Cardiovascular responses of women to orthostatic stress, the effects of the menstrual cycle and age, and a comparison to men

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Young women are known to exhibit a greater incidence of orthostatic hypotension than men. The exact mechanisms for this are unclear and it has been proposed to be related to cardiac filling, peripheral resistance, and/or regional blood pooling. The sexually dimorphic effects of lower body negative pressure (LBNP) or upright posture were investigated throughout this study. Women could experience these changes due to effects of the sex hormones estrogen and progesterone. Chapters 3 and 4 in this thesis investigated the responses of women to LBNP in both the follicular and the luteal phase of the menstrual cycle (and age-matched men). Women at these points of the cycle have approximately equal levels of estrogen with high levels of progesterone in the luteal phase. Furthermore, Chapter 5 investigated the responses of pre-menopausal and post-menopausal women (and age-matched men) to sitting and standing. These studies will help to explain the effects of female sex hormones on cardiovascular responses to simulated or real orthostatic stress.

LBNP simulates an orthostatic stress by causing a caudal fluid shift and was used in Chapters 3 and 4 as a stimulus to optimize the position of the participants for cardiovascular measurements. A supine-to-sit-to-stand test (i.e. actual orthostatic stress) was used in Chapter 5 as a stimulus. Head-down bed-rest (HDBR) is a model used to simulate microgravity and induces a fluid shift away from the legs towards the head. It has been shown to augment the responses to LBNP and was thus used to enhance the cardiovascular and hormonal responses of men and women to LBNP. A seated control (SEAT) was also used in an attempt to control for the equivalent period of inactivity and circadian rhythm.

Blood pressure responses to LBNP were not different between menstrual phases although the physiological mechanisms may be somewhat different. Women in the luteal phase had higher portal vein resistance index which would contribute to moving splanchnic blood pools to maintain venous return during an orthostatic stress. When comparing women in the follicular phase to men, there was a decrease of blood pressure in women during LBNP which was not observed in men. This decrease was likely a result of reduced venous return as evidenced by a greater loss of central venous pressure and a greater increase of thoracic impedance during LBNP. This could have been a result of 1) splanchnic blood pooling in women as men had a greater increase of portal vein resistance index during LBNP, and/or 2) attenuated activation of the renin-angiotensin-aldosterone pathway in women during LBNP.

After considering the effects of circadian rhythm and inactivity in all participants, HDBR resulted in 1) higher heart rate with a greater increase during LBNP, 2) a greater decrease of stroke volume during LBNP, 3) a greater increase of thoracic impedance during LBNP, 4) smaller inferior vena cava diameter, 5) lower norepinephrine, and 6) lower blood volume. These changes indicate that after 4-hours of HDBR resting venous return and venous return during LBNP was lower in all participants. However, the mechanisms by which each sex or menstrual phase responded were different. After HDBR men had higher pelvic impedance, higher vasopressin, and higher endothelin-1 compared to women in the follicular phase. After HDBR women in the luteal phase also had higher vasopressin and higher pelvic impedance compared to women in the follicular phase.

During the supine-to-sit-to-stand protocol young women (follicular phase) exhibited a greater increase of heart rate during the 3rd minute of each posture likely due to reduced stroke volume compared to young men and post-menopausal women. During the transitions to sitting or standing young women also had an impaired ability to maintain stroke volume and cardiac output compared to post-menopausal women and age-matched men. These results imply that young women had lower venous return than older women and age-matched men during an orthostatic stress. In comparison to older men, post-menopausal women also had slightly reduced venous return, but the difference was smaller than that seen in the younger groups. There were no differences in middle cerebral artery blood flow velocity when comparing younger and older groups of men and women.

The results of this investigation have outlined how men respond to an orthostatic stress differently than women (i.e. via a decrease in splanchnic pooling and a greater increase of vasoconstrictors), and have helped to outline a role for female sex hormones in the cardiovascular responses to an orthostatic stress (i.e. post-menopausal women exhibit greater venous return during an orthostatic stress compared to younger cycling women).

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List of Abbreviations

AngII – Angiotensin II
ANP – Atrial natriuretic peptide
AVP - Vasopressin
bpm – Beat per minute
BVR – Brachial vascular resistance
CO – Carbon monoxide
CPBR – Cardiopulmonary baroreceptor
CPP – Cerebral perfusion pressure
DBP – Diastolic blood pressure
Endo-1 – Endothelin-1
$ETCO_2$ – End-tidal carbon dioxide
F – Follicular phase of the menstrual cycle
HDBR – Head-down bed-rest
HR – Heart rate
HRT – Hormone replacement therapy
IVC – Inferior vena cava
kg - Kilogram
L – Luteal phase of the menstrual cycle (Appendix III)
LBNP – Lower body negative pressure
M - Menstruation
MAP – Mean arterial pressure
MCA – Middle cerebral artery
mmHg – Millimeters of mercury
OC – Oral contraceptives
PI – Pelvic impedance
Post – Indicates the LBNP test after HDBR or SEAT
Pre – Indicates the LBNP test before HDBR or SEAT
PVR – Portal vein resistance index
Q – Cardiac output
RAAS – Renin-angiotensin-aldosterone system
SBP – Systolic blood pressure
SEAT – Seated control
SV – Stroke volume
TI – Thoracic impedance
TPR – Total peripheral resistance

CHAPTER 1

Literature Review

I. Literature Review

The purpose of this literature review is to review some important topics that are discussed within this thesis and to briefly review some of the important methodologies used. These topics include discussion of the use of lower body negative pressure (LBNP) as an orthostatic stress and the use of head-down bed-rest (HDBR) as a model of microgravity to augment the cardiovascular responses to LBNP. This literature review will also cover the sexually dimorphic responses to LBNP, HDBR and a seated control as well as the influence of the menstrual cycle and the changes of the cardiovascular response to orthostatic stress due to age.

1.1 Cardiovascular homeostasis

The heart pumps blood through the ascending aorta to large conduit arteries to smaller resistance arterioles. These arterioles control total peripheral resistance (TPR) via constriction and dilation (i.e. increases and decreases TPR, respectively). Mean arterial pressure is a product of cardiac output and total peripheral resistance, and homeostasis is partially maintained by pressure sensors in the vessels.

Small decreases in venous return reduce pressure in the right side of the heart reducing the firing of the cardio-pulmonary baroreceptors (CPBR) leading to increased sympathetic nervous activity and greater peripheral resistance. Large increases in pressure stimulate arterial baroreceptors in the aorta and carotid body. Activation of arterial baroreceptors in the aorta and carotid body results in reduced sympathetic output leading to lower peripheral resistance (reviewed in (152)).

1.2 Cardiovascular responses to orthostatic stress

Standing is an orthostatic stress which causes a hydrostatic gradient resulting in unequal blood pressure in the body, higher in the low body and lower in the head (82). Lower body negative pressure (LBNP) is a model of orthostatic stress resulting in similar responses. A typical response to an orthostatic challenge is an increase in heart rate and total peripheral

resistance (153) in order to restore cardiac output and mean arterial pressure. These increases are due to increases in plasma concentrations of catecholamines (norepinephrine and epinephrine; primarily vasoconstricts and increases heart rate and stroke volume, respectively) (58; 200), renin (cleaves angiotensinogen to create angiotensin I), angiotensin II (a vasoconstrictor and releases aldosterone from the adrenal medulla), endothelin-1 (a vasoconstrictor), and vasopressin (causes water reabsorption and vasoconstriction) (62; 76; 200). If mean arterial pressure is not maintained, this is referred to as "orthostatic hypotension." If blood pressure continues to fall dizziness, faintness, and syncope may follow due to reduced brain blood flow.

1.3 Outline of brain blood flow

There are two pathways for brain blood flow. First, blood flow to the middle and anterior brain travels through the common carotid artery to the internal carotid and finally to the middle cerebral artery. Second, blood flow to the posterior brain travels through the vertebral artery to the basilar artery and finally to the posterior middle cerebral artery (169). Brain blood flow is regulated by a process called autoregulation and by responses to blood gases. Autoregulation is the maintenance of steady cerebral blood flow across a wide range of changing cerebral perfusion pressures via vasomotor tone (111). Blood gases are extremely important in regulating cerebral blood flow. For example, a high level of carbon dioxide or a low level of oxygen will elicit vasodilation of cerebral arteries to increase cerebral blood flow and oxygen supply to the brain (111).

1.4 Head-down bed rest (HDBR) as a model of microgravity

In microgravity there is an absence of a hydrostatic pressure gradient leading to equal blood pressure at all levels of the body. After a period of exposure the body adapts and when an orthostatic challenge is applied there is an even greater return of blood to the lower body resulting in reduced cardiac return and brain blood flow (82). Models for simulating microgravity include water immersion and head-down bed-rest (HDBR). Water immersion centralizes blood from the periphery, and HDBR involves fluid shifts towards the head from

the lower body. Long-term HDBR is a model commonly used to simulate microgravity conditions experienced by astronauts; however adaptations can also occur after only 4-hours of HDBR (22; 62).

In men, space flight and HDBR (7-28 days) result in decreased vasopressin, nitric oxide metabolites (a vasodilator), and atrial natriuretic peptide (causes sodium loss) with increases in renin, angiotensin II, aldosterone (causes sodium retention), natriuresis (over the first few days only), and plasma osmolality (11; 73; 77; 95; 175; 199). Ertl et al. have also shown higher muscle sympathetic nerve activity (MSNA) and plasma norepinephrine during space flight (56). Similarly, Kamiya et al. have shown higher MSNA after 14-days of HDBR (95), yet bed rest studies have found a reduction in plasma norepinephrine (73). Some of these hormonal changes could have been a result of a loss in plasma volume (73; 93; 199); however there are adaptations after only 4-hours of HDBR where plasma volume has been shown not change (21; 62).

In response to an orthostatic challenge after exposure to 30 days of HDBR, men exhibit a higher heart rate response and lower mean arterial pressure (73). Women also exhibit these changes after 14-16 days of microgravity, yet they are greater than those seen in men (78; 200). Changes in orthostatic responses after 4-hours of HDBR have also been observed. Fischer et al. found lower baseline renin and angiotensin II which increased in response to LBNP (no increase was observed before HDBR). They also found that the normal reduction in portal vein flow seen with LBNP was smaller after HDBR (62), indicating that splanchnic pooling occurs during an orthostatic challenge after 4-hours of HDBR. These short-term HDBR experiments have not been completed in women.

1.5 Sexual dimorphic responses to orthostatic stress

Women are known to exhibit greater hypotension upon an orthostatic stimulus than men. Female astronauts (n=5) experience pre-syncopal symptoms 100% of the time during a stand test upon return to Earth (compared to 20% occurrence in men) (200). Women also have a greater susceptibility to postural orthostatic tachycardia syndrome (POTS) (75; 176) which is described as an increase in heart rate by more than 30 bpm or to a heart rate greater than 120 bpm within 10 minutes of standing (159).

1.5.1 Sexually dimorphic hemodynamics and anatomy

It has been shown in previous studies that men and women exhibit some different baseline cardiovascular measurements. Women display lower mean, systolic, and diastolic blood pressure and higher heart rate than men (78; 200). However, Fu et al. found that when normalized to body surface area there are no differences in stroke volume, cardiac output, or total peripheral resistance (69).

There are also sexually dimorphic differences in regard to cardiovascular anatomy and the responses to stimuli. For example, women have smaller left ventricular mass (normalized to body surface area) yet no difference in left ventricle diameter (43). Also, the uterus and the ovaries have an arterial anastomosis and a venous plexus (169) leading to an additional pool of blood in the pelvis. Women have lower sympathetic output than men due to a greater inhibitory arterial baroreflex (86), and Hogarth et al. found that the vasoconstrictor effect of sympathetic innervation was attenuated in women. Together, these differences would attenuate increases in peripheral resistance and pool blood during an orthostatic challenge. No significant differences between men and women have been observed in cardiopulmonary baroreceptor function (39).

1.5.2 Sexually dimorphic release of vasoactive factors and water/sodium balance

There are few differences between men and women when observing baseline measurements of vasoactive factors. There are no differences regarding resting levels of norepinephrine, epinephrine, vasopressin, renin, or angiotensin II (34; 58; 126; 127; 200), yet men are known to have higher resting endothelin-1 (a vasoconstrictor) (187).Women have higher resting levels of atrial natriuretic peptide (elicits sodium loss) and higher levels of aldosterone (causes sodium retention) during the luteal phase of the menstrual cycle (33). One may infer that some of these differences could be due to differences in plasma volume and/or blood volume, yet no significant differences are seen when normalized to body size (200). There are also few differences in regard to water and sodium balance. There are no baseline differences in urodilatin (created in kidney; results in sodium loss upon increased venous volumes) (127; 198) or urine osmolarity (34). However, Edgell and Kaufman have shown higher plasma osmolarity in male rats (53).

1.5.3 Sexually dimorphic resting brain blood flow

Women have a smaller brain volume than men (not normalized to body size), but greater cerebral blood flow (79; 80). This may be a function of increased internal carotid blood flow due to higher levels of estrogen (104) especially in light of the fact that men have higher arterial CO_2 at rest (118) which would lead to greater brain blood flow. It is possible that this higher carotid blood flow may occur due to higher levels of nitric oxide (a vasodilator) in women as estrogen is known to stimulate the production of nitric oxide and its endothelial synthase (155; 174). Indeed, when estrogen is high during certain phases of the menstrual cycle levels of endothelial nitric oxide synthase increase (182).

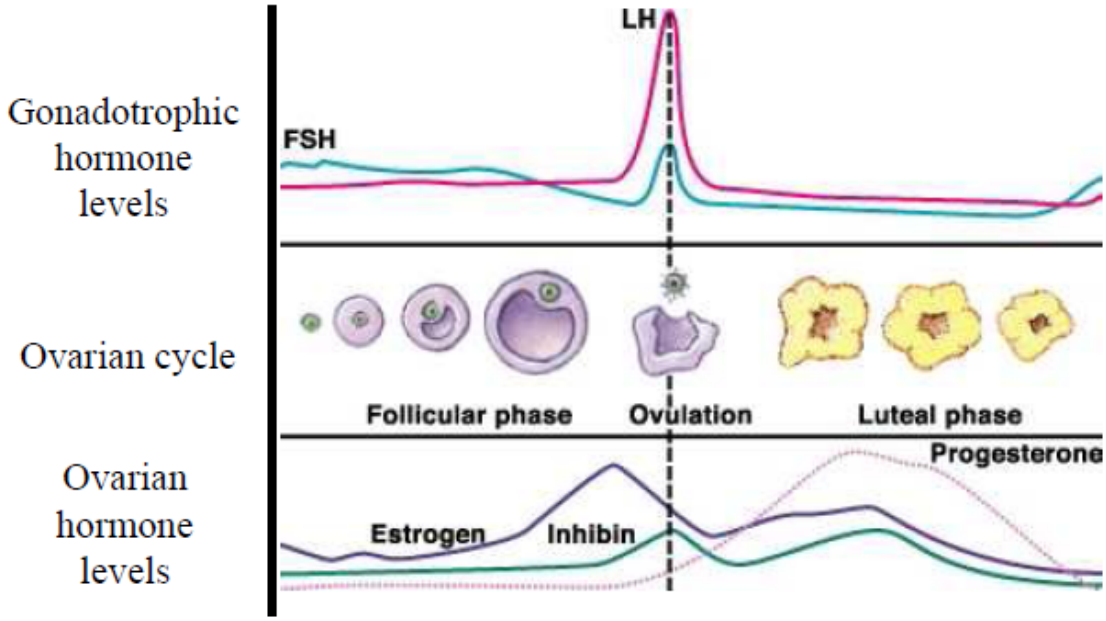
1.5.4 Sexually dimorphic responses to orthostatic stress and microgravity

While there are few baseline cardiovascular differences between men and women, there are some differences between their responses to orthostatic stress and microgravity. For example, after exposure to microgravity, non-presyncopal male astronauts exhibit greater norepinephrine release upon standing while women (and pre-syncopal men) exhibit greater epinephrine release (7). Also, both men and women have lower plasma volume after 5-16 days of spaceflight, but the loss is greater in women (200). This is not likely due to changes in vasopressin (i.e. water reabsorption) as there are no sex differences in vasopressin levels after 7 days of HDBR (although women tended to show an increase which should actually be protective of plasma volume)(127).

Changing venous compliance may also affect tolerance to an orthostatic stress by changing venous return. Grenon et al. observed greater baseline leg venous compliance in men which does not change after exposure to 14-16 days of HDBR (37). There are no sex differences in baseline central venous pressure or its responses to microgravity or orthostatic stress (18; 65). Therefore, women likely exhibit lower leg venous compliance than men as a function of reduced volume. Grenon et al. suggest the possibility that this may contribute to better orthostatic tolerance in men due to greater activation of sympathetic and vasoconstrictive responses (78).

There is contradictory evidence as to the role of splanchnic blood pooling during an orthostatic stress. It has been shown that portal vein blood flow does not differ between men and women and that LBNP results in decreasing portal flow in men (62; 177; 180; 185). However, after separating the sexes and by using pelvic bio-impedance measurements (between the iliac crest and xyphoid process), it has been shown that LBNP increases pelvic blood volume and that women display a 83% greater increase (202). These data support higher splanchnic blood pooling in women with an orthostatic stress. If even greater pooling is seen in women after HDBR, this could help to explain the greater orthostatic hypotension seen after exposure to microgravity (or bed-rest) seen in women as there would be a reduction in venous return and thus cardiac output.

1.6 Menstrual cycle and female sex hormones



(Adapted from Silverthorn, 2003)

Estrogen and progesterone change in concentration throughout a woman's menstrual cycle. Levels of estrogen are high immediately before ovulation (follicular phase) without any increase in progesterone. After ovulation (luteal phase), estrogen and progesterone are both high (167). Androgens are secreted in both men and women (from the testes and the ovaries

respectively) although in concentrations approximately 20 times greater in men (reviewed in (59)).

Estradiol, progesterone, and testosterone have all been shown to inhibit the contraction of rat aorta to phenylephrine (an adrenergic $\alpha 1$ receptor agonist causing vasoconstriction) (29). The mechanisms by which this occurs could be either genomic or non-genomic via sex hormone receptors that are both membrane bound and found in the cytosol of the cell. Membrane bound receptors elicit the non-genomic effects, and cytosolic receptors translocate to the nucleus where they act as a transcription factor for certain genes (reviewed in (59; 99)) such as angiotensinogen (115; 138). Therefore, sex hormones can affect many different pathways including cell growth, differentiation, angiogenesis, and apoptosis (reviewed in (55)).

1.6.1 Effects of the menstrual cycle on hemodynamics

There are few baseline hemodynamic differences through the menstrual cycle, but those that do exist are important to consider when examining orthostatic hypotension. For example, women in the luteal phase exhibit lower mean arterial pressure and total peripheral resistance (30; 52). This might imply a greater chance of hypotension given an orthostatic stress; however, very recent studies have shown that there are no differences in orthostatic responses between the luteal and follicular phases in regard to heart rate, mean arterial pressure, stroke volume, or cardiac output (28; 67). (These researchers examined the very early part of the follicular phase (1-3 days from the onset of menstruation) when there is very little circulating estrogen and progesterone). These results may imply that there are no differences in orthostatic tolerance throughout the menstrual cycle. Indeed, this has been shown (35; 121); however, there was also higher total muscle sympathetic nerve activity in the luteal phase during the orthostatic challenge (28; 67). Therefore, there exists the possibility that even though orthostatic tolerance does not differ between phases of the menstrual cycle, the mechanisms to maintain blood pressure may be different. No data could be found that tested the responses through the menstrual cycle after exposure to microgravity or bed-rest.

1.6.2 Effects of the menstrual cycle on vasoactive factors

There are conflicting results with respect to differences in resting norepinephrine or epinephrine levels due to the menstrual cycle. Some groups have observed no differences (116), while others have observed higher resting norepinephrine in luteal phase (30; 128). However, during an orthostatic stress there are no differences in catecholamine responses (67). Slightly higher endothelin-1 (188) and lower levels of nitric oxide metabolites (74) have been observed in the luteal phase, yet no information could be found on the effects of orthostatic stress through the menstrual cycle for these factors.

1.6.3 Effects of the menstrual cycle on water/sodium balance

In the luteal phase, women exhibit lower serum sodium and serum osmolality which leads to higher renin, angiotensin II, and aldosterone (1; 30; 124; 179; 188). One may expect from these observations that differences in blood volume may be present, yet Chapman et al. did not observe any differences in plasma volume, blood volume, or hematocrit when comparing the luteal phase to the follicular phase (30). However, Fortney et al. found that after 2-3 days of bed-rest there was a trend for greater plasma volume in luteal phase women (63). A greater plasma volume after bed-rest in the luteal phase could result in greater stroke volume and cardiac output which would reduce orthostatic hypotension.

1.6.4 Effects of the menstrual cycle on brain blood flow at rest and during an orthostatic stress

There is lower cerebral vascular resistance in the middle cerebral artery of women during the follicular phase of the menstrual cycle (16) which could be a result of vasodilation due to 10% greater arterial levels of carbon dioxide (118). However, there is no difference in the resistance index of the internal carotid artery between the follicular and luteal phases (104). Claydon et al. also found that there was no difference in the relationship between middle cerebral artery velocity (as an index of brain blood flow) and cerebral perfusion pressure during an orthostatic challenge indicating that autoregulation was the same in both phases (35).

Therefore, regulation of brain blood flow does not appear to be involved in orthostatic responses in different phases.

1.7 Menopause and Age

Menopause is the loss of endogenous estrogen and progesterone in women at an older age. A woman is known to be post-menopausal once menstruation has ceased for at least 12 months which most commonly occurs around the age of 50 (reviewed in (20)).

1.7.1 Effects of age on hemodynamics and vasoactivity

Resting heart rate and cardiac output do not change with age, yet peripheral resistance increases. However, catecholamine responsiveness of the heart to increase rate is blunted as is arterial baroreceptor sensitivity (reviewed in (60)). This blunted response could lead to the higher levels of plasma norepinephrine that are observed with age (no difference between men and women) (209). Indeed, in response to LBNP older male participants show an attenuated increase of heart rate, a smaller drop in blood pressure, a smaller drop in forearm vascular resistance, and a smaller increase in peripheral resistance (110; 139). Responses of post-menopausal women were not observed.

1.7.2 Effects of age on the arteries

Aging is associated with a loss of endothelial function, remodeling of the vascular wall, increased arterial stiffness, reduced large artery compliance, and reduced peak flow velocity within the aorta (reviewed in (60; 70)) possibly due to a larger aortic root (71). One might expect from these changes that the resultant higher peripheral resistance would lead to reduced orthostatic hypotension, yet the opposite has been observed (119). Mattace-Raso et al. found that arterial stiffness in the elderly is positively associated with orthostatic hypotension (119). They hypothesize that this is due to a reduced heart rate response due to baroreceptor dysfunction. An investigation of sex differences was not performed.

1.7.3 Effects of age on the veins

There is a reduction in calf venous compliance and capacitance with age in men (139) which is not likely due to a change in smooth muscle tone or reactivity (tested in mixed sex population)(206). In women however, there is no decline in venous compliance or capacitance with age, but there is a decrease in capillary filtration (117) which is not seen in men (110). This lower leg venous capacitance seen in men could lead to higher venous return during an orthostatic stress which could help to explain the smaller increase in heart rate described above. It could also help to explain the attenuation of vascular resistance responses as the cardiopulmonary and arterial baroreceptors would be activated to a larger degree and therefore inhibit sympathetic output. Similarly, the reduced capillary filtration seen in older women could help to attenuate orthostatic hypotension as more fluid would be retained in the venous circulation and returned to the heart rather than entering the interstitial spaces.

1.7.4 Effects of age on brain blood flow

There are no changes of the diameter of the middle cerebral artery with age in both men and women (133). However, velocity of blood traveling through this artery declines with age in both men and women to the same degree (2; 45). (There are no changes in cerebral autoregulation (204)). These results indicate that with age cerebral blood flow is lower in both men and women.

1.8 Overview of impedance and ultrasound measurements

Two techniques used in this thesis to help describe regional blood flow are impedance and ultrasound measurements. Impedance was used to determine fluid shifts in the thoracic and pelvic regions and ultrasound was used for blood flow measurements through a particular vessel. The use of impedance cardiography as a method of monitoring cardiac function was described by Kubicek et al. (106-108). Since then impedance cardiography has also been used to indicate approximate segmental volumes, in particular the splanchnic or pelvic region (130; 185; 202). While these investigators used impedance to determine stroke or segmental volume,

in this thesis this method is used simply as an index of fluid shifts as in Butler et al. (21). Ultrasound techniques are used in this thesis to determine flow through a particular vessel. Ultrasound has been used to determine cardiac output and splanchnic blood flow by calculation of the flow through the ascending aorta (54; 166) or portal vein (8; 62; 88), respectively. Flow is determined by multiplying the cross-sectional area of the vessel by the velocity of the blood traveling through it.

1.9 Purpose of thesis

The purpose of this thesis is to explore the mechanisms that underlie the greater incidence of orthostatic hypotension in women compared to men (e.g. (39; 200)) and to determine the effect of the menstrual cycle on the cardiovascular responses to orthostatic stress. There is a growing body of evidence that orthostatic responses throughout the menstrual cycle can vary. However, quite often orthostatic studies that are specifically investigating sex differences do not control for phase of the menstrual cycle, or they do not report it (68; 69; 166). For this study, women were tested in two different phases of the menstrual cycle (follicular and luteal) to determine if changing female sex hormones indeed affect cardiovascular responses to an orthostatic stress.

Many researchers have examined the sexually dimorphic effects of orthostatic stress in the past and have attributed the greater orthostatic hypotension observed in women to 1) decreased cardiac filling (68), 2) an attenuated increase in peripheral resistance (42; 200), or 3) increased splanchnic blood pooling (202). The first thesis project was designed to compare the responses of men to women during an orthostatic stress (i.e. LBNP) and to compare the responses of women through the menstrual cycle. However, after completion there were still some unclear responses.

Since four hours of HDBR has previously been shown to decrease orthostatic tolerance in men (22) this model was used in Chapter 4 to augment the orthostatic responses in men and women in order to clarify any changes that were particularly subtle. Furthermore, the inherent inactivity and circadian rhythm change of a 4-hour test was controlled for by repeating all of the LBNP tests before and after a 4-hour seated control period (SEAT). Any differences due to SEAT were attributed to inactivity and/or circadian rhythm, and differences between SEAT

and HDBR will be attributed to the posture change (i.e. the head-ward fluid shift of HDBR). Men were compared to women in the follicular phase to match the investigation of Convertino (39). It would be statistically improper to compare men to women in both phases of the menstrual cycle (a repeated measure).

To further investigate the effects of female sex hormones and to observe the effects of age on cardiovascular and cerebrovascular responses to an orthostatic stress (i.e. standing), young women, young men, post-menopausal women, and age-matched men were examined in Chapter 5. Previous studies investigating the effects of orthostatic stress in men and women have had a wide range of ages and have observed differing results. For example, Fu et al. studied LBNP responses in young women (aged 30±2), and they observed a greater heart rate response to LBNP compared to men (68). However, Convertino also examined LBNP responses in women, but used women aged 36±2 (closer to the perimenopausal period). He did not observe the same higher heart rate response to LBNP (39). Thus, this final study was designed to examine two cohorts of women approximately 30 years apart in age to determine the effects of age and menopause.

CHAPTER 2

Materials and Methods for Chapters 3 and 4

2.1 Ethics

All experimental procedures were approved by the Office of Research Ethics at the University of Waterloo. Every participant was aware of his/her right to withdraw from the study at any time.

2.2 Participant description

Ten men and twelve women between the ages of 25 and 32 participated (Male BMI: 24.8 ± 1.1; Female BMI: 24.1 ± 1.0). Women participated in day 8-11 of the menstrual cycle (follicular phase) and in day 18-24 (luteal phase). Participants were requested to drink 5mL/kg of water the night before testing and the morning before testing to ensure a consistent level of hydration. They were also asked to have the same breakfast on each testing day 2 hours prior to testing. Participants refrained from exhaustive exercise and alcohol for 24 hours prior to testing and refrained from caffeinated beverages for 12 hours prior to testing. (Note: one woman did not complete the follicular days of testing).

2.3 Arm volume measurement

Arm volume was measured with water displacement in order to normalize brachial blood flow to the size of the arm. First, hand volume was measured in duplicate, followed by forearm displacement in duplicate. The arm volume used is the difference of the two. Women (653.2 ± 63.6mL), Men (848.1 ± 50.7mL).

2.4 Body surface area

For the purposes of normalization of data to body size we used the Dubois and Dubois formula. This formula has been shown to be an accurate measurement of body surface area (197). Women (1.72 ± 0.07m^2), Men (1.94 ± 0.06m^2).

2.5 Blood volume

Participants arrived at the laboratory at 7am and were requested to empty their bladders before height and weight measurements. They rested in a seated position and a 20-gauge catheter (BD Instyle, BD Medical Systems, Sandy, UT, U.S.A.) was inserted into the antecubital vein of the right arm. Baseline hematocrits were taken immediately and participants then moved to the blood volume measurement station. As described in 1995 (19), subjects remained seated and breathed normally from a mouth-piece attached to a filtered open circuit system (containing soda lime to absorb exhaled CO_2) with a steady supply of 100% medical grade O_2 for 10 minutes. The subjects were then transferred to a closed loop system containing 100% medical grade O_2. Subjects breathed from this closed loop system until they were comfortable breathing from this new circuit. At this point, a priming dose (~20mL) of carbon monoxide (CO) was injected into the closed loop system. After 5 minutes, a blood sample was taken for analysis with a co-oximeter. A test dose (~70mL) was then injected into the closed loop system. After 5 minutes, another blood sample was taken for analysis. (The actual dose of CO was recorded for future calculations).

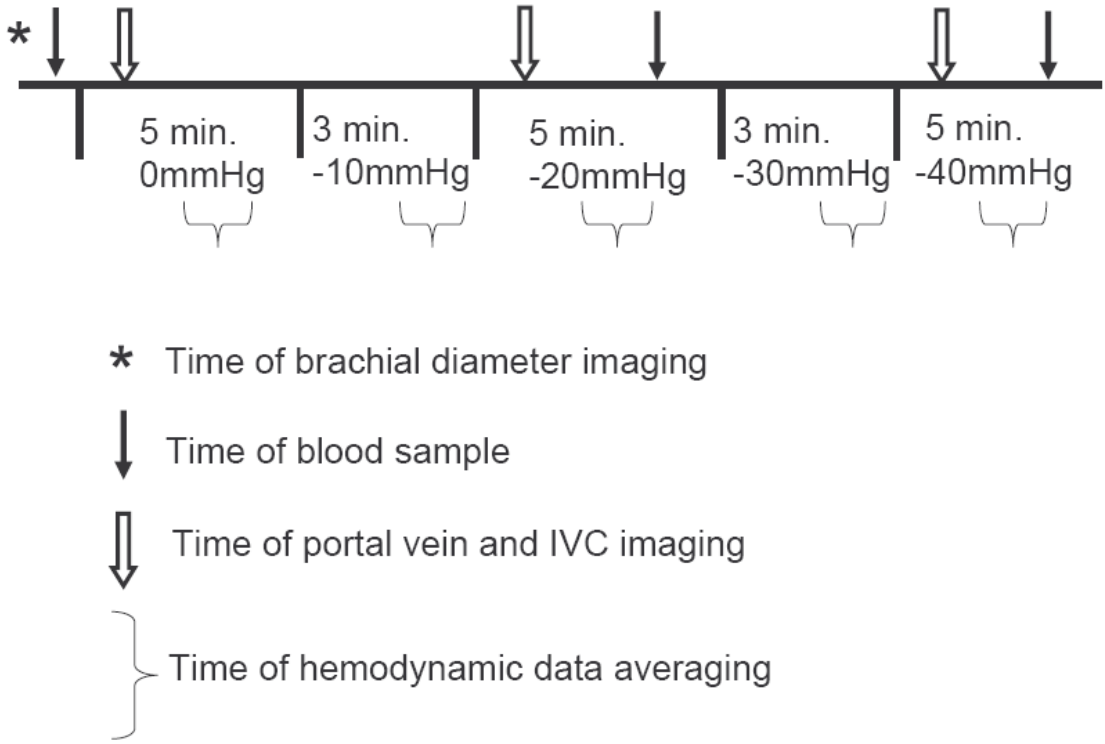
The co-oximeter analyzer (Nova Biomedical, Waltham, MA, U.S.A.) uses spectrophotometry to determine the concentration of hemoglobin (Hb) and carbon monoxide bound Hb. Together with baseline hematocrit, blood and plasma volume can be determined according to Burge and Skinner. Hematocrit measurements throughout the bed-rest and the seated protocol are used to determine changes in blood volume.

2.6 Lower body negative pressure protocol

Lower body negative pressure (LBNP) was used as a model for orthostatic stress in order to permit measurements of central venous pressure by the dependent arm technique (72). Individuals remained lying down and the entire LBNP box (with the participant) was tilted to the right to ensure that the right arm was dependent throughout the test. This ensured the absence of arm vein compression which is necessary for accurate measurement of central venous pressure. Central venous pressure was measured via the catheter in the right antecubital vein (inserted for blood volume measurements above) to which a line of saline and a pressure

transducer (TranStar, Medex Inc., Carlsbad, CA, U.S.A.) were attached. The transducer was attached to a laser level maintained at the level of the heart. All signals were output at 1000 Hz to a PowerLab (ADInstruments, Australia) and recorded onto a computer running Chart 5.5.1 for future analysis. The level at which the transducer was set was indicated on the body with a marker in order to match this height for subsequent tests.

Lower body negative pressure (LBNP) was achieved using a tightly sealed horizontal rectangular plywood box with an opening at one end for subject entry. Subjects lay on their backs while in the box to the level of their pelvis. The subject wore a customized neoprene kayak skirt attached to a metal tube at the entrance of the LBNP box. A leather belt was securely fastened around the subject's pelvis and the kayak skirt in order to ensure a good seal on the neoprene and to achieve an air-tight seal. Inside the box was a bicycle seat secured to the base of the box to prevent subjects from being pulled inside the box during suction. Suction was accomplished by the attachment of a household vacuum to a sealed orifice at the caudal end of the box. The level of suction was regulated using a voltage regulator and was measured using a pressure manometer.



As above, the LBNP protocol consisted of a 5 minute baseline period followed by 3 minutes of -10mmHg, 5 minutes of -20mmHg, 3 minutes of -30mmHg, and 5 minutes of -40mmHg. The LBNP was stopped if systolic pressure dropped to <70mmHg or at any time if the participant experienced nausea, dizziness or light-headedness. The test was terminated early for these reasons in the morning pre-test 0/20 time in the men, 3/22 times in the follicular phase, and 2/24 times in the luteal phase. After the seated protocol (described below), the LBNP test was terminated early in 0/10 men, 1/11 follicular phase women, and 2/12 luteal phase women. After the head-down bed-rest (HDBR) protocol (described below), the LBNP test was terminated early in 1/10 men, 5/11 follicular phase women, and 3/12 luteal phase women. All participants reached -40mmHg for every test (the minimum length of time was 2.3 minutes for one participant). Blood samples (black arrows in figure above) were taken at 3.5 minutes of -20mmHg, and at 3.5 minutes of -40mmHg. Means for hemodynamic variables were taken from the start of the third minute until either the end of the LBNP level (-10, -30mmHg) or the beginning of blood sampling (-20, -40mmHg). Means do not include the first minute after changing the LBNP level in order to avoid transitional changes skewing the data. Portal vein and inferior vena cava imaging (white arrows in figure above) were done in the second minute of baseline, -20mmHg, and -40mmHg.

2.7 Head-down bed rest (HDBR) and seated control (SEAT) protocol

Head-down bed rest (HDBR) was used to induce a fluid shift towards the head as a model of microgravity. The seated control (SEAT) was used to control for time of day effects and/or inactivity effects. Participants were randomly assigned to the SEAT protocol or the HDBR protocol on the first day of participation. After blood volume measurement and the morning LBNP protocol, participants started 4-hours of sitting or 4-hours of head-down bed rest in the lab. A second LBNP protocol was completed after the 4-hour period.

2.8 Food and water intake during testing

For the first 2 hours of the first protocol (HDBR or SEAT) subjects were allowed to drink water *ad libitum*. This amount was matched within 25% for the subsequent protocols.

Within one hour after completion of the morning LBNP protocol a whole-wheat turkey sandwich with mayonnaise, lettuce, tomato, and cucumber was provided to each participant for lunch.

2.9 Imaging

Cross sectional area of the portal vein, aorta, brachial artery and inferior vena cava were obtained with ultrasound imaging (Micromaxx, Sonosite Inc., Bothell, WA, USA) using a HFL38 transducer (6-13MHz; brachial artery) and a P17 transducer (1-5MHz; portal vein, aorta, inferior vena cava). All images and velocities captured by the Sonosite during the experiment were transferred to video tape using a Sony Handycam (Model DCR-HC42) and Panasonic digital video cassettes. Videos were digitized using Adobe Premier 6.5 for post-analysis. The diameter of the portal vein and the inferior vena cava were determined at baseline and after one minute of -20mmHg and -40mmHg of LBNP (Microsoft Paint 5.1; Adobe Fireworks CS4). Velocity of the blood within the portal vein was obtained and measured at the same time points (Micromaxx, Sonosite Inc., Bothell, WA, USA; MATLAB 5.3 GDM application (© Hanif M. Ladak 1998-1999)). Portal vein flow was found from the velocity and the cross sectional area of the vessel [Portal flow (mL/min) = velocity*(π*radius^2)*60] and this was normalized to body surface area. Portal vein resistance index was determined by dividing portal flow by mean arterial pressure.

Brachial vascular resistance was used as an index for limb vascular resistance. Portal vein resistance index was used as an index for splanchnic vascular resistance. The portal vein receives blood from the inferior mesenteric vein, superior mesenteric vein, splenic vein, and gastric veins. There is only a small amount of splanchnic blood that does not pass through the portal vein (i.e. it returns to the heart through vena caval tributaries). The diameter of the inferior vena cava was normalized to the volume of blood within each person.

2.10 Beat-by beat measurements

Blood pressure was measured using finger-cuff plethysmography (Finometer; Finapres Medical Systems, Arnheim, The Netherlands), and heart rate was measured from the R-R

interval of the electrocardiogram (COLIN PILOT, Colin Medical Instruments Corp., San Antonio, TX, USA). Blood flow velocity from the aortic root and the brachial artery were recorded using 2MHz and 4MHz probes, respectively (Neurovision Transcranial Doppler System Model 500M, Multigon Industries Inc., Yonker, NY, USA). Stroke volume (SV) was determined from the cross-sectional area of the aorta and the blood velocity of the aorta [SV (mL) = velocity*(π*radius^2)*R-R interval]. Cardiac output was calculated as stroke volume multiplied by heart rate divided by 1000. Total peripheral resistance was calculated as mean arterial pressure minus central venous pressure divided by cardiac output. Brachial arterial flow was determined from the cross-sectional area of the brachial artery and the blood velocity [Brachial flow (mL/min) = velocity*(π*radius^2)*60]. Brachial vascular resistance was calculated as flow divided by mean arterial pressure. Cardiac output and stroke volume were normalized to body surface area, and brachial vascular flow was normalized to arm volume.

2.11 Impedance

Biological impedance was measured in the thoracic and pelvic regions (Minnesota Impedance Cardiograph, Instrumentation for Medicine Inc., Greenwich, CT, USA). Electrocardiogram (ECG) electrodes were placed at the level of the neck and xyphoid for thoracic measurements (21) and at the xyphoid and iliac crest for pelvic measurements (178). A constant alternating current of 4mA at 100kHz was passed through the region between the outer electrodes. The voltage was detected by the inner electrodes and was a product of this current and the regional impedance (Z_0). All impedance measurements were normalized to the length of the respective region.

2.12 Inferior vena cava compliance

Inferior vena cava (IVC) compliance was determined using IVC diameters (as an index of volume) and central venous pressure (CVP) measurements during the LBNP protocol (baseline, -20mmHg, and -40mmHg). Compliance = ΔVolume / ΔPressure.

2.13 Cardiopulmonary baroreceptor (CPBR) function

Cardiopulmonary baroreceptor (CPBR) function was determined using brachial vascular resistance (BVR) and central venous pressure (CVP) measurements during the LBNP protocol (baseline, -20mmHg, -40mmHg). (Note: This data is found only in Appendix II).

2.14 Blood collection

At baseline, -20mmHg, and -40mmHg of the LBNP protocols blood samples were taken for analysis. All analyses were performed at the University of Waterloo. Blood was either let sit for 10 minutes (serum samples: sodium, osmolality, aldosterone, estrogen, progesterone) or added to tubes containing appropriate anticoagulants (catecholamines: 25μL/mL EDTA with glutathione; atrial natriuretic peptide (ANP): 25μL/mL EDTA with aprotinin; angiotensin II: 25μL/mL EDTA with 20μL/mL bestatin; endothelin-1, nitrates/nitrites, vasopressin (AVP), renin: 25μL/mL EDTA). All tubes were then centrifuged at 2500rpm for 10 minutes at room temperature. Plasma and serum were transferred to 1.5mL Eppendorf tubes and frozen at -80^{o}C for future analysis.

2.15 Blood analysis

Radioimmunoassay kits were completed for measurements of renin (Active Renin IRMA, Diagnostic Systems Laboratories, Inc., Webster, TX, USA), aldosterone (Coat-A-Count Aldosterone, Diagnostics Products Corporation, Los Angeles, CA, USA), AVP (Vasopressin Direct RIA, ALPCO Diagnostics, Windham, NH, USA), estradiol (Coat-A-Count Estradiol, Diagnostics Products Corporation, Los Angeles, CA, USA), progesterone (Coat-A-Count Progesterone, Diagnostics Products Corporation, Los Angeles, CA, USA), and ANP (Atrial Natriuretic Peptide (ANP) RIA, ALPCO Diagnostics, Windham, NH, USA). Enzyme immunoassay kits were performed for measurements of endothelin-1 (Human Endothelin-1 Immunoassay, R & D Systems, Inc., Minneapolis, MN, USA) and angiotensin II (Angiotensin II Enzyme Immunoassay Kit, Societe de Pharmacologie et d'Immunologie – BIO, Montigny Le Bretonneux, France). A colorimetric assay was performed to determine nitrate and nitrite

levels (metabolites of nitric oxide; Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI, USA). Analysis by HPLC with electrochemical detection was performed for measurement of norepinephrine and epinephrine according to Weicker et al. (201).

2.16 Urine collection and analysis

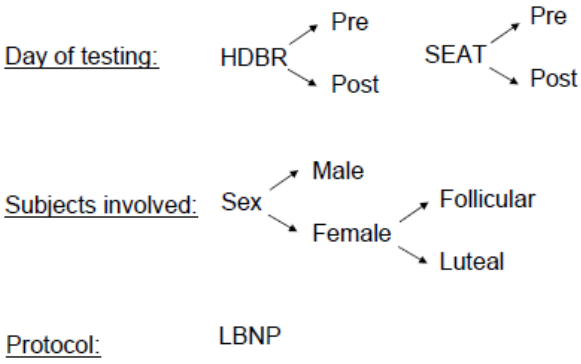
Morning urine samples were collected by participants at home and brought into the lab for processing. Urine was collected and sampled throughout the test day as required. It was weighed and samples were taken for sodium, osmolality, and urodilatin measurements. Urodilatin was measured with a radioimmunoassay (Urodilatin/ANP (95-126) – RIA Kit, Phoenix Pharmaceuticals Inc., Burlingame, CA, USA).

2.17 Sodium and osmolality measurements

Serum samples were obtained before and after the 4 hours of HDBR or SEAT. Urine samples were obtained before and throughout testing. Sodium concentrations in 250μL of serum were measured in duplicate using a Micro-Combination Sodium Electrode (Thermo Electron Corporation, Waltham, MA, USA). Sodium concentrations in 2mL of urine were measured in duplicate using an Orion ROSS™ Sodium Electrode (Thermo Electron Corporation, Waltham, MA, USA). Osmolality was measured in 10μL of serum or urine using a vapor pressure osmometer (model 5100C, Wescor Inc., Logan, UT, USA).

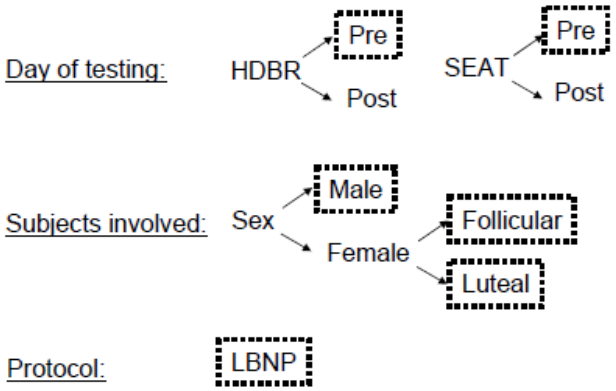
2.18 Design and statistical analysis

The current study was designed to examine the effects of 4-hr HDBR on responses to LBNP in women during both the follicular and luteal phases of the menstrual cycle, and in men. Control conditions were established by 4-hr of inactive upright seated posture (SEAT). Testing days, subject groups, and protocol are diagrammed here.



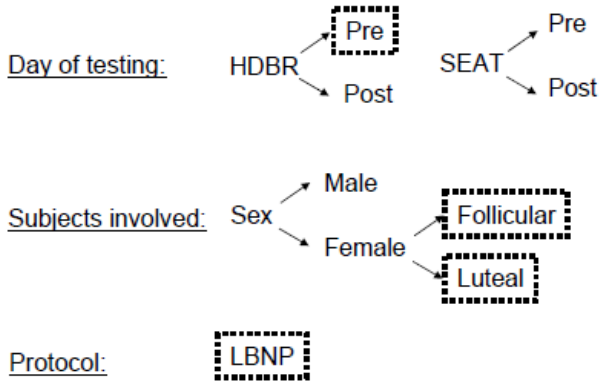
In Chapter 3 and Appendix II, various comparisons were planned to determine responses to LBNP: 1) reproducibility of responses to LBNP (Appendix II), 2) responses to LBNP in different phases of the menstrual cycle, 3) responses to LBNP between men and women.

1) To determine if LBNP was reproducible on two different days, statistical analysis was done within each group of subjects (i.e. Male, Female Follicular and Female Luteal) with two-way repeated measure ANOVAs with two repeated factors (Day of testing (e.g. pre-HDBR and pre-SEAT) and LBNP).



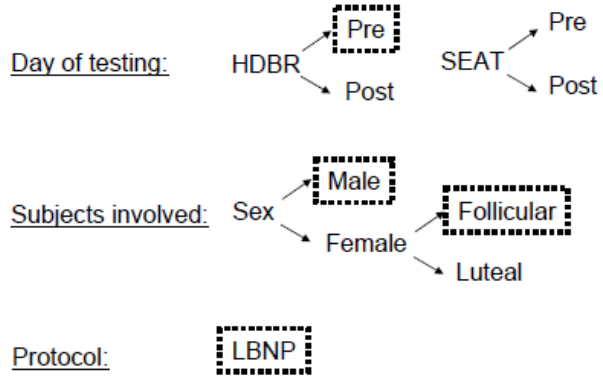
(Each outlined factor is used in the above comparison)

2) To determine differences in response to LBNP in different phases of the menstrual cycle, statistical analysis was done with two-way repeated measure ANOVAs with two repeated factors (Menstrual phase and LBNP)



(Each outlined factor is used in the above comparison)

3) To determine any sexually dimorphic differences in response to LBNP, statistical analysis was done with two-way repeated measure ANOVAs with one repeated factor (LBNP). Men were compared to the follicular phase only in order to match relevant literature that investigated sex differences (e.g. (39)). A comparison of all 3 "groups" (i.e. men, follicular phase, and luteal phase) is statistically improper as the women repeated the test in each phase and the men did not have this repeated measure.

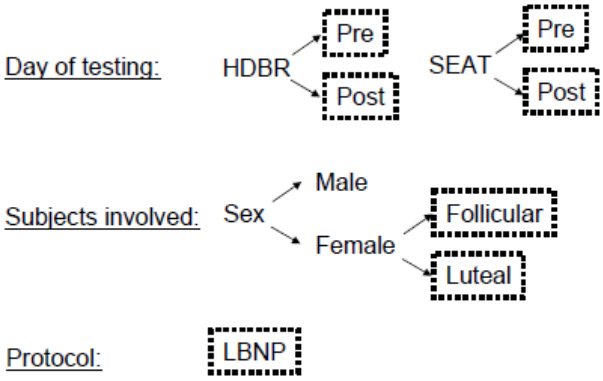


(Each outlined factor is used in the above comparison)

In Chapter 4, the effects of 4-hr HDBR were contrasted with the effects of 4-hr SEAT. This was done to determine if any changes observed were an effect of the body position (HDBR) or if they were related to inactivity or circadian factors. These comparisons were planned to determine: 1) effects of HDBR or SEAT on the response to LBNP in different phases of the

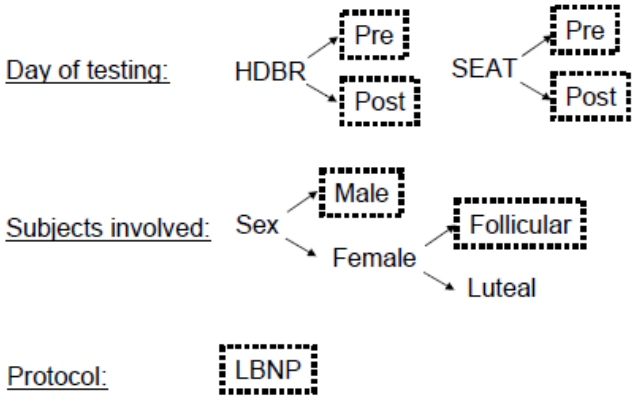
menstrual cycle, 2) effects of HDBR or SEAT on the response to LBNP in men and women, and 3) effects of HDBR or SEAT on venous compliance and water/sodium balance.

1) To determine the effects of HDBR or SEAT in different phases of the menstrual cycle and to determine if the responses to LBNP change due to HDBR or SEAT, statistical analysis was done with three-way ANOVAs with three repeated measures. Factors were LBNP, HDBR or SEAT, and phase of menstrual cycle.



(Each outlined factor is used in the above comparison)

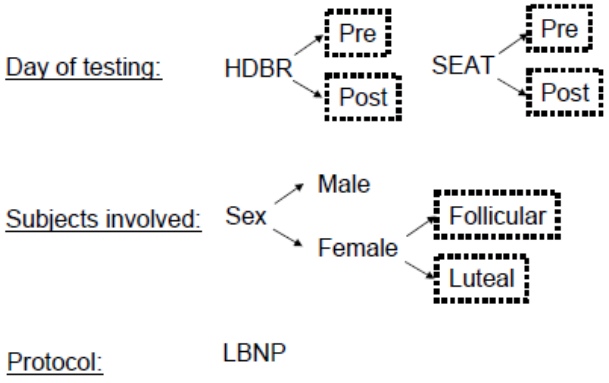
2) To determine the effects of HDBR or SEAT in men and women and to determine if the responses to LBNP change due to HDBR or SEAT, statistical analysis was done with three-way ANOVAs with two repeated measures. Factors were LBNP, HDBR or SEAT, and sex.



(Each outlined factor is used in the above comparison)

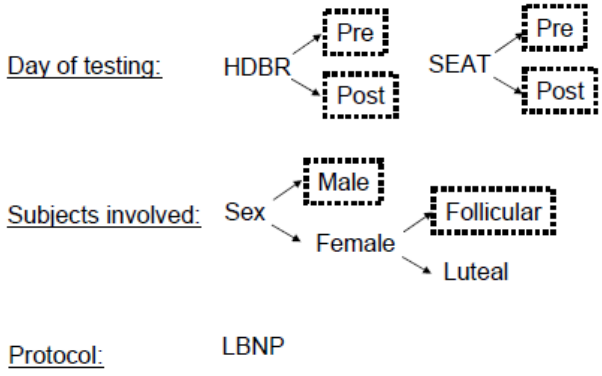
3) Analysis of compliance, blood volume, urinary measurements, serum osmolality and serum sodium was done as follows:

   a. To determine the effects of HDBR or SEAT in different phases of the menstrual cycle, statistical analysis was done with two-way repeated measures ANOVAs with two repeated factors. Factors were HDBR or SEAT and phase.



(Each outlined factor is used in the above comparison)

   b. To determine the effects of HDBR or SEAT in men and women, statistical analysis was done with two-way repeated measures ANOVAs with one repeated factor. Factors were HDBR or SEAT and sex.



(Each outlined factor is used in the above comparison)

Analysis was completed using SAS 9.1.3 analysis software (Cary, USA). *P*-values less than 0.05 are indicated as significant, and *P*-values less than 0.10 are noted throughout.

2.19 Limitations in study design/subject recruitment

Recruitment of women not taking oral contraceptives (OC) was difficult. Only five of twelve women recruited were not on any OC. Of the remaining seven women, three were taking cyclic types of OC (i.e. increasing levels of progesterone analog per week; similar to normal physiology) and four were taking a non-cyclic type of OC (i.e. the same level of progesterone analog every day). A recent study has shown that in women taking OC there are no differences in the cardiovascular responses to LBNP in women taking cyclic (n=9) versus non-cyclic OC (n=3) (27).

In the current study, all of the women taking cyclic-OC took Tricyclen-lo 21 (0.180 mg norgestimate and 0.025 mg ethinyl estradiol for the first week, 0.215 mg norgestimate and 0.025 mg ethinyl estradiol for the second week, and 0.250 mg norgestimate and 0.025 mg ethinyl estradiol for the third week; Ortho-McNeil Pharmaceutical, Raritan, NJ, USA). The women taking non-cyclic OC all took Alesse 21 (100 μg levonorgesterel and 20 μg ethinyl estradiol (Wyeth Canada, Montreal, QC, Canada)). All women taking OC had 7 hormone free days which would approximately correspond to days 25-4 of the menstrual cycle.

To determine the potential impact of this, women not on OC were grouped with those taking cyclic OC (creating one group with cycling hormones) and compared to women taking non-cyclic OC (Appendix I). The responses to LBNP in the follicular phase were compared. The follicular phase was chosen because the levels of hormones in these two groups at this time should differ (i.e. high progesterone/analog in the non-cyclic group compared to lower levels in the cyclic group).

There were no differences in heart rate, central venous pressure, or portal vein resistance index. Women taking non-cyclic OC had higher baseline stroke volume, higher baseline cardiac output, a smaller decrease in inferior vena cava diameter (due to a smaller baseline), higher baseline mean arterial pressure, lower baseline total peripheral resistance, and higher baseline brachial vascular resistance. With the small sample numbers involved (n=4 for non-cyclic) it is hard to conclude that these differences are from physiological effects of the OC. Some of these differences could be due to a greater level of athleticism in the non-cyclic group. Each individual's exercise capacity (VO_2max) was not determined. Future studies

should take this into account, and women who are taking non-cyclic OC should not be recruited.

2.20 Measurements of estrogen and progesterone

In women not-taking OC, appropriate levels of estrogen were observed in the follicular and luteal phases. As expected, a greater concentration of progesterone is observed in the luteal phase. Measuring appropriate levels of estradiol and progesterone in women taking OC was unsuccessful. According to the estradiol kit manufacturers, men should exhibit a range from 0-44 pg/mL, ovulating women in the follicular phase should have a range between 60-200 pg/mL, and ovulating women in the luteal phase should have a range between 60-260 pg/mL. According to the progesterone kit manufacturers, men should exhibit a range of 0.1-1.2 ng/mL, ovulating women in the follicular phase should have a range between 0.15-1.4 ng/mL, ovulating women in the luteal phase should have a range between 1.6-21 ng/mL, and women on oral contraceptives should have a range between 0.18-0.64 ng/mL.

Group	Phase	Posture	Estradiol (pg/mL)	Progesterone (ng/mL)
No-OC	Follicular	HDBR	67.2±16.9	0.8±0.2
(n=3)		SEAT	56.5±18.2	0.7±0.2
	Luteal	HDBR	69.6±20.0	5.3±2.9
		SEAT	89.0±29.1	9.2±4.1
Cyclic-OC	Follicular	HDBR	15.9±12.5	0.6±0.1
(n=3)		SEAT	13.2±6.1	0.6±0.1
	Luteal	HDBR	2.2 (n=1)	0.5±0.1
		SEAT	4.2 (n=1)	0.6±0.1
Non-cyclic OC	Follicular	HDBR	18.6±7.5	0.7±0.1
(n=4)		SEAT	35.7±13.4	0.9±0.1
	Luteal	HDBR	45.5±28.9	0.7±0.1
		SEAT	53.7±46.5	0.8±0.1
Men		HDBR	34.6±6.5	1.3±0.1
(n=10)		SEAT	38.1±5.8	1.3±0.1

According to the manufacturers of the estradiol radioimmunoassay, the ethinyl estradiol and norgestimate in OC should have “no clinical effect” and that they should not be detected with the kit. Cross-reactivity of the progesterone kit for ethinyl estradiol and norgestimate was not examined by the manufacturers. The lower progesterone levels in women taking OC are expected due to the absence of ovulation and creation of a corpus luteum (a major source of progesterone secretion in luteal phase). The lower estradiol levels in women taking OC could be due to the lower androgen levels seen in women taking OC (37) (i.e. androgens are a precursor to estradiol production).

CHAPTER 3

Cardiovascular effects of lower body negative pressure (LBNP) in men and women through the menstrual cycle

Lower body negative pressure (LBNP) is used to simulate orthostatic stress and elicit a fluid shift towards the legs. Typical physiological responses are those which maintain mean arterial pressure and include the release of hormones which elicit fluid retention, vasoconstriction and/or increase heart rate. These hormones include norepinephrine, the renin-angiotensin-aldosterone system (RAAS) and vasopressin (58; 62; 76; 200) which increase peripheral resistance and maintain mean arterial blood pressure. If this response is inadequate blood pressure will decrease (i.e. orthostatic hypotension) resulting in reduced perfusion of the brain leading to faintness, dizziness and syncope.

Women experience a greater incidence of orthostatic hypotension compared to men. This may be due to lower sympathetic output or an attenuated vasoconstrictor response (86). Lower body venous compliance may also play a role. Men have greater leg venous compliance (78) and leg blood volume during an orthostatic challenge (39). This may actually benefit them as greater volume load in the veins may elicit greater sympathetic responses. Indeed, Chen et al. have shown that forearm venous occlusion increases peroneal muscle sympathetic nerve activity (31). Greater splanchnic blood pooling may also be involved in the greater incidence of orthostatic hypotension in women. Orthostatic stressors decrease blood flow through the portal vein (indicating reduced blood volume of the splanchnia)(62); however women have greater splanchnic blood pooling with LBNP (as shown with impedance measurements)(202).

Orthostatic tolerance has been shown to be the same throughout the menstrual cycle (35), but there are many different baseline cardiovascular differences and some differences in the response to orthostatic stress. Women in the luteal phase (i.e. late in the cycle when both estrogen and progesterone levels are high) have lower blood pressure and total peripheral resistance (30; 52). There are no differences in blood or plasma volume (30), yet luteal phase women have lower serum sodium and osmolality resulting in greater activation of RAAS (1; 30; 124; 179; 188). In response to an orthostatic stress there is higher total muscle sympathetic nerve activity in the luteal phase (compared to early follicular when there is very low levels of both estrogen and progesterone), but there are no observed differences in heart rate, mean arterial pressure, stroke volume, or cardiac output (28; 67).

It was hypothesized that 1) The degree of orthostatic hypotension experienced by women will not be different in the luteal and follicular phase of the menstrual cycle; however the cardiovascular and endocrine responses to orthostatic stress will differ, and 2) In

comparison to men, women will experience a greater fall in blood pressure with an orthostatic stress and greater splanchnic blood pooling will be a contributing factor.

3.1 Materials and Methods

Refer to Chapter 2

3.2 Results

Cardiovascular and hormonal responses to LBNP: Women during the menstrual cycle

Main effects of Phase

There were no differences between the follicular and luteal phases for heart rate (P=0.622; Figure 1A), stroke volume (P=0.737; Figure 1B), cardiac output (P=0.902; Figure 1C), central venous pressure (P=0.852; Figure 2A) or inferior vena cava diameter (P=0.969; Figure 2B). Nor were there any phase differences for mean arterial pressure (P=0.935; Figure 3A), total peripheral resistance (P=0.906; Figure 3B), portal vein resistance index (P=0.381; Figure 3C), or brachial vascular resistance (P=0.982; Figure 3D). There were also no phase differences for norepinephrine (P=0.928; Figure 4A), vasopressin (P=0.989; Figure 4B), renin (P=0.763; Figure 5A), angiotensin II (P=0.656; Figure 5B), aldosterone (P=0.119; Figure 5C), epinephrine (P=0.255; Table 1), endothelin-1 (P=0.927; Table 1), nitrates and nitrites (P=0.531; Table 1), or atrial natriuretic peptide (P=0.664; Table 1). There were no phase differences for thoracic (P=0.383; Figure 6A) or pelvic impedance (P=0.858; Figure 6B).

Main effects of LBNP

In both phases of the menstrual cycle, lower body negative pressure (LBNP) increased heart rate (P<0.0001; Figure 1A), decreased stroke volume (P<0.0001; Figure 1B), decreased cardiac output (P<0.0001; Figure 1C), decreased central venous pressure (P<0.0001; Figure 2A) and decreased inferior cava diameter (P<0.0001; Figure 2B). There was a concurrent decrease in mean arterial pressure (P=0.009; Figure 3A), an increase in total peripheral resistance (P<0.0001; Figure 3B), and a slight increase in portal vein resistance index

(P=0.088; Figure 3C). There was no significant effect of LBNP on brachial vascular resistance (P=0.156; Figure 3D) in either phase. Lower body negative pressure increased norepinephrine (P=0.012; Figure 4A), did not change vasopressin (P=0.191; Figure 4B), increased renin (P=0.036; Figure 5A), did not change angiotensin II (P=0.541; Figure 5B), and decreased aldosterone (P=0.005; Figure 5C). There was a slight increase in epinephrine (P=0.098; Table 1) with no significant effects on endothelin-1 (P=0.518; Table 1), nitrates and nitrites (P=0.644; Table 1), or atrial natriuretic peptide (P=0.285; Table 1). Lower body negative pressure increased thoracic impedance (P<0.0001; Figure 6A) with no change in pelvic impedance (P=0.285; Figure 6B).

Interaction effects

There were no interaction effects between LBNP and menstrual cycle.

Cardiovascular and hormonal responses to LBNP: Women (follicular phase) compared to men

Main effects of Sex

There were no main sex effects for heart rate (P=0.812; Figure 1A), stroke volume (P=0.515; Figure 1B), cardiac output (P=0.581; Figure 1C), central venous pressure (P=0.689; Figure 2A), mean arterial pressure (P=0.100; Figure 3A), inferior cava diameter (P=0.189; Figure 2B), total peripheral resistance (P=0.924; Figure 3B), or portal vein resistance index (P=0.349; Figure 3C). Women had higher brachial vascular resistance (P=0.006; Figure 3D). There were no sex effects for norepinephrine (P=0.988; Figure 7A) or vasopressin (P=0.125; Figure 7B). There was higher renin in men (P=0.014; Figure 4A) yet no difference in angiotensin II (P=0.989; Figure 4B) or aldosterone (P=0.286; Figure 4C). There were no sex effects for epinephrine (P=0.834; Table 1), endothelin-1 (P=0.181; Table 1), nitrates and nitrites (P=0.974; Table 1), or atrial natriuretic peptide (P=0.977; Table 1). Women had higher thoracic impedance (P=0.002; Figure 6A), and higher pelvic impedance (P=0.039; Figure 6B) than men. (Interaction effects of sex are discussed below).

Main effects of LBNP

In both men and women, LBNP increased heart rate ($P<0.0001$; Figure 1A), decreased stroke volume ($P<0.0001$; Figure 1B), decreased cardiac output ($P<0.0001$; Figure 1C), decreased central venous pressure ($P<0.0001$; Figure 2A) and decreased inferior vena cava diameter ($P<0.0001$; Figure 2B). There was also a decrease in mean arterial pressure ($P=0.043$; Figure 3A), an increase in total peripheral resistance ($P<0.0001$; Figure 3B) and an increase in portal vein resistance index ($P=0.002$; Figure 3C), yet no significant changes in brachial vascular resistance ($P=0.278$; Figure 3D) were observed. Lower body negative pressure increased norepinephrine ($P=0.0002$; Figure 4A), did not change vasopressin ($P=0.130$; Figure 4B), increased renin ($P=0.002$; Figure 5A), did not change angiotensin II ($P=0.672$; Figure 5B), and slightly decreased aldosterone ($P=0.082$; Figure 5C). There were no changes in epinephrine ($P=0.158$; Table 1), endothelin-1 ($P=0.627$; Table 1), nitrates and nitrites ($P=0.273$; Table 1), or atrial natriuretic peptide ($P=0.441$; Table 1) due to LBNP. Lower body negative pressure increased thoracic impedance ($P<0.0001$; Figure 6A), but there was no change in pelvic impedance ($P=0.530$; Figure 6B). (Interaction effects of LBNP are discussed below).

Interaction effects

In women, LBNP resulted in a greater reduction of central venous pressure ($P=0.048$ (Sex*LBNP effect); Figure 2A) and a reduction in mean arterial pressure that was not seen in men ($P=0.047$ (Sex*LBNP effect); Figure 3A). Women did not have an increase in portal vein resistance index with LBNP ($P=0.004$, Sex*LBNP effect); Figure 3C) and had a greater increase of thoracic impedance during LBNP ($P<0.0001$ (Sex*LBNP effect); Figure 6A).

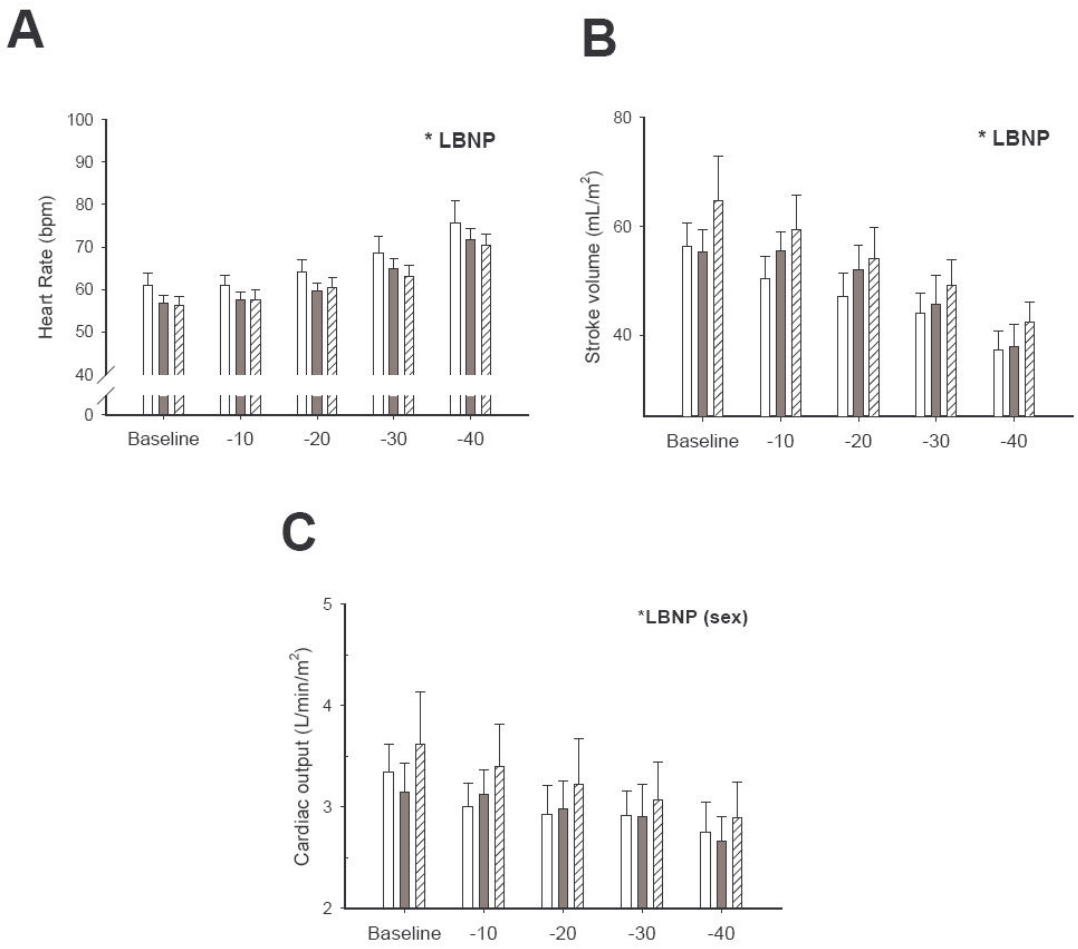


Figure 1: The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on heart rate (A), stroke volume (B), and cardiac output (C). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons. *LBNP (sex) indicates a significant main effect of LBNP for the sex comparison only.

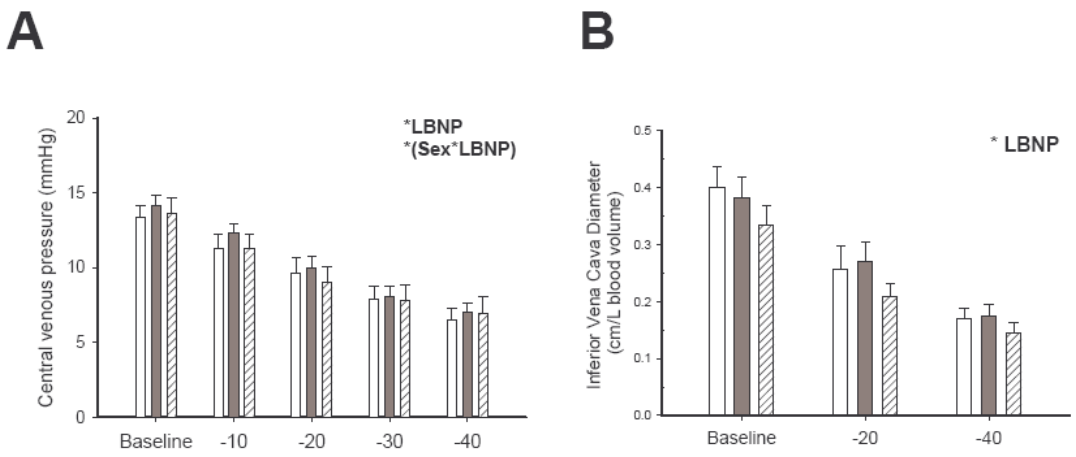


Figure 2: The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on central venous pressure (A), and inferior vena cava diameter (B). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons. *(Sex*LBNP) indicates a significant interaction effect between Sex and LBNP.

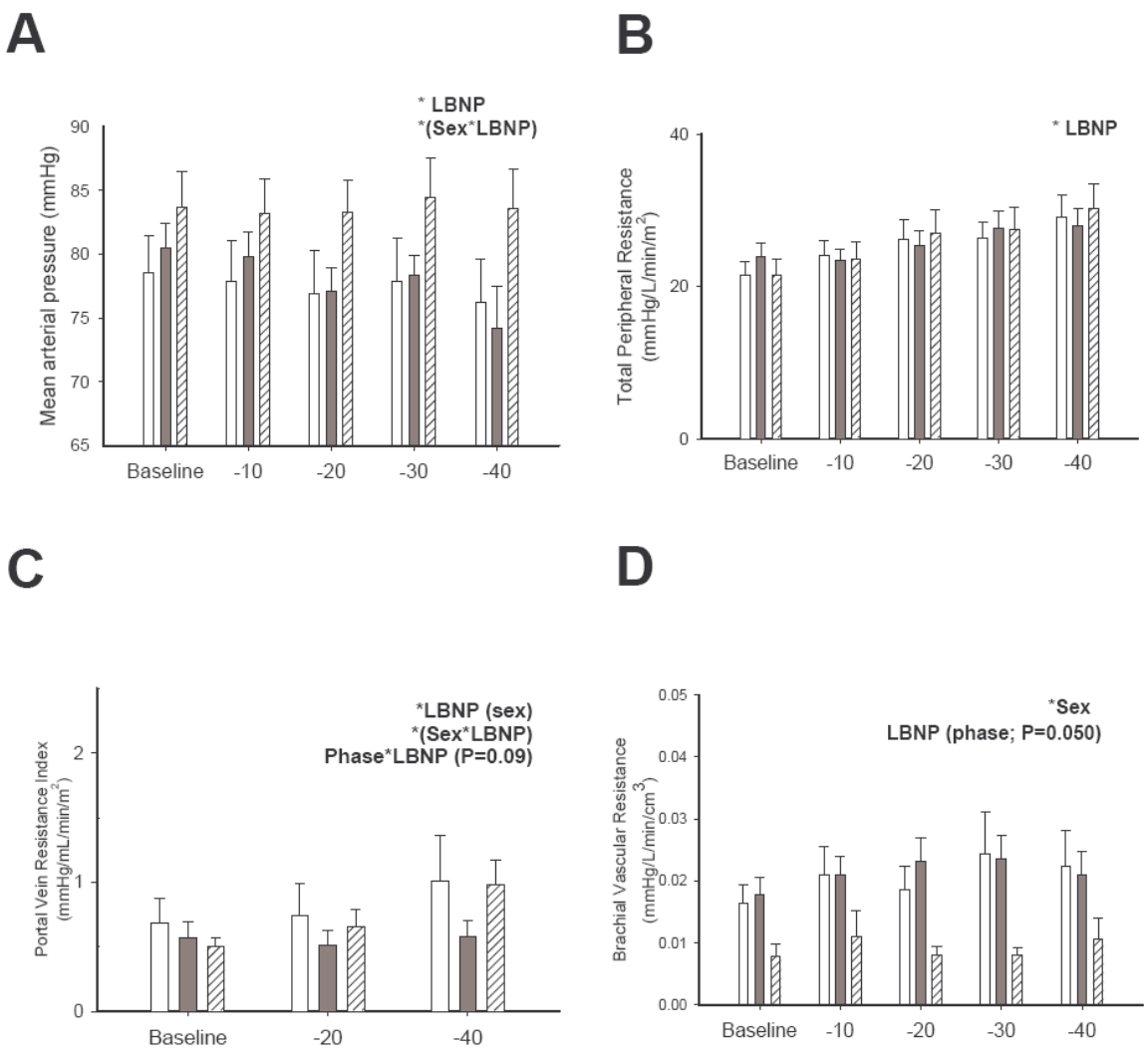


Figure 3: The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on mean arterial pressure (A), total peripheral resistance (B), portal vein resistance index (C), and brachial vascular resistance (D). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons. *LBNP (sex) indicates a significant main effect of LBNP for the sex comparison only. *LBNP (phase) indicates a main effect (P=0.050) of LBNP for the phase comparison only. *(Sex*LBNP) indicates a significant interaction effect between Sex and LBNP.

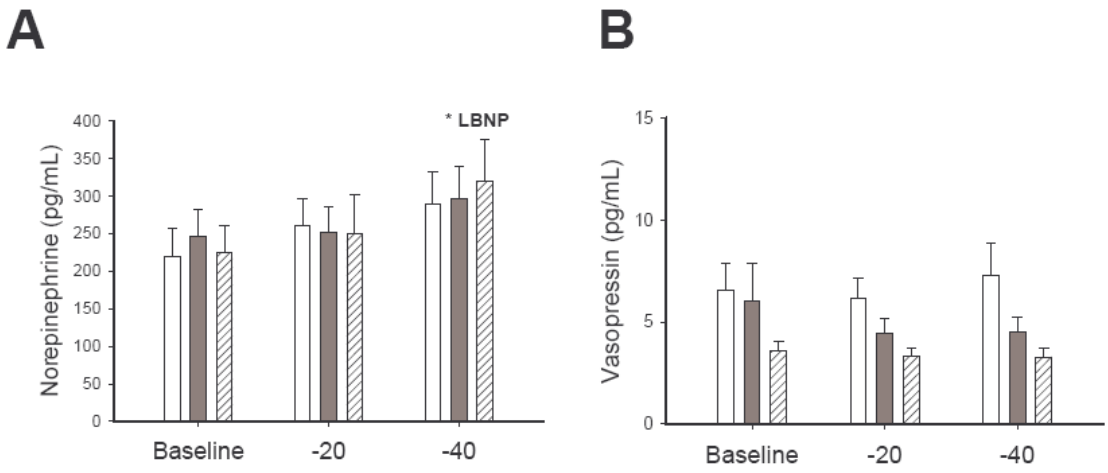


Figure 4: The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on plasma concentrations of norepinephrine (A) and vasopressin (B). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons.

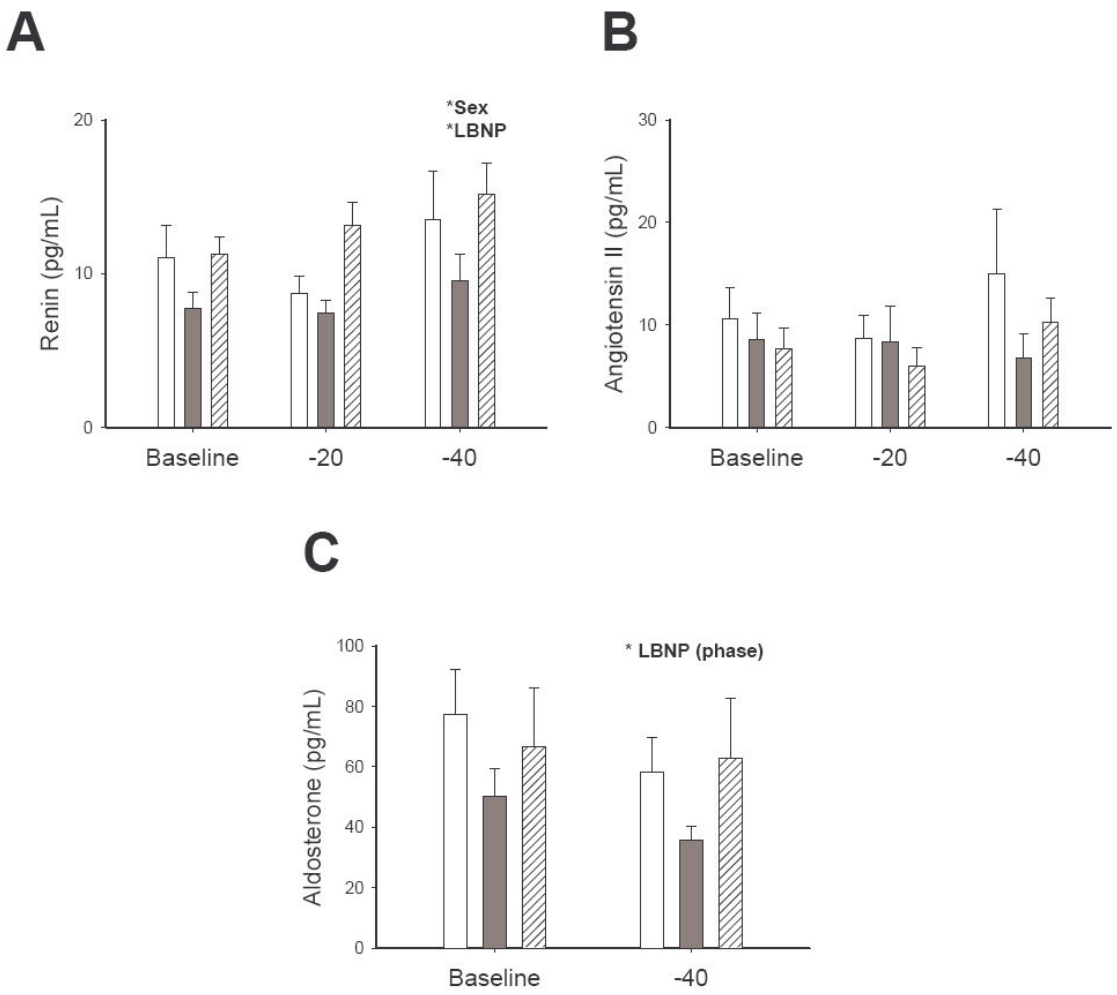


Figure 5: A. The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on plasma concentrations of renin (A), angiotensin II (B), and aldosterone (C). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons. *LBNP (phase) indicates a significant main effect of LBNP for the phase comparison only. *Sex indicates a significant main effect of Sex.

Table 1: Responses of other vasoactive factors in the luteal phase, follicular phase and men during lower body negative pressure (LBNP).

	Phase	0 mmHg	-20mmHg	-40mmHg
Epinephrine (pg/mL)	**Luteal**	14.2 ± 2.6	20.0 ± 2.0	21.4 ± 2.5
	Follicular	21.1 ± 3.0	17.3 ± 1.4	24.0 ± 3.7
	Male	22.6 ± 3.0	19.8 ± 5.0	22.8 ± 4.3
Endothelin-1 (pg/mL)	**Luteal**	1.5 ± 0.3	-	1.4 ± 0.3
	Follicular	1.4 ± 0.2	-	1.6 ± 0.5
	Male	2.2 ± 0.4	-	2.0 ± 0.4
Nitrates/Nitrites (µmol/L)	**Luteal**	7.3 ± 1.2	7.0 ± 1.1	8.1 ± 1.8
	Follicular	6.4 ± 1.5	6.1 ± 1.3	6.0 ± 1.1
	Male	6.2 ± 1.0	6.1 ± 1.0	6.0 ± 1.0
Atrial natriuretic peptide (pg/mL)	**Luteal**	25.8 ± 3.1	21.5 ± 3.8	23.4 ± 4.7
	Follicular	29.9 ± 3.3	29.5 ± 4.4	26.5 ± 4.6
	Male	29.4 ± 4.5	27.6 ± 4.1	27.7 ± 4.9

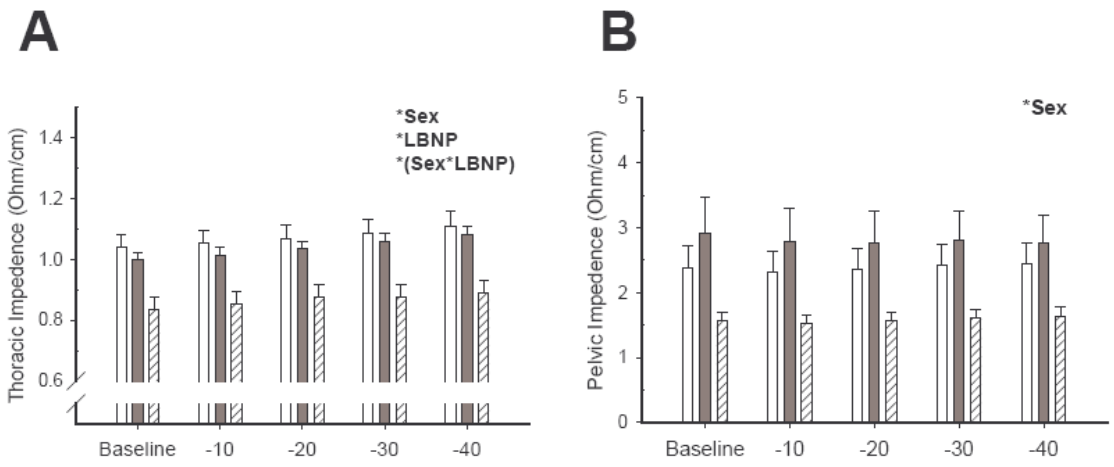


Figure 6: The effects of lower body negative pressure (LBNP) in women during the luteal phase (white bars) and the follicular phase (grey bars) and men (hatched bars) on thoracic impedance (A) and pelvic impedance (B). *LBNP indicates a significant main effect of LBNP for both the sex and phase comparisons. *Sex indicates a significant main effect of Sex. *(Sex*LBNP) indicates a significant interaction effect between Sex and LBNP.

3.3 Discussion

It was shown that in comparison to the luteal phase a tendency for splanchnic blood pooling exists in the follicular phase of the menstrual cycle during an orthostatic stress. Furthermore it was shown that women (in the follicular phase) experience greater splanchnic pooling during lower body negative pressure (LBNP) compared to men. Women also experience a greater loss of mean arterial pressure, central venous pressure and thoracic impedance with LBNP, and an attenuated release of renin with LBNP.

Effects of LBNP through the menstrual cycle

Data were presented here that have not been observed previously: central venous pressure, inferior vena cava diameter, portal vein resistance index, thoracic impedance and pelvic impedance (both baseline differences and responses to orthostatic stress). These new observations support the hypothesis that while orthostatic hypotension is the same in the follicular and luteal phases some responses tended to differ (i.e. splanchnic pooling tended to be greater in the follicular phase with LBNP). There were no differences in venous return indices, stroke volume, heart rate, cardiac output, or blood pressure/volume regulating hormones. These results confirm and extend those of Fu et al. and Carter et al. who also found no differences as a function of menstrual cycle phase in heart rate, mean arterial pressure, stroke volume, or cardiac output before and during LBNP (28; 67).

Portal vein resistance index has not been investigated previously in different phases of the menstrual cycle during an orthostatic stress. Portal vein resistance index was used as an index of splanchnic vascular resistance because most blood from the stomach, pancreas, spleen and intestines passes through the portal vein. A tendency ($P=0.09$) for women in the luteal phase to have higher portal vein resistance index with LBNP in comparison to the follicular phase was observed. This implies that there is a tendency for greater pooling of blood in the splanchnia in the follicular phase during an orthostatic stress; however the measurements of pelvic impedance (i.e. approximate measurement of the amount of blood in the pelvic region) did not show any differences between menstrual phases. The pelvic impedance measurement

also includes the bladder, bowels and pelvic organs, therefore this may be an inappropriate index of splanchnic blood pooling.

There were no baseline differences in brachial vascular resistance between phases or any differences in the response to LBNP. Lehtovirta also found no baseline difference between follicular and luteal phases for forearm blood flow (using venous plethysmography) (112), and Claydon et al. found similar brachial vascular resistance between phases with an orthostatic challenge (using Doppler ultrasound) (35). It has also been observed that there was no difference in forearm blood flow reactivity to angiotensin II or norepinephrine infusions between phases (15). Thus similar levels of these hormones (as observed in this study) would not be expected to elicit different levels of vascular constriction.

There were no baseline differences in vasopressin between the follicular and luteal phases which corresponded to the results of Altemus et al. who found no difference between the early follicular (minimal estrogen and minimal progesterone) and the luteal phase (3). In response to LBNP there was a small trend towards a greater increase of vasopressin in the luteal phase ($P=0.130$). Similarly, Altemus et al. examined the responses to treadmill exercise stress (90% of maximal oxygen consumption) and found that women in the luteal phase had a greater increase in vasopressin. The current study found no difference between phases in regard to baseline catecholamines or their response to LBNP. Fu et al. also found no differences in baseline levels or in the increase of catecholamines seen with head-up tilt between the early follicular and luteal phases (67).

These results confirm those of Usuki et al. who found that plasma atrial natriuretic peptide (ANP) levels did not change throughout the menstrual cycle (188). This study also observed no difference in baseline plasma endothelin-1 between phases, yet Usuki et al. observed slightly higher endothelin-1 levels in the luteal phase (188). This difference could be methodological as Usuki et al. measured endothelin-1 levels in a biopsy of the endometrium rather than a plasma sample. Resting nitric oxide (NO) metabolite plasma levels (nitrates and nitrites) have been shown to be lower in the luteal phase (74); however no difference between phases at rest was shown here. The finding that ANP, endothelin-1, and NO metabolites do not change due to LBNP in different phases of the menstrual cycle are unique.

There were no measurements of brain blood flow in this study, but this has previously been measured in the follicular and luteal phases both before and during an orthostatic stress.

Others have found that there is lower resting cerebral vascular resistance during the follicular phase (16).This outlines the possibility that even though there may be less blood returning from the periphery during an orthostatic stress (due to splanchnic pooling), there may be greater brain blood flow during the follicular phase (possibly due to higher arterial carbon dioxide (35; 118)). However, cerebral vascular resistance during an orthostatic stress was investigated by Claydon et al. and they found a slightly greater increase in cerebral vascular resistance in the follicular phase (35). They reported their results as an "autoregulation index" by which they divided the change in cerebral blood velocity by the change in calculated cerebral perfusion pressure. While they did not find a significant difference, the luteal phase appeared to have a lower correlation coefficient than the follicular phase (Correlation coefficients: 0.50±0.1 for luteal; 0.60±0.1 for follicular) indicating a tendency for a smaller drop in brain blood flow with a given change in cerebral perfusion pressure in the luteal phase. (They did not observe differences in blood pressure response between phases). Therefore, cerebral blood flow could be lower in the follicular phase during an orthostatic stress.

Chapman et al. observed greater resting renal vascular resistance in the follicular phase (30), and Kurjak et al. found greater resting uterine and ovarian artery resistance in the follicular phase (109). Esformes et al. have shown that resting calf vascular resistance is not different between early follicular, late follicular, and luteal phases (57) though Minson et al. found that there is a tendency for higher calf vascular resistance with increased sympathetic nerve activity (blood pressure was changed using pharmacological agents) in the early follicular compared to the luteal phase (128). These results imply that there is the potential for greater renal, uterine, ovarian, and calf vascular resistance during an orthostatic stress in the follicular phase. If this is indeed the case, this increase in vascular resistance could offset the absence of an increase in splanchnic vascular resistance seen in this study. While there were no direct measurements of blood flow to the legs, pelvic organs, or kidneys in this study, there were measurements of the diameter of the inferior vena cava (IVC). This is used as an index of blood flow from these regions. It was found that the IVC diameter is not different in size between the phases with or without LBNP implying no difference in the volume returning from the lower body. However, the IVC is not a precise circle (32), thus using 2-D longitudinal ultrasound images as an index of blood return from the legs and kidneys may not be accurate.

The observations from this study suggest that women in the follicular phase exhibit some pooling of the blood in the splanchnia during an orthostatic challenge. However, since the Phase*LBNP interaction for portal vein resistance index did not quite reach statistical significance (P=0.09), it is still unclear if splanchnic pooling is indeed different between phases with LBNP. Similarly, there seemed to be greater activation of the renin-angiotensin-aldosterone system (RAAS) and a greater vasopressin response in the luteal phase, yet these were not significant. Inadequate statistical power may have been a factor thus increasing error. In order to reduce this error and strengthen responses this protocol was followed with a 4-hour bed rest model (or a 4-hour seated control) in order to augment orthostatic responses (22). This is described in the next Chapter.

Effects of LBNP in men and women

Data were presented that support the hypothesis that women experience a greater fall in blood pressure with LBNP in comparison to men and that splanchnic blood pooling is likely a contributing factor. It was shown that women have a greater loss of central venous pressure with a concurrent greater increase in thoracic impedance with LBNP. These results indicate that women have a greater loss of venous return during LBNP which could be due (at least in part) to the greater degree of splanchnic blood pooling and perhaps to the attenuated activation of the renin-angiotensin-aldosterone pathway observed with LBNP.

In this study mean arterial pressure decreases with LBNP only in women. However, Convertino found that both men and women had a drop in blood pressure at -40mmHg LBNP (39). These discrepancies could be due to differences in protocol. Convertino used a protocol of 2min baseline, 10min at -15mmHg, 10min at -30mmHg, and further 10mmHg reductions every 3min until presyncope. Thus -40mmHg occurred after 20 minutes of LBNP, whereas in this study -40mmHg occurred after 11 minutes of LBNP (3min at -10mmHg, 5min at -20mmHg, and 3min at -30mmHg). Perhaps the longer period of LBNP led to greater orthostatic hypotension in men.

In this study, there were no baseline differences in heart rate, stroke volume, or cardiac output. However, Convertino observed higher baseline heart rate, lower baseline stroke volume, and equal baseline cardiac output in women. (39). The higher heart rate could be

explained by the lower fitness levels in the women, and the lower stroke volume could be explained by the absence of normalization to body size (cardiac output is also not normalized to body size). During LBNP, Convertino observed a greater increase in total peripheral resistance and a greater increase in thoracic impedance (this was used to calculate the greater loss in cardiac output which in turn was used to calculate total peripheral resistance) in women. In this study there were no differences in total peripheral resistance or cardiac output, but there was a greater increase in thoracic impedance in women. These discrepancies could be because cardiac output was calculated with beat-by-beat ultrasound measurements of flow through the aorta in the present study rather than with impedance. In this study, cardiac output was also estimated using the Modelflow algorithm implemented with the Finometer (data not shown), and those results were similar to those found by Convertino. However, it has been shown previously that the relationship between the measurements of cardiac output using the Finometer estimation and the ultrasound measurement becomes less precise with LBNP or head-up tilt ((190); N. Gagné, M.Sc. thesis). Further work is necessary in order to show which measurement of cardiac output (i.e. Doppler, impedance, or Finometer) is more accurate, particularly in women; however the observations that women experienced a greater loss in central venous pressure with LBNP support the greater increase of thoracic impedance and thus greater loss of venous return and cardiac output.

There were no baseline differences in portal vein resistance index, yet there was an attenuated increase in women with LBNP. This could result in greater splanchnic pooling in women during LBNP. Jarvis et al. observed higher baseline splanchnic blood flow in men (using an indocyanine green injection technique) but the data were not normalized to body size (94). Furthermore, in support of the current findings, they observed a greater decrease in splanchnic vascular conductance (the inverse of resistance) in men with head-up tilt. The increase in portal vein resistance index observed in the men of this study would lead to greater movement of splanchnic blood pools during an orthostatic stress assisting in the maintenance of venous return, cardiac output, and mean arterial pressure.

The portal vein resistance index data support the greater pelvic blood pooling previously seen in women with LBNP (202), yet the pelvic impedance data do not. This could be due to the fundamental limitations of the impedance measurement (described in Limitations). Briefly, the amount of blood in the inferior vena cava and gut contents may

obscure the changes in splanchnic blood pools as measured by impedance. The higher baseline impedance in women, observed in this study, has been observed before by Metry et al. who found greater thoracic impedance in women than in men (123). These differences could be due to body composition differences.

Higher brachial vascular resistance in women was observed which confirms previous studies (15; 103). This could be a function of both a smaller brachial artery and a greater arterial vasoconstrictor response to hormones such as vasopressin. In rat thoracic aorta, females have a higher contractile response to vasopressin than males (173). There was a trend for higher vasopressin in women (Sex effect: P=0.12; Figure 4B) therefore the combination of both higher sensitivity and slightly higher concentrations of vasopressin could lead to greater arterial constriction.

While norepinephrine increases due to LBNP in both sexes, there was neither the greater response in men nor the increase in epinephrine due to LBNP that was observed previously (39). Similarly, the increase in vasopressin due to LBNP that was observed by Convertino was not observed in this study (39). These discrepancies could be due to the fact that Convertino examined these hormone levels at the time of syncope rather than at -40mmHg. However, there was an increase in renin due to LBNP and a greater increase in men, similar to Convertino. After separating men with high orthostatic tolerance from those with low orthostatic tolerance, Greenleaf et al. observed that men with low tolerance had an attenuated increase of renin during LBNP (76). Perhaps the attenuated increase of renin seen in women can help to explain their reduced orthostatic tolerance.

Cutaneous blood flow accounts for approximately 5% of cardiac output (167). Therefore, nitric oxide was examined as it has been shown to inhibit cutaneous vasoconstrictor responsiveness to LBNP (164) and because of the possibility of greater nitric oxide production in women (17; 155; 174). It has also been shown that individuals with postural orthostatic tachycardia syndrome (POTS) exhibit reduced NO-dependent cutaneous vasodilation (120). In this study, no changes were found in plasma nitrates/nitrites (nitric oxide metabolites) between the sexes or due to LBNP. This could be due to the fact that the measurements in this study indicate whole-body levels of nitrates/nitrites rather than specifically investigating cutaneous content.

There were no effects of sex or LBNP on atrial natriuretic peptide. This is different from the results of Clark et al. and Clerico et al. who both found that pre-menopausal women had higher baseline levels than men (33; 36); however the lack of an LBNP effect is in line with the results of Imam et al. who found that atrial natriuretic peptide levels in men did not change with LBNP (90). (Note: Our mean levels are appropriate for the methods that we used (radioimmunoassay, ALPCO Diagnostics)). The higher levels of endothelin-1 observed in men compared to women support what has been seen previously (187); however the increase due to LBNP that was seen by Fischer et al. was not observed. White et al. also found increased endothelin-1 with head-up tilt but only in those individuals with higher orthostatic tolerance (203). Technical difficulties could be a factor in these discrepancies, particularly considering that the minimum detectable concentration for the assay used in the current study is <1.0pg/mL and low values of ~1.5-2.0pg/mL were measured (Note: these values are approximately double those of the aforementioned studies and therefore precision may be a factor in all of these studies).

The responses of renal and leg vasculature were not directly measured. These regions could play a role in orthostatic hypotension as 20% of cardiac output flows through the kidneys, and 21% flows through skeletal muscle (167). Convertino and Grenon et al. investigated leg volume and compliance between sexes with LBNP and found higher leg volume and leg venous compliance in men than in women during LBNP (39; 78). While this would result in less venous return from the legs, Chen et al. found that forearm venous occlusion increased muscle sympathetic nervous activity (31). Therefore, this higher volume in the legs could result in higher resistance in other areas of the body in order to increase venous return from those pools to maintain cardiac output and mean arterial pressure. Not much is known about renal vascular resistance and orthostatic stress, yet Conboy et al. recently observed an approximately 30% decrease in renal vascular conductance with head-up tilt (all but one of these participants were women, but were not controlled for menstrual phase) (38). They also observed a smaller decrease in renal vascular conductance with head-up tilt after 8-weeks of endurance training. They suggest that this greater renal conductance contributes to the higher incidence of orthostatic intolerance seen in endurance trained athletes (38). Perhaps greater renal conductance could play a role in the higher incidence of orthostatic hypotension in women versus men.

Summary and Conclusions

The different cardiovascular responses to LBNP in different phases of the menstrual cycle were discussed, and it was shown that while there was no difference in blood pressure response, there was a trend towards an increase in portal vein resistance index in the luteal phase. This would assist in moving splanchnic blood pools in order to maintain venous return. Furthermore, based on previous studies, there could be an increase in renal and leg vascular resistance in the follicular phase in order to maintain venous return. These variables should be measured in future studies. These different pathways to maintain venous return could lead to similar orthostatic tolerance in each menstrual phase as seen by Claydon et al. (35) and in Appendix III.

It was also shown that there were different cardiovascular responses to LBNP in men and women. Women had lower mean arterial pressure with LBNP which was not seen in men. Women also exhibited a greater loss of central venous pressure and a greater increase in thoracic impedance with LBNP. These results describe reduced venous return during LBNP in women. Men increased portal vein resistance index with LBNP which would help to reduce splanchnic blood pooling and thus maintain venous return, cardiac output and mean arterial pressure. Furthermore, there was an attenuated release of renin in women during LBNP. This attenuated activation of the renin-angiotensin-aldosterone pathway along with the higher splanchnic pooling could be partially responsible for greater orthostatic hypotension in women.

Limitations

1) Two different methods of determining splanchnic blood pooling were used, ultrasound imaging and electrical impedance. There are limitations to both of these methods. Portal vein imaging does not measure blood return from the sex organs, bladder and kidneys which is returned via the inferior vena cava (169). Therefore, there may be splanchnic pooling that is not indicated by portal vein imaging. Furthermore, the rat portal vein has been shown to exhibit both tonic and phasic contractions *in vitro* with adrenergic stimulation (3; 10; 19; 30; 31). These phasic contractions (if present in humans) might obscure measurements as the diameter could change spontaneously from moment to moment. These phasic contractions

have been observed to be greater in amplitude in female rats (Edgell and Rush, unpublished observations) and therefore could possibly obscure the results from women to a greater degree. Pelvic impedance measures the electrical impedance of the entire pelvic cavity (between the xyphoid process and iliac crest) including the inferior vena cava, bladder and gut contents. Participants were encouraged to urinate before the LBNP sessions; however this did not always occur.

2) The bicycle seat inside the LBNP box could not be moved towards the opening. Therefore, the neoprene skirt that was sealed around the participant and attached to the LBNP box was sometimes higher than the iliac crest, particularly in shorter participants. Thus at times the level of suction was above the iliac crest. This could have led to greater pooling of blood in the pelvic region. This could have been a factor in the sex comparisons of pelvic impedance, but should not have been be a factor to consider in the comparison of menstrual phase as they were repeated measures on the same women. Nor should this have affected the repeated measures of LBNP, HDBR or SEAT.

Chapter 4

Cardiovascular effects of lower body negative pressure (LBNP) before and after 4-hours of head-down bed-rest (HDBR) and a 4-hour seated control (SEAT) in men and women through the menstrual cycle

This Chapter investigates the cardiovascular responses to lower body negative pressure (LBNP) in men and women throughout the menstrual cycle before and after 4-hours of head-down bed-rest (HDBR). Furthermore, a 4-hour sitting (SEAT) model is used to control for circadian rhythm and inactivity.

An orthostatic stress, such as sitting or standing, results in movement of blood towards the lower body (82). In order to maintain blood pressure higher heart rate, higher total peripheral resistance, and higher levels of vasoconstrictive factors are necessary (58; 62; 76; 153; 199; 200). Orthostatic hypotension occurs if blood pressure is not maintained leading to dizziness, faintness and eventually to syncope.

In microgravity, or in situations that simulate microgravity such as head-down bed-rest, there is a fluid shift towards the head (82). The cardiovascular system adapts quickly, even after only 4-hours of exposure (22; 62; 62). These adaptations can lead to augmented symptoms of orthostatic hypotension on return to an upright posture, particularly in women (22; 42; 73; 78; 93; 200). In response to an orthostatic stress there is a higher heart rate response, lower mean arterial pressure, higher vasopressin, and higher renin-angiotensin-aldosterone activation (11; 73; 76; 77; 95; 175; 199).

Many studies that examine exposure to microgravity do not investigate the equivalent period of inactivity or even the time-of-day effects. Pavy-Le Traon et al. studied the effects of 4-days of confinement or 4-days of HDBR in men (142). They found that HDBR increased heart rate, reduced baroreflex sensitivity, decreased parasympathetic activity, and increased sympathetic activity. Confinement elicited the same results to a lesser degree. Sigaudo et al. also investigated 4-days of HDBR versus 4-days of confinement. In this study, HDBR and confinement both elicited lower atrial natriuretic peptide (ANP) and higher renin implying that these changes are due to the inherent confinement of bed-rest and not the HDBR position itself. Changes unique to confinement include lower vasopressin and lower urine sodium (i.e. the HDBR does not change vasopressin or urine sodium from baseline). However, these results imply that in comparison to the decreases elicited by confinement alone the lack of change seen with the HDBR position could actually be higher vasopressin and higher urine sodium.

These confinement studies allowed participants to walk around a hospital department as long as “no muscular exercise was performed.” Hughson et al. compared 10-hours of sitting and 10-hours of HDBR (87). When comparing this level of inactivity to HDBR (a more

accurate comparison of activity levels) many differences were observed. There were no differences in resting heart rate, but HDBR resulted in lower sympathetic activity, higher ANP, and lower renin in comparison to sitting. These results are in the opposite direction of the comparison between 4-days of HDBR and confinement. These differences could be a result of the duration of HDBR or due to the degree of inactivity.

Another concern for short-term HDBR studies is that of diurnal variation. Schiedermaier et al. observed circadian rhythm of portal blood flow in patients with liver cirrhosis (157). They found peak flow around noon (+10%) and nadir of flow at approximately midnight (-8%). However, there did not appear to be a difference between 8am and 2pm (the testing times for this study). They also observed a greater post-prandial increase in portal vein flow in the morning. Renin and aldosterone also cycle throughout the day (89) with peak levels in the early morning (4:00am and 5:30am, respectively) and falling to a nadir at approximately midnight. In men, there are also lower levels of vasopressin, lower whole-body impedance, higher urodilatin, and higher urine sodium later in the day (50; 168). All of these differences highlight the importance of controlling for time of day effects. No information on the circadian changes in central venous pressure, inferior vena cava (IVC) diameter, or IVC compliance could be found.

Not much is known about sexually dimorphic cardiovascular responses to sitting, short-term HDBR (<24 hours), or diurnal variation. However, a recent paper investigating endothelial function and stress levels in male and female police officers has found a greater awakening level of cortisol in women which inversely correlates to endothelial function of the brachial artery during a flow-mediated dilation test (194). There are no known studies investigating the responses to sitting, short-term HDBR, or diurnal variation in different phases of the menstrual cycle.

It was hypothesized that 4-hours of HDBR in men and women throughout the menstrual cycle will elicit cardiovascular and hormonal responses to counter the cephalic fluid shift, and that the cardiovascular and hormonal responses to orthostatic stress (LBNP) will be augmented after HDBR in order to maintain mean arterial pressure. It was also hypothesized that some of the cardiovascular and hormonal responses previously attributed to HDBR will actually be due to circadian rhythm or inactivity (as shown by the SEAT protocol).

4.1 Materials and Methods

Refer to Chapter 2

4.2 Results

4.2.1 *Effects of LBNP, SEAT, and HDBR through the menstrual cycle*

Main effects of Phase

There were no main effects of menstrual phase, but interaction effects with SEAT/HDBR and LBNP are described below.

Main effects of LBNP

In both phases of the menstrual cycle, lower body negative pressure (LBNP) resulted in increased heart rate ($P<0.0001$, $P<0.0001$; Figure 7A and 7B), decreased stroke volume ($P<0.0001$, $P<0.0001$; Figure 8A and 8B), decreased cardiac output ($P<0.0001$, $P<0.0001$; Figure 9A and 9B), decreased central venous pressure ($P<0.0001$, $P<0.0001$; Figure 10A and 10B) and decreased inferior cava diameter ($P<0.0001$, $P<0.0001$; Figure 11A and 11B). There was also a significant decrease in mean arterial pressure on the HDBR testing day ($P=0.479$, $P=0.0001$; Figure 12A and 12B; SEAT and HDBR day), an increase in total peripheral resistance ($P<0.0001$, $P<0.0001$; Figure 13A and 13B), an increase in portal vein resistance index ($P=0.025$, $P=0.006$; Figure 14A and 14B), and an increase in brachial vascular resistance but only on the HDBR testing day ($P=0.260$, $P=0.029$; Figure 15A and 15B; SEAT and HDBR).

LBNP resulted in an increase in norepinephrine ($P=0.0008$, $P=0.016$; Figure 16A and 16B), no main effect on the SEAT testing day for vasopressin ($P=0.205$; Figure 17A) with increasing vasopressin in the luteal phase and decreasing vasopressin in the follicular phase HDBR testing day ($P=0.024$; Figure 17B), an increase in renin ($P=0.033$, $P=0.006$; Figure 18A and 18B), no change in angiotensin II ($P=0.247$, $P=0.172$; Figure 19A and 19B), and a decrease in aldosterone ($P=0.014$, $P=0.003$; Figure 20A and 20B). There was no effect on

epinephrine (P=0.425, P=0.114; SEAT and HDBR; Table 2) or endothelin-1 (P=0.715, P=0.486; SEAT and HDBR; Table 2). There was a slight decrease in nitrates and nitrites due to LBNP on the SEAT testing day (P=0.075, P=0.420; SEAT and HDBR; Table 2) and a decrease in atrial natriuretic peptide due to LBNP on the SEAT testing day (P=0.025, P=0.215; SEAT and HDBR; Table 2).

LBNP resulted in an increase in thoracic impedance (P<0.0001, P<0.0001; Figure 21A and 21B) and pelvic impedance (P=0.003, P=0.021; Figure 22A and 22B). (Interaction effects of LBNP are discussed below).

Main effects of SEAT/HDBR

Heart rate did not change with SEAT (P=0.945, Figure 7A), but increased with HDBR (P=0.002, Figure 7B). Stroke volume also did not change with SEAT (P=0.400, Figure 8A) yet there was a trend for lower stroke volume after HDBR (P=0.091; Figure 8B). There were no main effects of SEAT or HDBR on cardiac output (P=0.345, P=0.649; Figure 9A and 9B). Central venous pressure decreased with SEAT (P=0.043, Figure 10A) with no main effect of HDBR (P=0.148; Figure 10B). The diameter of the inferior vena cava was smaller after both SEAT and HDBR (P=0.012, P=0.030; Figure 11A and 11B). Mean arterial pressure was reduced after SEAT (P=0.003, Figure 12A) but not after HDBR (P=0.383, Figure 12B). There were no effects of SEAT or HDBR on total peripheral resistance (P=0.160, P=0.561: Figure 13A and 13B), yet portal vein resistance index increased with both SEAT and HDBR (P=0.073, P=0.038; Figure 14A and 14B). Brachial vascular resistance had a tendency to decrease after both SEAT and HDBR (P=0.066, P=0.075; Figure 15A and 15B).

There was no main effect of SEAT on norepinephrine yet it was significantly reduced after HDBR (P=0.233, P=0.022; Figure 16A and 16B). There were no main effects of SEAT or HDBR on vasopressin (P=0.194, P=0.661; Figure 17A and 17B). There was no main effect of SEAT on renin yet it was lower after HDBR (P=0.470, P=0.008; Figure 18A and 18B). Angiotensin II was significantly higher after SEAT (P=0.036; Figure 19A), but not after HDBR (P=0.456; Figure 19B). Both SEAT and HDBR protocols caused a reduction in aldosterone (P=0.063, P=0.0004; Figure 20A and 20B). There were no main effects of SEAT or HDBR on epinephrine (P=0.386, P=0.178; SEAT and HDBR; Table 2). There was a

reduction in endothelin-1 after both SEAT (P=0.09, Table 2) and HDBR (P=0.038, Table 2). There were no main effects of SEAT or HDBR on nitrates/nitrites (P=0.291, P=0.588; SEAT and HDBR; Table 2). There was a tendency for SEAT to decrease atrial natriuretic peptide (P=0.065; Table 2) with no main effect of HDBR (P=0.355; Table 2). Thoracic impedance increased with SEAT (P=0.011; Figure 21A) with no main effect of HDBR (P=0.866; Figure 21B). There were no main effects of SEAT or HDBR on pelvic impedance (P=0.143, P=0.158; Figure 22A and 22B).

Blood volume did not change after SEAT (P=0.950, Table 3), but decreased after HDBR (P=0.019; Table 3). There were no changes in urine output after SEAT (P=0.670; Table 3) or HDBR (P=0.676; Table 3). There was a trend for urine osmolality to decrease after HDBR (P=0.074; Table 3) but not after SEAT (P=0.814; Table 3). There were no effects of SEAT or HDBR on urine sodium (P=0.220, P=0.374; Table 3), serum osmolality (P=0.898, 0.695; Table 3), or serum sodium (P=0.987, P=0.779; Table 3). Urodilatin tended to decrease after both SEAT (P=0.109; Table 3) and HDBR (P=0.063; Table 3). .There were no effects of SEAT or HDBR on the compliance of the inferior vena cava (P=0.331, P=0.953; Table 4). (Interaction effects of SEAT and HDBR are described below).

Interaction effects

After HDBR there was a greater increase in heart rate ((HDBR*LBNP) effect P<0.0001; Figure 7B) and a greater loss in stroke volume ((HDBR*LBNP) effect P=0.010; Figure 8B) during LBNP in both phases. In the follicular phase there was a greater loss in central venous pressure with HDBR, particularly at higher levels of LBNP ((Phase*HDBR*LBNP) effect, P=0.060; Figure 10B). After HDBR, there was a greater increase in portal vein resistance index ((HDBR*LBNP) effect; P=0.013; Figure 14B) in both phases.

On both testing days there was a trend towards an increase in vasopressin release with LBNP in the luteal phase and a decrease in vasopressin in the follicular phase ((Phase*LBNP effect); P=0.094, P=0.070; SEAT and HDBR; Figure 17A and 17B). After SEAT there was an increase in baseline vasopressin, particularly in the follicular phase ((Phase*SEAT*LBNP) P=0.051; Figure 17A). After HDBR, there was an increase in vasopressin in the luteal phase

and a decrease in the follicular phase ((Phase*HDBR) effect; P=0.055; Figure 17B). After SEAT, there was a tendency for an increase in renin in the luteal phase at higher levels of LBNP ((Phase*SEAT*LBNP) effect; P=0.095; Figure 18A). Both SEAT and HDBR caused a smaller reduction in aldosterone due to LBNP ((SEAT*LBNP) and (HDBR*LBNP) effects; P=0.008, P=0.021; Figure 20A and 20B) which was particularly evident in the follicular phase after SEAT ((Phase*SEAT*LBNP) effect; P=0.028; Figure 20A).

Before the SEAT protocol, LBNP caused a reduction in nitrates and nitrites in the follicular phase ((Phase*SEAT*LBNP effect); P=0.017; Table 2). After HDBR, there was a tendency for LBNP to cause a reduction in nitrates and nitrites ((HDBR*LBNP) effect; P=0.069; Table 2). After the HDBR protocol, there was a significant reduction in atrial natriuretic peptide with LBNP in the follicular phase ((Phase*HDBR*LBNP) effect; P=0.034; Table 2).

Head-down bed-rest (HDBR) led to a greater loss of thoracic impedance with LBNP ((HDBR*LBNP) effect; P=0.026; Figure 21B). It also led to a slight increase in pelvic impedance in the luteal phase with a decrease in the follicular phase which was particularly evident at baseline ((Phase*HDBR*LBNP) effect; P=0.001; Figure 22B).

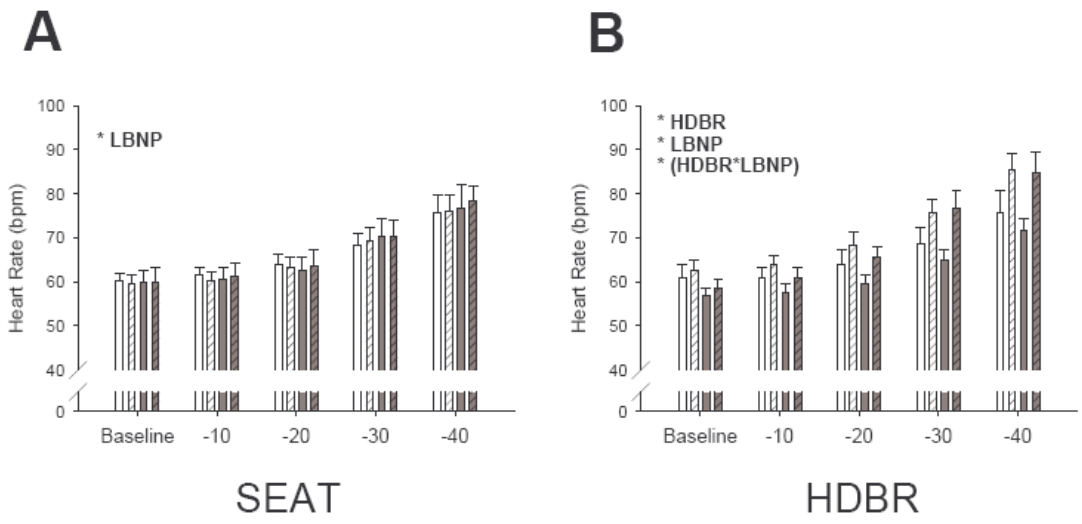


Figure 7: Heart rate responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *HDBR*LBNP indicates a significant interaction effect of HDBR and LBNP.

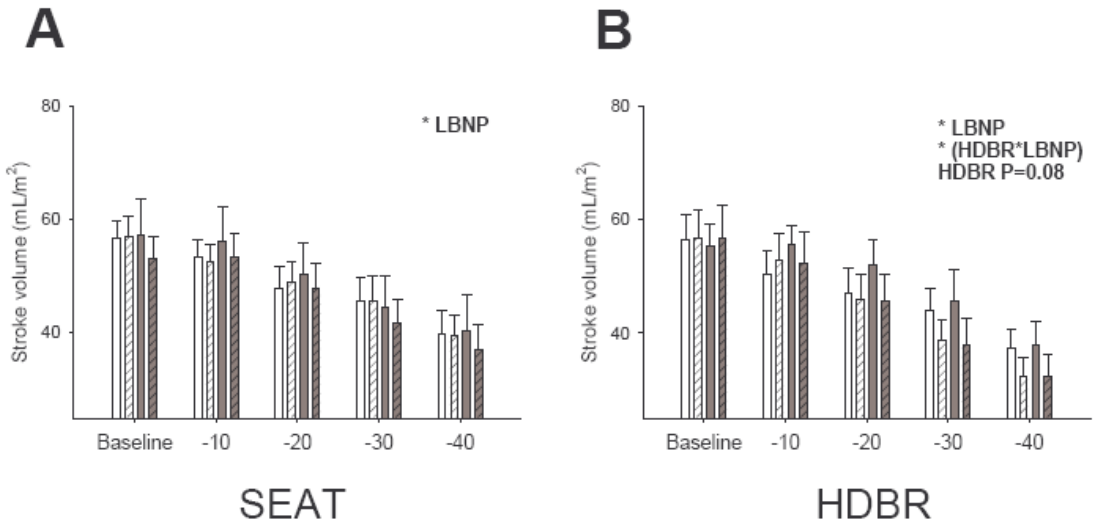


Figure 8: Stroke volume responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR*LBNP indicates a significant interaction effect of HDBR and LBNP.

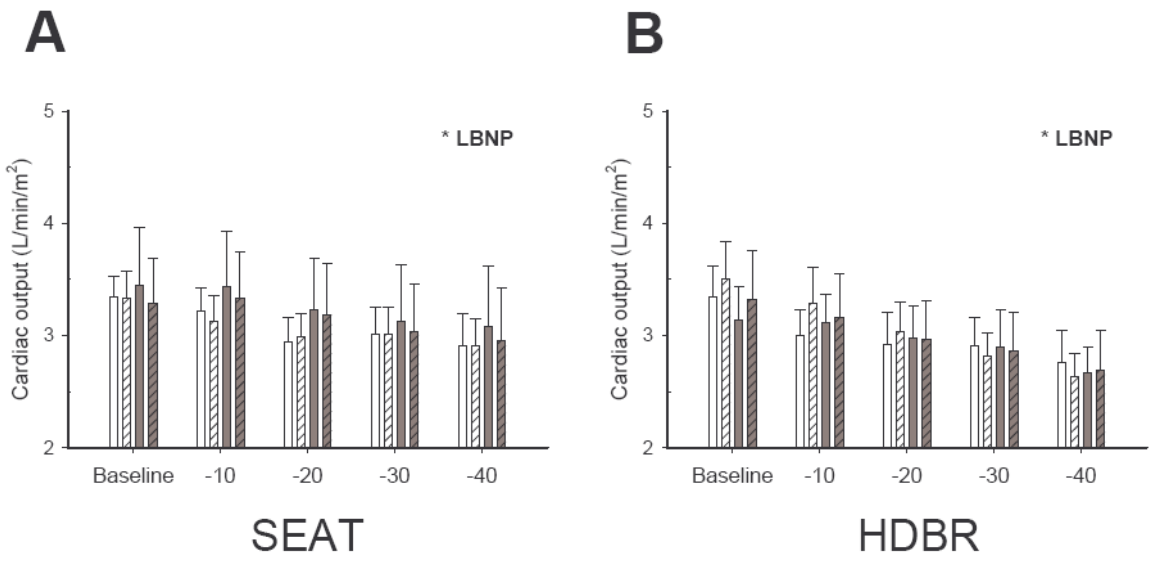


Figure 9: Cardiac output responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP.

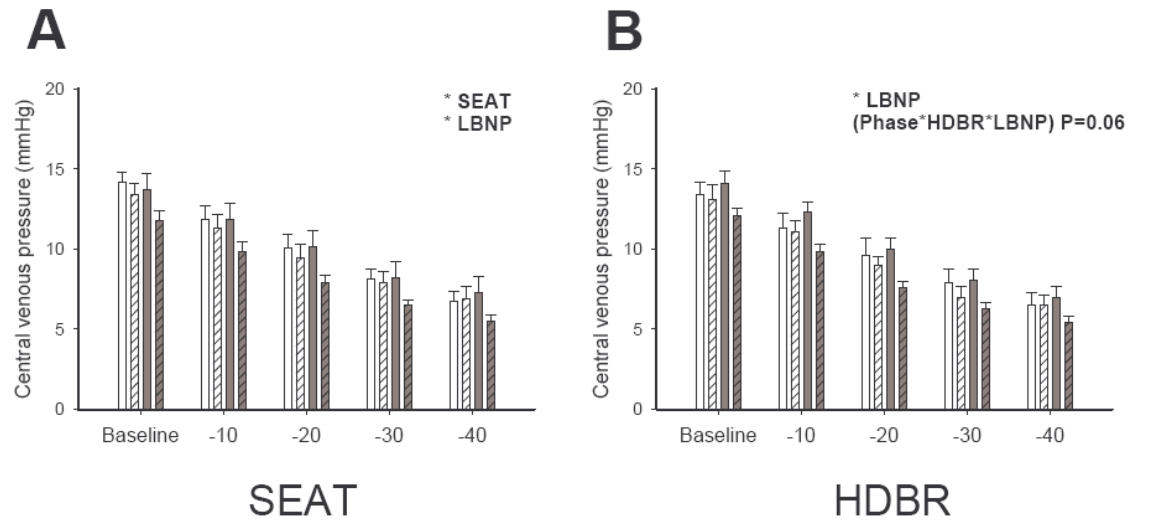


Figure 10: Central venous pressure responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT.

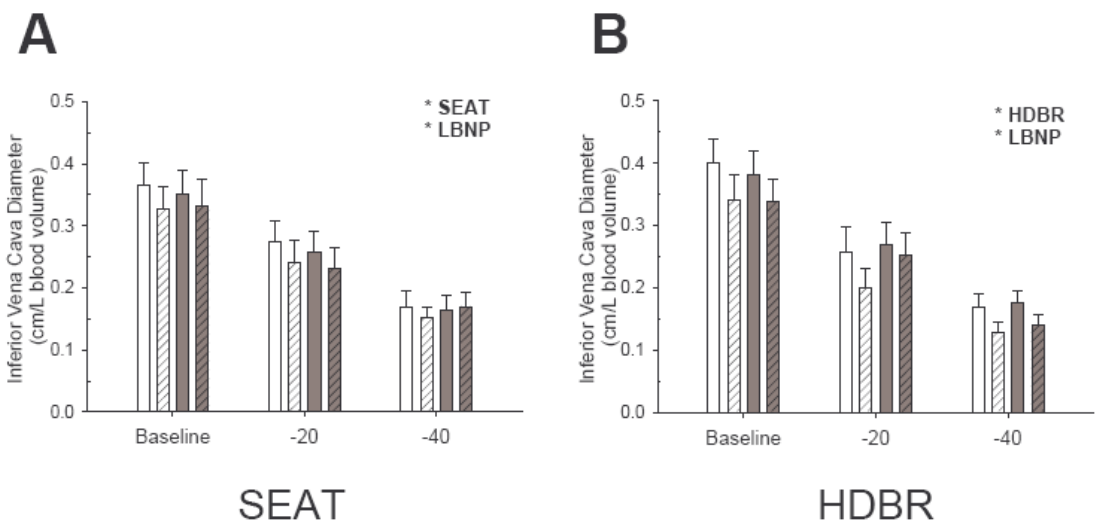


Figure 11: Inferior vena cava diameter responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT. *HDBR indicates a significant main effect of HDBR.

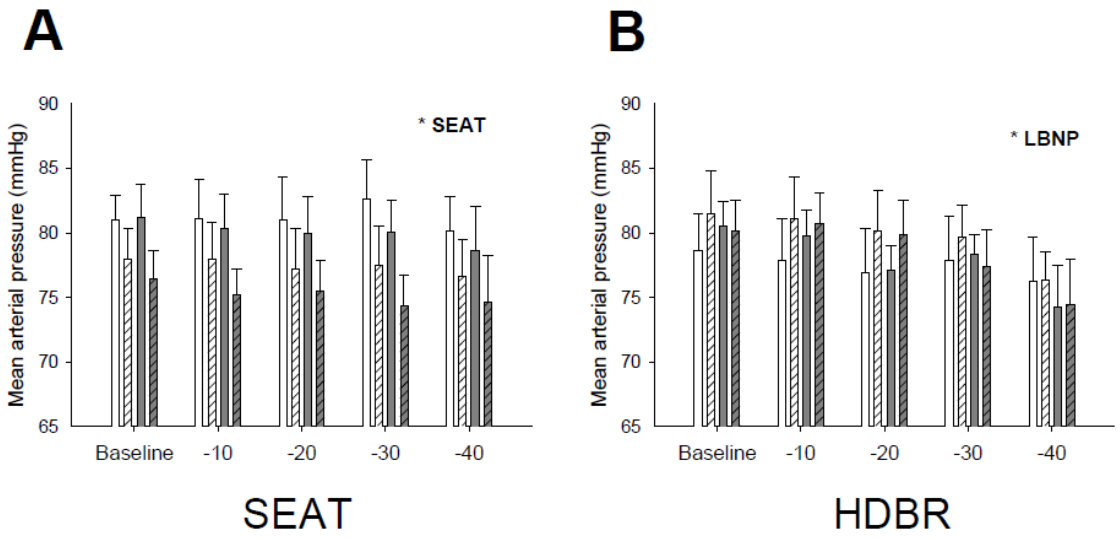


Figure 12: Mean arterial pressure responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT.

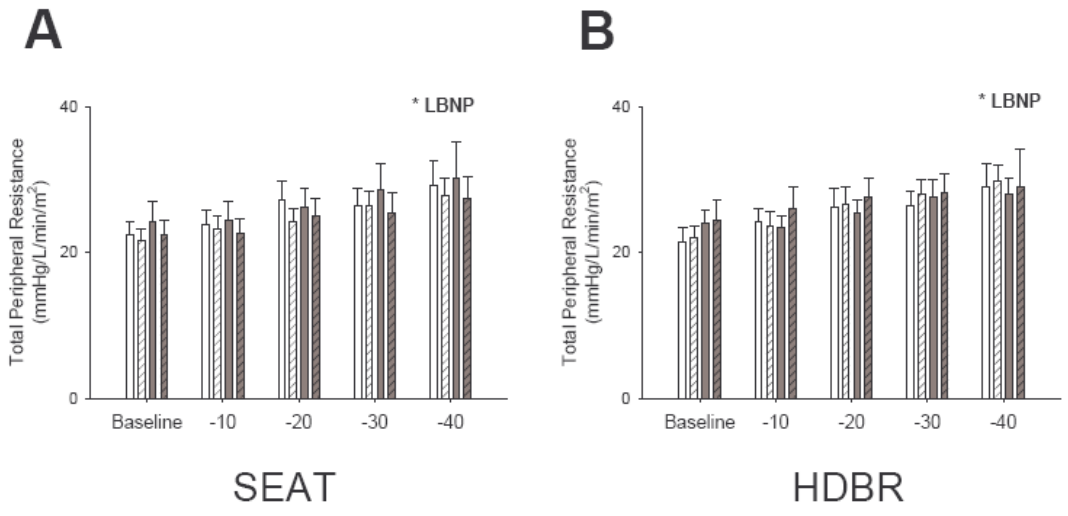


Figure 13: Total peripheral resistance responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP.

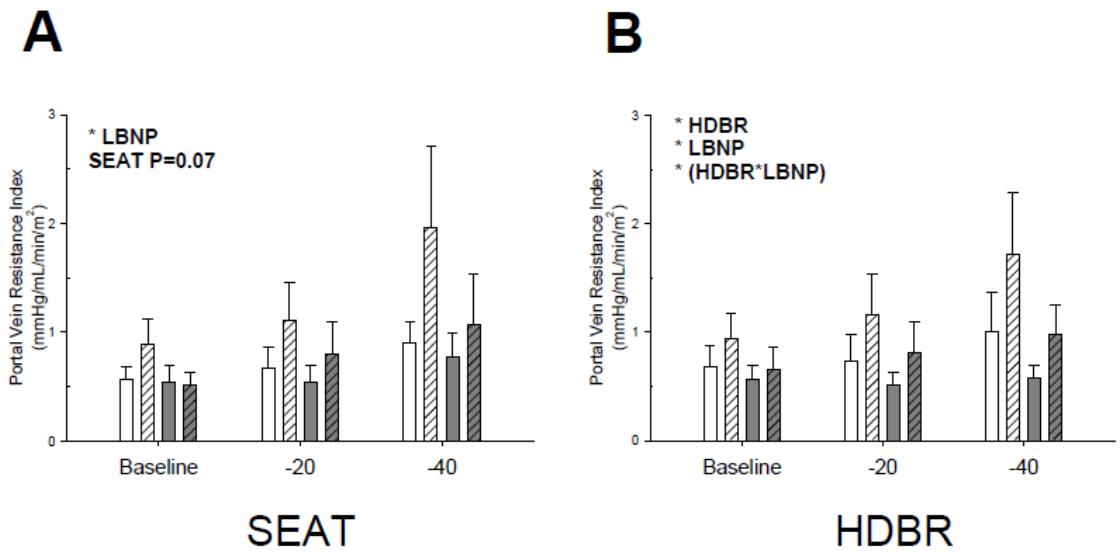


Figure 14: Portal vein resistance index responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *(HDBR*LBNP) indicates a significant interaction effect of HDBR and LBNP.

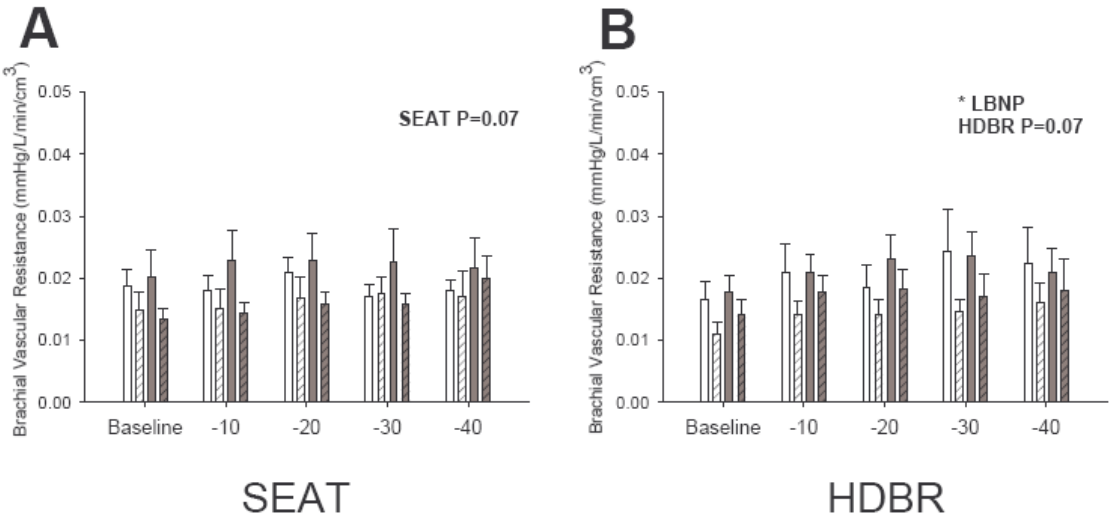


Figure 15: Brachial vascular resistance responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP.

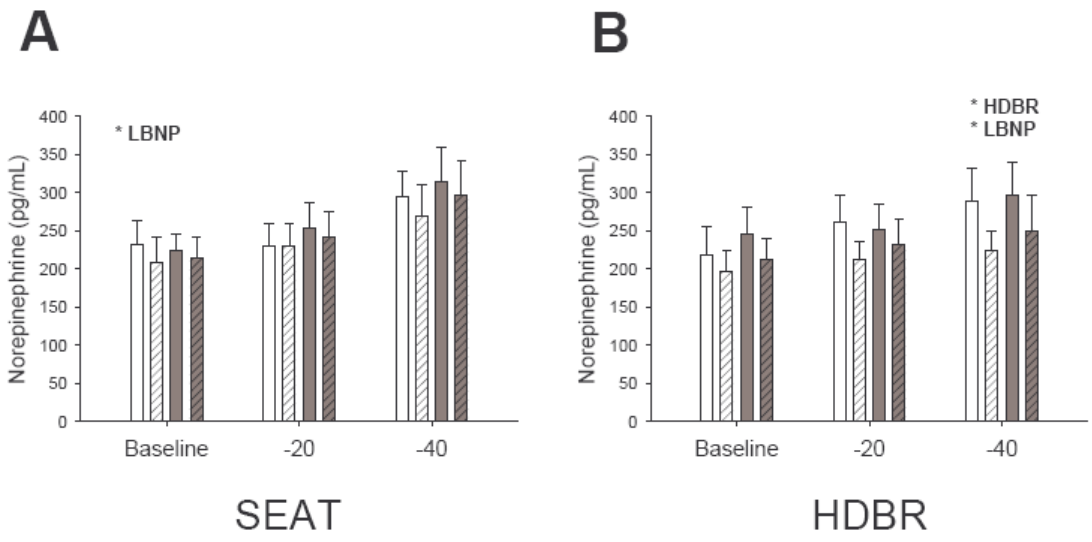


Figure 16: Plasma norepinephrine responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR.

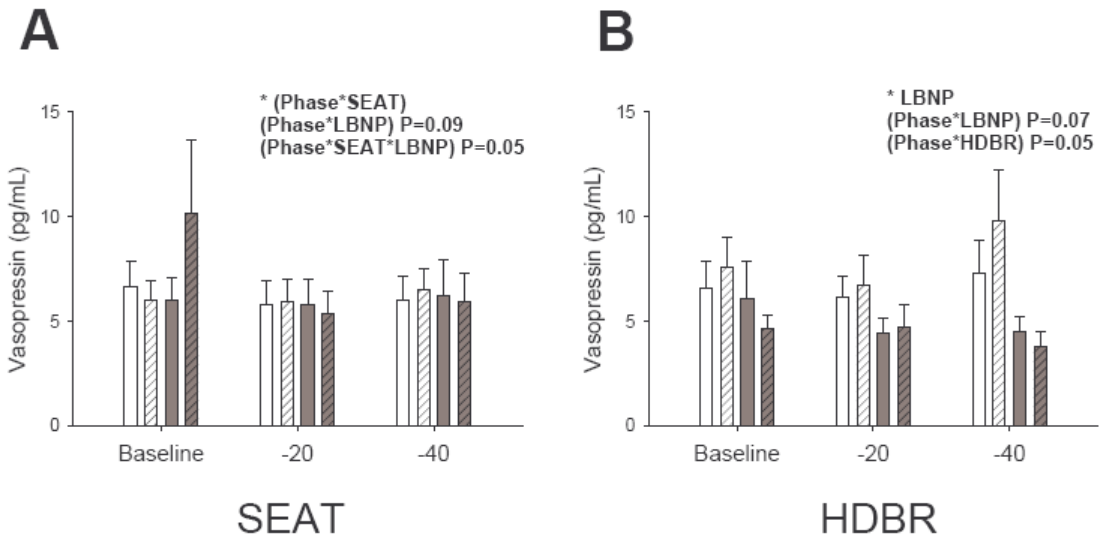


Figure 17: Plasma vasopressin responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Phase*SEAT) indicates a significant interaction effect of phase and SEAT.

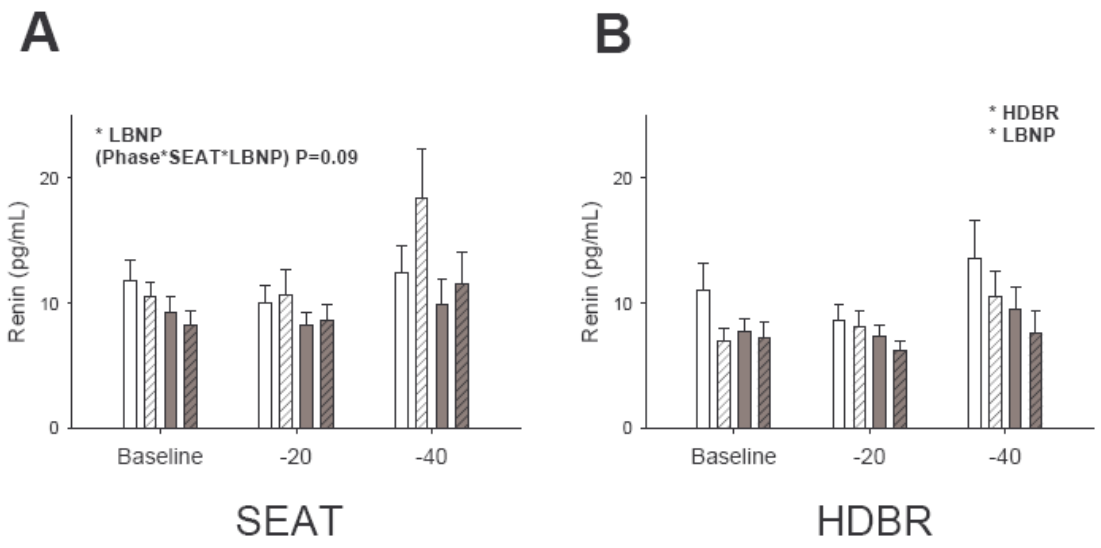


Figure 18: Plasma renin responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR.

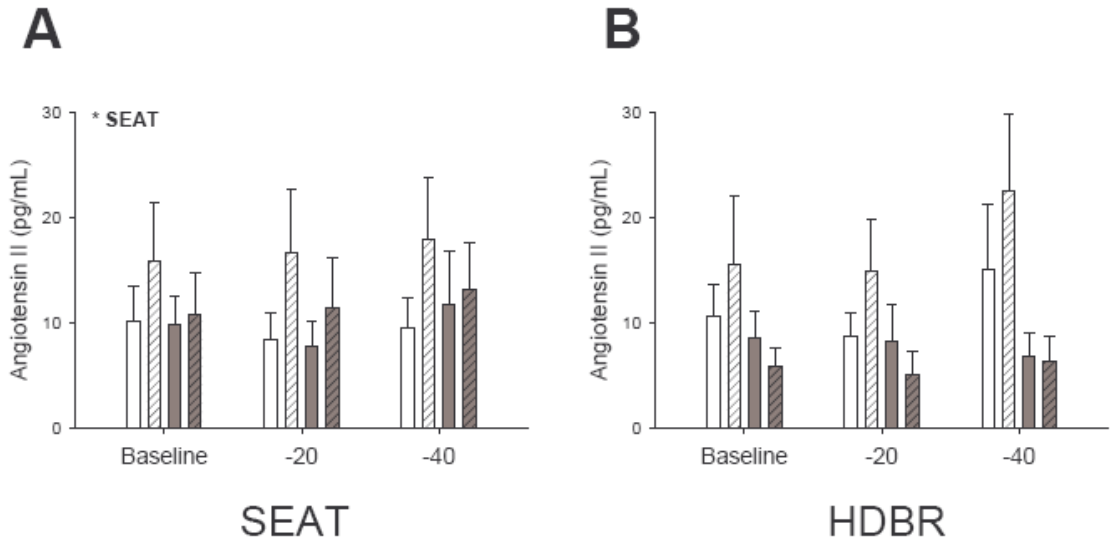


Figure 19: Plasma angiotensin II responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *SEAT indicates a significant main effect of SEAT.

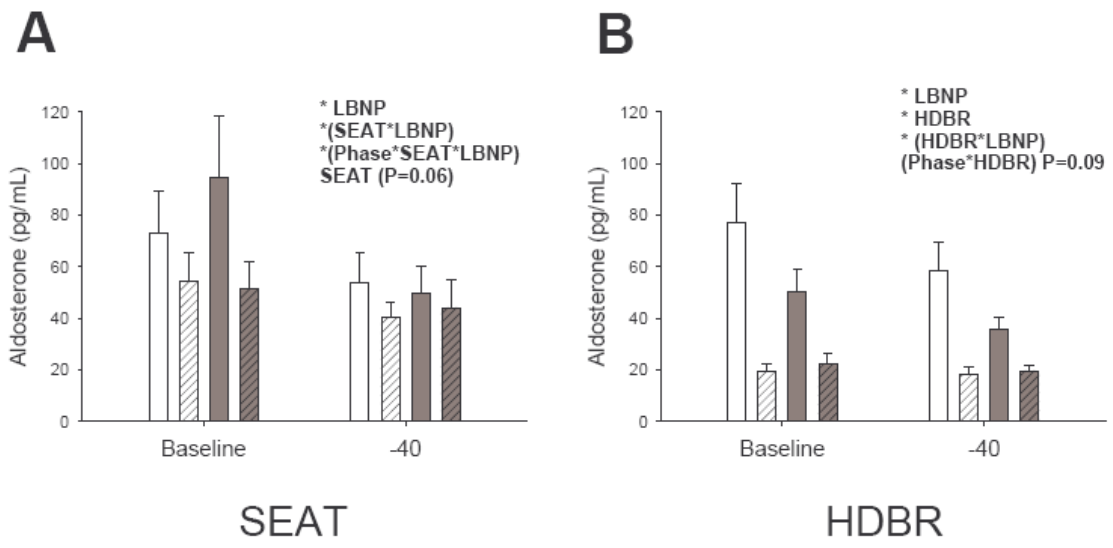


Figure 20: Plasma aldosterone responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *HDBR*LBNP indicates a significant interaction effect of HDBR and LBNP. *SEAT*LBNP indicates a significant interaction effect of SEAT and LBNP. *Phase*SEAT*LBNP indicates a significant interaction effect of phase, HDBR and LBNP.

Table 2: Responses of other vasoactive factors in luteal and follicular phase during lower body negative pressure before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Phase			**0 mmHg**	**-20mmHg**	**-40mmHg**
Epinephrine (pg/mL)	**Luteal**	**Pre**	**HDBR**	14.2 ± 2.6	20.0 ± 2.0	21.4 ± 2.5
		Post	**HDBR**	15.6 ± 1.9	20.5 ± 2.7	25.7 ± 5.3
	Follicular	**Pre**	**HDBR**	21.1 ± 3.0	17.3 ± 1.4	24.0 ± 3.7
		Post	**HDBR**	23.5 ± 3.1	23.0 ± 3.7	21.8 ± 4.5
	Luteal	**Pre**	**SEAT**	19.1 ± 2.4	17.5 ± 1.4	20.8 ± 5.1
		Post	**SEAT**	16.8 ± 1.9	18.7 ± 2.5	23.1 ± 4.2
	Follicular	**Pre**	**SEAT**	22.6 ± 3.0	19.8 ± 5.0	22.8 ± 4.3
		Post	**SEAT**	19.3 ± 3.3	19.2 ± 3.7	26.9 ± 3.8
Endothelin-1 (pg/mL)	**Luteal**	**Pre**	**HDBR**	1.5 ± 0.3	-	1.4 ± 0.3
		Post†	**HDBR**	1.4 ± 0.3	-	1.5 ± 0.2
(SEAT P=0.09)	**Follicular**	**Pre**	**HDBR**	1.4 ± 0.2	-	1.6 ± 0.5
		Post†	**HDBR**	1.3 ± 0.3	-	1.7 ± 0.3
	Luteal	**Pre**	**SEAT**	1.5 ± 0.2	-	1.3 ± 0.2
		Post	**SEAT**	1.2 ± 0.2	-	1.2 ± 0.2
	Follicular	**Pre**	**SEAT**	1.5 ± 0.2	-	1.4 ± 0.2
		Post	**SEAT**	1.5 ± 0.2	-	1.2 ± 0.3
Nitrates/ Nitrites (µmol/L)	**Luteal**	**Pre**	**HDBR**	7.3 ± 1.2	7.0 ± 1.1	8.1 ± 1.8
		Post	**HDBR**	7.2 ± 0.8	7.4 ± 0.6	6.9 ± 1.3
	Follicular	**Pre**	**HDBR**	6.4 ± 1.5	6.1 ± 1.3	6.0 ± 1.1
(LBNP P=0.07;		**Post**	**HDBR**	6.1 ± 0.8	6.5 ± 0.7	5.7 ± 1.0
SEAT test day)	**Luteal**	**Pre**	**SEAT**	6.8 ± 0.8	7.0 ± 0.7	6.8 ± 1.0
(HDBR*LBNP		**Post**	**SEAT**	7.0 ± 0.7	6.7 ± 0.7	6.6 ± 0.7
P=0.07)	**Follicular**	**Pre**	**SEAT**	6.9 ± 0.9	6.4 ± 0.8	5.9 ± 0.8‡
		Post	**SEAT**	6.2 ± 0.5	6.5 ± 0.3	6.3 ± 0.4
Atrial natriuretic peptide (pg/mL)	**Luteal**	**Pre**	**HDBR**	25.8 ± 3.1	21.5 ± 3.8	23.4 ± 4.7
		Post	**HDBR**	29.9 ± 4.8	31.2 ± 6.1	24.2 ± 5.8
	Follicular	**Pre**	**HDBR**	29.9 ± 3.3	29.5 ± 4.4	26.5 ± 4.6
		Post	**HDBR**	30.7 ± 6.0	28.3 ± 5.6	21.7 ± 3.4‡
(SEAT P=0.07)	**Luteal**	**Pre**	**SEAT**	30.0 ± 3.6	24.8 ± 3.4	27.4 ± 3.6*
		Post	**SEAT**	25.0 ± 5.6	22.4 ± 4.8	19.6 ± 2.1*
	Follicular	**Pre**	**SEAT**	25.4 ± 2.3	20.9 ± 2.7	21.7 ± 2.5*
		Post	**SEAT**	22.7 ± 2.6	23.7 ± 2.8	20.9 ± 1.8*

(* denotes a significant main effect of LBNP; † denotes a significant main effect of HDBR; ‡ denotes a significant interaction effect of phase, HDBR/SEAT, and LBNP)

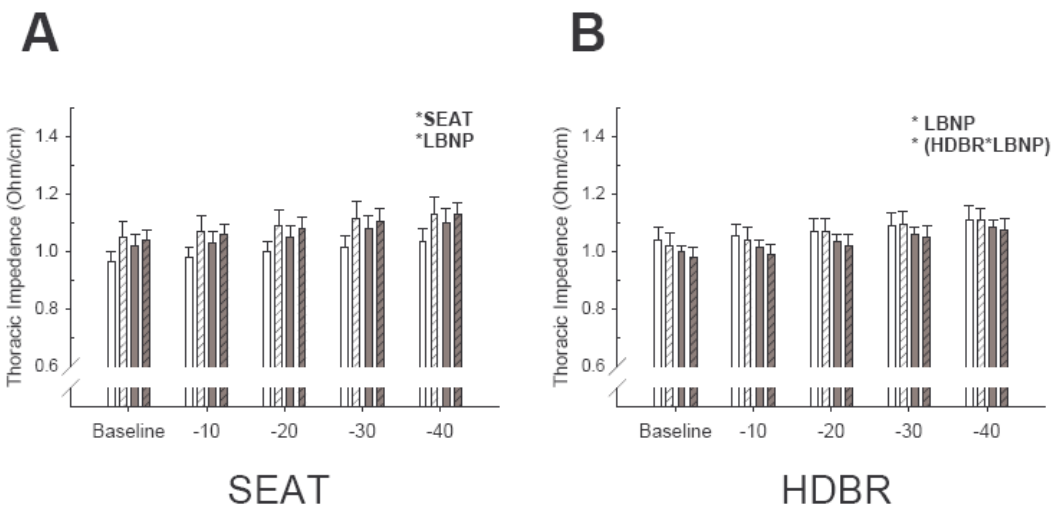


Figure 21: Thoracic impedance responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT. *HDBR*LBNP indicates a significant interaction effect of HDBR and LBNP.

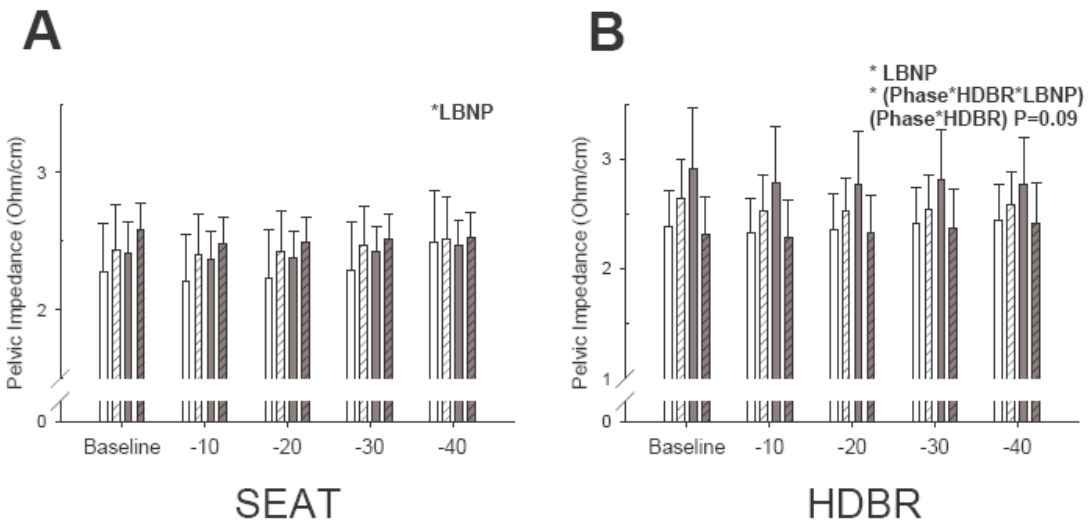


Figure 22: Pelvic impedance responses to lower body negative pressure (LBNP) in women in the luteal (white bars) and follicular phases (grey bars) of the menstrual cycle before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Phase*HDBR*LBNP) indicates a significant interaction effect of phase, HDBR, and LBNP.

Table 3: Water and sodium balance in luteal and follicular phase before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Phase		Pre	Post
Blood volume (mL)	**Luteal**	**HDBR**	4387.8 ± 470.7	4316.4 ± 494.1‡
	Follicular	**HDBR**	4836.2 ± 337.1	4715 ± 347.0‡
	Luteal	**SEAT**	4509.2 ± 235.2	4610.4 ± 234.7
	Follicular	**SEAT**	4821.0 ± 463.8	4810.3 ± 480.0
Water intake (mL)	**Luteal**	**HDBR**	-	324.9 ± 44.9
	Follicular	**HDBR**	-	314.2 ± 51.3
	Luteal	**SEAT**	-	369.5 ± 49.8
	Follicular	**SEAT**	-	324.7 ± 57.7
Urine output (mL)	**Luteal**	**HDBR**	525.9 ± 117.0	661.6 ± 109.2
	Follicular	**HDBR**	497.6 ± 156.5	567.0 ± 83.4
	Luteal	**SEAT**	510.8 ± 79.3	525.9 ± 100.0
	Follicular	**SEAT**	448.7 ± 90.1	461.5 ± 75.5
Urine Osmolality (mmol/kg)	**Luteal**	**HDBR**	575.6 ± 59.0	425.1 ±56.2 (P=0.07)
	Follicular	**HDBR**	558.2 ± 79.1	377.3 ± 46.9 (P=0.07)
	Luteal	**SEAT**	582.6 ± 69.8	530.1 ± 70.0
	Follicular	**SEAT**	515.2 ± 82.8	504.6 ± 68.6
Urine sodium (mM)	**Luteal**	**HDBR**	148.8 ± 66.7	213.5 ± 126.8
	Follicular	**HDBR**	153.6 ± 72.3	214.8 ± 130.4
	Luteal	**SEAT**	163.9 ± 69.3	277.1 ± 144.6
	Follicular	**SEAT**	104.0 ± 35.6	214.8 ± 130.4
Urodilatin (pg/mL)	**Luteal**	**HDBR**	45.9 ± 13.8	17.8 ± 4.3 (P=0.06)
	Follicular	**HDBR**	32.5 ± 8.6	12.9 ± 4.0 (P=0.06)
	Luteal	**SEAT**	47.9 ± 11.6	25.8 ± 5.5
	Follicular	**SEAT**	35.8 ± 12.5	18.9 ± 5.6
Serum Osmolality (mmol/kg)	**Luteal**	**HDBR**	277.1 ± 2.5	274.7 ± 2.9
	Follicular	**HDBR**	278.6 ± 1.6	279.4 ± 2.7
	Luteal	**SEAT**	276.7 ± 2.8	277.7 ± 2.4
	Follicular	**SEAT**	280.9 ± 1.8	280.7 ± 1.8
Serum Sodium (mM)	**Luteal**	**HDBR**	124.9 ± 2.0	125.2 ± 3.1
	Follicular	**HDBR**	127.4±2.0	126.6±2.6
	Luteal	**SEAT**	126.4 ± 1.9	125.4 ± 2.1
	Follicular	**SEAT**	125.4 ± 2.0	126.8 ± 2.2

(‡ denotes a significant main effect of HDBR)

Table 4: Compliance of the inferior vena cava in luteal and follicular phase during lower body negative pressure before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Phase		**Pre**	**Post**
Compliance	**Luteal**	**HDBR**	0.15 ± 0.03	0.14 ± 0.04
(cm/mmHg)	**Follicular**	**HDBR**	0.14 ± 0.02	0.11 ± 0.02
	Luteal	**SEAT**	0.12 ± 0.02	0.12 ± 0.02
	Follicular	**SEAT**	0.12 ± 0.02	0.10 ± 0.03

4.2.2 *Effects of LBNP, SEAT, and HDBR in men and women (follicular phase)*

Main effects of Sex

No main effects for sex were observed for heart rate (P=0.111, P=0.644; Figure 23A and 23B), stroke volume (P=0.255, P=0.390; Figure 24A and 24B), cardiac output (P=0.896, P=0.541; Figure 25A and 25B), central venous pressure (P=0.181, P=1.0; Figure 26A and 26B), or inferior vena cava diameter (P=0.852, P=0.705; Figure 27A and 27B). There were no main effects of sex for mean arterial pressure (P=0.219, P=0.211; Figure 28A and 28B), total peripheral resistance (P=0.914, 0.758; Figure 29A and 29B), or portal vein resistance index (P=0.730, P=0.666; Figure 30A and 30B). Women exhibited greater brachial vascular resistance on both the SEAT (P=0.011) and the HDBR (P=0.003) protocol days (Figure 31A and B).

There were no main effects of sex on norepinephrine (P=0.965, P=0.952; Figure 32A and 32B) or vasopressin (P=0.114, P=0.432; Figure 33A and 33B). On the HDBR protocol day, women had lower renin concentrations than men (P=0.019) but this was not clear on the seated control testing day (P=0.699; Figure 34A and B). There were no main effects of sex on angiotensin II (P=0.215, P=0.915; Figure 35A and 35B) On the SEAT testing day women had slightly higher aldosterone than men (P=0.087; Figure 36A); this was not observed on the HDBR testing day (P=0.203; Figure 36B). Women tended to have lower levels of endothelin-1 (P=0.057, P=0.086; SEAT and HDBR; Table 5), and slightly lower atrial natriuretic peptide on the SEAT testing day (P=0.084, P=0.938; SEAT and HDBR; Table 5). There were no main effects of sex on epinephrine (P=0.923, P=0.957; SEAT and HDBR; Table 5), or nitrates/nitrites (P=0.973, P=0.933; SEAT and HDBR; Table 5).

On both testing days, women had higher thoracic impedance (P=0.0007; P=0.0017; Figure 37A and B) and higher pelvic impedance (P=0.004; P=0.058; Figure 38A and B). There were no main sex effects on blood volume (P=0.130, P=0.298; SEAT and HDBR; Table 6), water intake (P=0.653, P=0.741; SEAT and HDBR; Table 6), urine output (P=0.178, P=0.706; SEAT and HDBR; Table 6), urine osmolality (P=0.244, P=0.109; SEAT and HDBR; Table 6), urine sodium (P=0.789, P=0.507; SEAT and HDBR; Table 6), or urodilatin (P=0.145, P=0.850; SEAT and HDBR; Table 6). Serum sodium was higher in women compared to men

on the HDBR test day (P=0.105, P=0.053; SEAT and HDBR; Table 6). There were no main effects of sex on serum osmolality (P=0.556, P=0.550; SEAT and HDBR; Table 6) or inferior vena cava compliance (P=0.276, P=0.608; SEAT and HDBR; Table 7). (Interaction effects of sex are described below).

Main effects of LBNP

Lower body negative pressure (LBNP) caused an increase in heart rate (P<0.0001, P<0.0001; Figure 23A and 23B), a decrease in stroke volume (P<0.0001, P<0.0001; Figure 24A and 24B), a decrease in cardiac output (P<0.0001, P<0.0001; Figure 25A and 25B), a decrease in central venous pressure (P<0.0001, P<0.0001; Figure 26A and 26B), and a decrease in inferior vena cava diameter (P<0.0001, P<0.0001; Figure 27A and 27B). LBNP also decreased mean arterial pressure (P=0.009, P=0.003; Figure 28A and 28B), increased total peripheral resistance (P<0.0001, P<0.0001; Figure 29A and 29B), increased portal vein resistance index (P=0.008, P<0.0001; Figure 30A and 30B), and increased brachial vascular resistance on the SEAT collection day (P=0.001, P=0.185; Figure 31A and 31B; SEAT and HDBR).

Lower body negative pressure (LBNP) increased norepinephrine (P<0.0001, P<0.0001; Figure 32A and 32B), had no main effect on vasopressin (P=0.114, P=0.432; Figure 33A and 33B), increased renin (P=0.063, P<0.0001; Figure 34A and 34B), increased angiotensin II on the SEAT testing day (P=0.038, P=0.141; Figure 35A and 35B), and decreased aldosterone (P=0.013, P=0.027; Figure 36A and 36B). There was also a slight reduction in nitrates and nitrites with LBNP (P=0.088, P=0.044; SEAT and HDBR; Table 5). No main effects of LBNP were seen with epinephrine (P=0.480, P=0.178; SEAT and HDBR; Table 5), endothelin-1 (P=0.411, P=0.735; SEAT and HDBR; Table 5), or atrial natriuretic peptide (P=0.143, P=0.676; SEAT and HDBR; Table 5).

LBNP caused increased thoracic impedance (P<0.001, P<0.0001; Figure 37A and 37B) and increased pelvic impedance (P=0.012, P=0.023; Figure 38A and 38B). (Interaction effects of LBNP are described below).

Main effects of SEAT/HDBR

Heart rate did not change with SEAT (P=0.111, Figure 23A), but did increase with HDBR (P<0.0001, Figure 23B). Stroke volume also did not change with SEAT (P=0.255; Figure 24A), but tended to decrease with HDBR (P=0.091; Figure 24B). There were no effects of SEAT or HDBR on cardiac output (P=0.896, P=0.541; Figure 25A and 25B). There was no main effect of SEAT on central venous pressure, but HDBR caused a decrease (P=0.140, P=0.001; Figure 26A and 26B). SEAT did not affect inferior vena cava diameter (P=0.120; Figure 27A), yet HDBR decreased it (P=0.0003; Figure 27B).There were no main effects of SEAT or HDBR on mean arterial pressure (P=0.219, P=0.211; Figure 28A and 28B), or total peripheral resistance (P=0.249, P=0.961; Figure 29A and 29B). There was no significant effect of SEAT on portal vein resistance index (P=0.101; Figure 30A), yet HDBR significantly increased it (P=0.032; Figure 30B). SEAT and HDBR both reduced brachial vascular resistance (P=0.013, P=0.048; Figure 31A and 31B).

There were no effects of SEAT on norepinephrine (P=0.234; Figure 32A), but a significant decrease due to HDBR (P=0.030; Figure 32B). There were no significant main effects of SEAT or HDBR on vasopressin (P=0.057, P=0.364; Figure 33A and 33B). The SEAT protocol did not affect renin (P=0.338; Figure 34A), yet HDBR decreased it (P=0.016; Figure 34B). There were no main effects of SEAT or HDBR on angiotensin II (P=0.470, P=0.283; Figure 35A and 35B). Both SEAT and HDBR decreased aldosterone (P=0.033, P=0.004; Figure 36A and 36B). There were no main effects of SEAT or HDBR on epinephrine (P=0.363, P=0.547; SEAT and HDBR; Table 5), endothelin-1 (P=0.276, P=0.327; SEAT and HDBR; Table 5), nitrates/nitrites (P=0.658, P=0.819; SEAT and HDBR; Table 5), or atrial natriuretic peptide (P=0.116, P=0.692; SEAT and HDBR; Table 5). The SEAT protocol increased thoracic impedance (P=0.009; Figure 37A), while there were no main effects of HDBR (P=0.663; Figure 37B). There were no main effects of SEAT or HDBR on pelvic impedance (P=0.491, P=0.096; Figure 38A and 38B).

There was no main effect of SEAT on blood volume (P=0.130; Table 6), urine output (P=0.630; Table 6), urine osmolality (P=0.463; Table 6), urodilatin (P=0.107; Table 6), or serum sodium (P=0.906; Table 6). The SEAT protocol tended to increase urine sodium (P=0.091; Table 6) and decrease serum osmolality (P=0.090; Table 6). The HDBR protocol

resulted in reduced blood volume (P=0.001; Table 6), reduced urine osmolality (P=0.005; Table 6), and reduced urodilatin (P=0.005; Table 6). There was no main effect of HDBR on urine output (P=0.267; Table 6), urine sodium (P=0.302; Table 6), serum osmolality (P=0.834; Table 6), or serum sodium (P=0.302; Table 6). There were no main effects of SEAT or HDBR on inferior vena cava compliance (P=0.780, P=0.205; SEAT and HDBR; Table 7). (Interaction effects of SEAT and HDBR are described below).

Interaction effects

Women had a greater heart rate response to LBNP on the SEAT testing day ((Sex*LBNP) P=0.045; Figure 23A). Head-down bed-rest (HDBR) augmented the heart rate increase ((HDBR*LBNP) P<0.0001; Figure 23B) and the stroke volume decrease ((HDBR*LBNP) P=0.008; Figure 24B) due to LBNP in both men and women. Women had a smaller loss of cardiac output with LBNP on the SEAT testing day ((Sex*LBNP) P=0.009; Figure 25A). SEAT attenuated the loss of central venous pressure seen with LBNP ((SEAT*LBNP) P=0.029; Figure 26A) as did HDBR ((HDBR*LBNP) P=0.007; Figure 26B)). At the highest level of LBNP women tended to have a lower central venous pressure after HDBR than men ((Sex*HDBR*LBNP) P=0.084; Figure 26B). Head-down bed-rest (HDBR) resulted in an attenuated decrease of inferior vena cava diameter with LBNP ((HDBR*LBNP) P=0.055; Figure 27B).

After SEAT, women had lower mean arterial blood pressure ((Sex*SEAT) P=0.069; Figure 28A). On the HDBR testing day, women had a greater decrease in mean arterial pressure than men during LBNP ((Sex*LBNP) P=0.011; Figure 28B), and this corresponded to a smaller increase in total peripheral resistance ((Sex*LBNP) P=0.009; Figure 29B). Women show a smaller increase in portal vein resistance index with LBNP on the HDBR testing day ((Sex*LBNP) P=0.023; Figure 30B), and after HDBR both men and women have a greater increase in portal vein resistance index in response to LBNP ((HDBR*LBNP) P=0.039; Figure 30B).

The seated control (SEAT) tended to increase vasopressin levels in women at the baseline period ((Sex*SEAT*LBNP) P=0.093; Figure 33A). Head-down bed-rest (HDBR) resulted in higher levels of vasopressin in men and lower levels in women ((Sex*HDBR)

P=0.003; Figure 33B). Head-down bed-rest (HDBR) also resulted in lower renin but only in men ((Sex*HDBR) P=0.068; Figure 34B). The seated control (SEAT) decreased angiotensin II in men and increased it in women ((Sex*SEAT) P=0.030; Figure 35A). On both the SEAT and HDBR testing days, women had a greater drop in aldosterone due to LBNP ((Sex*LBNP) P=0.068, P=0.062; Figure 36A and 36B), and in women SEAT attenuated this drop ((Sex*SEAT*LBNP) P=0.089; Figure 36A). Head-down bed-rest (HDBR) tends to augment the increase of endothelin-1 due to LBNP ((HDBR*LBNP) P=0.093; Table 5). On the SEAT day women decrease nitrates and nitrites during LBNP more than men ((Sex*LBNP) P=0.067; Table 5), but this difference is not seen after SEAT ((Sex*SEAT*LBNP) P=0.093; Table 5). The seated control (SEAT) decreases baseline atrial natriuretic peptide in men ((Sex*SEAT*LBNP) P=0.091; Table 5).

Women had a greater increase of thoracic impedance with LBNP on both testing days ((Sex*LBNP) P<0.0001, P0.011; Figure 37A and 37B). This increase was slightly augmented after SEAT in women, slightly attenuated after SEAT in men ((Sex*SEAT*LBNP) P=0.075; Figure 37A) and augmented after HDBR in both men and women ((HDBR*LBNP) P=0.011; Figure 37B). The seated control (SEAT) resulted in a decrease in pelvic impedance in men and an increase in women ((Sex*SEAT) P=0.043; Figure 38A); the opposite effect was seen due to HDBR ((Sex*HDBR) P=0.026; Figure 38B). After SEAT, women decreased pelvic impedance with LBNP (as opposed to the increase seen before SEAT and in men) ((Sex*SEAT*LBNP) P=0.014; Figure 38A).

The seated control (SEAT) tended to decrease plasma osmolality in men, but this was not seen in women ((Sex*SEAT) P=0.044; Table 6). SEAT increased inferior vena cava compliance in men, but decreased it in women ((Sex*SEAT) P=0.043; Table 7).

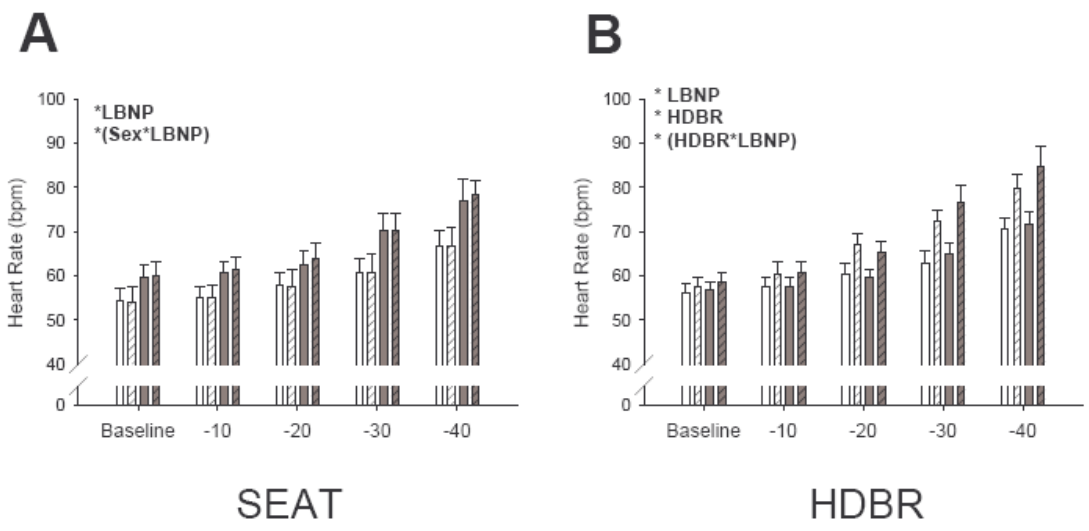


Figure 23: Heart rate responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP. *(HDBR*LBNP) indicates a significant interaction effect of HDBR and LBNP.

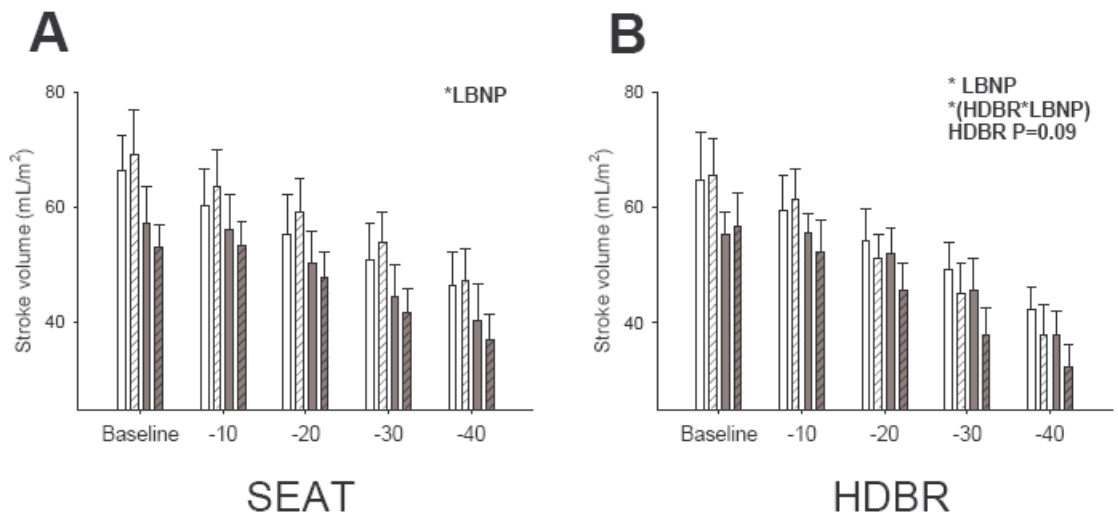


Figure 24: Stroke volume responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(HDBR*LBNP) indicates a significant interaction effect of HDBR and LBNP.

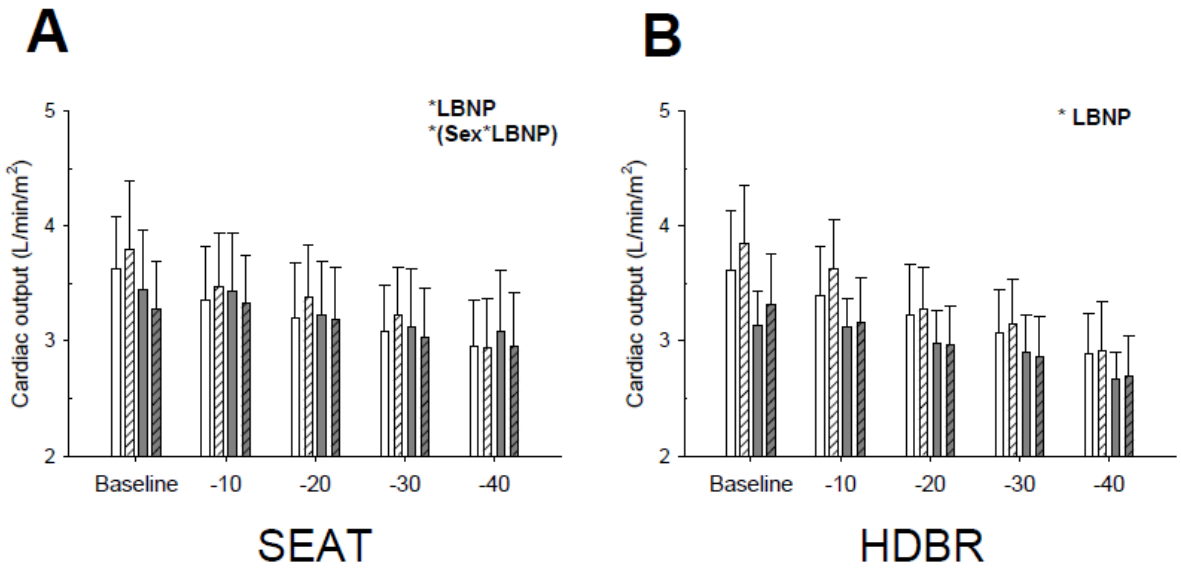


Figure 25: Cardiac output responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP.

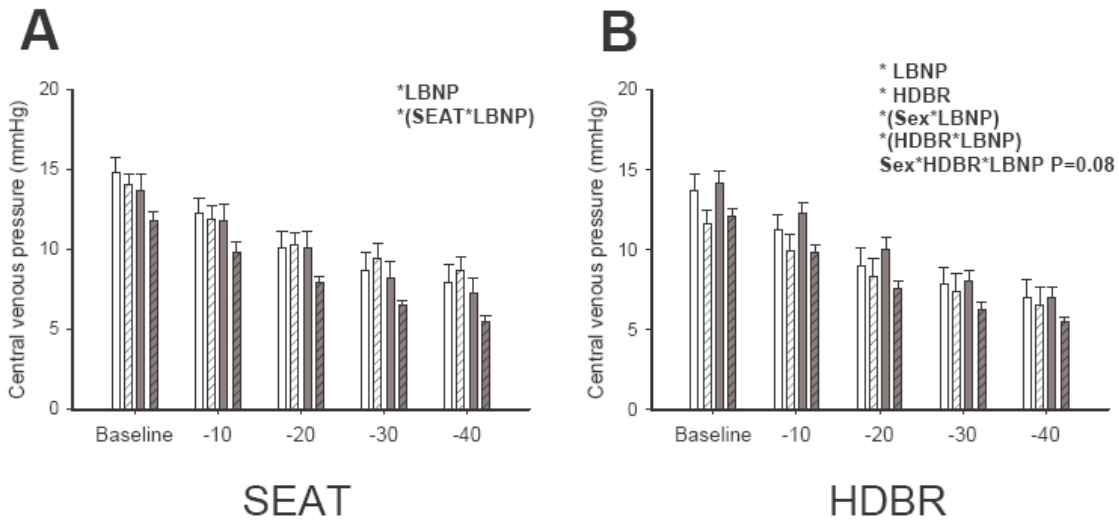


Figure 26: Central venous pressure responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP. *(SEAT*LBNP) indicates a significant interaction effect of SEAT and LBNP. *(HDBR*LBNP) indicates a significant interaction effect of HDBR and LBNP.

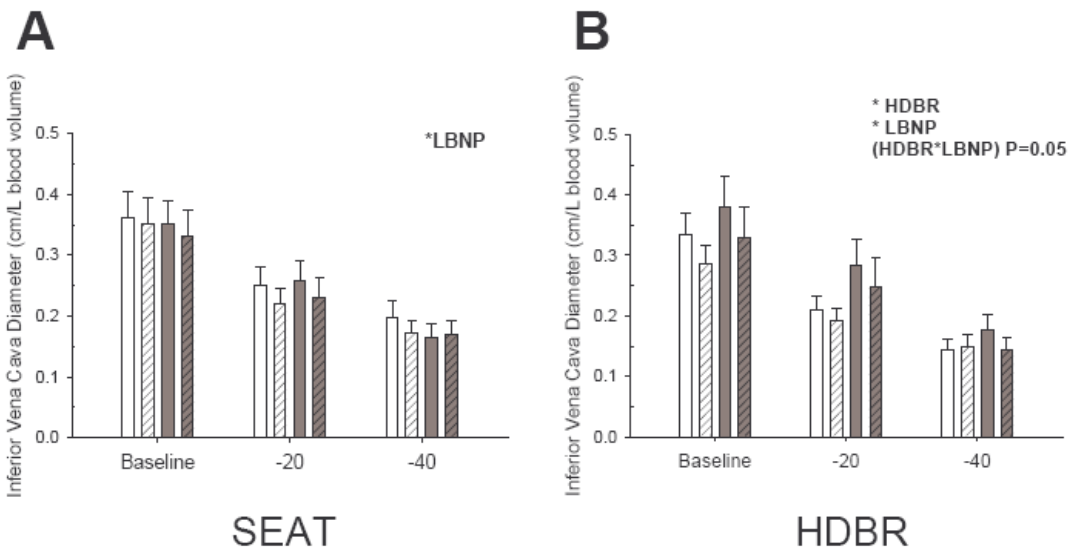


Figure 27: Inferior vena cava diameter responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR.

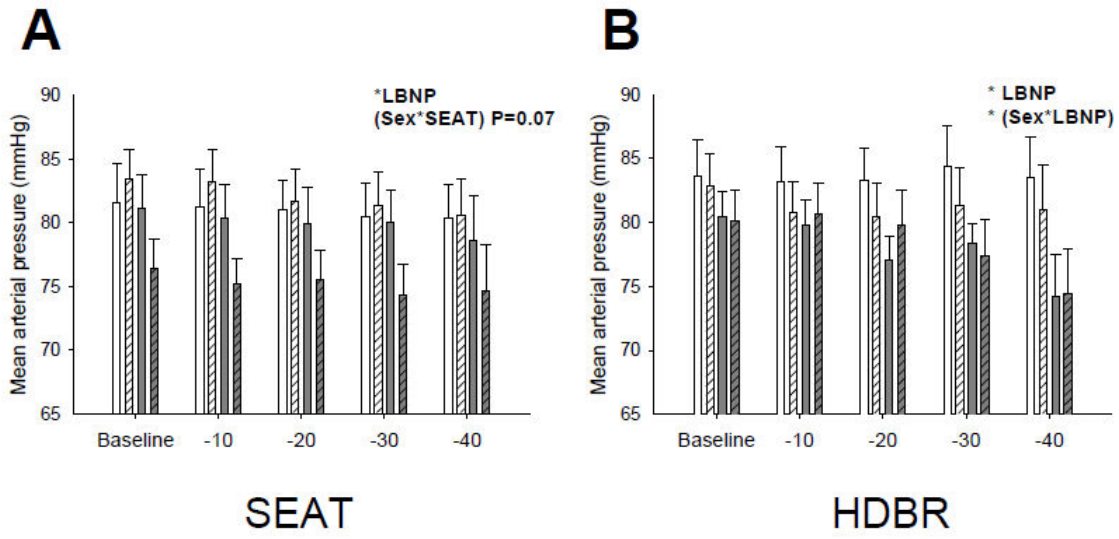


Figure 28: Mean arterial pressure responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP.

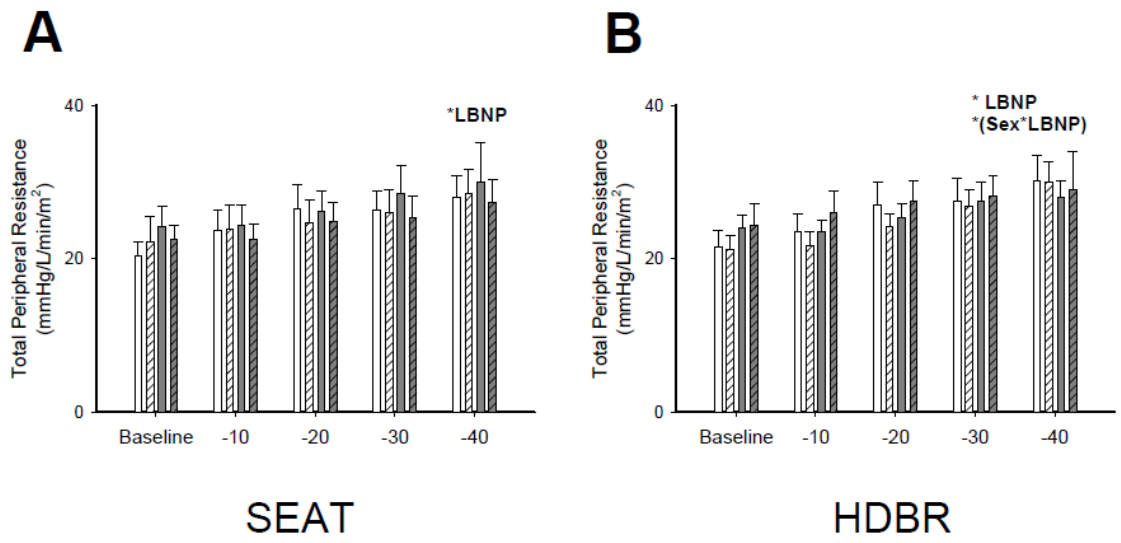


Figure 29: Total peripheral resistance responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP.

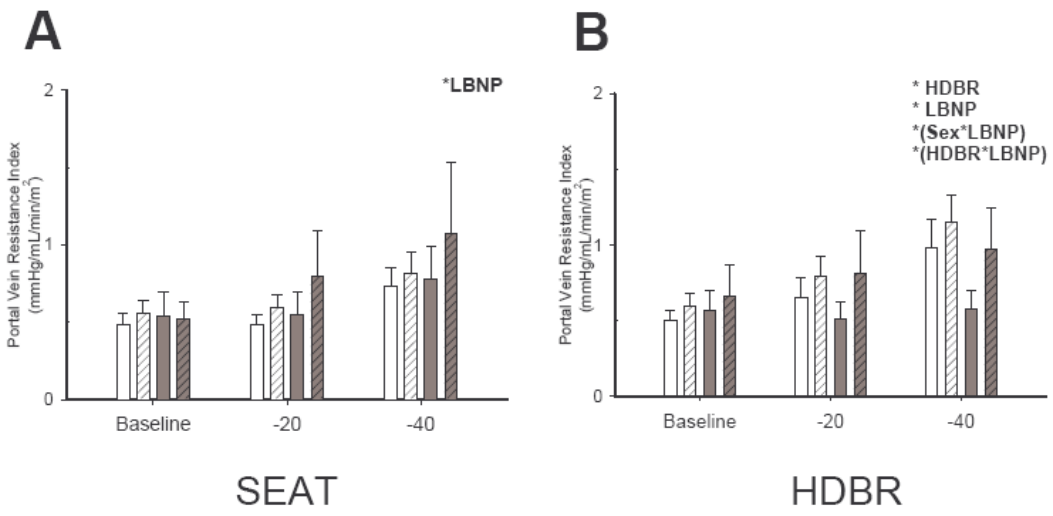


Figure 30: Portal vein resistance index responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of LBNP. *Sex*LBNP indicates a significant interaction effect of sex and LBNP. *HDBR*LBNP indicates a significant interaction effect of HDBR and LBNP.

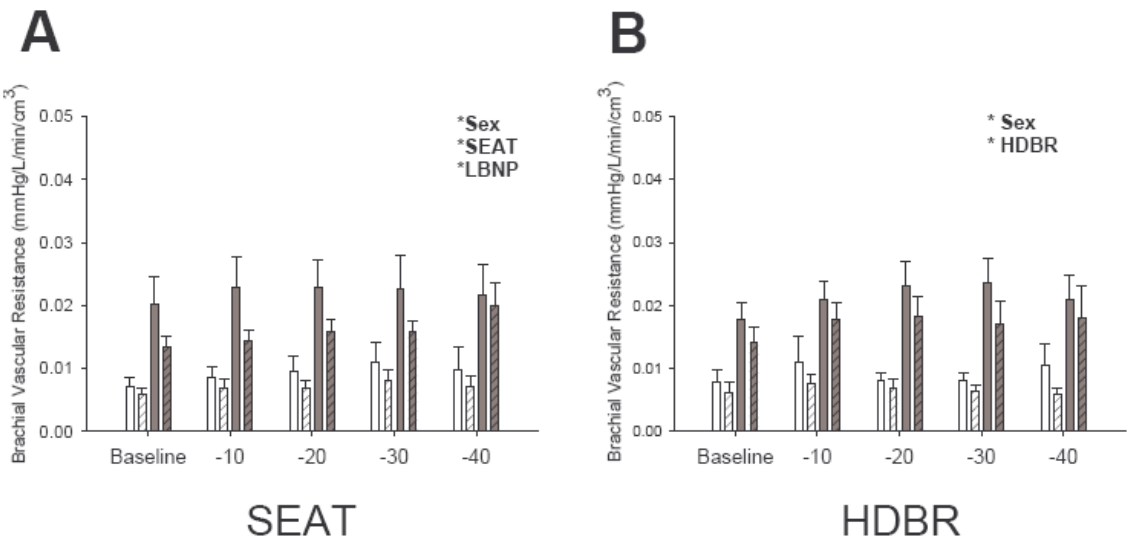


Figure 31: Brachial vascular resistance responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *SEAT indicates a significant main effect of SEAT. *Sex indicates a significant main effect of sex.

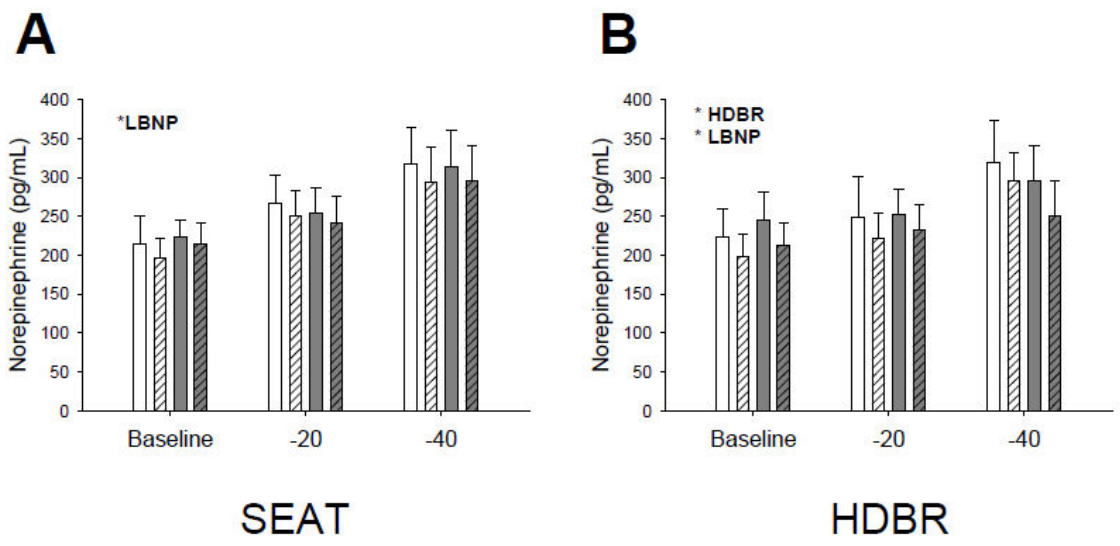


Figure 32: Plasma norepinephrine responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR.

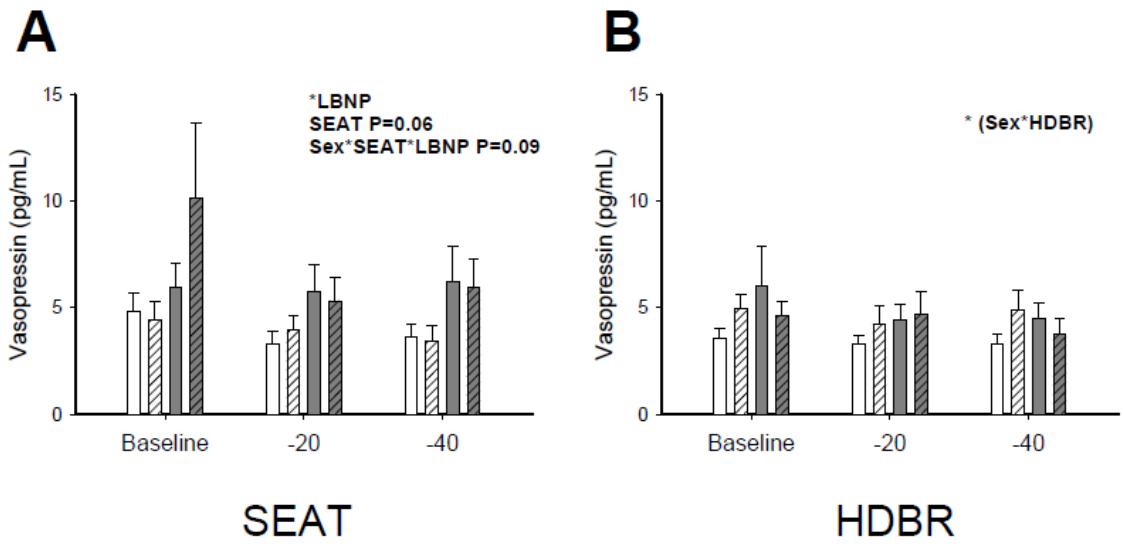


Figure 33: Plasma vasopressin responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Sex*HDBR) indicates a significant interaction effect of sex and HDBR.

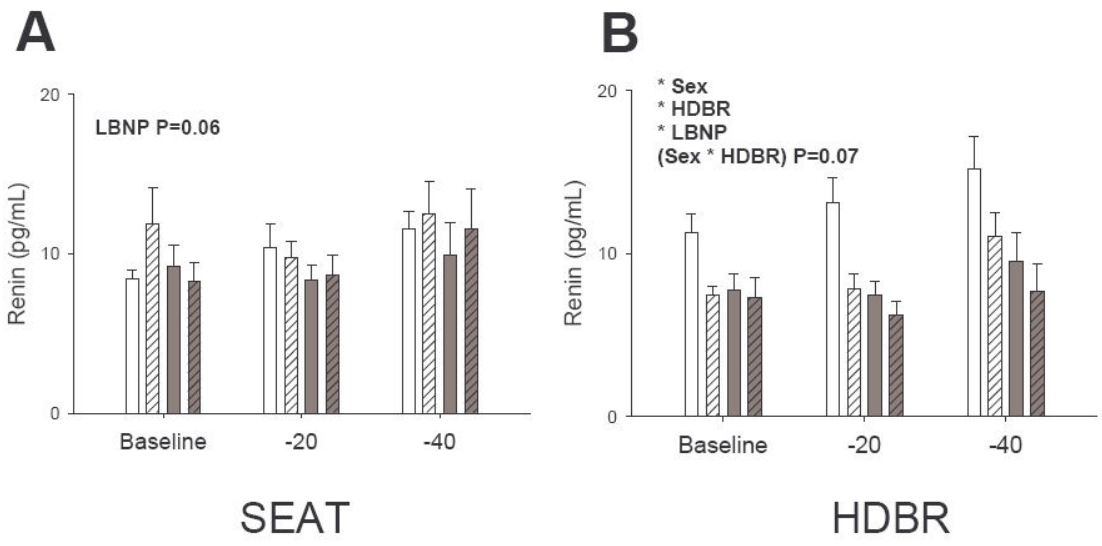


Figure 34: Plasma renin responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *HDBR indicates a significant main effect of HDBR. *Sex indicates a significant main effect of sex.

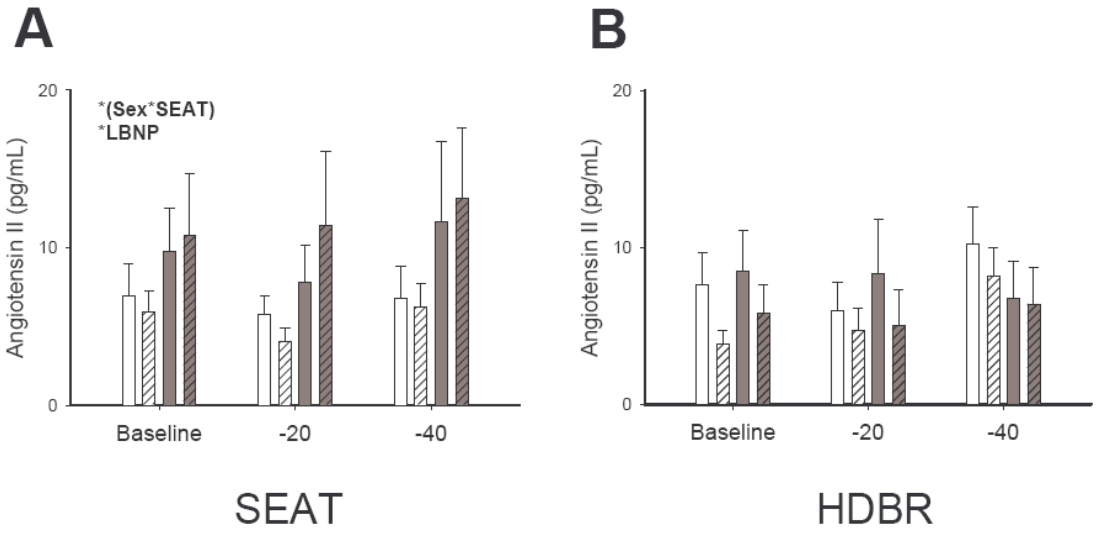


Figure 35: Plasma angiotensin II responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *(Sex*SEAT) indicates a significant interaction effect of sex and SEAT.

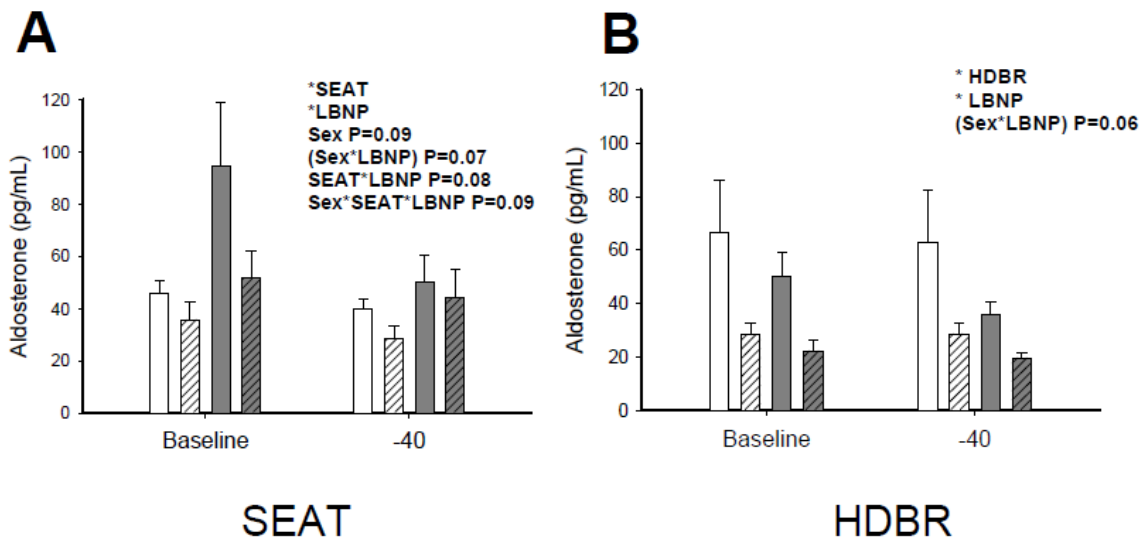


Figure 36: Plasma aldosterone responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT. *HDBR indicates a significant main effect of HDBR.

Table 5: Other vasoactive factors in men and women in the follicular phase during lower body negative pressure (LBNP) before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Sex			0 mmHg	-20mmHg	-40mmHg
Epinephrine (pg/mL)	**Male**	**Pre**	**HDBR**	22.6 ± 3.0	19.8 ± 5.0	22.8 ± 4.3
		Post	**HDBR**	19.3 ± 3.3	19.2 ± 3.7	26.9 ± 3.8
	Female	**Pre**	**HDBR**	21.1 ± 3.0	17.3 ± 1.4	24.0 ± 3.7
		Post	**HDBR**	23.5 ± 3.1	23.0 ± 3.7	21.8 ± 4.5
	Male	**Pre**	**SEAT**	18.6 ± 4.4	17.7 ± 2.5	15.4 ± 1.6
		Post	**SEAT**	18.9 ± 2.7	18.7 ± 3.3	19.7 ± 2.6
	Female	**Pre**	**SEAT**	22.6 ± 3.0	19.8 ± 5.0	22.8 ± 4.3
		Post	**SEAT**	19.3 ± 3.3	19.2 ± 3.7	26.9 ± 3.8
Endothelin-1 (pg/mL)	**Male**	**Pre**	**HDBR**	2.2 ± 0.4	-	2.0 ± 0.4
		Post‡‡	**HDBR**	2.4 ± 0.3	-	2.2 ± 0.3
(Sex P=0.06 SEAT day)	**Female**	**Pre**	**HDBR**	1.4 ± 0.2	-	1.6 ± 0.5
		Post‡‡	**HDBR**	1.3 ± 0.3	-	1.7 ± 0.3
(HDBR*LBNP P=0.09)	**Male**	**Pre**	**SEAT**	2.3 ± 0.3	-	2.1 ± 0.4
		Post	**SEAT**	2.1 ± 0.3	-	2.1 ± 0.2
	Female	**Pre**	**SEAT**	1.5 ± 0.2	-	1.4 ± 0.2
		Post	**SEAT**	1.5 ± 0.2	-	1.2 ± 0.3
Nitrates/ Nitrites (µM)	**Male**	**Pre**	**HDBR**	6.2 ± 1.0	6.1 ± 1.0	6.0 ± 1.0
		Post	**HDBR**	6.3 ± 1.0	6.9 ± 0.9	6.0 ± 1.0
LBNP P=0.09	**Female**	**Pre**	**HDBR**	6.4 ± 1.5	6.1 ± 1.3	6.0 ± 1.1
(Sex*LBNP) P=0.07, SEAT day		**Post**	**HDBR**	6.1 ± 0.8	6.5 ± 0.7	5.7 ± 1.0
	Male	**Pre**	**SEAT**	7.1 ± 0.6	6.3 ± 0.6	6.7 ± 0.7*
		Post	**SEAT**	6.7 ± 1.0	5.7 ± 0.5	6.0 ± 0.6*
(Sex*SEAT* LBNP) P=0.09	**Female**	**Pre**	**SEAT**	6.9 ± 0.9	6.4 ± 0.8	5.9 ± 0.8*
		Post	**SEAT**	6.2 ± 0.5	6.5 ± 0.3	6.3 ± 0.4*
Atrial natriuretic peptide (pg/mL)	**Male**	**Pre**	**HDBR**	29.4 ± 4.5	27.6 ± 4.1	27.7 ± 4.9
		Post	**HDBR**	30.2 ± 3.1	28.8 ± 2.5	30.2 ± 2.9
	Female	**Pre**	**HDBR**	29.9 ± 3.3	29.5 ± 4.4	26.5 ± 4.6
		Post	**HDBR**	30.7 ± 6.0	28.3 ± 5.6	21.7 ± 3.4
(Sex P=0.08 SEAT day)	**Male**	**Pre**	**SEAT**	36.2 ± 5.9	34.8 ± 4.7	29.8 ± 4.0
		Post	**SEAT**	28.1 ± 3.2	26.5 ± 2.7	29.7 ± 4.7
(Sex*SEAT* LBNP P=0.09)	**Female**	**Pre**	**SEAT**	25.4 ± 2.3	20.9 ± 2.7	21.7 ± 2.5
		Post	**SEAT**	22.7 ± 2.6	23.7 ± 2.8	20.9 ± 1.8

(* denotes a significant main effect of LBNP; ‡‡ denotes a significant Sex*HDBR interaction)

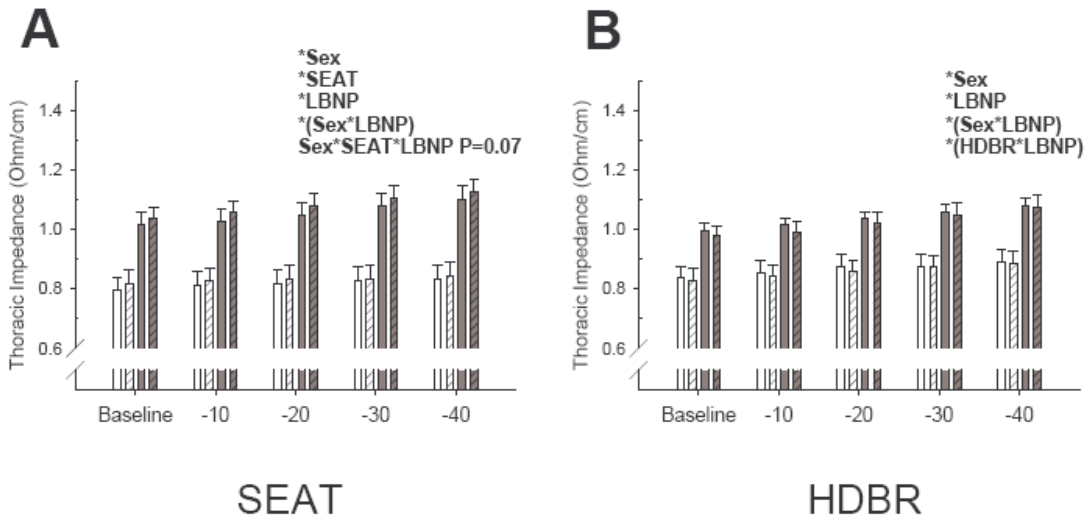


Figure 37: Thoracic impedance responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *Sex indicates a significant main effect of sex. *LBNP indicates a significant main effect of LBNP. *SEAT indicates a significant main effect of SEAT. *(Sex*LBNP) indicates a significant interaction effect of sex and LBNP. *(HDBR*LBNP) indicates a significant interaction effect of HDBR and LBNP.

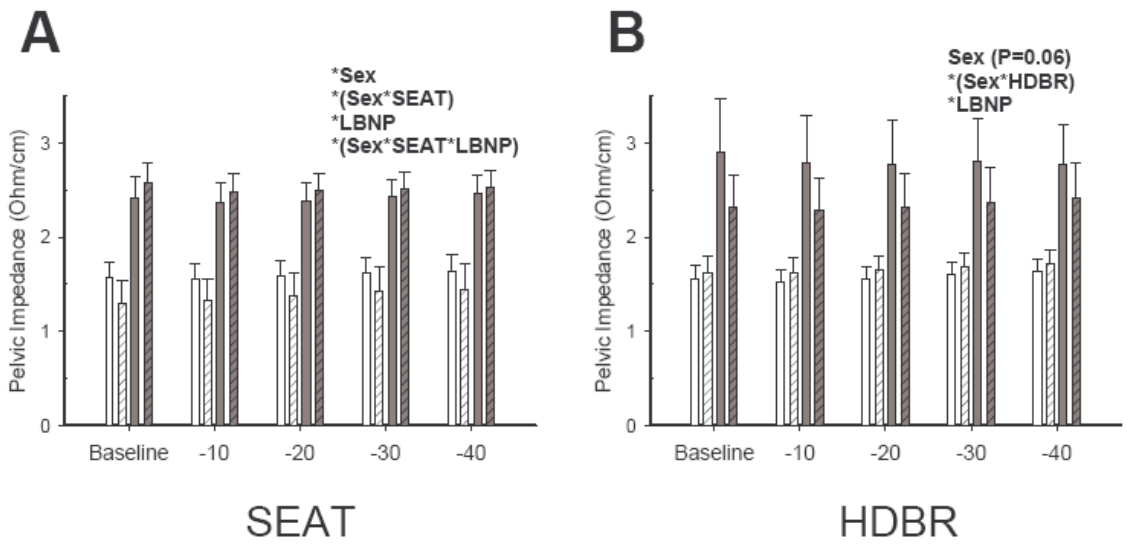


Figure 38: Pelvic impedance responses to lower body negative pressure (LBNP) in men (white bars) and women in the follicular phase (grey bars) before (solid bars) and after (hatched bars) 4-hours of seated control (SEAT; A) or 4-hours of head-down bed-rest (HDBR; B). *Sex indicates a significant main effect of sex. *LBNP indicates a significant main effect of LBNP. *(Sex*SEAT) indicates a significant interaction effect of sex and SEAT. *(Sex*HDBR) indicates a significant interaction effect of sex and HDBR. *(Sex*SEAT*LBNP) indicates a significant interaction effect of sex, SEAT and LBNP.

Table 6: Water and sodium balance in men and women in the follicular phase before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Sex		Pre	Post
Blood volume (mL)	**Male**	**HDBR**	5665.1 ± 697.3	5485.6 ± 690.9‡
	Female	**HDBR**	4836.2 ± 337.1	4715 ± 347.0‡
	Male	**SEAT**	6311.9 ± 805.4	6244.1 ± 766.0
	Female	**SEAT**	4821.0 ± 463.8	4810.3 ± 480.0
Water intake (mL)	**Male**	**HDBR**	-	343.0 ± 70.2
	Female	**HDBR**	-	314.2 ± 51.3
	Male	**SEAT**	-	364.0 ± 64.0
	Female	**SEAT**	-	324.7 ± 57.7
Urine output (mL)	**Male**	**HDBR**	470.1 ± 112.1	689.0 ± 121.5
	Female	**HDBR**	497.6 ± 156.5	567.0 ± 83.4
	Male	**SEAT**	601.8 ± 114.2	597.8 ± 117.4
	Female	**SEAT**	448.7 ± 90.1	461.5 ± 75.5
Urine osmolality (mmol/kg)	**Male**	**HDBR**	718.6 ± 78.5	503.4 ± 78.4‡
	Female	**HDBR**	558.2 ± 79.1	377.3 ± 46.9‡
	Male	**SEAT**	589.9 ± 69.0	649.9 ± 70.8
	Female	**SEAT**	515.2 ± 82.8	504.6 ± 68.6
Urine sodium (mmol/L)	**Male**	**HDBR**	110.3 ± 13.1	119.5 ± 21.1
(SEAT P=0.09)	**Female**	**HDBR**	153.6 ± 72.3	214.8 ± 130.4
	Male	**SEAT**	108.6±15.5	147.8 ± 18.3
	Female	**SEAT**	104.0 ± 35.6	191.5 ± 97.8
Urodilatin (pg/mL)	**Male**	**HDBR**	29.6 ± 9.2	13.1 ± 2.2‡
	Female	**HDBR**	32.5 ± 8.6	12.9 ± 4.0‡
	Male	**SEAT**	19.3 ± 3.7	11.8 ± 1.9
	Female	**SEAT**	35.8 ± 12.5	18.9 ± 5.6
Serum osmolality (mmol/kg)	**Male**	**HDBR**	280.9 ± 2.5	280.6 ± 2.0
	Female	**HDBR**	278.6 ± 1.6	279.4 ± 2.7
(SEAT P=0.09)	**Male**	**SEAT**	282.0 ± 2.2	278.4 ± 2.0‡‡
	Female	**SEAT**	280.9 ± 1.8	280.7 ± 1.8‡‡
Serum sodium (mmol/L)	**Male**	**HDBR**	122.7 ± 0.7	122.1 ± 1.1
(Sex P=0.05; HDBR test day)	**Female**	**HDBR**	127.4±2.0	126.6±2.6
	Male	**SEAT**	123.0 ± 1.0	122.4 ± 1.0
	Female	**SEAT**	125.4 ± 2.0	126.8 ± 2.2

(‡ denotes a significant main effect of HDBR; ‡‡ denotes a significant Sex*SEAT interaction)

Table 7: Compliance of the inferior vena cava in men and women in the follicular phase during lower body negative pressure (LBNP) before and after 4-hr head-down bed rest (HDBR) or seated control (SEAT).

	Sex		**Pre**	**Post**
Compliance	**Male**	**HDBR**	0.15 ± 0.03	0.13 ± 0.02
(cm/mmHg)	**Female**	**HDBR**	0.14 ± 0.02	0.11 ± 0.02
	Male	**SEAT**	0.13 ± 0.02	0.15 ± 0.02‡‡
	Female	**SEAT**	0.12 ± 0.02	0.10 ± 0.03‡‡

(‡‡ denotes a significant Sex*SEAT interaction)

4.3 Discussion

The effects of LBNP in men and women throughout the menstrual cycle have been described in depth in Chapter 3. Those data are the baseline conditions of the head-down bed-rest (HDBR) investigation described here. This discussion will focus on the cardiovascular and hormonal effects of 4-hours of HDBR and how this position changes the responses to lower body negative pressure (LBNP) in men and women in both the follicular and luteal phases of the menstrual cycle. Furthermore, this discussion will describe the effect of 4-hours of sitting (SEAT) as a control for circadian rhythm and inactivity. This section will first discuss the comparison between menstrual phases and will then discuss the comparison between men and women in the follicular phase.

Summary of results (menstrual cycle comparison)

These results support the hypotheses and show that in women throughout the menstrual cycle (follicular and luteal phases) HDBR elicits cardiovascular and hormonal changes that counter-act the cephalic fluid shift, and that HDBR augments some of the cardiovascular and hormonal responses to LBNP in order to maintain mean arterial pressure. Furthermore, the results indicate that some of the effects seen with HDBR are in fact due to inactivity and/or circadian rhythm (as shown by similar effects seen with SEAT).

Systemic cardiovascular changes

Compared to the baseline condition: 1) the increase of heart rate during LBNP was augmented after HDBR but not after SEAT, 2) the decrease of stroke volume during LBNP was augmented after HDBR but not after SEAT, 3) responses of cardiac output to LBNP did not change with either HDBR or SEAT, 4) central venous pressure tended to be lower after HDBR ($P=0.148$) and was lower after SEAT, 5) inferior vena cava diameter was smaller after both HDBR and SEAT, 6) inferior vena cava compliance did not change with either HDBR or SEAT, 7) mean arterial pressure was not different after HDBR yet was lower after SEAT, 8) responses of total peripheral resistance during LBNP did not change with either HDBR or

SEAT, 9) portal vein resistance index increased after both HDBR and SEAT (P=0.07), and 10) brachial vascular resistance decreased after both HDBR (P=0.08) and SEAT (P=0.07).

Hormonal changes

Compared to the baseline condition: 1) norepinephrine was lower after HDBR but not after SEAT, 2) vasopressin was higher after HDBR in the luteal phase, lower after HDBR in the follicular phase, and there was higher vasopressin after SEAT in the follicular phase (there was also a trend for vasopressin to increase with LBNP in the luteal phase and to decrease with LBNP in the follicular phase), 3) renin was lower after HDBR but not after SEAT (there was also a trend for renin to increase more during LBNP after SEAT in the luteal phase), 4) angiotensin II did not change after HDBR but was higher after SEAT, 5) aldosterone was lower after HDBR and SEAT and due to this smaller baseline the loss of aldosterone due to LBNP was smaller, 6) epinephrine did not change after HDBR or SEAT, 7) endothelin-1 was lower after HDBR and tended to be lower after SEAT (P=0.09), 8) nitrates/nitrites were not different after HDBR or SEAT but there was a smaller loss of nitrates/nitrites during LBNP after SEAT in the follicular phase, and 9) atrial natriuretic peptide was lower with LBNP after HDBR in the follicular phase (there was also a trend for atrial natriuretic peptide to be lower after SEAT (P=0.07)).

Blood volume, fluid shifts, and water/sodium balance

Compared to the baseline condition: 1) the increase of thoracic impedance during LBNP was augmented after HDBR and thoracic impedance increased after SEAT, 2) pelvic impedance increased in the luteal phase after HDBR, decreased in the follicular phase after HDBR, and did not change after SEAT, 3) blood volume decreased after HDBR but not after SEAT, 4) urine output did not change after HDBR or SEAT, 5) urine osmolality tended to be lower after HDBR (P=0.07) but not after SEAT, 6) urine sodium did not change after HDBR or SEAT, 7) urodilatin tended to decrease after HDBR (P=0.06) to a greater degree than after SEAT (P=0.109), 8) serum osmolality did not change after HDBR or SEAT, and 9) serum sodium did not change after HDBR or SEAT.

Inactivity and circadian rhythm through the menstrual cycle

As hypothesized, the 4-hours of sitting elicited multiple cardiovascular changes in women throughout the menstrual cycle. However, these changes could be due to either circadian rhythm or to the seated posture. This section will discuss each variable in order to determine if the changes are primarily due to circadian rhythm (according to previous investigations) or to sitting. One major challenge in comparing this study to those previously investigating circadian rhythm is that most previous data were collected in men. Therefore, the possibility exists that there are sexually dimorphic circadian changes.

There are no circadian changes in mean arterial pressure or central venous pressure (48; 125; 165), yet there was a decrease in both variables after 4-hours of sitting in women. It is unknown if the diameter of the inferior vena cava changes throughout the day, yet smaller inferior vena cava diameters were observed after 4-hours of sitting. Brachial vascular resistance was also lower after sitting which is likely due to circadian rhythm (141). Brachial vascular resistance was used as an index of limb vascular resistance as Kitano et al. found that both limbs increase resistance to the same degree with LBNP (102). Therefore, these results are interpreted to mean that leg vascular resistance was also lower after sitting. This could have led to increased pooling of blood in the legs and therefore lower venous return and less blood in the inferior vena cava of the pelvic and thoracic region. This was confirmed by the measurements of higher pelvic and thoracic impedance (Note: higher impedance implies less blood in a given section), smaller inferior vena cava diameter, lower central venous pressure, and lower atrial natriuretic peptide (due to less atrial stretch (154); not a diurnal effect (50)). Therefore, all of these measurements could be at least partially due to circadian rhythm. The lower limb vascular resistance and the lower mean arterial pressure could also be a function of the small trend towards lower norepinephrine (not a diurnal effect (50)) and the slightly lower endothelin-1 (not a diurnal effect (64)).

After 4-hours of sitting, no change in vasopressin was observed in the luteal phase with an increase in the follicular phase. Drummer et al. observed lower vasopressin in the afternoon. These results would therefore translate into higher vasopressin in the afternoon in both phases which could have been a response to the lower mean arterial and/or central venous pressure seen in women. In the follicular phase after SEAT, there was a tendency towards lower central

venous pressure compared to the luteal phase. Perhaps in response to this, women in the follicular phase had higher baseline vasopressin in an attempt to restore blood pressure. Women in the luteal phase did not exhibit the same decrease in central venous pressure after sitting and this could have been in part due to slightly higher levels of angiotensin II and greater activation of renin with an orthostatic stress. A constant low-dose infusion of angiotensin II has indeed been shown to increase mean circulatory filling pressure (an index of venous pressure) in male rats (101).

In women there was no change in renin after sitting (this has previously been observed in men (87)), yet an increase in angiotensin II was observed. There were also lower levels of aldosterone. These differences could have been due to different clearance rates. However, bearing in mind that renin and aldosterone have peak levels in the morning due to diurnal variation (50; 89), our afternoon post-SEAT tests should have had lower levels of renin and aldosterone (it is assumed that angiotensin II would also be lower). This means that sitting elicited a stimulus for renin secretion, and therefore a stimulus for angiotensin II creation (particularly in the luteal phase). Alternatively, renin may have been higher after sitting, not directly from the seated position, but from activation due to the small amount of time (~10 seconds) that individuals stood before moving to the LBNP table. This was a limitation of our study listed below. In either case, this increase in angiotensin II may not yet have translated into an increase in aldosterone and thus we observed a lower level due to time of day only.

In patients with liver cirrhosis, there are no diurnal changes in portal vein flow beyond that due to meals (157). Schiedermaier et al. found that in the morning, meals increased portal vein flow with a return to baseline after 4-hours. The participants in the current study ate breakfast at ~6am, lunch at ~10am, and the afternoon test was at ~2pm. Therefore, these tests should not have a post-prandial effect. Higher portal vein resistance index (i.e. lower flow) was observed in the afternoon (particularly in the luteal phase) which could have been due to the higher levels of angiotensin II as the vasculature of the splanchnia is responsive to angiotensin II. This was observed by Stadeager et al. who showed that angiotensin converting enzyme inhibitors attenuated the increase in splanchnic vascular resistance seen with LBNP (172).

In men, Drummer et al. found higher urine sodium, higher urodilatin, and lower urine osmolality in the afternoon compared to the morning (50). In women after SEAT, a similar tendency was observed for higher urine sodium, lower urodilatin, and no change in urine

osmolality were observed. Therefore (and assuming circadian changes are the same in women), the 4-hour period of sitting did not affect urine sodium levels, decreased urodilatin, and increased urine osmolality. The observed decrease in urodilatin may have been due to the lower levels of atrial natriuretic peptide (193) or perhaps due to reduced renal blood flow due to the vasoconstriction resulting from observed higher levels of angiotensin II (84). Changes in urine osmolality could be attributable to changes in many other solutes besides sodium such as potassium, calcium, urea, and proteins.

Whole body impedance decreases with meals, and this is a cumulative effect throughout the day (168). This meal effect lasts from 2-4 hours, but never returns to baseline. If this whole body measurement of impedance translates into thoracic and/or pelvic segmental measurements, the afternoon tests of this study should have observed lower thoracic and pelvic impedance. However, an increase in thoracic and pelvic impedance after SEAT was observed indicating that there is a fluid shift from the thorax and pelvis, presumably towards the legs. Considering these diurnal and post-meal effects on whole body impedance, this fluid shift is expected to be an effect of inactivity.

There are no circadian changes in endothelin-1 (64), therefore the reduction in women that is seen in the afternoon of this study ($P=0.09$ for SEAT) was likely due to inactivity. This reduction could have helped to improve endothelial function as inhibition of endothelin-1 improves endothelium-dependent vasodilation (49). Indeed, Violanti et al. suggest that endothelial function of women is impaired in the morning (194). Perhaps the drop in central venous pressure and mean arterial pressure seen in women after sitting could have been partially due to greater endothelial function in the afternoon.

From all of these observations and after considering the effects of circadian rhythm, it was shown that in women throughout the menstrual cycle 4-hours of sitting caused: 1) lower mean arterial pressure, 2) higher portal vein resistance index, 3) lower central venous pressure (particularly in the follicular phase), 4) smaller inferior vena cava, 5) higher vasopressin (augmented in the follicular phase), 6) higher renin and a greater increase with LBNP in the luteal phase, 7) higher angiotensin II (tendency for higher in the luteal phase), 8) lower atrial natriuretic peptide, 9) tendency for lower endothelin-1, 10) lower nitrates/nitrites after SEAT in the follicular phase, 11) higher thoracic impedance, 12) higher pelvic impedance, 13) lower urodilatin, and 14) higher urine osmolality. These changes were due to inactivity and/or

exposure to 4-hours of orthostatic stress (i.e. sitting). These results indicate that mean arterial pressure and central venous pressure are lower after 4-hours of sitting in women which is likely due to pooling of blood in the legs (as indicated by higher thoracic and pelvic impedance). Also, although mean arterial pressure and cardiac output are similar between the different phases of the menstrual cycle after 4-hours of sitting, there is a reduced ability to maintain central venous pressure in the follicular phase which could stem from attenuated vasoconstriction (lower activation of the renin-angiotensin-aldosterone system) during an orthostatic stress leading to greater splanchnic blood pooling.

Effects of HDBR through the menstrual cycle

These results supported the hypothesis and showed that 4-hours of HDBR in women (regardless of menstrual phase) elicited cardiovascular and hormonal responses to counter the cephalic fluid shift and augmented some of the responses to LBNP. Furthermore, some of the changes observed after HDBR were indeed attributable to inactivity (as shown by the seated control), such as higher portal vein resistance index, lower central venous pressure, smaller inferior vena cava diameter, higher angiotensin II, and lower endothelin-1. Some of the changes observed after HDBR were also attributable to circadian rhythm (as shown by previous studies) such as lower brachial vascular resistance, lower renin, lower aldosterone, lower urine osmolality, and lower urodilatin (50; 89; 141).

Head-down bed rest resulted in higher heart rate and an augmented increase during LBNP, an augmented reduction of stroke volume during LBNP, an augmented increase of thoracic impedance during LBNP, and lower plasma volume. The increased heart rate response to LBNP, the lower stroke volume during LBNP, and the higher thoracic impedance with LBNP all point towards reduced venous return during LBNP after HDBR in women. This may also be partially due to the reduced blood volume observed after HDBR; however another study investigating blood volume after 4-hours of HDBR in men found no change in blood volume with similar changes in heart rate (62). Changes in heart rate could have been attributable to any changes in epinephrine, yet there were no observed changes in epinephrine due to SEAT, HDBR, or LBNP. There are also no circadian changes in epinephrine (25; 158).

Venous return may also be reduced due to the observed reduction of norepinephrine which may lead to reduced vascular resistance in regions that were not directly measured in this study (i.e. renal, leg, pelvic organs). The lower norepinephrine after HDBR was also observed after 4-weeks of HDBR in men (73); however it was not previously observed after 4-hours of HDBR in men (62). In the Fischer et al. study, participants drank 125mL water 1 hour before the afternoon test. In the current study participants were required to stop drinking 2 hours before the afternoon test. Drinking water has been shown to elicit a pressor effect and increase muscle sympathetic nerve activity and plasma norepinephrine (23; 160). This could have obscured the decrease in norepinephrine due to the HDBR in the Fischer et al. investigation.

After comparing the changes (or the lack thereof) due to HDBR to the changes due to SEAT, after HDBR there also appears to be an increase in mean arterial pressure, an increase in atrial natriuretic peptide, and a decrease in thoracic impedance (i.e. the decrease of mean arterial pressure, decrease of atrial natriuretic peptide, and increase of thoracic impedance observed with sitting were not seen with HDBR). Mean arterial pressure did not drop as seen with SEAT. Therefore either the drop in mean arterial pressure seen after SEAT was not a function of better endothelial function in the afternoon as suggested in the last section of this Chapter, or there could have been a balancing increase in mean arterial pressure after HDBR from the tendency towards higher portal vein resistance index in the luteal phase (and perhaps higher renal and leg vascular resistance after HDBR in the follicular phase as was discussed in Chapter 3). The higher atrial natriuretic peptide was likely a result of atrial stretch during the HDBR (i.e. the position induces a fluid shift towards the heart). It was suggested in the last section that lower levels of urodilatin may be in part due to lower levels of atrial natriuretic peptide after SEAT. However, after HDBR higher levels of atrial natriuretic peptide were observed with lower levels of urodilatin, thus the drop in urodilatin could have been a result of reduced renal blood flow. The lower thoracic impedance was also a function of the fluid shift towards the thorax (i.e. the higher volume of blood in this region would lead to lower thoracic impedance). Lower thoracic impedance after 4-hours of HDBR has been previously observed in men (21).

There was higher vasopressin after HDBR in the luteal phase and lower vasopressin after HDBR in the follicular phase. Lower vasopressin was expected due to circadian rhythm

(50), and it has been previously shown that exercise stress increases vasopressin more in the luteal phase than the follicular phase (3). There is a lower osmolality threshold for vasopressin release in the luteal phase (195), so perhaps stressors such as exercise or HDBR and LBNP will also have a lower threshold for release. After SEAT there was higher vasopressin in the follicular phase which was not seen after HDBR. It was suggested in the last section that this may be in response to lower mean arterial or central venous pressure. As mean arterial pressure was not different from baseline after HDBR and the higher vasopressin was not present, the increase seen after SEAT was likely due to the drop in mean arterial pressure not the drop in central venous pressure.

After HDBR, there was an increase of pelvic impedance in the luteal phase (inactivity effect) and a decrease in pelvic impedance in the follicular phase (circadian effect). These results imply that there was greater blood volume in the pelvic region in the follicular phase after HDBR and smaller blood volume in the pelvic region in the luteal phase after HDBR. This potential higher level of pooling in the follicular phase could help to explain why central venous pressure tends to be lower after HDBR compared to the luteal phase, and therefore could also help to explain the greater loss of atrial natriuretic peptide with LBNP after HDBR in the follicular phase (due to lower venous return). The inactivity induced movement of pelvic pools in the luteal phase could be a result of the tendency for higher angiotensin and the higher vasopressin after HDBR.

From all of these observations and after considering the effects of the 4-hours of sitting and circadian rhythm, it was shown that in women throughout the menstrual cycle 4-hours of HDBR caused: 1) higher heart rate and a greater increase due to LBNP, 2) a greater decrease of stroke volume due to LBNP, 3) no decrease in mean arterial pressure (as seen with SEAT), 4) lower norepinephrine, 5) higher vasopressin in luteal phase, 6) higher atrial natriuretic peptide, 7) a greater increase of thoracic impedance due to LBNP, 8) lower pelvic impedance in the follicular phase, 9) lower blood volume, and 10) lower urodilatin. These results indicate that women experience reduced venous return during an orthostatic stress after 4-hours of HDBR despite mechanisms to counter the inactivity-induced decrease in mean arterial pressure. Furthermore, there appears to be greater pelvic blood pooling in the follicular phase after HDBR which could partially stem from the lower vasopressin and angiotensin II.

Summary of results (sex comparison)

These results support the hypotheses and show that men and women (follicular phase) have cardiovascular and hormonal responses to the cephalic fluid shift of 4-hours of HDBR and that some of the responses to LBNP are augmented. Furthermore, the results show that some of these changes are in fact due to circadian rhythm and/or inactivity (as shown by the responses to SEAT). Due to the extensive discussion of the responses of men and women to LBNP found in Chapter 3, this discussion will focus on the effects of HDBR or SEAT and the changes in the responses to LBNP.

Systemic cardiovascular changes

Compared to the baseline condition: 1) heart rate was higher and the response to LBNP was augmented after HDBR but not after SEAT, 2) stroke volume was lower during LBNP after HDBR but not after SEAT, 3) cardiac output was not different after HDBR or SEAT, 4) central venous pressure was lower after HDBR and SEAT in women but only after HDBR in men, and there was a smaller loss of central venous pressure during LBNP after both HDBR and SEAT, 5) inferior vena cava diameter was lower after HDBR but not after SEAT leading to a smaller reduction of diameter during LBNP after HDBR, 6) inferior vena cava compliance was not different after HDBR but was higher after SEAT in men, 7) mean arterial pressure was lower after SEAT in women, 8) total peripheral resistance did not change after HDBR or SEAT, 9) portal vein resistance index is higher after HDBR and tends to be higher after SEAT ($P=0.101$) and there is a greater increase during LBNP after HDBR, and 10) brachial vascular resistance was lower after both HDBR and SEAT.

Hormonal changes

Compared to the baseline condition: 1) norepinephrine was lower after HDBR but not after SEAT, 2) vasopressin was higher after HDBR in men and lower after HDBR in women (there was a trend for higher vasopressin in women after SEAT), 3) renin was lower after HDBR but not after SEAT, particularly in men, 4) angiotensin II is lower after SEAT in men,

angiotensin II is higher after SEAT in women, and the increase of angiotensin II seen during LBNP on the SEAT testing day is not observed on the HDBR testing day, 5) aldosterone is lower after HDBR and SEAT leading to a smaller reduction during LBNP, 6) epinephrine was not different after HDBR or SEAT, 7) endothelin-1 was higher after HDBR in men and was lower after HDBR in women, 8) nitrates/nitrites were not different after HDBR or SEAT, and 9) atrial natriuretic peptide was not different after HDBR and was slightly lower after SEAT in men.

Blood volume, fluid shifts, and water/sodium balance

Compared to the baseline condition: 1) thoracic impedance was higher after SEAT but not HDBR but there was a greater increase of thoracic impedance during LBNP after HDBR, 2) pelvic impedance was lower after HDBR and higher after SEAT in women, pelvic impedance was higher after HDBR and lower after SEAT in men, and pelvic impedance decreased in women during LBNP after SEAT (as opposed to the increase seen during LBNP before SEAT and in men), 3) blood volume was lower after HDBR but not after SEAT, 4) urine output was not different after HDBR or SEAT, 5) urine osmolality was lower after HDBR but not after SEAT, 6) urine sodium tended to be higher after SEAT but not after HDBR, 7) urodilatin was lower after HDBR and tended to be lower after SEAT, 8) serum osmolality was lower after SEAT but not after HDBR in men and serum osmolality was not different after either HDBR or SEAT in women, and 9) serum sodium was not different after HDBR or SEAT.

Circadian rhythm and inactivity in men and women (follicular phase)

These results show that 4-hours of sitting (SEAT) elicited cardiovascular and hormonal changes in men and women. These changes could be due to either circadian rhythm or to the seated posture. Therefore, each variable will be discussed to clarify the reasons for changes observed after SEAT.

This study has shown that there are no changes in heart rate, stroke volume, cardiac output, total peripheral resistance, or portal vein resistance index after 4-hours of SEAT in men

and women. This corresponds to previous investigations that have shown no diurnal changes in these variables (at least when comparing 8am to 2pm, the approximate testing times of this study) (4; 48; 165). It was also observed that there was lower brachial vascular resistance in the afternoon after SEAT which corresponds to the results of Panza et al. who observed significantly lower brachial vascular resistance in the afternoon compared to the morning (141). Similarly, no changes in atrial natriuretic peptide, norepinephrine, epinephrine, endothelin-1, and plasma nitrates were observed after 4-hours of sitting and previous studies have confirmed that these substances do not change throughout the day (50; 64; 148; 150). Therefore, we can conclude from these observations that both SEAT and circadian rhythm do not affect these systemic measurements or hormones in men and women.

In women, lower mean arterial pressure was observed after 4-hr SEAT; however there are no diurnal changes in mean arterial pressure (48; 165). It is presumed from this that the seated position itself is responsible for this change. Similarly, in this study lower central venous pressure was observed after SEAT while previous studies have not observed circadian changes (125). After SEAT, men and women also had a smaller loss of central venous pressure with LBNP (which is likely due to the smaller baseline), and since Middlekauff and Sontz found no circadian difference in the central venous pressure response to low-level LBNP (up to -20mmHg), it is likely that there are no circadian effects on central venous pressure and that the differences observed are due to the seated posture.

There were no changes of vasopressin levels after SEAT in men and there were higher levels after SEAT in women, yet vasopressin levels are naturally lower in the afternoon (50). Therefore, it appears that there was higher vasopressin in both sexes after 4-hours of sitting with a greater increase in women. Simulated orthostatic stress (LBNP) has been shown to increase vasopressin in men, and there are higher levels in those men with greater susceptibility to orthostatic hypotension (76). Therefore, the increase of vasopressin could be due to the exposure to a constant orthostatic stress (4-hours of sitting); however the concurrent inactivity may also play a role in the increase of vasopressin. The greater increase of vasopressin in women could be a function of lower mean arterial pressure and central venous pressure observed after SEAT. Alternatively, since vasopressin release is stimulated by hyperosmotic plasma (12), the release of vasopressin due to SEAT could be attenuated in men due to the

observed lower serum osmolality in the afternoon. (There are no circadian differences in regard to serum sodium or osmolality (92).)

In both men and women there were no changes in renin levels after SEAT, lower angiotensin II was observed in men after SEAT, and higher angiotensin II was observed in women after SEAT. However, plasma concentrations of renin and aldosterone are lower in the afternoon than in the morning (it is assumed that the levels of angiotensin II are also lower in the afternoon) (50; 89). Therefore, it appears that there was actually higher renin in men and women after SEAT. It also appears that the reduction in angiotensin II in men after SEAT was due to normal circadian rhythm, yet the increase of angiotensin II in women was due to SEAT. The lower baseline level of aldosterone observed in the afternoon in both sexes was expected due to circadian rhythm and led to the observed result of a smaller decrease due to LBNP. Perhaps the exposure to 4-hours of sitting (orthostatic stress and inactivity) resulted in the higher renin in both sexes and the greater angiotensin II in women, or perhaps it was the few steps required to move to the LBNP apparatus (~10 seconds of standing and walking; a limitation described below). In either case, an orthostatic stress resulted in greater levels of renin in both sexes and angiotensin II in women.

After 4-hours of sitting there was higher thoracic impedance in both men and women, and higher pelvic impedance in women. After 4-hours of sitting there was lower pelvic impedance in men, but there was higher pelvic impedance in women. Electrical impedance of the whole body decreases throughout the day due mainly to meal consumption (157). Assuming that lower whole body impedance also equals lower thoracic and pelvic impedance, it appears that after 4-hours of sitting there was less blood in the thorax of both sexes and less blood in the pelvic region in women. However, the portal vein resistance index data indicated that there was no sexually dimorphic splanchnic blood pooling after 4-hours of sitting. Therefore, the higher pelvic impedance in women could have been a result of less blood in the inferior vena cava as it passed through the pelvic cavity. In support of this assumption, higher inferior vena cava compliance after SEAT was observed in men and lower compliance after SEAT was observed in women. No studies could be found that investigated the circadian rhythm of inferior vena cava diameter or compliance.

After SEAT there were no observed differences in blood volume in either men or women, yet plasma volume and blood volume have been observed to be slightly higher (~3%)

later in the day (41; 61) (in these referenced studies the activity level of the participants before the afternoon test is unknown). It is therefore presumed that SEAT actually results in slightly lower blood volume in men and women. This could be due to higher blood pressure in the lower body (from the hydrostatic gradient) causing greater plasma extravasation into the extravascular space at the level of the capillary.

When comparing the overnight/morning urine volume (Pre-SEAT) to the afternoon volume (Post-SEAT), there was no difference; however, these data were not normalized for time (duration of the overnight collection was not obtained from participants). Drummer et al. observed urine flow to be greater in the afternoon than in the morning (50). These data were normalized to a 6-hour period in the afternoon and an 8-hour period in the morning. Had the data in this study been normalized to time (~6-hours for the afternoon and ~8-hours for the morning) these results would have also exhibited greater urine flow after SEAT, and the ratio (morning to afternoon) would have been greater than the results of Drummer et al. Considering this, 4-hours of SEAT resulted in lower urine flow which could have been a result of the presumed greater plasma extravasation into the extravascular space due to the hydrostatic gradient. This could also have resulted from the observed increase of vasopressin.

After SEAT, lower urine flow was observed with no change in urine osmolality. Therefore, there was a smaller loss of urinary solutes after 4-hours of SEAT. Drummer et al. observed greater urine flow with lower urine osmolality in the afternoon (50). Therefore, the amount of solute loss should not change due to circadian rhythm. Drummer et al. also observed higher urine sodium flow in the afternoon compared to the morning (50). These data were again normalized to 6-hours in the afternoon and 8-hours in the morning. In the current study, there was a tendency towards higher urine sodium after SEAT. If the data were normalized to time (~6-hours for the afternoon and ~8-hours for the morning), these results would result in a ratio (morning to afternoon) approximately equal to that of Drummer et al. and therefore there were no changes in sodium output beyond circadian rhythm. Thus, there must be lower urinary levels of other unmeasured solutes such as urea or creatinine.

There was lower serum osmolality in men after 4-hours of sitting, and there are no circadian effects (50). These differences due to SEAT could be due to changes in unmeasured substances in the plasma. For example, lower levels of urea and creatinine in the blood have been observed after 4-hours of sitting in men (87). If this is true of women also, the reduced

serum osmolality from the loss of urea and creatinine could be balanced by the observed higher levels of the hormones vasopressin and angiotensin II leading to no change in serum osmolality in women.

Lower urinary output of urodilatin was observed after SEAT, yet Drummer et al. observed higher urinary flow output of urodilatin in the afternoon compared to the morning (50). Thus, the SEAT protocol was responsible for decreasing urodilatin concentration in the urine. Reduced renal blood flow from the orthostatic stress of sitting could have occurred and been partially responsible for the increase in renin and the decrease in urodilatin, but it was not measured. Lower urodilatin should reduce sodium output, but this was not observed. Conflicting signals from another source causing an increase of sodium output could have been responsible. For example, aldosterone was lower in the afternoon (due to circadian rhythm) and this would have elicited greater sodium output compared to the morning.

From all of these observations and after considering the effects of circadian rhythm, we have shown that compared to the baseline condition 4-hours of sitting resulted in: 1) lower mean arterial pressure in women, 2) a tendency toward lower central venous pressure (particularly in women) and a smaller loss with LBNP (likely due to lower baseline), 3) higher vasopressin in men and women, with a greater increase in women, 4) higher renin in men and women, 5) higher angiotensin II in women, 6) smaller loss of aldosterone with LBNP (likely due to the lower baseline from normal circadian rhythm), 7) higher thoracic impedance in men and women, 8) higher pelvic impedance in women, 9) higher inferior vena cava compliance in men and lower compliance in women 10) lower blood volume in men and women, 11) lower urine flow, 12) smaller loss of urinary solutes, 13) lower serum osmolality in men, and 14) lower urodilatin in both men and women. These changes are due to inactivity and/or exposure to 4-hours of orthostatic stress (i.e. sitting). It was shown that women experience difficulty maintaining mean arterial pressure and central venous pressure after 4-hours of sitting despite higher vasopressin, higher angiotensin II, and higher pelvic impedance.

Head-down bed-rest in men and women (follicular phase)

The sexually dimorphic cardiovascular effects of 4-hours of head-down bed-rest (HDBR) have not previously been investigated, and those studies that have been completed in

men have not always taken into consideration the effects of both circadian rhythm and inactivity. The changes after 4-hours of HDBR seen in this study that are actually attributable to circadian rhythm and/or inactivity (according to the changes due to SEAT) are: 1) lower central venous pressure (particularly in women) and a smaller loss due to LBNP (SEAT effect), 2) lower brachial vascular resistance (circadian effect), 3) lower vasopressin in women (circadian effect), 4) lower renin in men (circadian effect), 5) tendency for lower angiotensin II (circadian effect), 6) lower aldosterone and therefore a smaller drop due to LBNP (circadian effect), 7) thoracic impedance is lower (circadian effect), 8) pelvic impedance is lower in women (circadian effect), 9) higher urine output (circadian effect), 10) lower urine osmolality (circadian effect), 11) slightly higher urine sodium (circadian effect), and 12) lower urodilatin (SEAT effect).

Bearing in mind these effects of circadian rhythm and inactivity, the observed effects of 4-hours of HDBR in men and women were: 1) higher heart rate and a greater increase due to LBNP, 2) a greater decrease of stroke volume due to LBNP, 3) lower central venous pressure in men, 4) smaller inferior vena cava diameter with a smaller decrease due to LBNP, 5) higher portal vein resistance index with a greater increase due to LBNP, 6) lower norepinephrine, 7) higher vasopressin in men, 8) higher renin in women, 9) higher endothelin-1 in men and lower in women, 10) a greater increase of thoracic impedance due to LBNP, 11) higher pelvic impedance in men, and 12) lower blood volume.

As discussed in the previous section of this Chapter, central venous pressure was lower after SEAT in both men and women; however this was particularly evident in women. This decrease after SEAT was similar in magnitude to the decrease observed after HDBR in women. However, in men, there appeared to be a greater decrease in baseline central venous pressure after HDBR which has been previously observed in men (62). Fischer et al. suggested that the reason for this loss of central venous pressure after HDBR could be increased central vein compliance and/or changes in blood flow distribution leading to reduced venous return. After HDBR we did not observed any change in inferior vena cava compliance in either men or women as volume (i.e. inferior vena cava diameter) changed proportionally with pressure. The drop in central venous pressure after SEAT in women could have been due to attenuated venous return as shown by the reduction in inferior vena cava compliance. Indeed, there was a slight reduction in stroke volume and an increase of pelvic impedance in women after SEAT

perhaps indicating greater movement of blood to the legs. The drop in central venous pressure in women and men after HDBR was also likely due to reduced venous return, but perhaps for different reasons.

The augmented changes in heart rate, stroke volume and thoracic impedance with LBNP indicated that venous return is smaller during an orthostatic stress after HDBR in both men and women. The smaller diameter of the inferior vena cava (caudal to the junction with the hepatic veins) further supported this hypothesis. Another contributing factor to reduced venous return could be the observed reduction in blood volume in both men and women. While there could be some fluid extravasation in the upper limbs with HDBR, it is not likely that this would be greater than that seen in the legs during sitting due to the higher blood pressure in them (i.e. there are more capillary beds in the legs due to greater muscle volume). Another explanation could be that of splenic extravasation. Portal hypertension has been shown to decrease splenic venous flow without changing splenic arterial flow in male rats (98). This would increase intra-splenic pressure, and higher intrasplenic pressure has been shown to increase fluid extravasation into the lymphatic system of rats (5). If this is true of humans as well, the observed increase in portal vein resistance index after HDBR could lead to splenic extravasation. Enlarged spleens have indeed been observed after 6-8 hours of 10^{o} HDBR (9).

Higher portal vein resistance index was observed after 4-hours of HDBR indicating that blood is not pooling in the splanchnia as shown by Fischer et al. They found greater portal vein flow (i.e. reduced resistance) after 4-hours of HDBR in men (62). One possible protocol difference is that Fischer et al. allowed participants to drink water closer to the afternoon test, but water drinking has previously been shown not change portal vein flow (156). There were differences in the placement of the ultrasound probe that may also be important to consider. Fischer et al. found the diameter of the portal vein from a ventral position on the rib cage and the velocity was determined from a lateral position on the rib cage. This could have led to a source of error as the two sites of insonation may not have been the same. The data in the current study imply that the observed changes in portal vein resistance index should have actually been protective of venous return in both men and women rather than detrimental.

After 4-hours of HDBR, lower norepinephrine was observed in both men and women. However, men exhibited higher endothelin-1 and higher vasopressin. The higher levels of these hormones could have counteracted the lower norepinephrine in order to maintain peripheral

resistance and mean arterial pressure in men. There was no increase of vasoconstrictor hormones in women after HDBR (i.e. endothelin-1, vasopressin, angiotensin II) that could counteract the decrease of norepinephrine. Thus, with lower levels of norepinephrine and no compensatory increase in another measured vasoactive hormone, women may not be able to increase peripheral resistance sufficiently to maintain mean arterial pressure during an orthostatic stress.

Lastly, men exhibited higher pelvic impedance after HDBR which indicated that there is less blood in the pelvic region. However, there are no sexually dimorphic differences in regard to portal vein resistance index after HDBR indicating that flow from the mesentery, stomach and spleen is not different. Since volume, pressure and compliance of the inferior vena cava, which runs through the pelvic cavity, is also not different between men and women, the difference in blood pooling could be in the pelvic organs. If men are mobilizing pools of blood from the pelvic organs this could help to maintain venous return. Flow of the internal iliac should be investigated in future studies.

Conclusions

The importance of proper controls has been demonstrated for studies done over multiple hours in terms of menstrual cycle, circadian rhythm, inactivity, and posture. The results have shown some important sexually dimorphic differences when investigating a 4-hour orthostatic stress/inactivity model (SEAT) or a 4-hour simulated microgravity model (HDBR). The former model is increasing in importance as society is becoming more sedentary and sitting for 4-hours is commonplace (i.e. watching television, traveling, or working at a computer). The 4-hour head-down bed-rest model is relevant as both a model for short-term exposure to microgravity and as a model for supine bed-rest. The head-down position merely accelerates the cardiovascular changes that normally occur with supine rest (i.e. removal of the hydrostatic pressure gradient). It was shown that both of these postural situations (SEAT and HDBR) change the normal cardiovascular response to an orthostatic stress in both men and women.

It was shown that women during the follicular phase experience lower central venous pressure after 4-hours of SEAT in comparison to the luteal phase. Therefore, women in the

follicular phase of the menstrual cycle may have reduced venous return in comparison to the luteal phase after 4-hours of SEAT, perhaps stemming from an attenuated increase in portal vein resistance index resulting in splanchnic blood pooling. After HDBR, women in the follicular phase exhibited pelvic blood pooling (i.e. lower pelvic impedance) which could be a function of lower vasopressin and angiotensin II.

Lower mean arterial pressure was observed during LBNP in women before SEAT or HDBR (as discussed in Chapter 3); however, neither intervention changed the pressure response to LBNP. After 4-hours of SEAT and in comparison to men, women have lower mean arterial pressure and central venous pressure despite higher vasopressin and angiotensin II. This could be due to lower inferior vena cava compliance and unmeasured peripheral pooling in women after SEAT. After HDBR men have higher levels of the vasoconstrictor hormones vasopressin and endothelin-1. These changes are not observed in women, in fact, women decrease endothelin-1. This could decrease the ability to maintain peripheral resistance in the face of an orthostatic challenge in women, and thus decrease the ability to maintain blood pressure. Furthermore, there is higher pelvic impedance in men after HDBR which could indicate a greater movement of blood pools from pelvic organs into the inferior vena cava in an attempt to maintain venous return. This would be protective of venous return in order to maintain mean arterial pressure.

Future Directions

Research in this area will continue to investigate the sexually dimorphic cardiovascular responses to simulated microgravity and orthostatic stress while controlling for menstrual cycle and inactivity. It might also be prudent to ensure that circadian rhythm does not differ between the sexes. This could be done by examining the baseline measurements and the cardiovascular responses of men and women to LBNP before and after a 4-hour period of "normal" activity.

There are some vascular beds that were not investigated as a part of this study. For example, investigation of the femoral, renal and internal iliac artery would give a more robust description of blood pooling throughout the body. The discussion of the changes in regional blood flow throughout the menstrual cycle described the potential for differences in the leg and renal vasculature between phases during an orthostatic stress. Furthermore, studies done with

longer term bed-rest have found that leg vascular resistance decreases in women after 56-days of HDBR (54) and increases in men after 14-days of HDBR (40). While these periods of time are vastly different it allows for the possibility of sexually dimorphic differences if leg vascular resistance were studied in men and women over similar periods of time in the same investigation. Flow of the internal iliac artery would describe any blood pooling that might exist in the pelvic organs.

There could be changes in the urine and plasma that were not measured in this study. Measurements of potassium, urea and creatinine could help to explain changes in serum and urine osmolality. Similarly, measurements of more vasoactive substances in the blood, such as prostanoids, reactive oxygen species and dopamine, could help to further explain sexually dimorphic differences. There is also evidence that receptors for vasoactive compounds can be changed by sex hormones (i.e. estrogen downregulates angiotensin II receptors while progesterone upregulates the receptors (137)) or even just by circadian rhythm (141). Thus, future studies investigating sexually dimorphic cardiovascular responses during receptor agonist infusion or receptor blockades could be insightful.

Limitations

1) In order to move participants from the chair to the LBNP apparatus participants stood up and walked approximately 4 steps. When participants moved from HDBR to the LBNP apparatus they moved straight from one bed to the other to avoid the orthostatic stress of standing, but some exertion was necessary. Both transitions took approximately 5-10 seconds.

2) We investigated the effects of 4-hours of either a constant orthostatic stress (i.e. sitting) or simulated microgravity (i.e. head-down bed-rest); however we are testing the effects of LBNP on the participants while lying down. Thus the changes that occur due to sitting or bed-rest need to persist into this new posture. For example, the measurements of atrial natriuretic peptide may not be reflective of the SEAT or HDBR position as it took approximately 30 minutes of instrumentation after lying down to take the first blood sample. Thus while supine after SEAT, this period would be an atrial load eliciting higher atrial natriuretic peptide, and after HDBR this period would be unloading the atrium eliciting lower atrial natriuretic peptide.

3) Subjects were asked to eat a small breakfast at 6:00am before coming to the lab to start our study at 7:00am. The first portal vein images would have been taken at approximately 8:30am. Participants may have eaten later than asked, and if this is the case, morning portal vein flow may be higher than it should be due to a postprandial increase of flow.

4) The measurements of plasma nitrates/nitrites reflect the levels of the whole body and not just a localized area such as the leg or skin to determine if endothelial function changes in a particular area.

5) Before starting the afternoon LBNP test, participants were strongly encouraged to urinate; however some participants elected to wait until after the LBNP test when they could use a washroom to collect the urine sample. Those participants that elected this route would have had lower pelvic impedance due to the urine in the bladder. Men and women chose this option equally (~25% of the time).

Chapter 5

Cardiovascular responses to sitting and standing in younger and post-menopausal women and age-matched men

This chapter investigates the cerebrovascular and cardiovascular responses of younger and older women and men during the transition from supine to upright posture. Previous chapters of this thesis investigated the cardiovascular responses of young women and men to simulated orthostatic stress (LBNP) yet did not include direct measurements of brain blood flow because there were only small changes in mean arterial pressure anticipated. This study was designed to investigate both the sexually dimorphic responses to orthostatic stress and the effects of female sex hormones by comparing pre- and post-menopausal women to each other and to age-matched men. This design allowed for examination of the effects of the loss of endogenous female sex hormones after menopause while minimizing the effects of ageing.

The erect posture elicits a hydrostatic gradient for blood pressure and results in higher pressure in the legs and lower arterial pressure in the head (82). The active transition to an upright posture results in a transient drop of peripheral vascular resistance due to vasodilation and thus pooling of blood in the legs. This also leads to a transient reduction of mean arterial pressure. To compensate for this, heart rate and total peripheral resistance increase (via vagal withdrawal, sympathetic activation, and vasoconstriction) in order to restore mean arterial pressure (reviewed in (152)). Fainting, or orthostatic hypotension, results from an inability to maintain blood pressure with a consequent reduction in brain blood flow; this is known to be particularly common in young women (151).

Female astronauts exhibit greater orthostatic hypotension upon return to Earth (200), and women have a greater propensity towards postural orthostatic tachycardia syndrome (POTS) (75; 176). There are many hypotheses in the literature for the reasons behind the greater incidence of orthostatic hypotension in women compared to men including greater splanchnic blood flow (202), lower sympathetic output (86), or reduced cardiac filling leading to an inability to maintain sufficient cardiac output (66). Brain blood flow regulation itself may also play a role considering that women have a greater cerebrovascular reactivity to changes in carbon dioxide (97) and that post-pubescent girls (aged 10-16) have impaired cerebral autoregulation (191). No information could be found investigating autoregulation in women between the ages of 16-70.

Before and during a simulated orthostatic stress (lower body negative pressure; LBNP), post-menopausal women exhibit higher systolic and mean arterial blood pressure with an attenuated increase in heart rate compared to pre-menopausal women (83). Therefore, female

sex hormones could be partially responsible for the greater incidence of orthostatic hypotension in young women. Indeed, after estrogen replacement therapy, post-menopausal women exhibit lower systolic, diastolic, and mean arterial pressure before and during LBNP (83).

Orthostatic hypotension is common in the elderly (~34% incidence (85)); however, studies of "elderly" participants usually involve participants who are over the age of 70. In response to standing (i.e. within seconds of the postural transition), elderly participants (a mixed sex group >70 years old) exhibit a greater decrease in mean arterial pressure possibly due to a smaller compensatory increase in heart rate, yet they also exhibit a smaller decrease of MCA velocity (170) (i.e. preserved brain blood flow) which could partially be a function of lower resting flow. The velocity of blood in the middle cerebral artery (MCA) declines with age in men and women to the same degree (2; 45) indicating that there is lower resting cerebral blood flow in older populations. This is especially true considering that with age the diameter of the MCA either gets smaller (autopsy)(140) or stays the same (image analysis of cerebral angiograms)(133). The current study uses a group of participants 57.2 ± 1.7 years old and was designed in an attempt to isolate the effects of menopause while minimizing the effect of age.

It was hypothesized that during standing young women would exhibit a greater decrease in mean arterial pressure with a greater reduction in brain blood flow compared to both young men and post-menopausal women due to decreased venous return and impaired autoregulation (i.e. a greater decrease of brain blood flow for a given decrease of cerebral perfusion pressure). It was further hypothesized that there will be few differences in the cerebrovascular and cardiovascular responses to orthostatic stress between post-menopausal women and age-matched men.

5.1 Materials and Methods

Participant description

Forty men and women participated in this study, ten young women, eleven young men, ten older women, and nine older men. The group of young women all participated in the follicular phase of the menstrual cycle (day 8-11) and were either not taking oral contraceptives (OC, n = 3) or were taking cyclic-OC (n = 7). The older women were each post-menopausal for at least one year (6.2 ±1.3 years; Range: 2-15 years). Age: (Young women: 23.5±0.8; Young men: 24.5±1.2; Older women: 57.2±1.7; Older men: 57.0±1.4). BMI: (Young women: 24.2±1.5; Young men: 25.8±0.8; Older women: 23.9±1.3; Older men: 26.8±1.1). Participants refrained from exhaustive exercise and alcohol for 24 hours prior to testing and refrained from caffeinated beverages for 12 hours prior to testing.

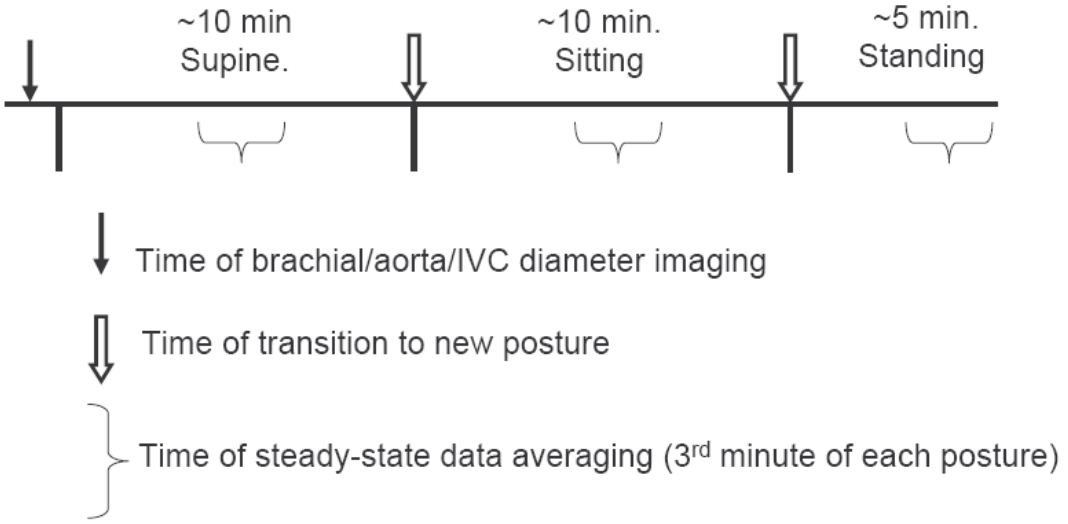
Arm volume measurement

Arm volume was measured with water displacement. First, hand volume was measured in duplicate, followed by forearm displacement in duplicate. The arm volume used is the difference of the two. (Young women: 633.3±65.1mL; Young men: 988.6±74.3mL; Older women: 564.6±35.5mL; Older men: 927.6±47.5mL).

Body surface area

For the purposes of normalization of data to body size the Dubois and Dubois formula was used to determine body surface area. (Young women: 1.75±0.07m^2; Young men: 1.99±0.06m^2; Older women: 1.68±0.04m^2; Older men: 2.05±0.04m^2).

Posture change protocol



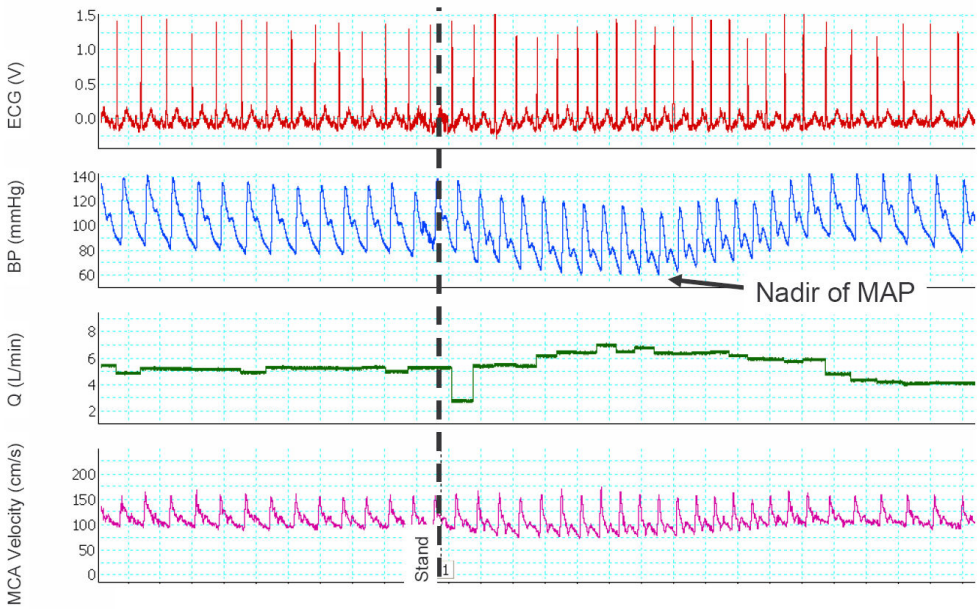
Participants were instrumented with ultrasound probes (transcranial, brachial, and aorta), ECG electrodes, impedance electrodes, nasal cannula, and the Finometer while supine. Images of the inferior vena cava, aortic diameter and brachial diameter, and baseline measurements of heart rate, mean arterial pressure, cardiac output, brachial vascular resistance, thoracic and pelvic impedance were taken while supine (Impedance data are found in Appendix IV). The participants then moved to a seated position (this moment is referred to as "Sit" for transition graphs) on the side of the bed in one fluid motion with the physical assistance of a researcher (to avoid displacement of the transcranial Doppler and the finger blood pressure probes). A chair back was placed behind them for their comfort and relaxation. Measurements were taken again in this position. The participants stood up and were offered a pole for balance purposes (this moment is referred to as "Stand" for transition graphs). Measurements were again taken. The participants were asked to sit down immediately if systolic pressure dropped to <70mmHg or at any time the participant experienced nausea, dizziness or light-headedness. One young woman could not maintain the standing posture for 5 minutes.

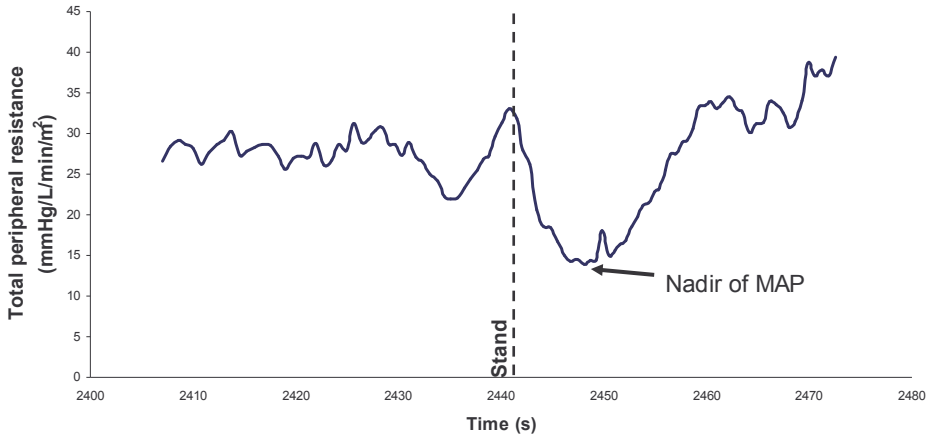
Steady-state data

Steady-state measurements of brachial vascular resistance, heart rate, stroke volume, cardiac output, mean arterial pressure, total peripheral resistance, and middle cerebral artery velocity were averaged during the 3rd minute of each posture. Measurements of ET-CO_2 and respiration rate were averaged for the 3rd and 4th minute of each posture.

Transitional periods

Transitional data for mean arterial pressure, cardiac output, stroke volume, heart rate, total peripheral resistance, and middle cerebral artery velocity were taken at the time of the nadir of mean arterial pressure (as shown in the figures below).





Imaging

Cross sectional area of the aorta, brachial artery and inferior vena cava were obtained in the supine position with ultrasound imaging (Micromaxx, Sonosite Inc., Bothell, WA, USA) using a HFL38 transducer (6-13MHz; brachial artery) or a P17 transducer (1-5MHz; aorta, inferior vena cava). Diameters were obtained using the caliper function on the Micromaxx Sonosite.

Systemic beat-by beat measurements

Blood pressure was measured using finger-cuff plethysmography (Finometer; Finapres Medical Systems, Arnheim, The Netherlands), and heart rate was measured from the R-R interval of the electrocardiogram (COLIN PILOT, Colin Medical Instruments Corp., San Antonio, TX, USA). Blood flow velocity from the aortic root and the brachial artery were recorded using a 2MHz and 4MHz probe, respectively (Neurovision Transcranial Doppler System Model 500M, Multigon Industries Inc., Yonkers, NY, USA). All signals were output at 1000 Hz to a PowerLab (ADInstruments, Australia) and recorded onto a computer running Chart 5.5.1 for future analysis.

Using the baseline velocity of the aorta, stroke volume was determined with the calculation SV (mL) = velocity*(π*radius^2)*R-R interval. Cardiac output was then determined by multiplying heart rate by stroke volume. This value of cardiac output was used to normalize the beat-by-beat cardiac output from the Finometer. The cardiac output from the Finometer

was used for data presented because of technical limitations in maintaining the aortic velocity signal during postural changes. Total peripheral resistance was calculated as mean arterial pressure divided by cardiac output. Brachial arterial flow was determined from the cross-sectional area of the brachial artery and the blood velocity [Brachial flow (mL/min) = velocity*(π*radius^2)*60]. Brachial vascular resistance was used as an index of limb vascular resistance and calculated as flow divided by mean arterial pressure. Brachial vascular resistance was no measured during the postural transitions due to technical limitations maintaining the velocity measurements. Cardiac output and stroke volume were normalized to body surface area, and brachial vascular flow was normalized to arm volume.

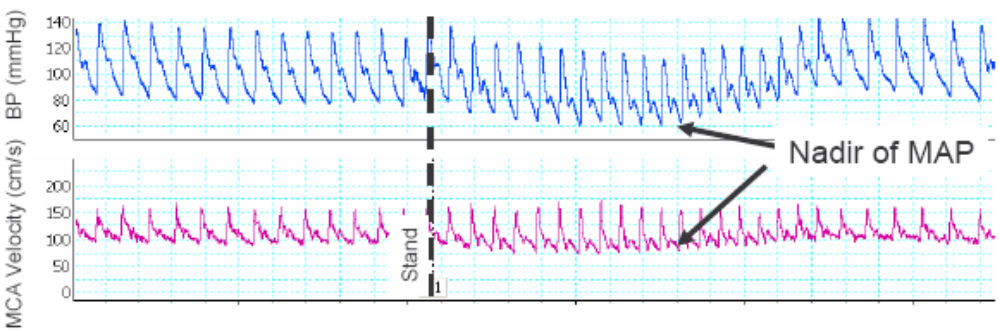
Transcranial Doppler and Cerebral autoregulation

Blood flow velocity from the middle cerebral artery (MCA) was recorded using a 2MHz probe (Neurovision Transcranial Doppler System Model 500M, Multigon Industries Inc., Yonker, NY, USA) held at the temple with an adjustable head-band. The sample size varied for this measurement due to technical difficulties. For the steady state measurements, n=10 for young women, n=11 for young men, n=8 for older women, and n=9 for older men. For the Sit transition, n=7 for young women, n=8 for young men, n=5 for older women, and n=5 for older men. For the Stand transition, n=10 for young women, n=11 for young men, n=8 for older women, and n=9 for older men.

The distance between the location of the transcranial Doppler and the heart was measured (centimeters). This value was converted into mmHg to account for the hydrostatic gradient in an erect posture (1 cmH_2O = 0.735 mmHg) and was subtracted from mean arterial pressure to equal cerebral perfusion pressure (CPP). At the sit-to-stand transition the percent change in middle cerebral artery (MCA) velocity from the seated position to the time point of the nadir of mean arterial pressure was divided by the change in cerebral perfusion pressure from the seated position to the time point of the nadir of mean arterial pressure to give an index of cerebral autoregulation (CA) for each participant as described previously (171).

$$CA = \%\Delta MCA/\%\Delta CPP*100\%$$

(Note: as per van Beek et al. a uniformly used methodology for measuring dynamic cerebral autoregulation does not yet exist (189). This calculation was used to match Sorond et al. whom used a similar sit-to-stand technique).

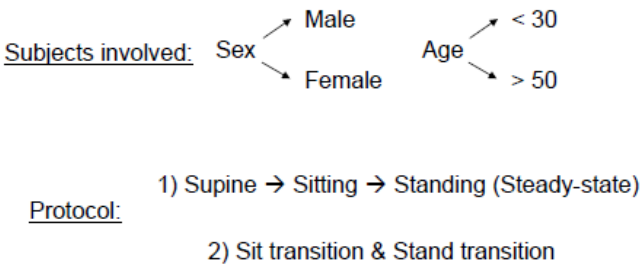


End-tidal CO_2 (ET-CO_2) and respiration rate

Breath-by-breath measurements of ET-CO_2 were measured using a nasal cannula connected to a COLIN PILOT (Colin Medical Instruments Corp., San Antonio, TX, USA). Maximum levels of CO_2 per expiration and duration of time between breaths were calculated using a Chart 5.1.1 macro. Respiration rate was calculated as duration between breaths divided by 60 seconds/minute.

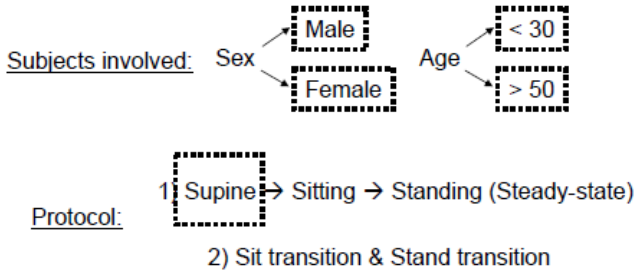
Design and Statistical analysis

The current study was designed to examine the effects of sex and age on the cardiovascular responses to posture change. Data at a steady-state during each different posture and data at the time of transition to a new posture were analyzed separately. Subject groups and the protocol are diagrammed here.



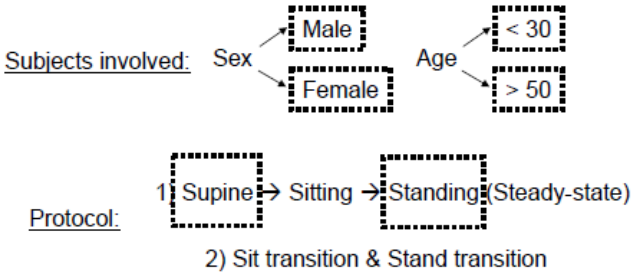
In Chapter 5, various comparisons were planned to determine the effects of sex and age on: 1) baseline differences in vessel size, 2) the change in thoracic and pelvic impedance from supine to standing (Appendix IV), 3) cerebral autoregulation, 4) cardiovascular measurements in different postures, and 5) cardiovascular responses to changing postures.

1. To determine any differences in brachial, aorta, and inferior vena cava diameter due to sex and age, statistical analysis was done with two-way ANOVAs. Factors were sex and age.



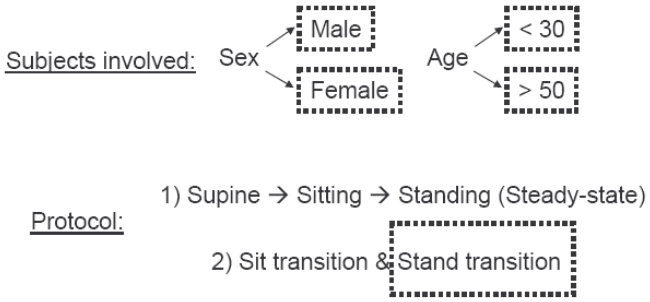
(Each outlined factor is used in the above comparison)

2. To determine any effects of sex and age on the change in thoracic and pelvic impedance (from supine to standing), statistical analysis was done with two-way ANOVAs. Factors were sex and age. (Appendix IV).



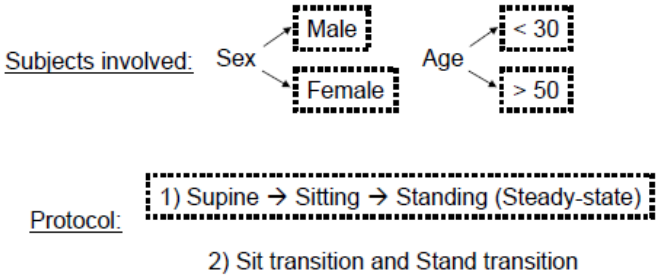
(Each outlined factor is used in the above comparison)

3. To determine the effect of sex and age on cerebral autoregulation during the sit-to-stand transition, statistical analysis was done with a two-way ANOVA. Factors were sex and age.



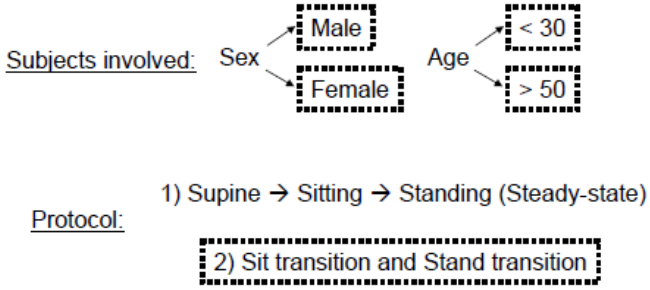
(Each outlined factor is used in the above comparison)

4. To determine the effect of sex and age on cardiovascular measurements in different postures (steady-state measurements), statistical analysis was done with three-way ANOVA s with one repeated measure. Factors were sex, age, and posture.



(Each outlined factor is used in the above comparison)

5. To determine the effect of sex and age on cardiovascular responses to changing postures (at the time of transition), statistical analysis was completed using a three-way ANOVA with one repeated measure. Factors were sex, age, and transition.



(Each outlined factor is used in the above comparison)

Analysis was completed using SAS 9.1.3 analysis software (Cary, USA). *P*-values less than 0.05 are indicated as significant, and *P*-values less than 0.10 are noted throughout.

5.2 Results

(Note: Transitional results in Part B of Figures 39-43 and 46 are found below the Steady-state results)

Steady-state results

Main effects of posture

Changing posture from supine to sitting to standing resulted in increasing mean arterial pressure (P=0.002; Figure 39A), increasing heart rate (P<0.0001; Figure 40A), decreasing stroke volume (P<0.0001; Figure 41A), decreasing cardiac output (P<0.0001; Figure 42A), increasing total peripheral resistance (P<0.0001; Figure 43A), and increasing brachial vascular resistance (P<0.0001; Figure 44). Posture did not affect respiration rate (P=0.225; Figure 45A), but decreased end-tidal CO_2 (P<0.0001; Figure 45B).Changing posture from supine to sitting to standing decreased middle cerebral artery velocity (P<0.0001; Figure 46A). The interaction effects seen with the posture will be described below.

Main effects of sex

There were no significant sexually dimorphic differences in mean arterial pressure (P=0.282; Figure 39A). Women had higher heart rates than men (P=0.029; Figure 40A) with no difference in stroke volume (P=0.517; Figure 41A). There were no sexually dimorphic differences in regard to cardiac output (P=0.708; Figure 42A) or total peripheral resistance (P=0.212; Figure 43A). Women had higher brachial vascular resistance (P=0.001; Figure 44) and a tendency for a higher respiration rate than men (P=0.096; Figure 45A), yet there was no effect of sex on end-tidal CO_2 (P=0.934; Figure 45B). Women exhibited higher middle cerebral artery velocity (P=0.002; Figure 46A). The interaction effects seen with sex will be described below.

Main effects of age

Mean arterial pressure was higher in the older participants (P=0.019: Figure 39A). There was no main effect of age on heart rate (P=0.165; Figure 40A), stroke volume (P=0.119; Figure 41A), cardiac output (P=0.368; Figure 42A), or total peripheral resistance (P=0.806; Figure 43A). Brachial vascular resistance decreased with age (P=0.016; Figure 44). There were no effects of age on respiration rate (P=0.462; Figure 45A), but the older participants had lower end-tidal CO_2 (P=0.026; Figure 45B). There was no effect of age on middle cerebral artery velocity (P=0.287; Figure 46A). The interaction effects seen with age will be described below.

Interaction effects

In response to posture change (particularly comparing the third minute of Sit to third minute of Stand), the older population increased mean arterial pressure compared to a decrease in the younger group (P=0.014; (Posture*Age); Figure 39A). Women had a greater increase of heart rate with posture change (P=0.0005; (Posture*Sex); Figure 40A). The increase in heart rate seen with posture change was attenuated with age ((Posture*Age) P<0.0001; Figure 40A), particularly in women ((Posture*Sex*Age) P=0.073; Figure 40A). The decrease in stroke

volume seen with changing postures was greater in women ((Posture*Sex) P=0.033; Figure 41A). In comparison to men, women exhibited a greater increase of brachial vascular resistance with changing postures (P=0.010; (Posture*Sex); Figure 44), particularly young women (P=0.028; (Posture*Age*Sex); Figure 44). Age attenuated the increase of brachial vascular resistance seen at standing ((Posture*Age) P=0.0006; Figure 44). There was a smaller drop in end-tidal CO_2 due to posture change in the older group which was likely due to a lower supine level ((Posture*Age) P=0.030; Figure 45B).

Transitional results

Main effects of posture transition

Cardiovascular responses during the transitions were sampled at the point of nadir in the arterial blood pressure response. During transitions, mean arterial pressure decreased (Figure 39B), heart rate increased (Figure 40B), stroke volume decreased in the young and increased in the old (Figure 41B), cardiac output increased (Figure 42B), total peripheral resistance decreased (Figure 43B), and middle cerebral artery velocity decreased (Figure 46B). The interaction effects seen with the postural transitions will be described below.

Main effects of sex

There were no effects of sex on the change in mean arterial pressure (P=0.894; Figure 39B) or heart rate (P=0.679; Figure 40B) seen at posture transition. Women had a greater loss (young group) or a smaller increase (older group) in stroke volume during posture transition (P=0.001; Figure 41B) and also exhibited a smaller increase in cardiac output (P=0.004; Figure 42B). Women had a smaller reduction in total peripheral resistance (P=0.021; Figure 43B), and there were no significant effects of sex on middle cerebral artery velocity (P=0.486; Figure 46B) during posture transition. There was no significant effect of sex on cerebral autoregulation index (P=0.303; Figure 47). The interaction effects seen with the sex will be described below.

Main effects of age

There are no main effects of age on the mean arterial pressure response to posture transitions (P=0.753; Figure 39B), yet older participants had smaller increases in heart rate (P<0.0001; Figure 40B). Older participants had an increase in stroke volume with posture transition (compared to the decrease seen in the younger group); however a significant main effect of age is not observed (P=0.412; Figure 41B). Age augmented the increase of cardiac output during posture transitions (P=0.0013; Figure 42B), yet there were no effects of age on total peripheral resistance (P=0.467; Figure 43B). Age led to a smaller decrease in middle cerebral artery velocity during posture transition (P=0.036; Figure 46B). There were no significant effects of age on cerebral autoregulation index (P=0.767; Figure 47). The interaction effects seen with the age will be described below.

Interaction effects

In the younger group the same drop in mean arterial pressure was seen in both the Sit and Stand transitions, but there was a smaller drop in pressure during the Stand transition in the older group ((Posture*Age) P=0.015; Figure 39B). In comparison to the Sit transition, the Stand transition resulted in a greater heart rate response in the younger group and a smaller heart rate response in the older group ((Posture*Age) P=0.001; Figure 40B). Stroke volume decreased during postural transitions in the younger group and increased in the older group ((Posture*Age) P=0.067; Figure 41B).

Anatomical differences

Compared to women, men had larger aortas (P=0.005; Table 8) and larger brachial arteries than women (P<0.0001; Table 8), but the size of the inferior vena cava was the same (P=0.143; Table 8). Age increased the diameter of the aorta (P=0.0002; Table 8) and brachial artery (P<0.0001; Table 8), but not the inferior vena cava (P=0.288). This increase of brachial artery diameter was greater in men ((Age*Sex) P=0.050; Table 8), and there was a tendency towards smaller inferior vena cavae in older women ((Age*Sex) P=0.093; Table 8).

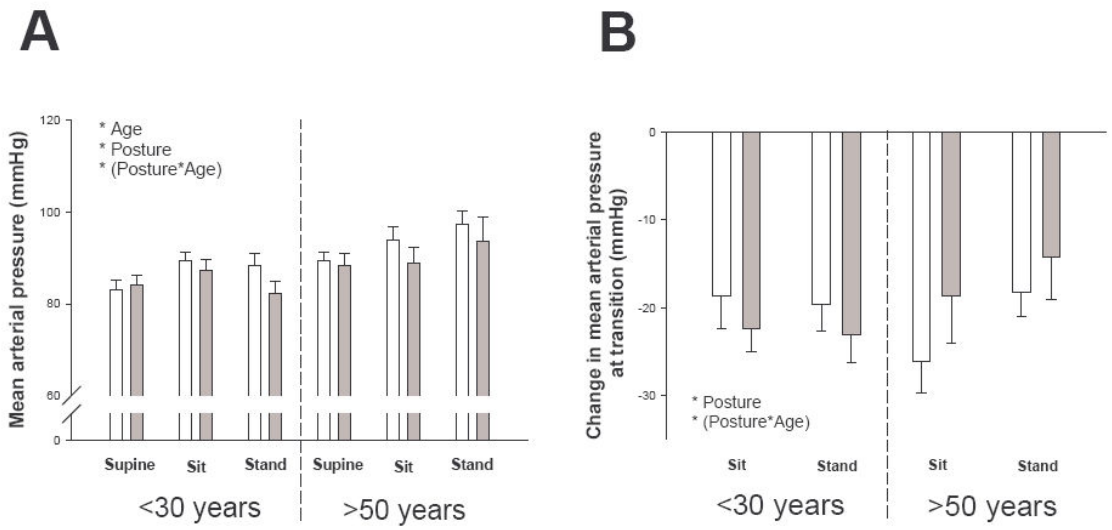


Figure 39: Mean arterial pressure during different postures in men and women. A) Mean arterial pressure while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in mean arterial pressure at the nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Age indicates a significant main effect of age. *Posture indicates a significant main effect of posture.*(Posture*Age) indicates a significant interaction effect between posture and age.

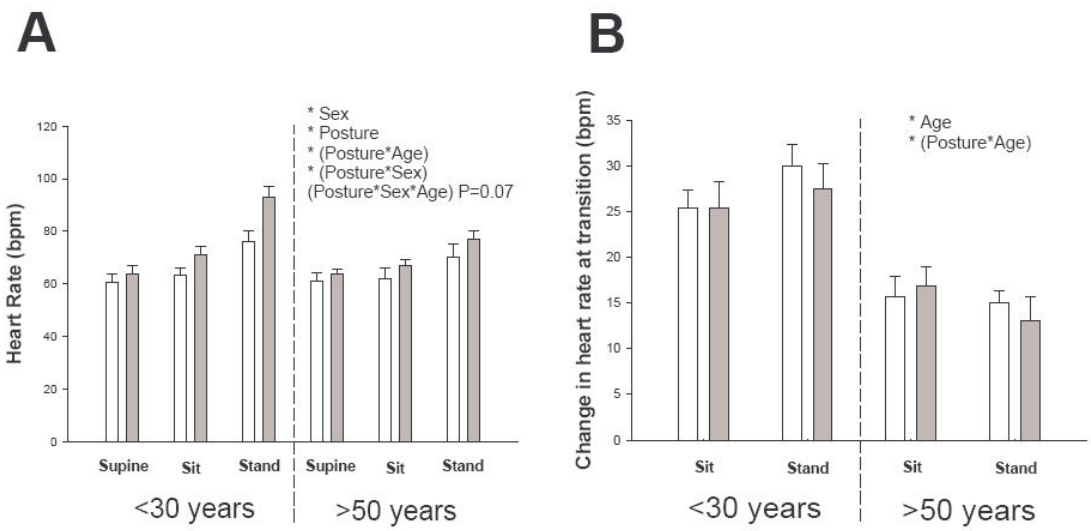


Figure 40: Heart rate during different postures in men and women. A) Heart rate while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in heart rate at nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Age indicates a significant main effect of age. *Sex indicates a significant main effect of sex. *Posture indicates a significant main effect of posture. *(Posture*Age) indicates a significant interaction effect between posture and age. *(Posture*Sex) indicates a significant interaction effect between posture and sex.

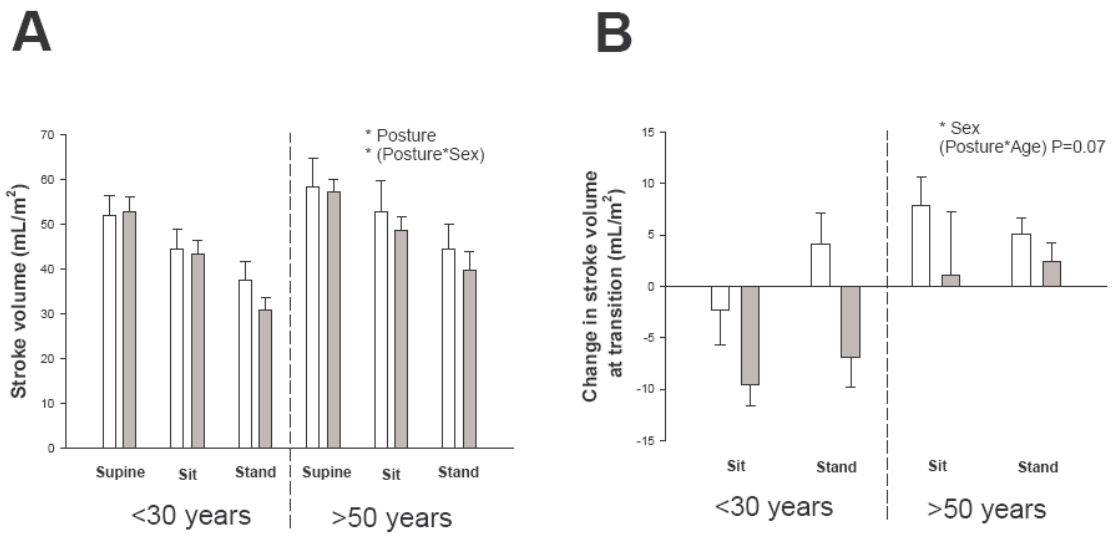


Figure 41: Stroke volume during different postures in men and women. A) Stroke volume while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in stroke volume at the nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Sex indicates a significant main effect of sex. *Posture indicates a significant main effect of posture. *(Posture*Sex) indicates a significant interaction effect between posture and sex.

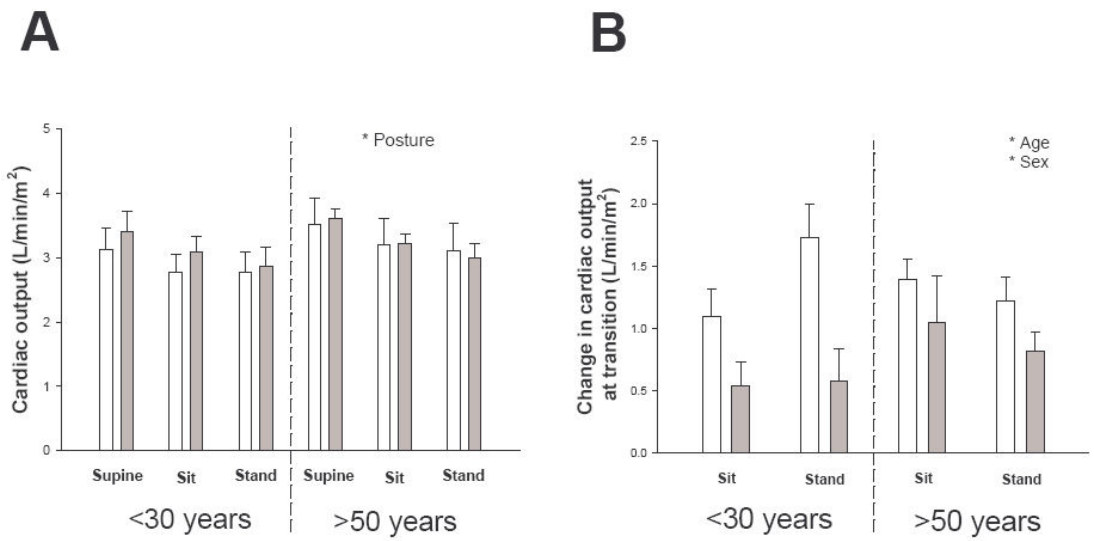


Figure 42: Cardiac output during different postures in men and women. A) Cardiac output while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in cardiac output at the nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Posture indicates a significant main effect of posture.*Age indicates a significant main effect of age. *Sex indicates a significant main effect of sex.

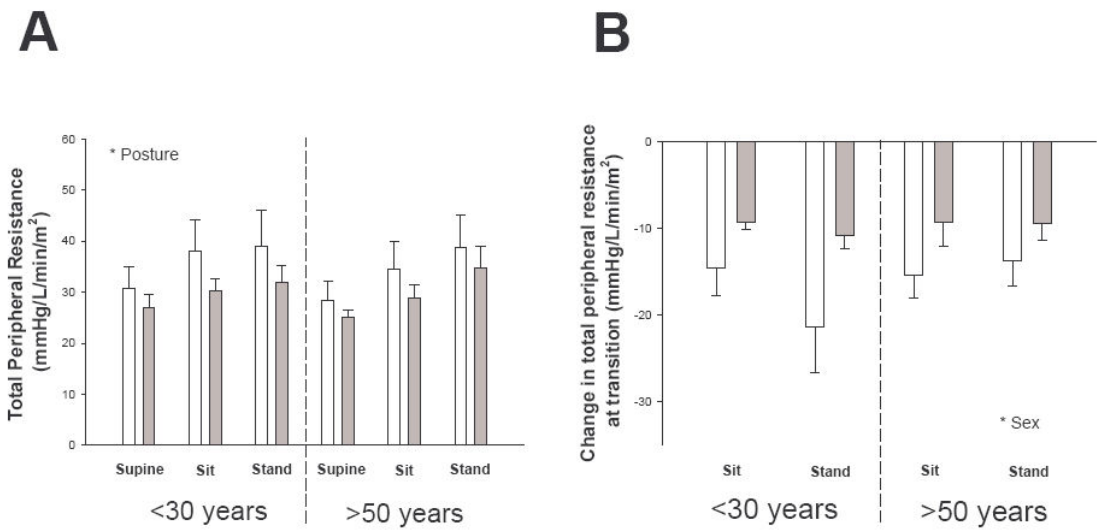


Figure 43: Total peripheral resistance during different postures in men and women. A) Total peripheral resistance while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in total peripheral resistance at the nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Sex indicates a significant main effect of sex. *Posture indicates a significant main effect of posture.

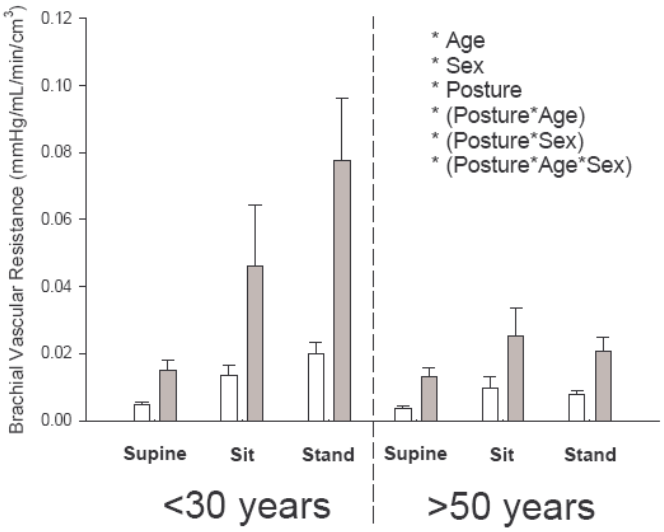


Figure 44: Brachial artery resistance response to posture change in younger and older men (white bars) and women (grey bars). *Age indicates a significant main effect of age. *Sex indicates a significant main effect of sex. *Posture indicates a significant main effect of posture. *(Posture*Age) indicates a significant interaction effect between posture and age. *(Posture*Sex) indicates a significant interaction effect between posture and sex. *(Posture*Age*Sex) indicates a significant interaction effect between posture, age, and sex.

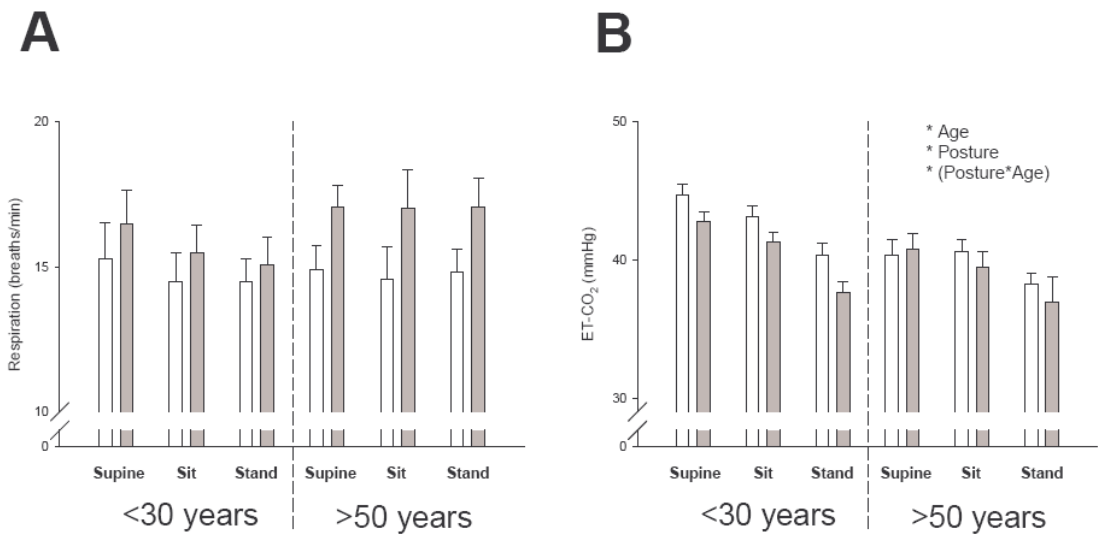


Figure 45: Respiration and end-tidal CO_2 (ET-CO_2) during different postures in men and women. A) Respiration rate while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) ET-CO_2 while supine, sitting and standing in younger and older men and women. *Age indicates a significant main effect of age. *Posture indicates a significant main effect of posture.*(Posture*Age) indicates a significant interaction effect between posture and age.

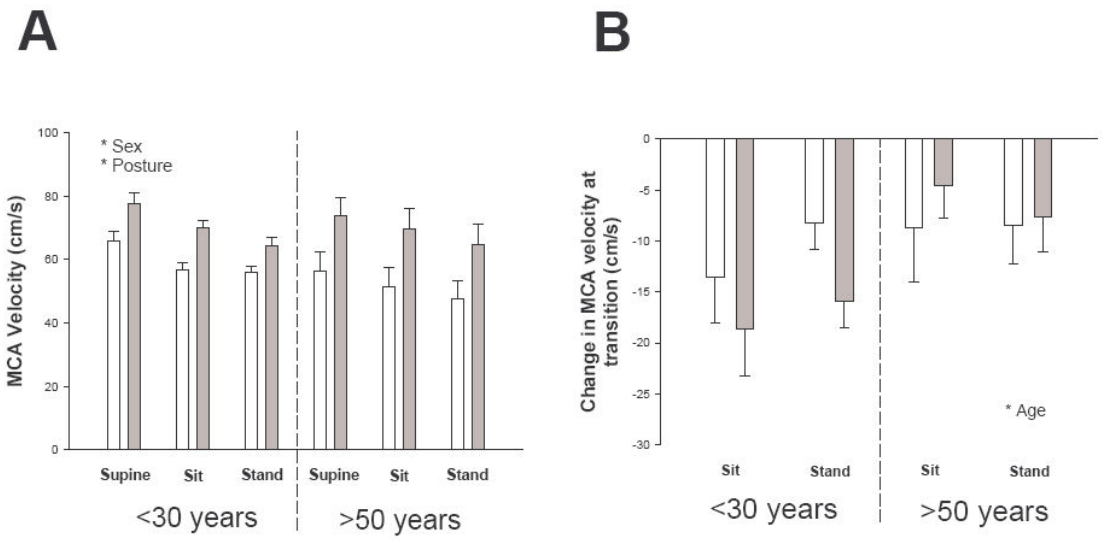


Figure 46: Middle cerebral artery (MCA) velocity during different postures in men and women. A) Velocity of blood in the MCA while supine, sitting and standing in younger and older men (white bars) and women (grey bars). B) Change in MCA velocity at the nadir of mean arterial pressure at the time of transition between positions in younger and older men and women. *Age indicates a significant main effect of age. *Sex indicates a significant main effect of sex. *Posture indicates a significant main effect of posture. (Figure A, n=10 for young women, n=11 for young men, n=8 for older women, and n=9 for older men. Figure B, Sit transition, n=7 for young women, n=8 for young men, n=5 for older women, and n=5 for older men; Stand transition, n=10 for young women, n=11 for young men, n=8 for older women, and n=9 for older men.)

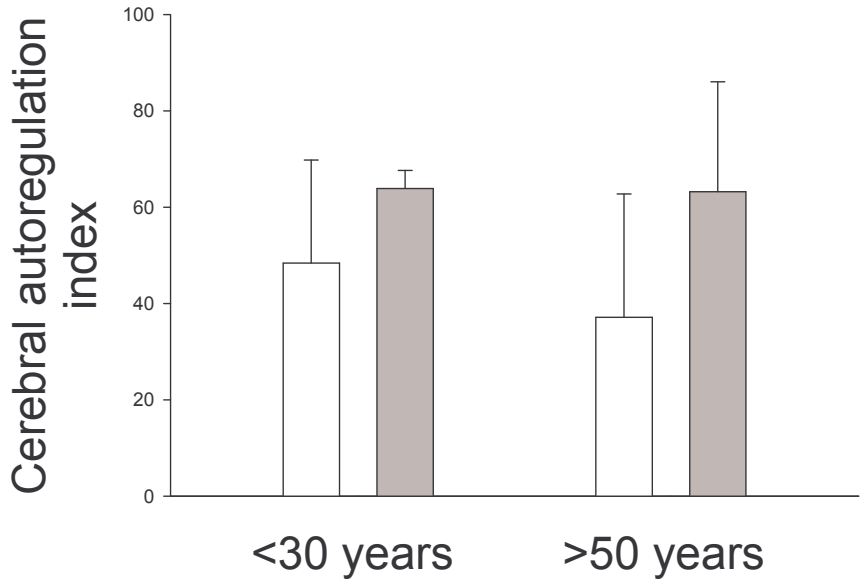


Figure 47: Cerebral autoregulation index in men (white bars) and women (grey bars) at the nadir of mean arterial pressure during the standing postural transition. n=10 for young women, n=11 for young men, n=8 for older women, and n=9 for older men.

Table 8: Vascular diameters in younger and older men and women

Sex	**Age**	**Aorta (cm)‡+**	**Inferior vena cava (cm)**	**Brachial artery (cm)‡+**
Male	< 30	2.32 ± 0.08	1.76 ± 0.16*	0.39 ± 0.01
	> 50	2.72 ± 0.05	1.86 ± 0.14	0.48 ± 0.02 *(Age*Sex) P=0.05*
Female	< 30	2.16 ± 0.09	1.79 ± 0.15*	0.30 ± 0.01
	> 50	2.42 ± 0.07	1.36 ± 0.16 *(Age*Sex) P=0.09*	0.34 ± 0.02

(* These data are from the baseline Pre-HDBR condition found in Chapter 4. ‡ indicates a significant main effect of Age; + indicates a significant main effect of Sex)

5.3 Discussion

Evidence was provided here that indicated that the mechanisms behind blood pressure regulation during either a steady-state or a transitional change in posture in younger and older men and women differed. It was hypothesized that young women would exhibit a greater decrease of mean arterial pressure and brain blood flow during an orthostatic stress. This was not observed in this study; however attenuated venous return indices in young women were observed during both the steady-state and the active transition to new postures. It was also hypothesized that there would be few differences in cardiovascular and cerebrovascular responses to orthostatic stress when comparing post-menopausal women to age-matched men. While this proved to be true for cerebrovascular responses, post-menopausal women still exhibited reduced venous return indices during both the steady-state and the active transition to new postures in comparison to age-matched men.

Systemic hemodynamics

In all participants, the active transitions from supine to sitting and from sitting to standing resulted in a decrease of total peripheral resistance and mean arterial pressure due to vasodilation and movement of blood to the lower body. Thus, there is a compensatory increase in heart rate and cardiac output in an attempt to maintain homeostasis. The decrease of total peripheral resistance was greater in men and could be due to greater leg volume and/or a greater number of dilated vessels. The drop in total peripheral resistance during active transitions was the same with ageing which could be due to similar body sizes of the participants (young vs. older). (Leg volume was not directly measured; however body surface area was not different with age).

During the postural transitions, despite a smaller decrease in total peripheral resistance (i.e. less vasodilation in the lower body), young women were unable to maintain stroke volume leading to a smaller increase of cardiac output in comparison to young men. This indicates an inability to maintain venous return during an orthostatic stress in young women. In comparison to the post-menopausal women during postural transition, young women had a similar decrease in total peripheral resistance, yet stroke volume and cardiac output were slightly lower. These

results indicate that venous return is better maintained during a postural change after menopause. In comparison to the age-matched men, post-menopausal women displayed a smaller drop in total peripheral resistance during the transitions with a smaller increase in stroke volume and cardiac output. Therefore, venous return is higher after menopause, but it still does not equate to that seen in age-matched men (likely due to an age-related increase in venous return in men as well).

In all participants, the changes due to the sitting and standing steady-state postures included increasing heart rate, decreasing stroke volume, decreasing cardiac output, and increasing total peripheral resistance. The hydrostatic gradient inherent with an erect posture results in higher blood pressure in the legs and lower blood pressure in the brain. Therefore, these changes occurred in order to maintain mean arterial pressure and cerebral perfusion pressure during the orthostatic stress. Women exhibited a greater increase of heart rate during steady-state orthostatic stress which was likely due to insufficient stroke volume. This was particularly evident in the younger women. Therefore, similar to the responses to active postural transitions, women exhibit reduced venous return.

This attenuated venous return in women compared to men could be due to the presence of estrogen. The post-menopausal women studied in this investigation were young (57.2±1.7 years old) and were only required to be menopausal for one year (6.2 ± 1.3 years postmenopausal). Therefore, some residual estrogen could still be present in some participants (147), yet estrogen levels would be significantly lower than those seen in the younger cycling women. Higher estrogen levels could be responsible for greater vasodilation in vascular beds (reviewed in (122)) and thus greater blood pooling. This would decrease venous return to the heart. For example, it has previously been shown that a group of post-menopausal women taking hormone replacement therapy (HRT) have higher resting femoral blood flow (and shear stress) than post-menopausal women that are not taking HRT, yet they still have lower flow than pre-menopausal women (131).

Mean arterial pressure was higher in the older groups with steady-state standing and the drop of mean arterial pressure was attenuated during the transition to standing. Therefore, the stimulus for an increase of heart rate via the arterial baroreceptors was attenuated in the older groups. Indeed, it was observed that heart rate increased more during steady-state standing and during postural transitions in the younger groups. Baroreflex sensitivity declines in older

populations (143) which could also help to explain why there was a greater increase of brachial vascular resistance with standing in the younger participants. Similarly, there is an even greater increase of brachial vascular resistance in women which could be due to a more sensitive arterial baroreflex in women compared to men (86). Furthermore, women had a greater reduction of stroke volume during steady state orthostasis reflecting lower cardiac filling and reduced activation of the cardiopulmonary baroreceptors and thus higher sympathetic output and brachial vascular resistance. Young men also exhibited a greater increase in brachial vascular resistance compared to the older group which could have been due to slightly more sensitive cardiopulmonary baroreceptors (184). Tanaka et al. found that young men showed a greater inhibition of forearm resistance with a hypervolemic stimulus implying that young men would also show a greater increase of resistance with a hypovolemic stimulus such as an orthostatic stress (compared to older men). Older participants had lower baseline brachial vascular resistance which in part could have been due to larger brachial arteries (observed previously (13)) or arterioles. The arm volumes of the older participants were not different than the younger participants. Therefore, baseline differences due to age could be a function of vascular remodeling.

There were no significant differences in the supine diameter of the inferior vena cava. However, it tended to be smaller in the group of older women. This could have been a result of lower resting femoral and splanchnic blood flow in post-menopausal women (51; 131). Leg vein diameter, compliance and capacitance do not change with age in women during an orthostatic stress (105; 117); however, there is very little known about splanchnic blood flow with age and orthostatic stress. If post-menopausal women do not exhibit the same degree of splanchnic blood pooling as seen in young women with an orthostatic stress, this could be a contributing factor to the preserved stroke volume. Older men exhibit lower leg venous compliance and capacitance with LBNP (139) which may a play a role in their higher stroke volume and cardiac output due to better venous return.

Mattace-Raso et al. observed an association between arterial stiffness and orthostatic hypotension in individuals older than 55 (119). As greater orthostatic hypotension was not observed in the older groups (~57 years of age) it is speculated that arterial stiffness had not yet reached an appropriate level to affect incidence of orthostatic hypotension. In support of this Takahashi et al. found that there was no difference in arterial stiffness between pre-menopausal

women and post-menopausal women in their 50s (183). Therefore, arterial stiffness may play only a minimal role in this study. However, it is known that women have lower central and peripheral arterial stiffness than men until they are aged 70+ (129) which may play a role in the reduced venous return observed in women.

Brain blood flow

Higher middle cerebral artery (MCA) velocity in women was observed during steady-state postures compared to age-matched men. Velocity of the blood in the MCA was used as an index of brain blood flow (i.e. if velocity is higher it is assumed that flow is higher as the diameter of the middle cerebral artery does not change with simulated orthostatic stress (lower body negative pressure) (162)). Contrary to the hypothesis that women would experience a greater loss of brain blood flow with an orthostatic stress, there was no sexually dimorphic difference in the response to sitting or standing (both steady-state and transitional). Women of all ages are known to have smaller middle cerebral arteries and thus higher velocity (44; 133; 186; 191), yet it has also been previously shown that pre-menopausal women have greater resting brain blood flow compared to age-matched men (in spite of a smaller brain mass) (79; 80). Therefore, the observations in this thesis of higher velocity in women are not simply a function of diameter, they are indicative of greater flow.

Women did not exhibit different brain blood flow or mean arterial pressure with orthostatic stress compared to age-matched men, and there was no significant difference in the cerebral autoregulation index (the percent change in MCA velocity divided by the percent change in cerebral perfusion pressure) between sexes. It was hypothesized that women would exhibit a greater loss of brain blood flow for a given reduction in cerebral perfusion pressure (i.e. greater cerebral autoregulation index); however this was not the case. Perhaps with greater sample sizes a sexually dimorphic difference would become evident (i.e. women appear to have a greater autoregulation index than men). (Young women: 63.9±3.8; Older women: 63.2±22.8; Young men: 48.4±21.4; Older men: 37.1±25.6). Post-pubescent girls (aged 10-16) show a greater loss of blood flow in the middle cerebral artery with a given change in cerebral perfusion pressure compared to age-matched boys (191). If with greater statistical power the results of this thesis had shown that women had a greater cerebral autoregulation index (i.e.

poorer cerebral autoregulation similar to the results of Vavilala et al.), women would have had more difficulty maintaining brain blood flow in the face of an orthostatic challenge. Future studies will investigate this further.

There were no changes in baseline MCA velocity due to age, and there were no changes in the response of MCA velocity to steady-state periods of sitting and standing with age. However, with age there was an attenuated loss of MCA velocity during the transitions to sitting and standing. Changes in the diameter of the middle cerebral artery with age are contentious. A study investigating the cross-sectional area of the middle cerebral artery of deceased individuals indicates that the area decreases with age (comparing ages 25-44 to ages 45-64) in both men and women (140), and that the decrease in women is greater than the decrease in men. However, there were only 3 women studied in each group and this was a post-mortem investigation. Using 200 cerebral angiograms, Muller et al. found that in both men and women there were no changes in the diameter of the middle cerebral artery with age (133). Therefore, if it is accepted herein that the artery diameter doesn't change with age according to Muller et al. it can be assumed that the changes in the MCA velocity truly reflect changes in brain blood flow with ageing. The attenuated loss of brain blood flow during transition in the older groups could be due to the observed greater cardiac output and stroke volume preserving brain blood flow. These results correspond to those of Sorond et al. who found an attenuated loss of brain blood flow in an elderly group during the standing transition (170). There was no effect of age on the cerebral autoregulation index which agrees with previous studies in adult mixed sex populations (26; 170; 204).

Lower end-tidal CO_2 (ET-CO_2) and MCA velocity were observed with sitting and standing. Brain blood flow has been previously shown to decrease with lower arterial CO_2 (113) which decreases with ET-CO_2 during upright tilt or standing (91; 161). There are many hypotheses as to the reasons behind lower arterial CO_2 and ET-CO_2 with orthostatic stress including lower venous return reducing the delivery of CO_2 to the lungs (6). More recently, Serrador et al. and Richardson et al. suggest that the chemoreflex control of breathing is altered in the erect posture (149; 161). Kastrup et al. found that the relationship between MCA velocity and ET-CO_2 was greater in women than in men (mean age, 32) (97). Therefore, for a given drop in ET-CO_2 there should be a greater drop in MCA velocity in young women (there was some evidence of this during steady-state sitting versus standing (Young men sitting:

56.7±2.3cm/s, standing: 56.1±1.8cm/s; Young women sitting: 70.1±2.2cm/s, standing: 64.2±2.8cm/s)). Thus, young women should have a greater loss of brain blood flow with standing (compared to young men) due to greater CO_2 reactivity which could contribute to the greater incidence of orthostatic hypotension.

In the current study, ET-CO_2 decreased with age and there was a smaller decrease of ET-CO_2 with standing (which may have been due to the lower baseline). This decrease with age was observed previously (47). Dhokalia et al. suggest that this lower resting ET-CO_2 may represent a progressive metabolic acidosis with age and/or a poorer ability of the kidneys to excrete an acid load. Furthermore, after the age of 50 cerebrovascular reactivity to CO_2 is not different between men and women (i.e. reactivity stays the same in men and decreases in women) (96). Therefore in older men, a smaller decrease of ET-CO_2 with an orthostatic stress could lead to better maintenance of brain blood flow. In older women, both the smaller decrease of ET-CO_2 with an orthostatic stress and the lower reactivity to CO_2 could lead to better maintenance of brain blood flow.

Conclusions

It was hypothesized that young women would exhibit a greater decrease in both mean arterial pressure and brain blood flow during orthostatic stress than the other groups. This was not observed during this study. However, there was an inability to maintain venous return during orthostatic stress in young women compared to age-matched men and post-menopausal women. This was shown by lower stroke volume and higher heart rate during the steady-state comparisons and lower stroke volume and cardiac output during the transitions. The hypothesis that young women would exhibit impaired cerebral autoregulation was not directly supported by this study.

It was also hypothesized that there would be few differences in cardiovascular and cerebrovascular responses to orthostatic stress between the older men and women. After menopause women had greater venous return indices compared to younger women but still not equal to age-matched men. This was shown by lower stroke volume and higher heart rate during the steady-state comparisons and due to reduced stroke volume and cardiac output during the transitions to sitting and standing (compared to age-matched men). These results

indicate that in response to orthostatic stress women (particularly young women) exhibit a greater reduction in venous return, stroke volume and cardiac output. Furthermore, previous investigations suggest that it is possible that the reduction in end-tidal CO_2 observed with standing could result in a greater loss of brain blood flow in young women due to higher reactivity.

Future directions

This study investigated women aged 50+ who were not taking any hormonal replacement and compared their responses to those of younger women in order to determine the effects of female sex hormones on the cardiovascular system. In order to make any firm conclusions about the effects of estrogens and progestins, a group of post-menopausal women who are taking hormone replacement therapy would benefit this study as age would not be a confounding variable. Furthermore, an analysis of endogenous estrogen and progestin concentrations in the plasma will help to verify the conclusions. This study would also benefit from a more accurate description of regional blood flow during standing, in particular that of the splanchnic area. If splanchnic blood flow is greater in the pre-menopausal women during an orthostatic stress compared to men and postmenopausal women this could help to explain the reduced venous return. It would also be prudent to investigate CO_2 reactivity in younger and older men and women in order to determine if there are indeed sexually dimorphic and age-related differences affecting brain blood flow. Finally, future measurements of muscle sympathetic nerve activity at each posture and brachial vascular resistance during the postural transitions will help to clarify the actions of the baroreceptors with ageing.

Chapter 6

Summary and Perspectives

6.1 Summary

The purpose of this thesis was to investigate the cardiovascular mechanisms behind the greater incidence of orthostatic hypotension known to exist in young women. Many measurements that have not been observed previously during an orthostatic stress and/or during exposure to 4-hours of head-down bed-rest (HDBR) were used in this study, particularly considering sex and the menstrual cycle. The investigations within this thesis were also unique in the controls for circadian rhythm and inactivity which are not always completed with head-down bed-rest (HDBR) investigations. Head-down bed-rest (HDBR) was used to alter the cardiovascular system in order to augment the cardiovascular responses to lower body negative pressure (LBNP; a model to simulate orthostatic stress), and a seated control model (SEAT) was used to control for circadian rhythm and inactivity.

During LBNP, systemic cardiovascular measurements changed to the same degree in women when comparing the follicular to the luteal phase of the menstrual cycle. However, there was a tendency for greater splanchnic blood pooling during the follicular phase compared to the luteal phase (a unique finding). This could result in a reduction of venous return during an orthostatic stress in the follicular phase. However, previous studies have indicated that renal and leg vascular resistance may be higher in the follicular phase which would balance the lower splanchnic resistance and may lead to venous return equal to the luteal phase, and thus equal orthostatic tolerance. This thesis did not investigate resistance of the renal or leg vasculature.

When investigating the sexually dimorphic effects of LBNP (comparing men to women in the follicular phase), it was shown that women have greater splanchnic blood pooling, a reduction in mean arterial pressure, a greater loss of central venous pressure, a greater loss of thoracic impedance, and an attenuated release of renin. Therefore it was shown that in comparison to men, women have a greater loss of venous return from splanchnic blood pooling leading to reduced mean arterial pressure during an orthostatic stress. Furthermore, the splanchnic blood pooling could be a function of attenuated activation of the renin-angiotensin-aldosterone pathway in women during LBNP.

After 4-hours of HDBR, and considering the effects of circadian rhythm and inactivity, all participants exhibited lower norepinephrine and lower blood volume. The headward fluid

shift during HDBR would have led to higher venous return, greater stimulation of the cardiopulmonary baroreceptors and thus inhibition of sympathetic output (and therefore norepinephrine release). In response to LBNP after HDBR, all participants exhibited an augmented increase of heart rate, a greater decrease of stroke volume, and a greater increase of thoracic impedance during LBNP. These results imply that the 4-hours of HDBR resulted in reduced venous return during LBNP.

When comparing the follicular phase and the luteal phase after HDBR, higher vasopressin and higher pelvic impedance were observed during the luteal phase. The responses to LBNP after HDBR were not different between phases. This comparison indicates that women in the luteal phase responded to HDBR with higher vasopressin release which perhaps led to mobilization of splanchnic/pelvic blood pools. When comparing women in the follicular phase to men, men exhibited higher vasopressin, higher endothelin-1, and higher pelvic impedance. Therefore in men, HDBR resulted in greater release of the vasoconstrictors vasopressin and endothelin-1 perhaps leading to mobilization of splanchnic/pelvic blood pools.

The final Chapter of this thesis sought to investigate the cardiovascular and cerebrovascular effects of orthostatic stress in two groups of women (follicular phase (<30 years old) and post-menopausal (>50 years old)) and in age-matched men. The purpose of this final study was to support the findings of Chapters 3 and 4 in the younger groups (by comparing standing versus LBNP as an orthostatic stress) and to extend those results by investigating a group of women that lacked endogenous sex hormones while minimizing the effects of age. Furthermore, the final Chapter extended the results by using transcranial Doppler measurements as an index of brain blood flow during an orthostatic stress which was not previously measured.

The results of this study supported the hemodynamic changes that were found in the younger men and women in the previous Chapters. In comparison to young men, young women experienced a greater increase of heart rate during steady-state sitting and standing due to lower stroke volume and cardiac output. During the transitions to each posture young women similarly had an inability to maintain stroke volume and cardiac output. However, there were no differences in the response of middle cerebral artery velocity to orthostatic stress.

In the older men and women during an orthostatic stress there was higher venous return, higher stroke volume, and higher cardiac output leading to a smaller decrease of middle

cerebral artery velocity. In regard to venous return indices, the difference between sexes was not as great as it was in younger participants. Therefore, the inability to maintain venous return upon an orthostatic stress observed in young women was attenuated in post-menopausal women; however, it should be noted that post-menopausal women still had slightly reduced venous return in comparison to age-matched men. Residual sex hormones at this young age of menopause could have played a factor.

6.2 Perspectives

Throughout this thesis reduced venous return has been observed in young women during lower body negative pressure (LBNP) or standing, in comparison to age-matched men or post-menopausal women. There were different levels of the sex hormones estrogen and progesterone present in each of these comparisons. For the sex comparisons in the younger groups, women had a moderate level of estrogen with low progesterone. For the menstrual phase comparisons, women in the follicular phase had a moderate level of estrogen with low progesterone while women in the luteal phase had approximately equal levels of estrogen with higher levels of progesterone. For the age comparison, the post-menopausal women should have had minimal levels of both estrogen and progesterone. Therefore, differences observed between the follicular phase and men could be attributable to the presence of estrogen (if not to anatomical sex differences that have developed over time), differences seen in the luteal phase compared to the follicular phase could be attributable to the presence of progesterone, and differences seen between the younger follicular phase women and post-menopausal women could be attributable to the presence of estrogen (if not to ageing effects).

6.2.1 Effects of sex hormones

Estrogen, progesterone and testosterone can each affect the cardiovascular system in a genomic or a non-genomic fashion. Estrogen receptors, progesterone receptors or androgen receptors can be cytosolic for genomic responses or membrane bound for non-genomic responses. Once bound to the appropriate ligand, cytosolic receptors dimerize and translocate to the nucleus to act as a transcription factor for particular genes (reviewed in (59; 99)). The

non-genomic model supposes that membrane bound receptors bind to the appropriate ligand and then elicit responses via cell-signaling pathways (reviewed in (99)). There is some cross-reactivity between progesterone and androgen receptors. In the hypothalamus of the rat (*in vitro*) progesterone binds to androgen receptors with a 32% affinity (compared to 7% for estradiol) in competition with testosterone (134). In the pituitary there is 16% cross-reactivity between progesterone and androgen receptors (compared to 8% for estradiol) (135). While this indicates that women in the luteal phase could be exhibiting androgenic effects due to progesterone (as reviewed in (10)), a more recent review has cited literature stating that there are no effects of progesterone on androgenic receptors (136).

All of these sex hormones have been shown to have vasodilatory effects and inhibit contraction of isolated rat aortas to phenylephrine (29). This could partially be a function of the ability of each hormone to increase the activity of endothelial nitric oxide synthase (100; 174; 207). From these observations one might expect greater nitric oxide in the luteal phase. Though not statistically significant this appears to be the case, as seen in Table 1. Progesterone also has an inhibitory effect on the production of nitric oxide that is elicited by estrogen (208) which perhaps could explain why the differences were not significant.

Estrogen and testosterone, but not progesterone, affect the release and/or transcription of vasopressin. Both estrogen and testosterone increase the transcription of vasopressin mRNA in the brain, but estrogen elicits a greater increase (81). In support of this, in Figure 4 at baseline there was a tendency towards higher plasma vasopressin in women in both phases compared to men. Estrogen has also been shown to decrease the osmotic threshold of vasopressin release (i.e. vasopressin is released at a lower serum osmolality) (81), and since Table 6 shows no difference in serum osmolality of follicular phase women compared to men there could be a greater release of vasopressin in women due to estrogen. Progesterone has no effect on the osmotic threshold of vasopressin release (24).

Estrogen, progesterone, and testosterone affect different levels of the renin-angiotensin-aldosterone pathway. Six months of treatment with raloxifene (a selective estrogen receptor modulator) in post-menopausal women had no effect on plasma levels of renin or aldosterone. (132) implying that estrogen does not affect the release of either of these hormones. Progesterone increases the release of renin from cultured human endometrial cells (163) and increases the release of aldosterone from cultured rat zona glomerulosa cells (181). Similarly,

dihydrotestosterone has been shown to increase renin mRNA in the adrenal glands, submandibular glands, and brain of mice (196) and to increase aldosterone secretion from human adrenocortical cells (205). These effects of progesterone and testosterone on renin and aldosterone are supported by this thesis. Higher levels of renin and aldosterone were observed in women in the luteal phase and in men compared to women in the follicular phase (Figures 5A and 5C).The higher levels of renin in the follicular phase and in men should translate into higher levels of angiotensin II; however this is not observed in the plasma (Figure 5B). This discrepancy could be explained by the upregulation of the angiotensin receptor (AT(1)-R) caused by both progesterone and testosterone (estrogen downregulates the receptor)(114; 137). More protein bound to receptors will decrease the amount of free angiotensin II detected in the plasma. This would likely lead to greater vasoconstriction and aldosterone release in both women in the luteal phase and in men. The presumed greater vasoconstriction of women in the luteal phase and in men could help to explain the higher portal vein resistance index observed in these groups (Figure 3C), particularly considering the potency of angiotensin II as a vasoconstrictor in the splanchnic vessels (172).

For the comparison of women in the follicular phase to post-menopausal women, the absence of estrogen needs to be considered. Without estrogen, post-menopausal women should display a combination of reduced vasodilation, reduced nitric oxide bioavailabilty, higher levels of AT(1)-R, yet a reduced release of vasopressin. In support of this supposition, the results of Chapter 5 show that post-menopausal women have no differences in total peripheral resistance compared to younger women which could be an effect of the balance between higher AT(1)-R induced vasoconstriction, lower nitric oxide and lower vasopressin. The post-menopausal women did however exhibit better venous return during sitting and standing which could be a result of greater vasoconstriction of the splanchnic region from the possible upregulation of AT(1)-R.

6.2.2 Possible counter-measures to improve orthostatic hypotension in women

In order to maintain stroke volume in women (particularly young women) that are prone to fainting during an orthostatic stress there are some counter-measures that could be considered. Compressive clothing of the splanchnic and leg regions could help to maintain

circulation of blood from these regions. Indeed, astronauts use compressive clothing in order to maintain venous return and mean arterial pressure upon return to Earth (144), and lower limb and abdominal compression bandages have been shown to ameliorate orthostatic hypotension (46; 145). Current treatments for individuals diagnosed with postural orthostatic tachycardia syndrome (POTS) include increased water consumption and dietary sodium (to expand blood volume), and sometimes the use of pharmacological agents such as midodrine or fludrocortisone (increases vasoconstriction and sodium retention, respectively) (146). Breathing carbon dioxide has also been shown to increase brain blood flow and orthostatic tolerance (14), and thus could perhaps be used to help individuals prone to fainting. However, this option would not be very practical in a daily situation. The results of this thesis also suggest that testosterone or progesterone supplementation might also benefit some individuals prone to orthostatic hypotension. This supplementation could increase activation of the renin-angiotensin-aldosterone system and thus increase sodium retention to increase blood volume, increase vasoconstrictor potential to improve venous return, and perhaps decrease splanchnic blood pooling to further improve venous return.

6.3 Future directions

This thesis has discussed how changes in female sex hormones through the monthly cycle and with age have the potential to change cardiovascular responses to orthostatic stress. Therefore, more research should be done investigating the cardiovascular effects of sex hormones and potentially the use of them as a treatment for orthostatic hypotension. Furthermore, more research should be done investigating the cardiovascular effects of the wide variety of oral contraceptives and replacement hormones that are taken by women of all ages.

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Appendix I: Comparison of orthostatic responses in women taking cyclic oral contraceptives (OC) (and those not taking any oral contraceptives) to women taking non-cyclic oral contraceptives.

Women taking cyclic oral contraceptives (OC) were grouped with those not taking any OC (cyclic group; n = 3 and n = 4, respectively) and tested during the follicular phase (Day 8-11) to form a "low-hormone" group and they were compared to women taking non-cyclic OC (non-cyclic group; n = 4; a "high-hormone" group). The responses to lower body negative pressure (LBNP) were compared between groups. This comparison contrasts the responses of women with lower levels of progesterone/analogs to those with higher levels of progesterone/analogs.

There were no group differences for heart rate (Figure 1A); however the cyclic group had lower stroke volume (Figure 1B) and cardiac output (Figure 1C). There was no group difference for central venous pressure (Figure 2A); however there was a greater decrease of inferior vena cava diameter with LBNP (Figure 2B) in the cyclic group, likely due to higher baseline. These results imply that the lower stroke volume and cardiac output of the cyclic group are not likely due to lower venous return. The cyclic group had lower mean arterial pressure (Figure 3A), higher total peripheral resistance (Figure 3B), no difference in portal vein resistance index (Figure 3C), and lower brachial vascular resistance (Figure 3D). Some of these differences could be due to different levels of athleticism (i.e. lower stroke volume and cardiac output leading to lower mean arterial pressure); however the exercise capacity (VO_2max) of the participants was not measured. Future studies should have higher sample numbers in each group and should measure exercise capacity of participants. If the differences were not due to athleticism they could have been due to the higher level of progesterone in the non-cyclic group (i.e. perhaps the lower brachial vascular resistance is due to the vasodilatory effects of progesterone). A more in-depth investigation is required.

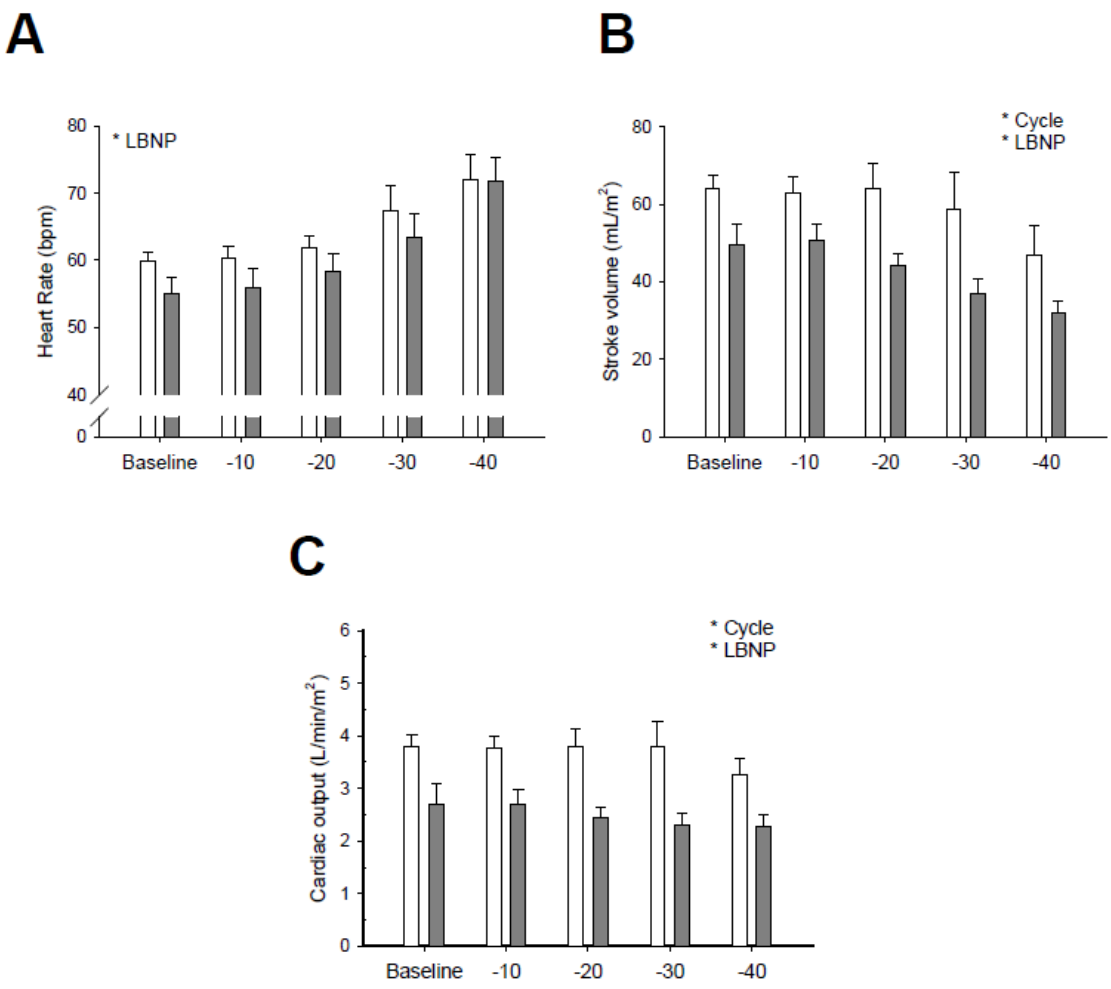

Figure 1: Effects of LBNP in women (follicular phase) that are taking non-cyclic OC (white bars) or are either not on OC or are taking cyclic OC (grey bars) on heart rate (A), stroke volume (B), and cardiac output (C). *LBNP indicates a significant main effect of LBNP. *Cycle indicates a significant main effect of cycling hormones.

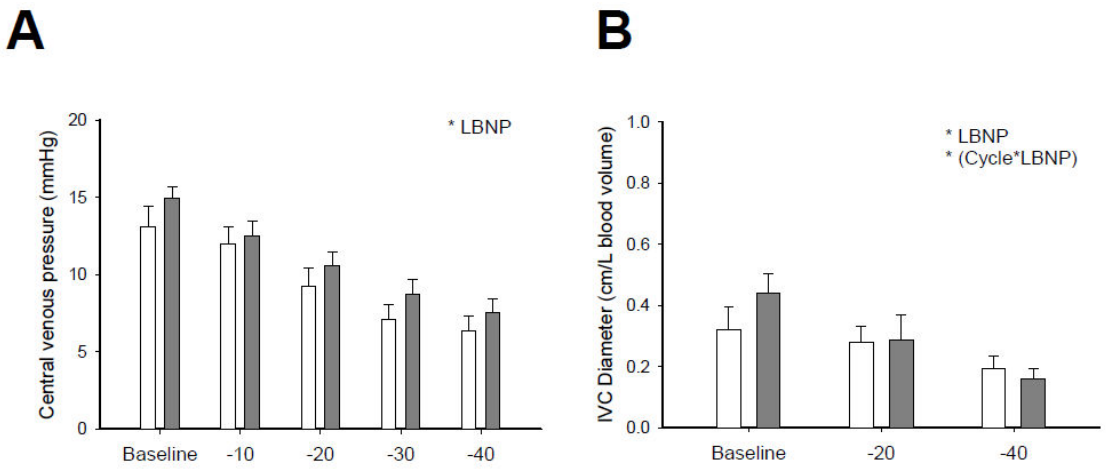

Figure 2: Effects of LBNP in women (follicular phase) that are taking non-cyclic OC (white bars) or are either not on OC or are taking cyclic OC (grey bars) on central venous pressure (A) and IVC diameter (B). *LBNP indicates a significant main effect of LBNP.*(Cycle*LBNP) indicates a significant interaction effect of LBNP and cycling hormones.

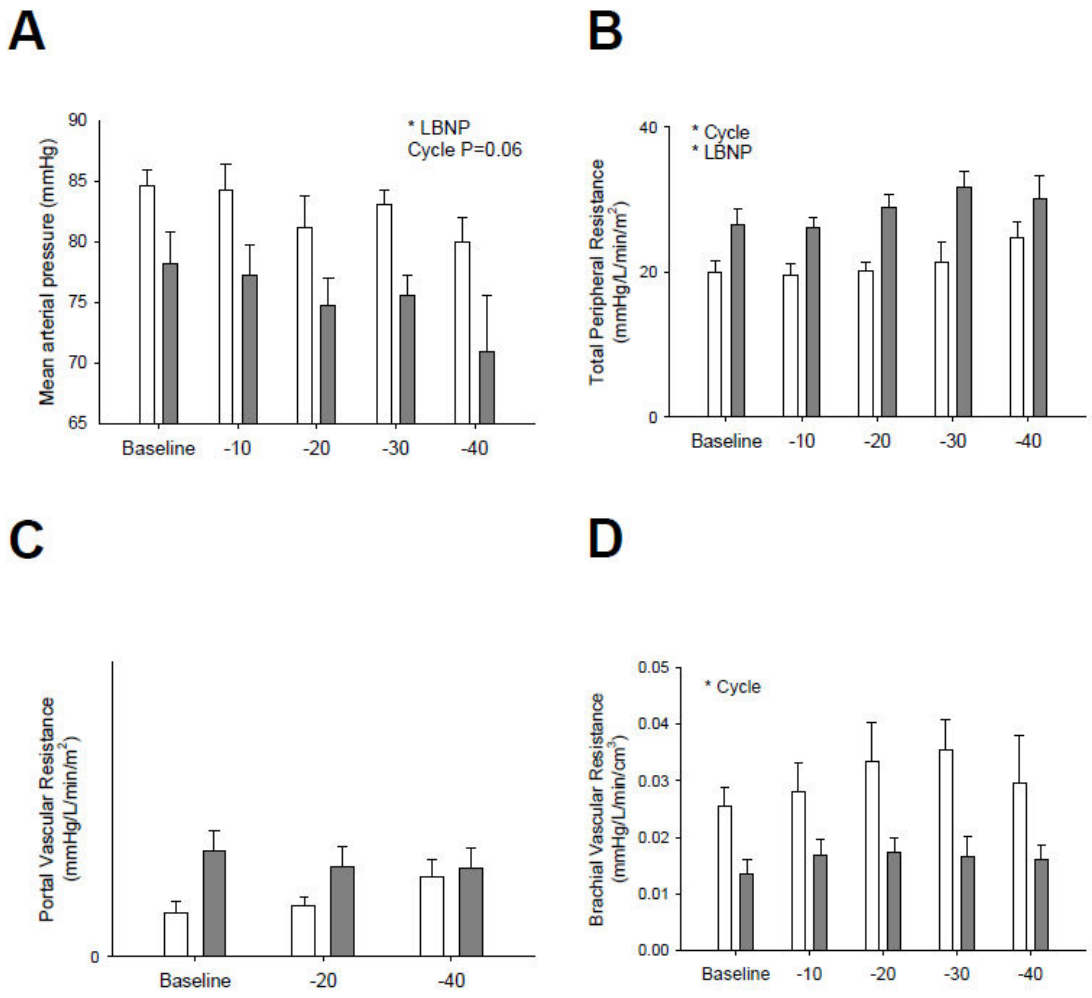

Figure 3: Effects of LBNP in women (follicular phase) that are taking non-cyclic OC (white bars) or are either not on OC or are taking cyclic OC (grey bars) on mean arterial pressure (A), total peripheral resistance (B), portal vein resistance (C), and brachial vascular resistance (D). *LBNP indicates a significant main effect of LBNP. *Cycle indicates a significant main effect of cycling hormones.

Appendix II: Reproducibility of lower body negative pressure in women through the menstrual cycle and men

Although lower body negative pressure (LBNP) is a commonly used method, the reproducibility of the LBNP protocol on two different days was tested to ensure that the results from day to day were consistent. If LBNP is indeed a reproducible measurement, the responses on two separate occasions should not differ. Appendix II compares the responses to LBNP before the head-down bed-rest (Pre-HDBR) protocol and before the seated control (Pre-SEAT) protocol.

For men, women in the follicular phase, and women in the luteal phase LBNP was reproducible in terms of heart rate (HR), central venous pressure (CVP), mean arterial pressure (MAP), stroke volume (SV), cardiac output (Q), total peripheral resistance (TPR), brachial vascular resistance (BVR), pelvic impedance (Pelvic Imped.), and thoracic impedance (Thoracic Imped) as shown in Table 1. LBNP was reproducible for portal vein resistance, inferior vena cava diameter, norepinephrine, vasopressin, renin, angiotensin II, and aldosterone (Table 2). LBNP was also reproducible for epinephrine, endothelin-1, nitrates and nitrites, and atrial natriuretic peptide (Table 3).

There were no differences between testing days for blood volume, urine output, urine osmolality, urine sodium, urodilatin, plasma osmolality, or plasma sodium (Table 4). The blood volume difference in men between days is a result of the absence of data on the pre-HDBR day for one individual. The difference between days is not significant (P=0.845; Table 4). On the two different testing days inferior vena cava compliance during LBNP is reproducible, but cardiopulmonary baroreceptor (CPBR) sensitivity during LBNP is not reproducible, particularly in follicular phase women (Table 5). Since this last measurement is not reproducible from day to day, it will not be discussed in this thesis.

Table 1: Hemodynamics in men and women during lower body negative pressure on two different days.

	Sex		0 mmHg	-10 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
HR	**Male**	**Pre-HDBR**	56.3 ± 2.0	57.6 ± 2.2	60.3 ± 2.6	63.0 ± 2.6	70.5 ± 2.7*
		Pre-SEAT	54.4 ± 2.9	54.9 ± 2.8	57.9 ± 2.9	60.9 ± 3.0	66.7 ± 3.4*
	Follicular	**Pre-HDBR**	56.8 ± 1.7	57.5 ± 2.0	59.6 ± 1.8	64.9 ± 2.5	71.8 ± 2.6*
		Pre-SEAT	59.8 ± 2.8	60.6 ± 2.7	62.6 ± 2.9	70.2 ± 4.1	76.8 ± 5.3*
	Luteal	**Pre-HDBR**	60.9 ± 2.9	60.9 ± 2.4	64.0 ± 3.1	68.6 ± 3.8	75.6 ± 5.2*
		Pre-SEAT	60.4 ± 1.7	61.6 ± 1.7	64.0 ± 2.1	68.4 ± 2.5	75.8 ± 3.8*
CVP	**Male**	**Pre-HDBR**	13.7 ± 1.0	11.3 ± 0.9	9.0 ± 1.1	7.8 ± 1.0	7.0 ± 1.1 *
		Pre-SEAT	14.8 ± 0.9	12.3 ± 0.9	10.1 ± 1.0	8.7 ± 1.1	8.0 ± 1.1*
	Follicular	**Pre-HDBR**	14.1 ± 0.7	12.3 ± 0.7	10.0 ± 0.7	8.0 ± 0.7	7.0 ± 0.7*
		Pre-SEAT	13.7 ± 1.0	11.8 ± 1.0	10.1 ± 1.1	8.2 ± 1.0	7.3 ± 1.0*
	Luteal	**Pre-HDBR**	13.4 ± 0.8	11.3 ± 1.0	9.5 ± 1.0	7.9 ± 0.9	6.9 ± 0.8*
		Pre-SEAT	14.2 ± 0.6	11.9 ± 0.8	10.1 ± 0.8	8.1 ± 0.6	6.7 ± 0.6*
MAP	**Male**	**Pre-HDBR**	83.7 ± 2.8	83.2 ± 2.7	83.3 ± 2.5	84.4 ± 3.1	83.6 ± 3.1
		Pre-SEAT	81.6 ± 3.1	81.2 ± 3.0	81.0 ± 2.4	80.5 ± 2.6	80.3 ± 2.7
	Follicular	**Pre-HDBR**	80.5 ± 1.9	79.8 ± 2.0	77.1 ± 1.9	78.3 ± 1.6	74.2 ± 3.2*
		Pre-SEAT	81.2 ± 2.6	80.3 ± 2.7	80.0 ± 2.8	80.1 ± 2.5	78.6 ± 3.4*
	Luteal	**Pre-HDBR**	78.6 ± 2.9	77.9 ± 3.2	76.9 ± 3.4	77.9 ± 3.4	76.2 ± 3.4
		Pre-SEAT	81.0 ± 1.9	81.1 ± 3.0	80.9 ± 3.4	82.6 ± 3.1	80.1 ± 2.7
SV	**Male**	**Pre-HDBR**	64.6 ± 8.3	59.4 ± 6.3	54.1 ± 5.6	49.1 ± 4.8	42.3 ± 3.8*
		Pre-SEAT	66.3 ± 6.2	60.3 ± 6.4	55.4 ± 6.9	50.9 ± 6.2	46.4 ± 5.9*
	Follicular	**Pre-HDBR**	55.2 ± 4.1	55.4 ± 3.5	52.0 ± 4.5	45.7 ± 5.4	38.0 ± 4.0*
		Pre-SEAT	57.1 ± 6.4	56.0 ± 6.3	50.3 ± 5.4	44.4 ± 5.7	40.5 ± 6.1*
	Luteal	**Pre-HDBR**	56.3 ± 4.4	50.4 ± 4.0	47.1 ± 4.3	44.0 ± 3.7	37.2 ± 3.6*
		Pre-SEAT	56.6 ± 3.1	53.2 ± 3.1	47.8 ± 4.0	45.7 ± 4.0	39.9 ± 4.0*
Q	**Male**	**Pre-HDBR**	3.6 ± 0.5	3.4 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	2.9 ± 0.4 *
		Pre-SEAT	3.6 ± 0.5	3.4 ± 0.5	3.2 ± 0.5	3.1 ± 0.4	3.0 ± 0.4*
	Follicular	**Pre-HDBR**	3.1 ± 0.3	3.1 ± 0.3	3.0 ± 0.3	2.9 ± 0.3	2.7 ± 0.2*
		Pre-SEAT	3.5 ± 0.5	3.4 ± 0.5	3.2 ± 0.5	3.2 ± 0.5	3.1 ± 0.5*
	Luteal	**Pre-HDBR**	3.3 ± 0.3	3.0 ± 0.2	2.9 ± 0.3	2.9 ± 0.2	2.8 ± 0.3*
		Pre-SEAT	3.3 ± 0.2	3.2 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	2.9 ± 0.3*
TPR	**Male**	**Pre-HDBR**	21.5 ± 1.1	23.6 ± 1.2	27.0 ± 1.5	27.5 ± 1.5	30.2 ± 1.7 *
		Pre-SEAT	20.3 ± 1.8	23.7 ± 2.7	26.4 ± 3.3	26.4 ± 2.4	28.1 ± 2.8*
	Follicular	**Pre-HDBR**	23.9 ± 1.8	23.4 ± 1.5	25.4 ± 1.8	27.8 ± 2.3	28.0 ± 2.2*
		Pre-SEAT	24.2 ± 2.7	24.4 ± 2.7	26.1 ± 2.8	28.5 ± 3.6	30.1 ± 5.1*
	Luteal	**Pre-HDBR**	21.4 ± 1.9	24.1 ± 1.9	26.1 ± 2.6	26.3 ± 2.1	29.1 ± 3.0*
		Pre-SEAT	22.5 ± 1.6	23.8 ± 2.0	27.1 ± 2.6	26.5 ± 2.2	29.1 ± 3.4*
BVR	**Male**	**Pre-HDBR**	0.0078 ± 0.0020	0.0110 ± 0.0042	0.0081 ± 0.0013	0.0081 ± 0.0012	0.0106 ± 0.0033
		Pre-SEAT	0.0072 ± 0.0015	0.0085 ± 0.0017	0.0095 ± 0.0024	0.0110 ± 0.0032	0.0097 ± 0.0036
	Follicular	**Pre-HDBR**	0.0178 ± 0.0026	0.0209 ± 0.0030	0.0232 ± 0.0037	0.0234 ± 0.0040	0.0210 ± 0.0038
		Pre-SEAT	0.0202 ± 0.042	0.0229 ± 0.0048	0.0227 ± 0.0045	0.0225 ± 0.0053	0.0216 ± 0.0050
	Luteal	**Pre-HDBR**	0.0165 ± 0.0029	0.0210 ± 0.0045	0.0186 ± 0.0037	0.0244 ± 0.0067	0.0223 ± 0.0059
		Pre-SEAT	0.0188 ± 0.0026	0.0181 ± 0.0024	0.0208 ± 0.0025	0.0171 ± 0.0020	0.0180 ± 0.0018
Pelvic	**Male**	**Pre-HDBR**	1.56 ± 0.14	1.53 ± 0.13	1.56 ± 0.13	1.60 ± 0.13	1.64 ± 0.13*
Imped.		**Pre-SEAT**	1.57 ± 0.17	1.55 ± 0.17	1.58 ± 0.17	1.61 ± 0.17	1.64 ± 0.17*
	Follicular	**Pre-HDBR**	2.91 ± 0.56	2.78 ± 0.51	2.76 ± 0.48	2.80 ± 0.46	2.77 ± 0.42
		Pre-SEAT	2.42 ± 0.22	2.37 ± 0.21	2.38 ± 0.19	2.43 ± 0.18	2.47 ± 0.18
	Luteal	**Pre-HDBR**	2.39 ± 0.33	2.32 ± 0.32	2.36 ± 0.32	2.42 ± 0.32	2.44 ± 0.32*
		Pre-SEAT	2.27 ± 0.35	2.20 ± 0.35	2.24 ± 0.35	2.29 ± 0.35	2.49 ± 0.38*
Thora.	**Male**	**Pre-HDBR**	0.84 ± 0.04	0.85 ± 0.04	0.87 ± 0.04	0.88 ± 0.04	0.89 ± 0.04*
Imped.		**Pre-SEAT**	0.80 ± 0.04	0.81 ± 0.05	0.82 ± 0.05	0.83 ± 0.05	0.83 ± 0.05*
	Follicular	**Pre-HDBR**	1.00 ± 0.02	1.02 ± 0.02	1.04 ± 0.02	1.06 ± 0.03	1.08 ± 0.03*
		Pre-SEAT	1.02 ± 0.04	1.03 ± 0.04	1.05 ± 0.04	1.08 ± 0.05	1.10 ± 0.05*
	Luteal	**Pre-HDBR**	1.04 ±0.04	1.05 ± 0.04	1.07 ± 0.05	1.09 ± 0.05	1.11 ± 0.05*
		Pre-SEAT	0.97 ± 0.03	0.98 ± 0.04	1.00 ± 0.04	1.02 ± 0.04	1.04 ± 0.04*

(* denotes significant main effect of LBNP)

Table 2: Hemodynamics and vasoactive factors in men and women during lower body negative pressure on two different days.

	Sex		0 mmHg	-20mmHg	-40mmHg
Portal vein Resistance (mmHg/mL/min/m^2)	**Male**	**Pre-HDBR**	0.50 ± 0.07	0.65 ± 0.13	0.98 ± 0.19*
		Pre-SEAT	0.49 ± 0.07	0.49 ± 0.06	0.73 ± 0.12*
	Follicular	**Pre-HDBR**	0.57 ± 0.13	0.51 ± 0.12	0.57 ± 0.12
		Pre-SEAT	0.54 ± 0.16	0.55 ± 0.15	0.77 ± 0.21
	Luteal	**Pre-HDBR**	0.68 ± 0.19	0.74 ± 0.25	1.01 ± 0.36*
		Pre-SEAT	0.57 ± 0.10	0.66 ± 0.20	0.90 ± 0.19*
Inferior vena cava Diameter (cm/L blood volume)	**Male**	**Pre-HDBR**	0.33 ± 0.03	0.21 ± 0.02	0.14 ± 0.02*
		Pre-SEAT	0.36 ± 0.04	0.25 ± 0.03	0.20 ± 0.03*
	Follicular	**Pre-HDBR**	0.38 ± 0.04	0.27 ± 0.03	0.18 ± 0.02*
		Pre-SEAT	0.35 ± 0.04	0.26 ± 0.03	0.16 ± 0.02*
	Luteal	**Pre-HDBR**	0.40 ± 0.04	0.26 ± 0.03	0.17 ± 0.02*
		Pre-SEAT	0.34 ± 0.04	0.20 ± 0.14	0.13 ± 0.02*
Norepinephrine (pg/mL)	**Male**	**Pre-HDBR**	223.8 ± 35.8	249.1 ± 52.5	319.8 ± 54.4*
		Pre-SEAT	215.0 ± 35.3	267.1 ± 35.7	317.8 ± 46.9*
	Follicular	**Pre-HDBR**	245.7 ± 35.8	251.9 ± 32.7	296.2 ± 43.7*
		Pre-SEAT	223.8 ± 35.8	249.1 ± 52.5	319.8 ± 54.4*
	Luteal	**Pre-HDBR**	219.3 ± 37.0	261.2 ± 35.2	289.4 ± 41.9*
		Pre-SEAT	232.5 ± 31.1	229.8 ± 30.5	294.2 ± 33.2*
Vasopressin (pg/mL)	**Male**	**Pre-HDBR**	3.59 ± 0.43	3.31 ± 0.40	3.28 ± 0.47 (P=0.052)
		Pre-SEAT	4.81 ± 0.88	3.31 ± 0.58	3.65 ± 0.62 (P=0.052)
	Follicular	**Pre-HDBR**	6.06 ± 1.80	4.44 ± 0.73	4.51 ± 0.73
		Pre-SEAT	5.98 ± 1.09	5.81 ± 1.21	6.23 ± 1.69
	Luteal	**Pre-HDBR**	6.58 ± 1.31	6.14 ± 0.98	7.27 ± 1.56
		Pre-SEAT	6.68 ± 1.22	5.80 ± 1.15	6.04 ± 1.14
Renin (pg/mL)	**Male**	**Pre-HDBR**	11.29 ± 1.10	13.13 ± 1.50	15.17 ± 2.02*
		Pre-SEAT	8.43 ± 0.57	10.43 ± 1.47	11.54 ± 1.12*
	Follicular	**Pre-HDBR**	7.71 ± 1.04	7.42 ± 0.85	9.54 ± 1.71
		Pre-SEAT	9.24 ± 1.28	8.32 ± 1.00	9.93 ± 2.03
	Luteal	**Pre-HDBR**	11.05 ± 2.09	8.69 ± 1.17	13.51 ± 3.17*
		Pre-SEAT	11.75 ± 1.72	9.99 ± 1.42	12.39 ± 2.22*
Angiotensin II (pg/mL)	**Male**	**Pre-HDBR**	7.66 ± 2.04	6.02 ± 1.81	10.25 ± 2.38*
		Pre-SEAT	6.96 ± 2.00	5.76 ± 1.23	6.77 ± 2.04*
	Follicular	**Pre-HDBR**	8.53 ± 2.61	8.32 ± 3.49	6.76 ± 2.37
		Pre-SEAT	9.79 ± 2.73	7.77 ± 2.42	11.68 ± 5.08
	Luteal	**Pre-HDBR**	10.55 ± 3.02	8.70 ± 2.26	14.98 ± 6.28 (P=0.07)
		Pre-SEAT	10.14 ± 3.37	8.39 ± 2.64	9.52 ± 2.90 (P=0.07)
Aldosterone (pg/mL)	**Male**	**Pre-HDBR**	66.6 ± 19.6	-	62.7 ± 19.8
		Pre-SEAT	45.8 ± 4.9	-	39.9 ± 3.5
	Follicular	**Pre-HDBR**	50.3 ± 8.9	-	35.9 ± 4.6*
		Pre-SEAT	94.8 ± 23.9	-	49.9 ± 10.4*
	Luteal	**Pre-HDBR**	77.2 ± 15.0	-	58.3 ± 11.3*
		Pre-SEAT	72.8 ± 16.7	-	53.6 ± 11.7*

(* denotes significant main effect of LBNP; P-value less than <0.07 noted)

Table 3: Other vasoactive factors in men and women during lower body negative pressure on two different days.

	Sex		0 mmHg	-20mmHg	-40mmHg
Epinephrine (pg/mL)	**Male**	**Pre-HDBR**	22.6 ± 3.0	19.8 ± 5.0	22.8 ± 4.3
		Pre-SEAT	18.6 ± 4.4	17.7 ± 2.5	15.4 ± 1.6
	Follicular	**Pre-HDBR**	21.1 ± 3.0	17.3 ± 1.4	24.0 ± 3.7*
		Pre-SEAT	22.5 ± 3.0	19.8 ± 5.0	22.8 ± 4.3*
	Luteal	**Pre-HDBR**	14.2 ± 2.6	20.0 ± 2.0	21.4 ± 2.5
		Pre-SEAT	19.1 ± 2.4	17.5 ± 1.4	20.8 ± 5.1
Endothelin-1 (pg/mL)	**Male**	**Pre-HDBR**	2.2 ± 0.4	-	2.0 ± 0.4
		Pre-SEAT	2.3 ± 0.3	-	2.12 ± 0.35
	Follicular	**Pre-HDBR**	1.4 ± 0.2	-	1.6 ± 0.5
		Pre-SEAT	1.5 ± 0.2	-	1.4 ± 0.2
	Luteal	**Pre-HDBR**	1.5 ± 0.3	-	1.4 ± 0.3
		Pre-SEAT	1.5 ± 0.2	-	1.33 ± 0.20
Nitrates/Nitrites (µmol/L)	**Male**	**Pre-HDBR**	6.2 ± 1.0	6.1 ± 1.0	6.0 ± 1.0*
		Pre-SEAT	7.1 ± 0.6	6.3 ± 0.6	6.7 ± 0.7*
	Follicular	**Pre-HDBR**	6.4 ± 1.5	6.1 ± 1.3	6.0 ± 1.1*
		Pre-SEAT	6.9 ± 0.9	6.4 ± 0.8	5.9 ± 0.8*
	Luteal	**Pre-HDBR**	7.3 ± 1.2	7.0 ± 1.1	8.1 ± 1.8*
		Pre-SEAT	6.8 ± 0.8	7.0 ± 0.7	6.8 ± 1.0*
Atrial natriuretic peptide (pg/mL)	**Male**	**Pre-HDBR**	29.4 ± 4.5	27.6 ± 4.1	27.7 ± 4.9
		Pre-SEAT	36.2 ± 5.9	34.8 ± 4.7	29.8 ± 4.0
	Follicular	**Pre-HDBR**	29.9 ± 3.3	29.5 ± 4.4	26.5 ± 4.6*
		Pre-SEAT	25.4 ± 2.3	20.9 ± 2.7	21.7 ± 2.5*
	Luteal	**Pre-HDBR**	25.8 ± 3.1	21.5 ± 3.8	23.4 ± 4.7*
		Pre-SEAT	30.0 ± 3.6	24.8 ± 3.4	27.4 ± 3.6*

(* denotes significant main effect of LBNP)

Table 4: Water and sodium balance in men and women on two different days.

	Sex	Pre-HDBR	Pre-SEAT
Blood volume (mL)	**Male**	5665.1 ± 697.3	6311.9 ± 805.4
	Follicular	4836.2 ± 337.1	4821.0 ± 463.8
	Luteal	4387.8 ± 470.7	4509.2 ± 235.2
Urine output (mL)	**Male**	470.1 ± 112.1	601.8 ± 114.2
	Follicular	497.6 ± 156.5	448.7 ± 90.1
	Luteal	525.9 ± 117.0	510.8 ± 79.3
Urine osmolality (mmol/kg)	**Male**	718.6 ± 78.5	589.9 ± 69.0
	Follicular	558.2 ± 79.1	515.2 ± 82.8
	Luteal	575.6 ± 59.0	582.6 ± 69.8
Urine sodium (mmol/L)	**Male**	110.3 ± 13.1	108.6±15.5
	Follicular	153.6 ± 72.3	104.0 ± 35.6
	Luteal	148.8 ± 66.7	163.9 ± 69.3
Urodilatin (pg/mL)	**Male**	29.6 ± 9.2	19.3 ± 3.7
	Follicular	32.5 ± 8.6	35.8 ± 12.5
	Luteal	45.9 ± 13.8	47.9 ± 11.6
Plasma osmolality (mmol/kg)	**Male**	280.9 ± 2.5	282.0 ± 2.2
	Follicular	278.6 ± 1.6	280.9 ± 1.8
	Luteal	277.1 ± 2.5	276.7 ± 2.8
Plasma sodium (mmol/L)	**Male**	122.7 ± 0.7	123.0 ± 1.0
	Follicular	127.4±2.0	125.4 ± 2.0
	Luteal	124.9 ± 2.0	126.4 ± 1.9

Table 5: Inferior vena cava compliance and CPBR sensitivity in men and women during lower body negative pressure on two different days.

	Sex	Pre-HDBR	Pre-SEAT
Compliance	**Male**	0.15 ± 0.03	0.13 ± 0.02
(cm/mmHg)	**Follicular**	0.14 ± 0.02	0.12 ± 0.02
	Luteal	0.15 ± 0.03	0.12 ± 0.02
CPBR slope	**Male**	-0.0001 ± 0.0004	-0.0004 ± 0.0003
(mmHg/mL/min/cm^3/mmHg)	**Follicular†**	-0.0016 ± 0.0004	0.0001 ± 0.0004
	Luteal	-0.0014 ± 0.0011	-0.0005 ± 0.0006

(* denotes significant main effect of LBNP; † denotes significant effect of testing day)

Appendix III: WISE-2005: Changes in Orthostatic Response Throughout Menstrual Cycle

As a small part of the collaborative 60-day bed-rest study, WISE-2005 (described in more detail in (54)), we examined the baseline cardiovascular responses to 80^{o} head-up tilt for 10 minutes followed by increasing levels of LBNP until pre-syncope in menstruating women (i.e. early follicular; n=5), follicular (n=8), and luteal phase (n=9) women. Heart rate, cardiac output, norepinephrine, epinephrine, and tolerance time were investigated. Baseline mean arterial pressure was not different between phases (Figure 1). Although not significant, there was a tendency for greater heart rate at pre-syncope in the follicular phase (Figure 2), no difference in cardiac output between phases (Figure 3), slightly higher norepinephrine at pre-syncope in the luteal phase (Figure 4), and slightly higher epinephrine in the follicular phase (Figure 5). Similar to the results of Claydon et al., there were no differences in orthostatic tolerance (Figure 6). These results indicate potential for different orthostatic responses between luteal and follicular phases.

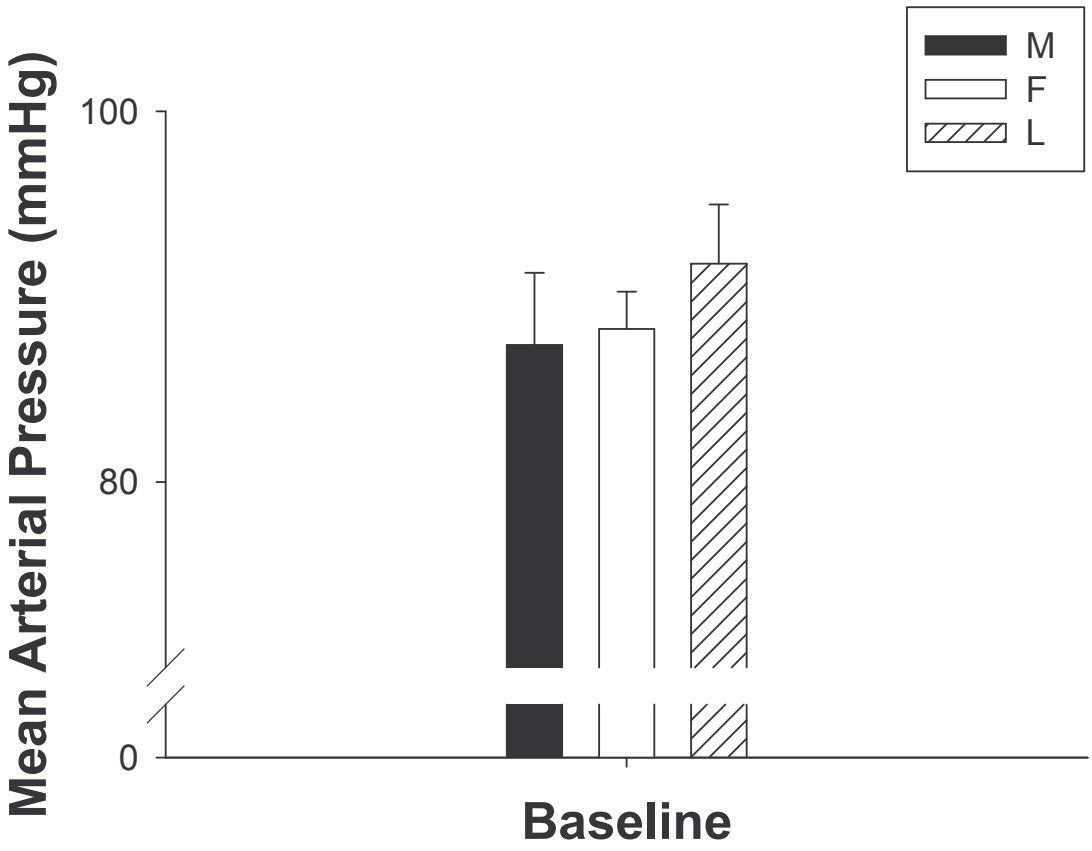

Figure 1: Mean arterial pressure (MAP) in supine position. Black bars are menstruating women (M; n=5), white bars are women in the follicular phase (F; n=8), and hatched bars are women in the luteal phase (L; n=9).

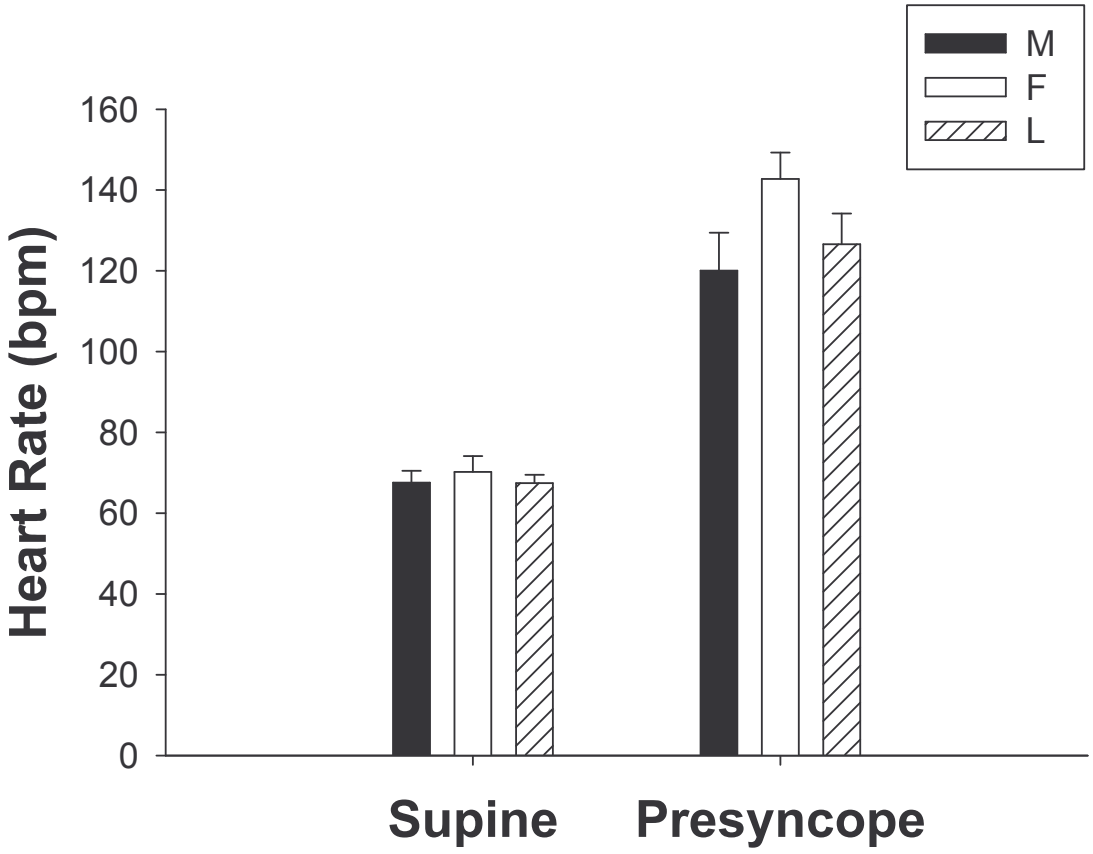

Figure 2: Heart rate (HR) in supine position and at presyncope. Black bars are menstruating women (M; n=5), white bars are women in the follicular phase (F; n=8), and hatched bars are women in the luteal phase (L; n=9). Presyncopal values are significantly higher than supine ($P<0.001$).

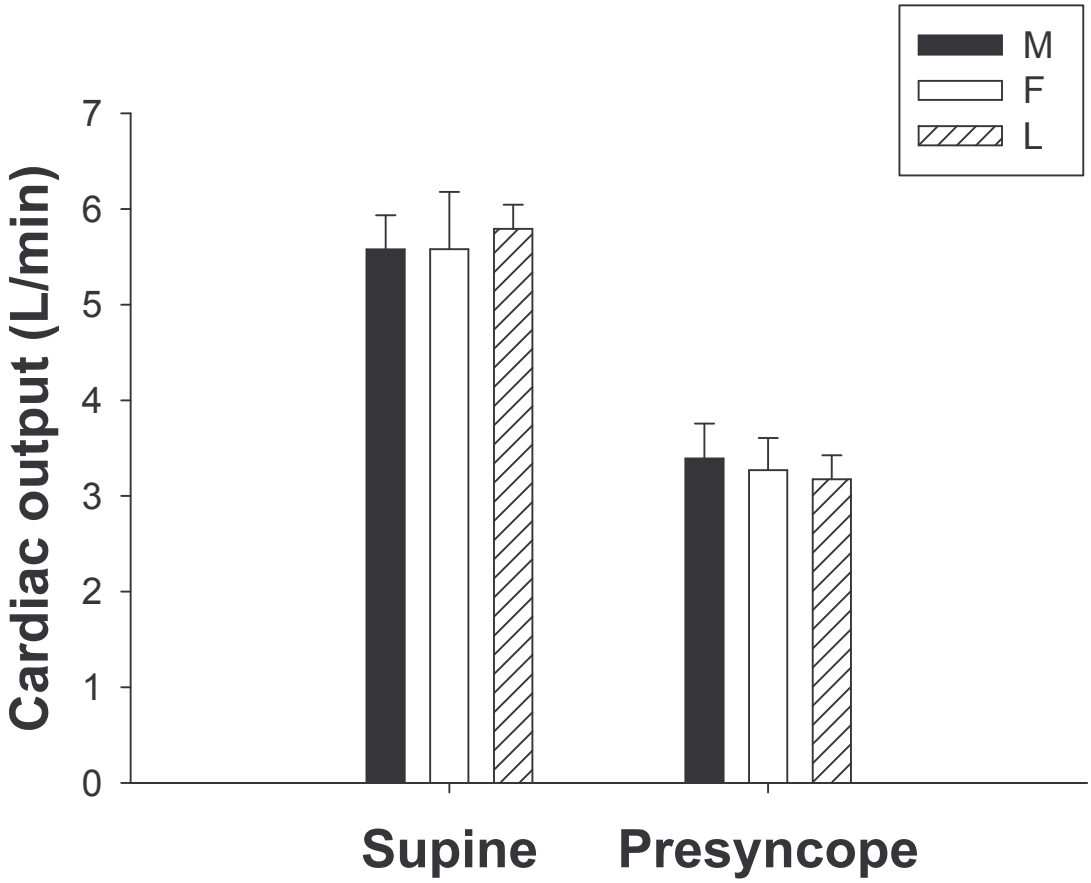

Figure 3: Cardiac output (Q) in supine position and at presyncope. Black bars are menstruating women (M; n=5), white bars are women in the follicular phase (F; n=8), and hatched bars are women in the luteal phase (L; n=9). Presyncopal values are significantly lower than supine ($P<0.001$).

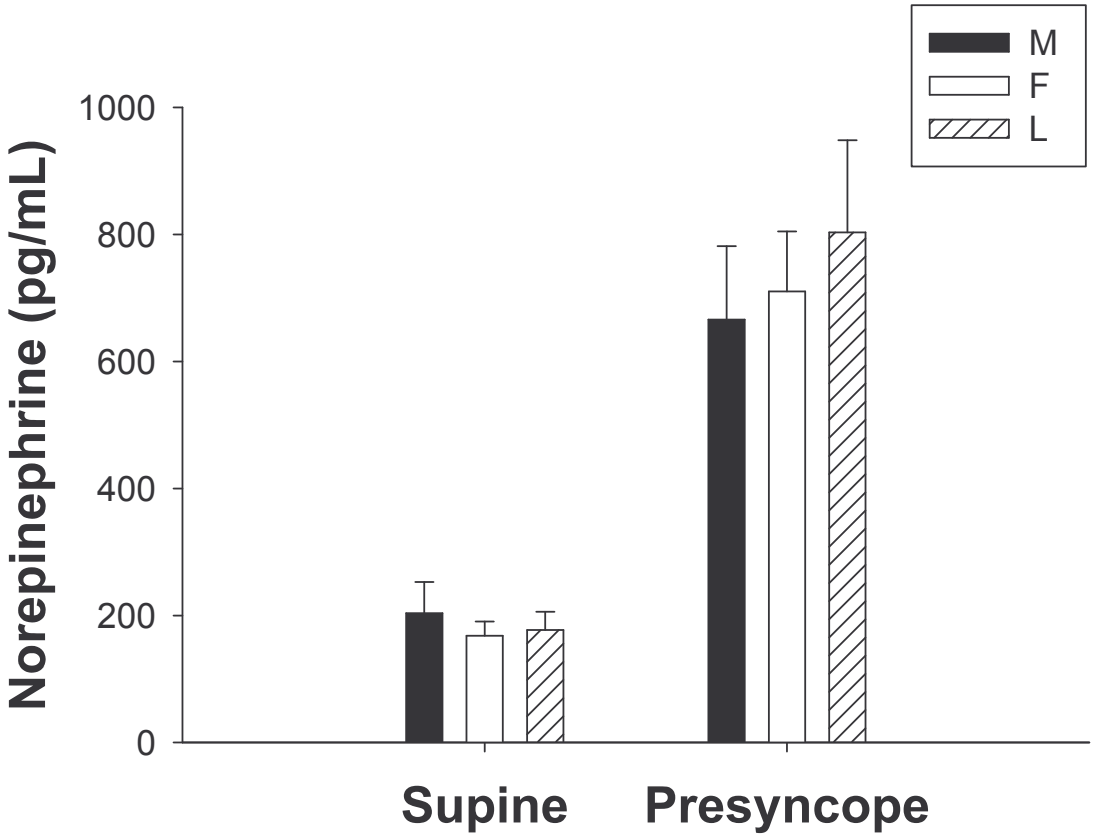

Figure 4: Norepinephrine concentration in supine position and at presyncope. Black bars are menstruating women (M; n=4), white bars are women in the follicular phase (F; n=7), and hatched bars are women in the luteal phase (L; n=7). Presyncopal values are significantly higher than supine ($P<0.001$).

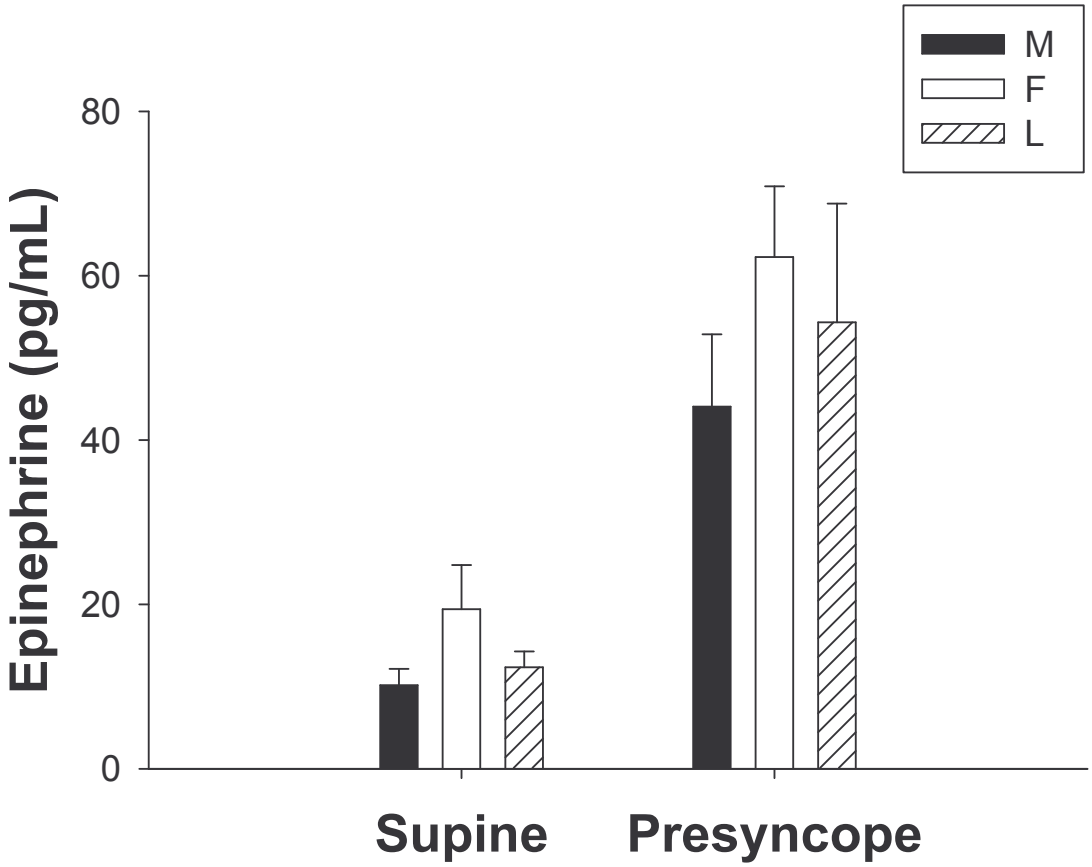

Figure 5: Epinephrine concentration in supine position and at presyncope. Black bars are menstruating women (M; n=4), white bars are women in the follicular phase (F; n=7), and hatched bars are women in the luteal phase (L; n=7). Presyncopal values are significantly higher than supine ($P<0.001$).

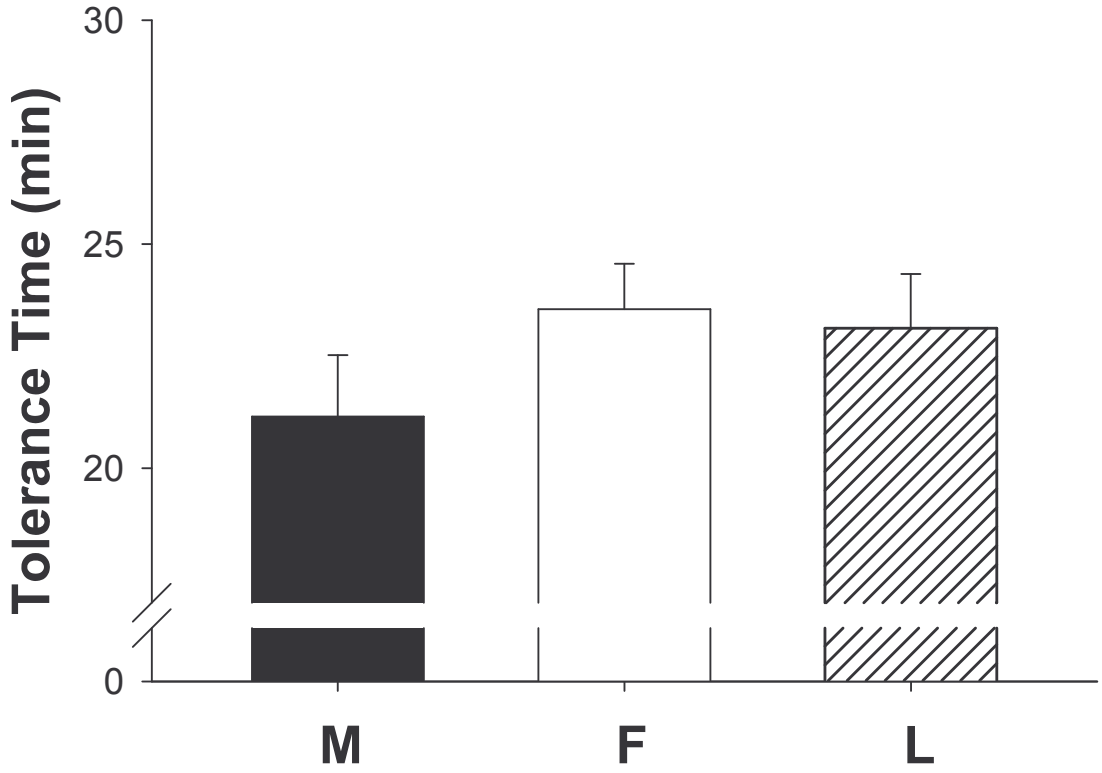

Figure 6: Duration of time to pre-syncope. Black bars are menstruating women (M; n=5), white bars are women in the follicular phase (F; n=8), and hatched bars are women in the luteal phase (L; n=9).

Appendix IV: Change in thoracic and pelvic impedance from supine to standing in young and older men and women

Unfortunately, the suggested reduced splanchnic blood pooling in postmenopausal women is not evident from our pelvic impedance measurements where pelvic impedance increases with standing in all four groups with no differences with sex or age. However, this region also includes the bladder, lower inferior vena cava, and pelvic organs. Changes in the fluid levels of these structures could obscure the meaning of this measurement. Furthermore, our impedance measurements (both thoracic and pelvic) are in doubt with posture changes because the level of abdominal breathing is higher in the supine position (192). This would pull the diaphragm more caudally and we would observe abnormally high impedance in the thorax with each breath. For this same reason pelvic impedance with changing postures may also be inappropriate.

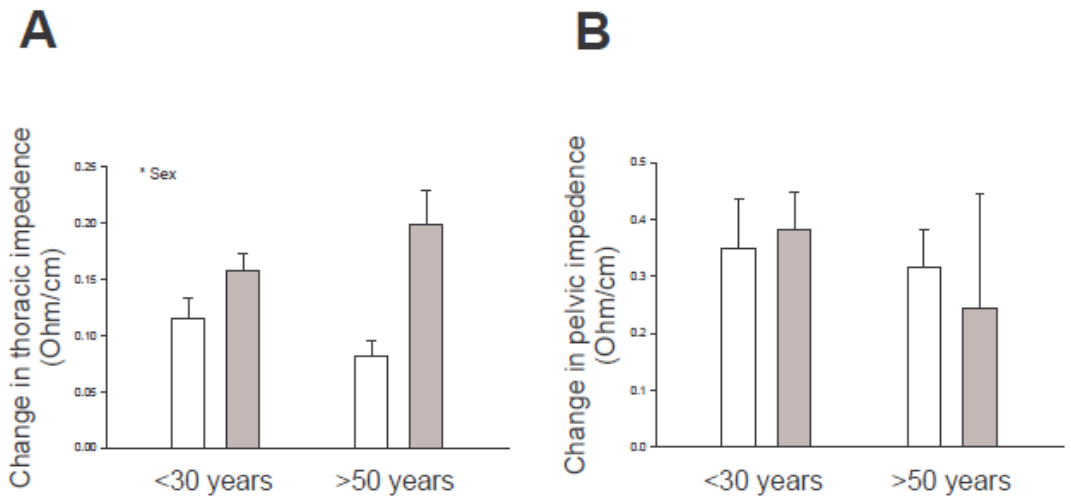

Figure 1: Thoracic and pelvic impedance responses to changing posture in men and women. A) Change in thoracic impedance between supine and standing in younger and older men (white bars) and women (grey bars). B) Change in pelvic impedance between supine and standing in younger and older men and women. *Sex indicates a significant main effect of sex.