limb, while a mild risk might be discomfort. The frequency of a risk is the chance that a problem will occur. In this section we have summarized the risks associated with the procedures used in this experiment. The risks in this experiment have different severity and frequency, and some could be life threatening. Please feel free to ask about the severity or frequency of these risks at any time. To help you decide whether or not you are willing to accept the risks associated with this experiment, the table below provides some commonly mentioned risks and the estimated chance they will occur to you:

Contracting meningitis while living in a dorm	1 in 20,000
Being struck by lightning in your lifetime	1 in 10,000
Contracting AIDS if you avoid "high risk" activity	1 in 3,000
Dying of liver disease if you drank one beer per day	1 in 1,000
Developing breast cancer by age 25	1 in 1,000
Contracting a disease caused by radon in your home	1 in 440
Being killed in a car accident in your lifetime	1 in 60
Contracting cancer at some point during your life	
Men	1 in 2
Women	1 in 3

General: If during the screening procedures we should detect any abnormality, we will inform you as soon as possible so that you can contact your personal doctor for treatment. Some people find medical procedures to be scary, and feel faint as a result (vasovagal response). To keep you comfortable and informed, we encourage you to share your concerns with us at any time.

Heart rate, blood flow, and blood pressure: The only known risk associated with measuring heart rate, blood flow, and blood pressure is the possibility that your skin will be irritated by the adhesive, cuffs, or tape. The blood pressure sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed.

Abdominal fluid measurement (bioimpedance): There are no risks involved with this procedure. The sticky tape may cause your skin to become irritated when we remove the tape.

Rebreathing cardiac output: Because you breathe slightly deeper than normal, there is a chance you will feel light headed for a few seconds during rebreathing or develop a slight headache after repeated measurements. There are no other known risks to this procedure.

Indocyanine Green Dye (ICG): ICG is a safe non-toxic substance that is easily cleared by the liver and can be safely injected with little risk when subjects are properly screened. **The only risk involved is when a person is sensitive or allergic to penicillin, iodides, or sulfa drugs**. Therefore, you will be asked if you have had any reactions to these types of medicines in the past, and if you have, you will not be allowed to participate in the project. This is a necessary requirement to insure that the slight possibility of an adverse response to ICG in a subject sensitive to the drugs listed above is alleviated. For those people that deny an allergy, there is a 1 in 250 risk of a mild to moderate reaction. Symptoms include lightheadedness, nausea, hives and itching. Rarely, (1 in 2000) a serious allergic reaction which affects the entire body (anaphylaxis)

may develop. An anaphylactic reaction can be life-threatening. You are free to discontinue these tests at any time.

Octreotide: Octreotide is very similar to a naturally occurring hormone called somatostatin. It is generally well tolerated, though about 1 in 10 people experience diarrhea or an upset stomach. Because this drug makes blood vessels narrow (constrict), your blood pressure will probably rise and your heart rate slow down during the injection. This might be accompanied by a flushed face or a feeling of heaviness in your chest. This is a natural result of a slow heart rate. Less often people become light-headed or tired (1 in 20). If your heart rate or blood pressure changes too much, or at your request, we will end the octreotide infusion. Any unusual sensations should last only a minute or two after the infusion ends.

Topical Anesthetic Cream: Numbing cream will not be used in those who have sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the treated area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling, skin redness or abnormal sensation may develop at the site of treatment. In clinical studies, no serious reactions resulted from the use of the cream. Allergic reactions can occur and can be managed by usual allergic means. Whole body adverse reactions following appropriate use are unlikely due to the small dose absorbed. If effects do occur, they are similar in nature to those seen with other local anesthetic agents and may include lightheadedness, nervousness, apprehension, dizziness, drowsiness, twitching, and vomiting. Reactions may be brief or not at all.

Blood sampling/venous catheter: The risks of a blood sample include bruising and/or discomfort from the needle, venous inflammation from the catheter, infection (less than 1 in 10,000), or the chance that you will become lightheaded. Should you feel this way we will stop the experiment, and you will be given fluids to drink (water or juice). We ask that you remain in the laboratory until we have checked your blood pressure and we are sure that you feel OK.

Head-up tilt: The risk of head-up tilt is that you become lightheaded, nauseous, or pass out. Should you feel this way or we suspect that see that your blood pressure is starting to fall, we will stop the procedure and you should begin to feel better within minutes. In one case (less than 1 in 10,000) a person developed an unusually slow heart rhythm that returned to normal with a drug (atropine) injected in their arm vein.

Treadmill: It is possible for you to stumble or fall on a treadmill leading to cuts, scrapes, dislocations, broken bones, head injury, abnormal cardiac rhythms, or even death. However, this risk is minimal. The risk of heart attack, although minor (1 in 15,000) does exist. You will be taught the safe use of the treadmill and watched closely during exercise. All changes in speed will be made gradually, and you will be assisted in mounting and dismounting.

4. a. Benefits to the subject:

None

b. Potential benefits to society:

This information will be used to help people or patients who have difficulty maintaining their blood pressure. An example is patients who have recently undergone dialysis, some elderly people after eating a meal, and some otherwise healthy people who

experience problems with lightheadedness. These problems are much more common in women.

5. Alternative procedures which could be utilized:
Recording heart rate, blood pressure, and blood flow is routine. Measuring your splanchnic (gut) blood flow can be estimated using sound waves (ultrasound), but this method is not as accurate as the green dye.

6. Time duration of the procedures and study:
This study will require two visits of two hours and two visits of four hours duration to the Noll Laboratory and the General Clinical Research Center (located next to Noll Laboratory).

7. Statement of confidentiality:
You have the right to privacy. All information that is obtained in connection with this study will remain confidential within the limits of State Law. Information gained from this study will be released only to the investigators, and if appropriate, to your physician and the sponsors of the study with your approval. Any information provided to the sponsors of the study will not include your identity. The following may review and copy records related to this research; The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The U.S. Food and Drug Administration (FDA) when applicable; the Penn State University Biomedical Institutional Review Board (IRB); The Penn State University Office for Research Protections. The results of this study may be published in scientific journals without identifying you by name.

8. Right to ask questions:
If you have any questions about the research or about your rights as a subject, we want you to ask us. If you have questions later, or if you wish to report a research-related injury, please contact Dr. Pawelczyk at (814) 865-3453 (W) or (814) 861-1379 (H). Questions regarding this statement or your rights as a subject of this research should be directed to the Office for Research Protections in 201 Kern Graduate Building, University Park, PA (814-865-1775). Please initial the statement below to indicate your understanding of this right.

___ I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction.

9. Compensation:
There will be no charge for any tests required for the study. You will receive $200 for participation in this investigation to compensate your travel and loss of time. If you choose to withdraw early from the investigation, this amount will be prorated accordingly. If you are an employee of Penn State University, the compensation you receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If you are not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Voluntary participation:
Participation in this research study is entirely voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your status (as a patient, student, employee, etc.), or the medical care that you will receive. Also, you may decline to answer any specific questions asked that you might be asked. Under certain circumstances the study may be discontinued by the sponsor or the investigator.

11. Event of Injury:
Medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators.

This is to certify that I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed copy of this consent form. I have read this form, and understand its contents.

_____
Volunteer Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____
Investigator Date

Informed consent for study 3 and 4

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY
The Pennsylvania State University
Protocol 1

Title of Project: Determination of the Human Volume Indifferent Point

Principal Investigator: Sara S. Jarvis
227 Noll Laboratory
(814) 865-0476
ssj120@psu.edu

Other Investigators: James A. Pawelczyk, PhD
107 Noll Laboratory
(814) 865-3453
jap18@psu.edu

Graduate Students: John P. Florian
Ellen E. Spiller

Research Assistants:

This is to certify that I,_____, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. James A. Pawelczyk.

1. **Purpose of the study:**
The purpose of this investigation is to locate your "volume indifferent point" or VIP, the point where volume in your veins does not change when you stand up. We suspect this point is closer to the feet in women than men, which causes women to pass out more often than men when they stand up. To measure the VIP we will place electrodes (sticky patches on your skin) then tilt you at different angles on a tilt table. We will repeat this measurement after we give you a drug called octreotide. Octreotide causes blood flow in your splanchnic (gut) region to decrease and should move your VIP closer to your head. A total of 32 volunteers will be included in this investigation (16 men and 16 women). To complete this study you will be asked to visit Noll Laboratory and/or the General Clinical Research Center (GCRC), on four separate occasions.

2. Procedures to be followed:
General information: A total of 4 days are required to complete this study. For female participants, study days 1 and 2 need to be scheduled around your menstrual cycle. Depending on your availability, this study could take up to three months to complete. These days include:

- Day 1 (Screening Day 1): Blood draw and electrocardiogram (1 tablespoon) (30 minutes)
- Day 2 (Screening Day 2): Physical exam and exercise test (2 hours)
- Day 3 (Study Day 1): VIP determination (3.5 hours)
- Day 4 (Study Day 2): VIP determination (3.5 hours)

- Either on Day 3 or Day 4: VIP determination following octreotide infusion (additional 1.5 hours)

Women of childbearing-age will submit a urine sample at the beginning of each visit for a pregnancy test. If you are pregnant, you will not be able to participate in the project.

A more detailed description of these days, and the procedures to be conducted, is provided below. For two days before each day of the study you should be sure to drink normal amounts of fluid (up to 8 glasses water, juice, or sports drinks per day). You should avoid using any type of stimulant (including cold medications and chocolate), drinking caffeine (coffee, tea, cola), and drinking alcohol after 9 PM the night before any procedure. You will not be allowed to eat or drink anything, except for water, after 9 PM the night before. You should not participate in strenuous physical activity for 12 hrs before the experiment.

Researchers of either sex will be available to administer tests if you so desire.

Screening: During your first visit we will give you a tour of Noll laboratory. You will complete a detailed medical history and a GCRC clinician will draw a small amount of blood from your arm (less than a tablespoon or 15 milliliters) with a needle in order to perform routine blood checks for anemia, kidney function, and liver function. We will record the electrical activity of your heart (electrocardiogram). On a second day, about 3 days later, you will receive a physical examination by a designated GCRC clinician. In addition, you will undergo a graded exercise test (GXT).

VIP determination: On the day of the study, you will be checked into the GCRC. A nurse will insert one catheter (a thin plastic tube) into a vein in your arm to draw blood during the study. If you choose, a numbing cream will be applied to your arm 15 minutes before the catheter is inserted to lessen discomfort. After the catheter is inserted, you will be taken to the lab for testing. During the experiment we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- blood flow in your forearm using blood pressure cuffs,
- your heart's pumping ability by breathing in and out of a bag containing a small amount of a gas called acetylene,
- hormone levels, blood sugar levels and hematocrit by drawing small amounts (about 3 tablespoons total) of blood from your arm,
- fluid shifts in your abdomen and legs using sticky patches that will be placed along your chest, abdomen, legs, and on your hand and foot.

These signals will be recorded as you lie flat on your back and while you are tilted for 12 minutes each at 15°, 30°, and 50° on a motorized tilt table. For comparison, lying on your back is 0 degrees, and standing up is 90 degrees. Once all measurements are taken at the different tilt angles, you will again lie flat on your back. We will repeat these measurements after giving you an injection of octreotide on one of the visits. You will return on another day for a repeat of the VIP determination.

Detailed description of the procedures to be used:
Please read the detailed description of each procedure below and initial each one to indicate that you have read and understand them.

____ Heart Rate. Electrodes (sticky patches) will be applied to the skin of your chest to measure your heart's electrical activity. Other than skin irritation from the sticky electrodes, there is no known risk involved with this procedure.

____ Blood Pressure. A wrist brace and sensor will be placed over your wrist and a cuff will be placed on your upper arm. The sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed. There is no known risk involved with this procedure.

____ Venous Catheter (antecubital). When you arrive to the GCRC a numbing lotion will be applied to your forearm. Once the anesthetic has taken effect the nurse will insert a soft plastic tube (catheter) into your arm vein. The catheter will remain in place during the experiment so that blood may be drawn without having to insert a needle each time. The catheter will be removed by a nurse before you are discharged from the GCRC.

____ Blood draw. Skilled GCRC staff will remove blood from the catheter in your arm. The staff uses standard safety measures and sterile techniques that are used in hospitals. A small amount of blood (less than 1/2 teaspoon each time) will be withdrawn 9 times during the VIP only determination study day and 22 times during the octreotide study day. This amount is much less than when you donate blood.

____ Blood Flow. Periodically we will measure blood flow to your forearm. Velcro blood pressure cuffs will be wrapped around your upper arm and around your wrist, and thin rubber tubes filled with mercury will be wrapped around the middle of your arm. The wrist cuff will be inflated to a pressure high enough to stop blood flow to your hand. This causes no problems for the 2-5 minute period the cuff will be inflated. While this cuff is inflated, the other cuff will be inflated to a much lower pressure for 10-15 seconds, about 3 times a minute. While this cuff inflates you may feel that your arm is swelling. This sensation will cease when the cuffs are released.

____ Abdominal and leg fluid measurement (bioelectrical impedance). We will place electrodes (sticky patches) on your chest, abdomen, legs, hand and foot. A cable will be connected to each patch. A very small current of electricity will flow through the electrode and through your body. You will not be able to detect the current as it passes through your body. For comparison, this measurement is similar to body fat scales available in gyms or for home use.

____ Cardiac Output (acetylene rebreathing). We will measure your heart's pumping rate by analyzing the air you rebreathe in and out of a bag using fairly deep breaths for 15-20 seconds. The bag will contain a small concentration of two gases, acetylene and helium. The concentrations of these gasses are so small that there is no risk of them catching fire. Some people report a slightly "tangy" taste from the rebreathing gas. Although you may become light-headed for a few seconds during rebreathing or develop a slight headache from repeated rebreathing, there are no other known risks to performing this procedure. The gas disappears from your lungs and blood in less than 5 minutes.

____ Octreotide. On either study day 1 or 2, 50 milliliters (a little more than 3 Tablespoons) of an octreotide/saline mix will be slowly infused through a tube (catheter) inserted into a vein in your forearm. Octreotide is generally well tolerated.

____ Head-up Tilt. You will lie on a table and then you will be tilted at an angle of 15°, 30°, and 50˚ on a tilt table for 12 min at each angle. During that time we ask that you relax and stay as motionless as possible. We will stop tilting if your blood pressure becomes too low, you experience an abnormal heart beat, or you feel light-headed or dizzy.

____ Graded Exercise Test (GXT). The GXT tests your fitness level and cardiovascular system. Your blood pressure and heart rate will be measured. During the test, you will wear a nose clip and breathe into a tube to measure the oxygen and carbon dioxide you breathe out. You will help the researcher adjust the harness that holds the tube so that you are comfortable. During the test, you will rate how hard you are working by using a numbered scale matched to short phrases (rating of perceived exertion or RPE scale). At first, you will warm up by walking at a comfortable pace on the treadmill for about 4 minutes. Then you will begin to run at a comfortable pace. The grade of the treadmill will increase a little every 2 minutes. The exercise will become harder. The test will be most accurate if you do your best to exercise for as long as you can. However, you can stop at any time. The test is 10-20 minutes long.

3. Discomforts and risks:

All procedures carry risk. Risk has two aspects: severity and frequency. Severe risk might threaten the loss of life or limb, while a mild risk might be discomfort. The frequency of a risk is the chance that a problem will occur. In this section we have summarized the risks associated with the procedures used in this experiment. The risks in this experiment have different severity and frequency, and some could be life threatening. Please feel free to ask about the severity or frequency of these risks at any time. To help you decide whether or not you are willing to accept the risks associated with this experiment, the table below provides some commonly mentioned risks and the estimated chance they will occur to you:

Contracting meningitis while you are at a large university	1 in 20,000
Being struck by lightning in your lifetime	1 in 10,000
Contracting AIDS if you avoid "high risk" activity	1 in 3,000
Dying of liver disease if you drank one beer per day	1 in 1,000
Developing breast cancer by age 25	1 in 1,000
Contracting a disease caused by radon in your home	1 in 440
Being killed in a car accident in your lifetime	1 in 60
Contracting cancer at some point during your life	
Men	1 in 2
Women	1 in 3

Please read the detailed description of each procedure below and initial each one to indicate that you have read and understand them.

____ General: If during the screening procedures we should detect any abnormality, we will inform you as soon as possible so that you can contact your personal doctor for treatment. Some people find medical procedures to be scary, and feel faint as a result (vasovagal response). To keep you comfortable and informed, we encourage you to share your concerns with us at any time.

___ Heart rate, blood pressure, and blood flow: The only known risk associated with measuring heart rate and blood pressure is the possibility that your skin will be irritated by the adhesive, cuffs, or tape. The blood pressure sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed.

___ Blood sampling/venous catheter: The risks of a blood sample include bruising and/or discomfort from the needle, venous inflammation from the catheter, infection (less than 1 in 10,000), or the chance that you will become lightheaded. Should you feel this way we will stop the experiment, and you will be given fluids to drink (water or juice). We ask that you remain in the laboratory until we have checked your blood pressure and we are sure that you feel OK.

___ Topical Anesthetic Cream: Numbing cream will not be used in those who have sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the treated area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling, skin redness or abnormal sensation may develop at the site of treatment. In clinical studies, no serious reactions resulted from the use of the cream. Allergic reactions can occur and will be managed. Whole body adverse reactions following appropriate use are unlikely due to the small dose absorbed. If effects do occur, they are similar in nature to those seen with other local anesthetic agents and may include lightheadedness, nervousness, apprehension, dizziness, drowsiness, twitching, and vomiting. Reactions may be brief or not at all.

___ Abdominal and leg fluid measurement (bioelectrical impedance): There are no risks involved with this procedure. The sticky electrodes may cause your skin to become irritated when we remove them.

___ Rebreathing cardiac output: Because you breathe slightly deeper than normal, there is a chance you will feel light headed for a few seconds during rebreathing or develop a slight headache after repeated measurements. There are no other known risks to this procedure.

___ Octreotide: Octreotide is very similar to a naturally occurring hormone called somatostatin. It is generally well tolerated, though about 1 in 10 people experience diarrhea or an upset stomach. Because this drug makes blood vessels narrow (constrict), your blood pressure will probably rise and your heart rate slow down during the injection, but this should not last more than a minute after stopping the infusion. This might be accompanied by a flushed face or a feeling of heaviness in your chest. This is a natural result of a slow heart rate. Less often people become light-headed or tired (1 in 20). If your heart rate or blood pressure changes too much, or at your request, we will end the octreotide infusion. Any unusual sensations should only last a minute or two after the infusion ends.

___ Head-up tilt: The risk of head-up tilt is that you become lightheaded, nauseous, or pass out. Should you feel this way or we suspect that your blood pressure is starting to fall, we will stop the procedure and you should begin to feel better within minutes. In one case (less than 1 in 10,000) a person developed an unusually slow heart rhythm that returned to normal with a drug (atropine) injected in their arm vein.

___ Treadmill: It is possible for you to stumble or fall on a treadmill leading to cuts, scrapes, dislocations, broken bones, head injury, abnormal cardiac rhythms, or even death. You will be taught the safe use of the treadmill and watched closely during exercise. All changes in speed will be made gradually, and you will be assisted in mounting and dismounting.

4. a. Benefits to the subject:

None

b. Potential benefits to society:

This information will be used to help people or patients who have difficulty maintaining their blood pressure. An example is patients who have recently undergone dialysis, some elderly people after eating a meal, and some otherwise healthy people who experience problems with lightheadedness. These problems are much more common in women.

5. Alternative procedures which could be utilized:

Recording heart rate, blood pressure, and blood flow is routine. Bioelectrical impedance analysis is the most non-invasive way to obtain these data.

6. Time duration of the procedures and study:

This study will require four visits (a total of about 10 hours) to Noll Laboratory and the General Clinical Research Center (a wing of Noll Laboratory). The screening visits will last about 2 hours and each study day will last about 4 hours each.

7. Statement of confidentiality:

You have the right to privacy. All information that is obtained in connection with this study will remain confidential within the limits of State Law. Information gained from this study will be released only to the investigators, and if appropriate, to your physician and the sponsors of the study with your approval. Any information provided to the sponsors of the study will not include your identity. The following may review and copy records related to this research The Office of Human Research Protections in the U.S. Dept. of Health and Human Services; The U.S. Food and Drug Administration (FDA); The Penn State University Biomedical Institutional Review Board (IRB); The Penn State University Office for Research Protections. Any information provided to the sponsors of the study will not include your identity. The results of this study may be published in scientific journals without identifying you by name.

8. Right to ask questions:

If you have any questions about the research or about your rights as a subject, we want you to ask us. If you have questions later, or if you wish to report a research-related injury, please contact Sara Jarvis at (814) 865-0476 (W) or (814) 441-2113 (H) or Dr. Pawelczyk at (814) 865-3453 (W) or (814) 861-1379 (H). Questions regarding this statement or your rights as a subject of this research should be directed to the Office for Research Protections in 201 Kern Graduate Building, University Park, PA (814-865-1775). Please initial the statement below to indicate your understanding of this right.

___ I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction**.**

9. Compensation:
There will be no charge for any tests required for the study. You will receive $50 after completing the first study day and $75 after completing the second study day (for a total of $125) for participation in this investigation to compensate your travel and loss of time. If you choose to withdraw early from the investigation, this amount will be prorated accordingly. Total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Voluntary participation:
Participation in this research study is entirely voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your status (as a patient, student, employee, etc.), or the medical care that you will receive. You may decline to answer specific questions. However, your acceptance into the study may be contingent upon answering these questions. Under certain circumstances the study may be discontinued by the sponsor or the investigator.

11. Event of Injury:
Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators.

12. Abnormal Test Results:
In the event that abnormal test results are obtained, you will be made aware of the results within 2 weeks and recommended to contact your private medical provider for follow-up.

This is to certify that I am 18 years of age or older and I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed and dated copy of this consent form. I have read this form, and understand its contents.

_____
Volunteer Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____
Investigator Date

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY
The **Pennsylvania State University**
Addendum to Protocol 1

Title of Project:	Determination of the Human Volume Indifferent Point
Principal Investigator:	Sara S. Jarvis 227 Noll Laboratory (814) 865-0476 ssj120@psu.edu
Other Investigators:	James A. Pawelczyk, PhD 107 Noll Laboratory (814) 865-3453 jap18@psu.edu
Graduate Students:	John P. Florian Ellen E. Spiller

This is to certify that I,_____, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. James A. Pawelczyk.

1. **Change in procedures:**
The purpose of this addendum is to remove the lower body negative pressure procedure from this study. A total of 4 visits are still required but either study day 1 or 2 will be 1.5 hrs shorter. Additionally, there will be fewer blood samples withdrawn (9 vs. 17) on one of those study days. The compensation will remain the same. All of the other procedures are the same as that which was outlined in the original consent form.

This is to certify that I am 18 years of age or older and I consent to and give permission for my continued participation as a volunteer in this program of investigation. I understand that I will receive a signed and dated copy of this addendum. I have read this form, and understand its contents.

_____
Volunteer Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____
Investigator Date

Informed consent fort study 5

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY
The **Pennsylvania State University**
Protocol 2

Title of Project: Experimental Manipulation of the Volume Indifferent Point with Lower Body Negative Pressure

Principal Investigator: Sara S. Jarvis
227 Noll Laboratory
(814) 865-0476
ssj120@psu.edu

Other Investigators: James A. Pawelczyk, PhD
107 Noll Laboratory
(814) 865-3453
jap18@psu.edu

Research Assistants: Sandy Smithmyer
201 Noll Laboratory
(814) 863-3182

Undergraduate Assistants: Dave Leone
Kylie Weaver

This is to certify that I,_____, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. James A. Pawelczyk.

1. **Purpose of the study:**
The purpose of this investigation is to locate your "volume indifferent point" or VIP, the point where volume in your veins does not change when you stand up. We suspect this point is closer to the feet in people susceptible to passing out when they stand up. To measure the VIP we will place electrodes (sticky patches on your skin) then tilt you at different angles on a tilt table. We will repeat this measurement during lower body negative pressure. Lower body negative pressure causes more blood to shift toward your feet and should move your VIP closer to your feet. We will also test your ability to control blood pressure while you are tilted on the tilt table. A total of 16 volunteers will be included in this investigation (8 men and 8 women). To complete this study you will be asked to visit Noll Laboratory and/or the General Clinical Research Center (GCRC), on five to six separate occasions.

2. Procedures to be followed:

General information: A total of 5 to 6 days are required to complete this study. For female participants, the blood volume determination visit and Study Days 1, 2, and 3 need to be scheduled during a certain time of your menstrual cycle (days 2-7). Depending on your availability, this study could take up to four months to complete. These days include:

- Day 1 (Screening Day 1): Blood draw (1 tablespoon), blood pressure (sitting and standing), height and weight, waist-to-hip ratio measurements, resting electrocardiogram, medical/family history (1 hour)
- Day 2 (Screening Day 2): Physical exam and lab familiarization (1 hr)
- Day 3 (Blood volume determination): Blood volume determination (1 ½ hours; this visit may be added to another visit or may be completed separately)
- Day 4 (Study Day 1, 2, or 3): VIP determination and VIP determination with lower body negative pressure (3 ½ hours)
- Day 5 (Study Day 1, 2, or 3): Tilt table tolerance test (2 ¾ hours)
- Day 6 (Study Day 1, 2, or 3): Tilt table tolerance test with lower body negative pressure (2 ¾ hours)

Women of childbearing-age will submit a urine sample at the beginning of day 2, 3, 4, 5, and 6 for a pregnancy test. If you are pregnant, you will not be able to participate in the project.

A more detailed description of these days, and the procedures to be conducted, is provided below. For two days before each day of the study you should be sure to drink normal amounts of fluid (up to 8 glasses water, juice, or sports drinks per day). You should avoid using any type of stimulant (including cold medications and chocolate), drinking caffeine (coffee, tea, cola), and drinking alcohol after 9 PM the night before any procedure. You will not be allowed to eat or drink anything, except for water, after 9 PM the night before. You should not participate in strenuous physical activity for 12 hrs before the experiments.

Screening: You will complete a detailed medical history and a GCRC nurse will draw a small amount of blood from your arm (less than a tablespoon or 15 milliliters) with a needle in order to perform routine blood checks for anemia, kidney function, and liver function. We will take measurements of height, weight, and your waist-to-hip ratio. We will record the electrical activity of your heart (electrocardiogram) and obtain blood pressure measurements during sitting and standing.

Physical exam and lab familiarization: About 3 days after your initial screening visit you will receive a physical examination by a designated GCRC clinician. After your physical exam you will attend a familiarization session in the lab (227 Noll Lab).

Blood volume determination: On the day of the study you will be checked into the GCRC. For female subjects this visit will need to be timed around your menstrual cycle. This visit may be added to another visit or can be completed separately. A nurse will insert one catheter (a thin plastic tube) into a vein in each arm to draw blood. If you choose, a numbing cream will be applied to your arms 20 minutes before the catheters are inserted to lessen discomfort. After the catheters are inserted, a small sample of blood will be drawn and then you will receive an injection of a dye, which will be followed by blood sampling every 3 min for 30 min.

During this visit we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- hematocrit by drawing a small amount (less than 1 teaspoon) of blood from your arm,
- disappearance of the dye from your blood by drawing small amounts of blood from your arm (about 3 tablespoons total)

VIP determination: On the day of the study you will be checked into the GCRC. A nurse will insert one catheter (a thin plastic tube) into a vein in your arm to draw blood during the study. If you choose, a numbing cream will be applied to your arm 20 minutes before the catheter is inserted to lessen discomfort. After the catheter is inserted, you will be taken to the lab for testing. During the experiment we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- your heart's pumping ability by breathing in and out of a bag containing a small amount of a gas called acetylene,
- hematocrit by drawing small amounts (about 1 tablespoon total) of blood from your arm,
- fluid shifts in your abdomen and legs using sticky patches that will be placed along your chest, abdomen, legs, and on your hand and foot.

These signals will be recorded as you lie flat on your back and while you are tilted for 6 minutes each at 15°, 30°, and 50° on a motorized tilt table. For comparison, lying on your back is 0 degrees, and standing up is 90 degrees. Once all measurements are taken at the different tilt angles, you will again lie flat on your back. We will repeat these measurements with lower body negative pressure.

Tilt table tolerance: On the day of the study you will be checked into the GCRC. A nurse will insert one catheter (a thin plastic tube) into a vein in your arm or hand. If you choose, a numbing cream will be applied 20 minutes before the catheter is inserted to lessen discomfort. After the catheter is inserted, you will be taken to the lab for testing. You will be placed on a tilt table and will be slowly raised to a tilt angle of 70˚ (almost standing). While you are lying flat and during the tilting, we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- your heart's pumping ability by breathing in and out of a bag containing a small amount of a gas called acetylene,

You will remain tilted for no more than 45 minutes. During that time you should stay as relaxed and motionless as possible. If your blood pressure begins to fall or you begin to feel like you may pass out, we will return you to a flat position and you will feel better within minutes. After the tilt test, you will be lowered to a flat position.

Tilt tolerance with LBNP: On the day of the study you will be checked into the GCRC. A nurse will insert one catheter (a thin plastic tube) into a vein in your arm or hand. If you choose, a numbing cream will be applied 20 minutes before the catheter is inserted to lessen discomfort. After the catheter is inserted, you will be taken to the lab for testing. You will be placed on a tilt table and will be slowly raised to a tilt angle of 70˚ (almost standing) while undergoing lower body negative pressure. While you are lying flat and during the tilting, we will measure:

- heart rate from sticky patches placed on your chest,
- blood pressure from your arm and a sensor on your wrist,
- your heart's pumping ability by breathing in and out of a bag containing a small amount of a gas called acetylene,

You will remain tilted for no more than 45 minutes. During that time you should stay as relaxed and motionless as possible. If your blood pressure begins to fall or you begin to feel like you may pass out, we will return you to a flat position and stop the lower body negative pressure and you will feel better within minutes. After the tilt test, you will be lowered to a flat position.

Detailed description of the procedures to be used:

Please read the detailed description of each procedure below and initial each one to indicate that you have read and understand them.

____ Heart Rate. Electrodes (sticky patches) will be applied to the skin of your chest to measure your heart's electrical activity. Other than skin irritation from the sticky electrodes, there is no known risk involved with this procedure.

____ Blood Pressure. A wrist brace and sensor will be placed over your wrist and a cuff will be placed on your upper arm. The sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed. There is no known risk involved with this procedure.

____ Venous Catheter. When you arrive to the GCRC a numbing lotion will be applied to your arm or hand. Once the anesthetic has taken effect the nurse will insert a soft plastic tube (catheter) into your vein. The catheter will remain in place during the experiment so that blood may be drawn without having to insert a needle each time. The catheter will be removed by a nurse before you are discharged from the GCRC.

____ Blood draw. Skilled GCRC staff will remove blood from the catheter in your arm. The staff uses standard safety measures and sterile techniques that are used in hospitals. Small amounts of blood will be drawn during screening (about 1 tablespoon), on the blood volume determination day (about 3 tablespoons), and on the VIP determination day (about 1 tablespoon). The total amount is much less than when you donate blood.

____ Indocyanine green (ICG). To determine your total blood volume we will look at the ability of your liver to remove a dye from your body. After 30 minutes of rest, this dye, indocyanine green (ICG), will be injected into a tube inserted into a vein in your forearm. Small samples of your blood will be taken from a second catheter inserted into a vein near your hand every three minutes for one-half hour. The total amount of blood removed will be about 3 tablespoons (45 mls). ICG is a safe non-toxic substance that is easily cleared by the liver and can

be safely injected with little risk when subjects are properly screened. **People who are sensitive or allergic to penicillin, iodides, shellfish, or sulfa drugs should not participate because they have a greater chance of being allergic to the dye.** Therefore, you will be asked if you have had any reactions to these types of medicines in the past, and if you have, you will not be allowed to participate in the project. This is a necessary requirement to reduce the slight possibility of an allergic reaction to ICG.

____ Abdominal and leg fluid measurement (bioelectrical impedance). We will place electrodes (sticky patches) on your chest, abdomen, legs, hand and foot. A cable will be connected to each patch. A very small current of electricity will flow through the electrode and through your body. You will not be able to detect the current as it passes through your body. For comparison, this measurement is similar to body fat scales available in gyms or for home use.

____ Cardiac Output (acetylene rebreathing). We will measure your heart's pumping rate by analyzing the air you rebreathe in and out of a bag using fairly deep breaths for 15-20 seconds. The bag will contain a small concentration of two gases, acetylene and helium. The concentrations of these gases are so small that there is no risk of them catching fire. Some people report a slightly "tangy" taste from the rebreathing gas. Although you may become light-headed for a few seconds during rebreathing or develop a slight headache from repeated rebreathing, there are no other known risks to performing this procedure. The gas disappears from your lungs and blood in less than 5 minutes.

____ Lower Body Negative Pressure. You will lie on your back during this procedure. From the waist down you will be sealed in a box-like chamber. Mild suction will be applied that will cause blood to shift into your legs similar to when you stand. We will stop the suction at your request or when we see that your blood pressure is starting to fall. Occasionally, light-headedness occurs and, rarely, a patient may faint. If this should happen, we will immediately stop the procedure, and you should begin to feel better in less than a minute.

____ Head-up Tilt. You will lie on a table and then you will be tilted to an angle of 15°, 30°, and 50˚ on a tilt table for 6 min at each angle. During that time we ask that you relax and stay as motionless as possible. We will stop tilting if your blood pressure becomes too low, you experience an abnormal heart beat, or you feel light-headed or dizzy. The tilt series will be repeated with lower body negative pressure.

____ Tilt Table Tolerance Test (with and without lower body negative pressure). You will lie on a table and then you will be tilted to an angle of 70° for up to 45 min. During that time we ask that you relax and stay as motionless as possible. We will stop tilting (and the lower body negative pressure) if your blood pressure becomes too low, you experience an abnormal heart beat, you feel light-headed or dizzy, or you ask to stop.

3. Discomforts and risks:

All procedures carry risk. Risk has two aspects: severity and frequency. Severe risk might threaten the loss of life or limb, while a mild risk might be discomfort. The frequency of a risk is the chance that a problem will occur. In this section we have summarized the risks associated with the procedures used in this experiment. The risks in this experiment have different severity and frequency, and some could be life threatening. Please feel free to ask about the severity or frequency of these risks at any time. To help you decide whether or

not you are willing to accept the risks associated with this experiment, the table below provides some commonly mentioned risks and the estimated chance they will occur to you:

Contracting meningitis while you are at a large university[2]	1 in 33,000
Dying from being struck by lightning in your lifetime[3]	1 in 30,000
Dying of liver disease if you drank one beer per day [3]	1 in 1,000
Contracting a disease caused by radon in your home[3]	1 in 440
Being killed in a car accident in your lifetime[3]	1 in 60
Contracting cancer at some point during your life[1]	
Men	1 in 2
Women	1 in 3

References:
[1] American Cancer Society. Statistics for 2008. www.cancer.org.
[2] Harrison LH, Dwyer DM, Maples CT, Billman L. (1999). Risk of Meningococcal Infection in College Students. *JAMA* 281:1906-1910, 1999.
[3] Walsh J. *True Odds: How Risk Affects Your Everyday Life.* 1996, p 16.

Please read the detailed description of each procedure below and initial each one to indicate that you have read and understand them.

____ General: If during the screening procedures we should detect any abnormality, we will inform you as soon as possible so that you can contact your personal doctor for treatment. Some people find medical procedures to be scary, and feel faint as a result (vasovagal response). To keep you comfortable and informed, we encourage you to share your concerns with us at any time.

____ Heart rate, blood pressure, and blood flow: The only known risk associated with measuring heart rate and blood pressure is the possibility that your skin will be irritated by the adhesive, cuffs, or tape. The blood pressure sensor will push down against your skin and may leave a mark when it is removed. The mark will go away within a few minutes after the sensor is removed.

____ Blood sampling/venous catheter: The risks of a blood sample include bruising and/or discomfort from the needle, venous inflammation from the catheter, infection (less than 1 in 10,000), or the chance that you will become lightheaded. Should you feel this way we will stop the experiment, and you will be given fluids to drink (water or juice). We ask that you remain in the laboratory until we have checked your blood pressure and we are sure that you feel OK.

____ Topical Anesthetic Cream: Numbing cream will not be used in those who have sensitivity to lidocaine. Eye contact should be avoided. When used, all sensations within the treated area are blocked. For this reason, unintentional trauma to the treated area, such as scratching, rubbing or exposure to hot or cold temperatures should be avoided until complete sensation has returned. During or immediately after application, mild swelling, skin redness or abnormal sensation may develop at the site of treatment. In clinical studies, no serious reactions resulted from the use of the cream. Allergic reactions can occur and will be managed. Whole body adverse reactions following appropriate use are unlikely due to the small dose absorbed. If effects do occur, they are similar in nature to those seen with other local anesthetic agents and may include

lightheadedness, nervousness, apprehension, dizziness, drowsiness, twitching, and vomiting. Reactions may be brief or not at all.

___ Abdominal and leg fluid measurement (bioelectrical impedance): There are no risks involved with this procedure. The sticky electrodes may cause your skin to become irritated when we remove them.

___ Rebreathing cardiac output: Because you breathe slightly deeper than normal, there is a chance you will feel light headed for a few seconds during rebreathing or develop a slight headache after repeated measurements. There are no other known risks to this procedure.

___ Indocyanine Green Dye (ICG): ICG is a safe non-toxic substance that is easily cleared by the liver and can be safely injected with little risk when subjects are properly screened. **The only known risk involved is when a person is sensitive or allergic to penicillin, iodides, or sulfa drugs**. Therefore, you will be asked if you have had any reactions to these types of medicines in the past, and if you have, you will not be allowed to participate in the project. This is a necessary requirement to insure that the slight possibility of an adverse response to ICG in a subject sensitive to the drugs listed above is alleviated. For those people that have no known allergy, there is a 1 in 250 risk of a mild to moderate reaction. Symptoms include lightheadedness, nausea, hives and itching. Rarely, (1 in 2000) a serious allergic reaction which affects the entire body (anaphylaxis) may develop. An anaphylactic reaction can be life-threatening. You are free to discontinue these tests at any time.

___ Lower body negative pressure: The risk of lower body negative pressure is that you become lightheaded, nauseous, or pass out. Should you feel this way or we see that your blood pressure is starting to fall, we will stop the procedure and you should begin to feel better within seconds. If we continued the suction you would develop an abnormally slow heart rhythm (bradyarrhythmia). This stops when suction is turned off, but in one case (less than 1 in 10,000) a person was injected with a drug (atropine) in their arm vein to restore their heart rhythm to normal. Because we observe your blood pressure closely, it is not likely (about 1 in 20) that you will develop a slow heart rhythm.

___ Head-up tilt: The risk of head-up tilt is that you become lightheaded, nauseous, or pass out. Should you feel this way or we suspect that your blood pressure is starting to fall, we will stop the procedure and you should begin to feel better within minutes. In one case (less than 1 in 10,000) a person developed an unusually slow heart rhythm that returned to normal with a drug (atropine) injected in their arm vein. Because we observe your blood pressure closely, it is not likely (about 1 in 20) that you will develop a slow heart rhythm.

4. a. Benefits to the subject:
None

b. Potential benefits to society:
This information will be used to help people or patients who have difficulty maintaining their blood pressure. An example is patients who have recently undergone dialysis, some elderly people after eating a meal, and some otherwise healthy people who experience problems with lightheadedness. These problems are much more common in women.

5. Alternative procedures which could be utilized:
Recording heart rate, blood pressure, and blood flow is routine. Bioelectrical impedance analysis is the most non-invasive way to obtain these data.

6. Time duration of the procedures and study:
This study will require five to six visits (a total of about 13 hours) to Noll Laboratory and the General Clinical Research Center (a wing of Noll Laboratory). The screening visit will last about 1 hour, the physical exam and familiarization visit about 1 hour, the blood volume determination visit about 1 ½ hrs, the VIP determination visit about 3 ½ hrs, and the tilt table tolerance visits up to 2 ¾ hours each.

7. Statement of confidentiality:
Your participation in this research is confidential. The data will be stored and secured in Noll Laboratory in a locked file. Electronic files will only identify you by a subject identification number, not by name. In the event of any publication or presentation resulting from this research, no personally identifiable information will be disclosed. Penn State's Office for Research Protections, the Biomedical Institutional Review Board, the U.S. Food and Drug Administration (FDA), the Office for Human Research Protections in the Department of Health and Human Services, and the National Aeronautics and Space Administration may review records related to this research study.

8. Right to ask questions:
Please contact Sara Jarvis at (814) 865-0476 (W) or (814) 441-2113 (H) or Dr. Pawelczyk at (814) 865-3453 (W) or (814) 861-1379 (H) with questions, complaints or concerns about the research. You can also call this number if you feel this study has harmed you. Questions about your rights as a research participant may be directed to Penn State University's Office for "Research Protections at (814) 865-1775.

Please initial the statement below to indicate your understanding of this right.

___ I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction**.**

9. Compensation:
There will be no charge for any tests required for the study. You will receive:

- $25 after completing the blood volume determination visit,
- $35 after completing the VIP determination visit,
- $30 after completing the first tilt table tolerance visit, and
- $35 after completing the second tilt table tolerance visit

(for a total of $125) for participation in this investigation to compensate your travel and loss of time. If you choose to withdraw early from the investigation, this amount will be prorated accordingly. Total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. Voluntary participation:
Participation in this research study is entirely voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your status (as a patient, student, employee, etc.), or the medical care that you will receive. You may decline to answer specific questions. However, your acceptance into the study may be contingent upon answering these questions. You may not be accepted into the study if you participate in other research studies, depending on what those studies involve. Under certain circumstances the study may be discontinued by the sponsor or the investigator.

11. Event of injury:
Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or the investigators.

12. Abnormal test results:
In the event that abnormal test results are obtained, you will be notified within 3 business days so that you can contact your private medical provider for follow up.

This is to certify that I am 18 years of age or older and I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed copy of this consent form. I have read this form, and understand its contents.

_____
Volunteer Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____
Investigator Date

VITA

Sara S. Jarvis

Education

Institution	Degree	Discipline	Date Completed
Pennsylvania State University (University Park, PA)	Ph.D.	Kinesiology Exercise Physiology	2009
University of Puget Sound (Tacoma, WA)	B.S.	Exercise Science Biology (minor)	2002

Assistantships/Fellowships

2004-2005 Graduate Assistant, Department of Kinesiology, Penn State University
2006-2009 NASA, Harriet G. Jenkins Pre-doctoral Fellow

Awards

2007 National Aeronautics and Space Administration Research Award
2005 Funding for Excellence in Graduate Recruitment

Professional Affiliations

American College of Sports Medicine
American Physiological Society

Publications

Jarvis SS, Florian JP, Curren MJ, Pawelczyk JA. Sex differences in vasoconstrictor reserve during 70° head-up tilt. [In review].

Jarvis SS and Pawelczyk JA. Identification of the human volume indifferent point. [In review].

Jarvis SS, Levine BD, Prisk GK, Shykoff BE, Elliott A, Rosow E, Blomqvist CG, Pawelczyk JA. Simultaneous determination of the accuracy and precision of closed-circuit cardiac output rebreathing techniques. *J Appl Physiol* 103(3): 864-74, 2007.

Parker BA, Smithmyer SL, **Jarvis SS**, Ridout SJ, Pawelczyk JA, Proctor DN. Evidence for reduced sympatholysis in the leg resistance vasculature of healthy older women. *Am J Physiol Heart Circ Physiol* 22(2):H1148-H1156, 2007.