Cerebrovascular and cardiovascular responses to lower body negative pressure and posture change in the elderly

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

Lower body negative pressure (LBNP) is frequently used in the research setting as a means to induce orthostatic stress. This tool is applicable to typical movements during daily living only if the associated responses are comparable to those of actual posture change. The purpose of this project was to investigate the correspondence between these two conditions in a group of elders with mean age of 74 ± 2 years. The primary hypothesis predicted very similar physiologic responses between LBNP and posture change. During LBNP, orthostatic stress was sufficient to reduce stroke volume (SV; p=0.0097), which was related to splanchnic pooling (r=-0.833; p=0.0020). Diastolic cerebral blood flow velocity (CBFV) was directly related to central venous pressure (r=0.683; p=0.0360), strengthening the notion that an important determinant of CBFV during LBNP is incoming pulsatile flow into the cerebral circulation. This is in contrast to what occurred during posture change (p=0.0355), where diastolic CBFV decreased significantly (p=0.0288) and was directly related to diastolic blood pressure (DBP; p=0.0427), highlighting the role of cerebral perfusion pressure (CPP) in maintenance of CBFV particularly during upright posture. In both conditions, DBP was related to cardiac output (Q) and total peripheral resistance (TPR). The relative heart rate (HR) response was higher during posture change than during LBNP (p=0.0397), suggesting that a negative pressure of up to 40 mmHg may not be adequate to elicit the same HR responses as true orthostasis in this elderly population. Contrary to the initial hypothesis, the HR and diastolic CBFV responses were different between the two conditions. Importantly, the results of this study support that in response to both actual orthostasis and simulated orthostasis, elderly persons experienced a drop in CBFV, but for different fundamental reasons, which were directly attributable to distinctions in the effects of gravity during the two postures. Recognizing the mechanistic differences in cerebro- and cardiovascular responses between true and simulated orthostasis is essential, and in this elderly population, there were important differences to consider.

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List of Abbreviations

Abbreviation	Explanation
AD	aortic diameter
AV	aortic velocity
AF	aortic flow
BD	brachial diameter
BF	brachial flow
BSA	body surface area
BV	brachial velocity
BVRi	brachial vascular resistance index
CBF	cerebral blood flow
CBFV	cerebral blood flow velocity
CPP	cerebral pulse pressure
CVP	central venous pressure
CVRi	cerebrovascular resistance index
ET_{CO_2}	end tidal carbon dioxide
Hb	hemoglobin
IVCD	inferior vena cava diameter
LBNP	lower body negative pressure
MSNA	muscle sympathetic nerve activity
PI	pulsatility index
PVD	portal vein diameter
P_{ETCO_2}	partial pressure of end-tidal carbon dioxide
Q	cardiac output
RAP	right atrial pressure
RI	resistance index
RR	respiratory rate
SV	stroke volume
SVR	systemic vascular resistance
TPR	total peripheral resistance
VR	venous return

1.0 Review of Literature

1.1 Introduction

During the transition in posture from supine to stand, a number of compensatory cardiovascular responses take place so that blood can still be pumped up to the brain despite the downward effects of gravity. In the elderly, these typical responses are altered compared to their younger counterparts. Aged individuals seem to be capable of maintaining blood flow to the brain; however, it is accomplished differently than their younger counterparts (Frey & Hoffler, 1988; Olsen *et al*., 2000; Gabbett *et al*., 2001). The exact mechanisms are still unclear. Studying postural changes is challenging because the shift from supine-to-standing requires a great deal of movement which complicates and precludes certain measurements. Consequently, surrogate techniques to induce a simulated gravity have been created. However, it is also unclear whether these alternative procedures are sufficiently similar to the organic sit-stand in terms of stimulating the same cardiovascular responses in elderly persons. There are two principal approaches to evaluating orthostatic tolerance. Head-up-tilt (Watenpaugh *et al.*, 2000) involves controlling the degree to which the person stands by altering the angle of the platform they are lying on from horizontal towards vertical. While this procedure draws on the natural effects of gravity to shift blood volume, lower body negative pressure (LBNP) is a purely motionless procedure, eliminating the difficulty in measuring a dynamic body. The stationary nature of LBNP makes it possible to record central venous pressure (CVP) during testing and adding substantial insight into the understanding of venous return (Grasser *et al*., 2009) during orthostatic challenge, and cardiovascular adaptations as a whole.

1.2 Orthostasis

Orthostasis refers to maintenance of an upright posture [*Ortho* (Greek) – Straight; *stasis* (Greek) – State of equilibrium or inactivity]. An orthostatic stress is a nonspecific term characterizing a challenge placed on the cardiovascular system due to the downward effects of gravity while standing, or simulates gravitational consequences during standing (Bradshaw & Edwards, 1986). The major gravitational effect of upright posture is an accumulation of blood in the venous system of the lower body, causing a drop in central venous pressure (CVP). With less blood arriving at the heart, cardiac output (Q) is compromised, leading to hypotension. If cardiovascular control mechanisms do not respond sufficiently, cerebral blood flow (CBF) may be in jeopardy.

1.2.1 Orthostatic intolerance

Orthostatic intolerance (OI) is inadequate cardiovascular adjustment to an orthostatic challenge, and is a very broad definition describing several conditions. OI, or orthostatic hypotension (OH) as it is often referred to, impacts a range of individuals, from young children to the elderly. Most individuals recognize the occasional sensations that develop when quickly rising from a seated or supine position, including transient light-headedness and possible visual disturbances due to a rapid drop in blood pressure (BP).

The estimates of OH prevalence are highly varied due to a number of factors. For example: 1) there is lack of consensus about defining OH, 2) there are differences in frequency of OH through the age continuum and with general health, 3) the impact of medications is not well documented, and 4) there are degrees of orthostatic stress that affect OH (Low, 2008). On the whole, in elders beyond the age of 65, prevalence has been reported at 5-30% (Mukai & Lipsitz, 2002; Raiha *et al*., 1995; Mathias & Kimber, 1999; Rutan *et al*., 1992; Lipsitz, 1989; Mader, 1989; Masaki *et al*., 1998; Tilvis *et al*., 1996). In nursing home

residents, OH has been seen in up to 50% of the population (Ooi *et al*., 1997). OH has been considered as an important risk factor for falling in the elderly (Rutan *et al*., 1992; Heitterachi *et al*., 2002), and Poon & Braun (2005) reported that 28% of geriatrics with OH in their study, experienced falls. Falling in the elderly increases morbidity and mortality, which are implicated in financial burden (Nurmi *et al*., 2004; Nurmi & Luthje, 2002) and decreased quality of life (Tinetti *et al*., 1994).

If OH persists without proper adjustment, blood flow to the brain may decrease. The relationship between BP and CBF is nonlinear, which means that BP cannot independently predict CBF. At present, an objective, non-invasive indicator of CBF has not been established. In up to 50% of elderly, typical symptoms (e.g. lightheadedness and visual blurring) often do not manifest, and the primary identifiable symptom is cognitive impairment (Low *et al.*, 1995). Blood flow to the brain must be maintained for adequate functioning of cognitive processes and maintenance of consciousness.

1.3 Gravitational influence on the circulation

1.3.1 Basic aspects of circulation

In simplified terms, the cardiovascular system can be thought of as circuit of enclosed tubing filled with fluid, and connected to a pump—the heart, whose function is to pump blood through the tubing. As the left ventricle contracts and ejects blood into the systemic circulation, the pressure generated by the contraction is partially absorbed by the elastic walls of the arteries and stored as energy. The volume of blood is then slowly released through recoil of the expandable walls. In this manner, blood flow is continuously propelled throughout the entire cardiac cycle. Pressure waves of blood travels through the vasculature and are reflected back towards the heart at the periphery. In young, healthy arteries, the

reflected wave arrives at the heart in time to overlap the diastolic portion of the next pulse wave.

At the lungs, oxygen infiltrates the blood and is dispersed through the circulation. A continuous source of oxygen for the cerebral cells is essential because when deficient in oxygen, cells undergo irreversible injury very quickly. The cerebral neurons are large consumers of oxygen and have metabolic demands that cannot be achieved through anaerobic means. Because brain tissue is especially susceptible to hypoxia, elaborate means of homeostatic regulation are always working to maintain cerebral blood flow (CBF). Without sufficient adaptive responses, including circulatory and autonomic reflexes, the gravitational strain incurred when maintaining an upright posture impairs perfusion to organs that are situated higher than the heart.

1.3.2 Translocation of blood volume in orthostasis

Upon standing, gravity causes circulatory stress. The degree to which blood volume is displaced to the dependent regions of the body is largely determined by the compliance of those regions. Relative to the arteries, the veins are 30 times more compliant (Rothe, 1983; Hainsworth, 1990). Accordingly, small changes in intravenous pressure result in comparatively larger changes in venous volume (Rowell, 1993). The rate at which the lower limb veins will fill with blood on standing is governed by the resistance in the preceding arterioles. Arterial constriction serves an important role in reducing the venous blood volume. With an increased resistance to flow into the compliant vascular bed, distending pressure in the veins is dramatically reduced. Upon upright posture, this action greatly reduces the volume of blood that will fill the veins of the skin and splanchnic regions, for example. Further, arterial constriction beyond supine resting tone can be seen as a mechanism for

translocation of blood volume from the splanchnic region back into the central circulation (Rowell, 1993) .

At any particular time, the portion of the venous system that lies below the level of the heart contains approximately 70% of the total blood volume (Rowell, 1993), while 18% is contained in the arteries, and 3% in the terminal arteries and arterioles (Gelman, 2008). In the healthy human body, total blood volume is roughly 70-75 ml/kg (Sjostrand, 1953; Levinson *et al.*, 1967). Once upright, approximately 10% of total blood volume rapidly descends from the thorax into the lower limbs (Smith & Porth, 1991). In fact, up to 700ml of blood may shift to the lower body (Mathias, 2002; Smit *et al.*, 1999; van Lieshout *et al.*, 2003).

The most compliant veins in the body are those of the splanchnic and cutaneous regions (Gelman, 2008). These areas serve as the major reservoirs for blood volume, as the veins of the extremities are less compliant and therefore contribute less to overall blood volume storage (Gelman, 2008). Unlike skeletal muscle veins, splanchnic and cutaneous veins are very responsive to adrenergic stimulation because their walls are covered in α_1- and α_2-adrenergic receptors. Since the sympathetic innervation of skeletal muscle veins is somewhat insignificant in comparison (Rowell, 1993), and because cutaneous venous flow is influenced predominantly by skin temperature, the splanchnic veins are primarily responsible for the mobilization of blood volume (Hainsworth, 1986; Rowell, 1993).

The dramatic shift in blood volume accompanying standing generally occurs in two phases (Sjostrand, 1953). The first stage commences immediately and is almost entirely complete within the first 2-3 minutes of orthostasis. This *fast* stage is characterized by rapid filling of the leg veins attributable to a sudden increase in hydrostatic pressure. Roughly 90% of the blood involved in the first phase of fluid shift is coming from the thorax (Smith & Porth, 1991). The second, or *slow* phase, may take up to 30 minutes to reach its conclusion, and consists of transcapillary fluid filtration into the interstitial tissues (Hagan *et al.*, 1978;

Thompson *et al*., 1928; Hinghofer-Szalkay & Moser, 1986; Asmussen *et al*., 1940; Brown & Hainsworth, 1999; Mathias, 2002; Thijs *et al*., 2007). These studies have indicated that during this phase of erect posture, about 2 to 3 ml/kg of plasma filtrate is sequestered in the lower body, and this diffusion tends to begin rapidly and then decrease exponentially.

Nearly 80% of the blood volume that becomes displaced once upright empties into the pelvic, buttock, and thigh regions, with smaller volumes entering the legs and feet (Ludbrook, 1966; Sjostrand, 1953; Wolthuis *et al*., 1974). The legs and feet naturally fill with arterial blood; however, because the pelvic and thigh areas are nearly devoid of venous valves, blood pooling here may be mostly venous in origin (Ludbrook, 1966; Smith J.J. & Ebert, 1990).

1.4 Orthostatic challenges

In general, two test types have been developed for examining an individual's capacity to tolerate an orthostatic challenge: Head up tilt (HUT) and lower body negative pressure (LBNP). For the purposes of medical diagnosis, the HUT test is used, though theoretical analyses (Fink et al., 2004) and experimental work (Hinghofer-Szalkay *et al.,* 2004) have revealed that the two tests are effectively equal and that both are suitable for orthostatic stress testing (Butler et al., 1993).

1.4.1 Lower body negative pressure

The lower body negative pressure (LBNP) technique was originally used in medical research over 40 years ago and has since demonstrated great utility as a means to simulate orthostatic stress. Responses to LBNP are a function of the length of time and the amount of negative pressure that is applied. Additionally, a number of factors will influence responses to LBNP, including age, sex, body surface area (BSA), fitness, and hydration (Convertino, 2001). The

inconsistency between experiments in techniques and details of methodology make it challenging to contrast LBNP-related cardiovascular and chemical changes. However, there is generally reasonable concurrence between results of similar experiments (Hisdal et al., 2001). Importantly, cardiovascular responses also tend to be reproducible within subjects measured repeatedly (Convertino, 2001). Before the availability of beat-to-beat data collection and analysis, cardiovascular responses were sampled just once or twice at each level of LBNP, illuminating steady-state changes in response to LBNP. In the last decade, technological progression has fostered the development of continuous monitoring, which now allows for transient cardiovascular changes to be carefully studied. Systematic accounts of time course studies of responses to LBNP are scant, and this is especially true in the elderly population. In designing a protocol for LBNP, it is critical to consider the responses/reflexes that one wants to examine. By keeping exposure time at each stage short (e.g. less than 5 minutes), the involvement of additional neural-hormonal factors can be avoided while ensuring that the desired physiological changes are taking place. Hisdal and colleagues (Hisdal *et al.,* 2001) have revealed that there are significant transient changes in mean arterial pressure (Matsuda et al., 1984), heart rate (HR), stroke volume (SV), cardiac output (Q), total peripheral resistance (TPR), acral skin blood flow, and brachial artery blood flow velocity in an adult population. Understanding fully the transitory adjustments that are made in response to orthostasis—in all populations—will clarify mechanisms responsible for preventing posture-related syncope and its associated symptoms.

1.4.2 Head-up-tilt

HUT table testing is another technique that is used as an orthostatic challenge. Since its inception over 50 years ago, HUT testing has earned its place, particularly, in the investigation of orthostatic intolerance and syncope of undiagnosed etiology (Benditt *et al.*,

1996; Grubb & Kimmel, 1998; Grubb & Kosinski, 1996; Grubb & Kosinski, 1997; Kapoor et al., 1994; Kapoor, 1999; Linzer et al., 1997; Taylor, 1994). Like LBNP, HUT has been shown to provoke similar cardiovascular and endocrine responses (Hinghofer-Szalkay *et al.*, 1996) as an active sit-to-stand maneuver, and, in fact, sympathetic activity and orthostatic stress increase with tilt angle (de Mey & Enterling, 1986; Montano et al., 1994; Segal *et al*., 1973; Smith *et al*., 1987; Stevens, 1966), which tends to be between 60 and 90 degrees (Benditt *et al.,* 1996; Segal *et al.,* 1973). During a graded head-up tilting maneuver, the amount of blood collecting in the periphery, and the adjustments in Q, SV, and muscle sympathetic nerve activity (MSNA) all seem to be predictably related to the sine of the tilt angle (Smith J.J. & Ebert, 1990). The two methods differ in their effects on splanchnic blood volume, where splanchnic volume decreases with LBNP and increases in HUT (Taneja *et al.,* 2007). Furthermore, HUT does not permit measurement of certain physiological variables, particularly because of the movement involved.

Because of the motionless nature of the LBNP test, it produces no otolith Gz stimulation. In contrast, HUT requires movement of the body in the sagittal plane, which stimulates the vestibular Gz system with minimal Gx and angular accelerations (Watenpaugh *et al.,* 2004), and is accompanied by erratic postural muscle contractions. During supine LBNP testing, when the individual is supported by a saddle seat rather than a footplate, extraneous skeletal muscle movement, facilitating physiological measurements. Currently, LBNP appears to be the best means to simulate orthostasis for research purposes.

1.5 Cardiovascular compensation as a result of gravity

The effect of gravity on the circulation is clearly observed when noting the considerable difference in transmural pressure in the feet between supine (~98 mmHg) and standing (~198

mmHg; Howard, 1977). In orthostasis, these pressure differences that exist throughout the circulation are a result of the gravitational force, the density of the blood, and the vertical distance separating the two points of interest. Hydrostatic forces generate a pressure differential of about 25 mmHg between the heart and the base of the brain (Howard, 1977). Logically, the vessels of the lower limbs are subject to higher pressure. The compliant venous vasculature contends with this increased pressure by expanding, and accommodating an increased blood volume. When LBNP is applied in the supine position, the pressure within the box surrounding the legs becomes reduced, which dilates the venous vessels, and causes an increase in their volume. The resultant drop in venous return and arterial pressure are similar to those produced naturally by gravitational force. Drops in CVP are directly associated with the degree of negative pressure elicited through LBNP (Wolthuis *et al.,* 1974) causing reduced cardiac filling and SV.

1.5.1 Compensatory orthostatic responses

Relative to the standing position, during supine rest, venous return is increased because gravitational forces are now perpendicular to the body. More blood is arriving at the heart (larger preload), and filling the right ventricle. Preload is the end volumetric pressure that stretches the right ventricle to its greatest geometric dimensions. Increasing preload increases sarcomere length, and increases troponin C calcium sensitivity, which increases the rate of cross-bridge formation and detachment, and subsequently, the amount of tension developed by the muscle fiber. This, in turn, increases the output of the heart per contraction, SV, through the Frank-Starling mechanism, which describes the length-tension and force-velocity relationship for cardiac muscle (Lakatta, 1992).

This relationship between preload and SV is nonlinear. Increases in preload only augment SV to a certain extent. There is a maximum ventricular fiber length at end diastole

where further fiber stretch no longer yields increases in SV. Harms and colleagues (2003) and other researchers (Bungaard-Nielson *et al.*, 2009) have determined that in a normal healthy heart, with adequate circulating blood volume, this occurs in the supine position. This is a function of the human anatomy, as the central blood volume that accumulates when the heart is at the same level as the vasculature results in the greatest venous return. In the clinical setting, preload is often assessed by the measurement of central venous pressure (CVP). In the steady state, venous return and Q remain essentially equal, so that blood does not accumulate in the central venous compartment or the peripheral vasculature. CVP has a significant positive impact on Q, and has the reverse influence on venous return (VR). Naturally, left and right outputs become unequal for short periods of time. This is especially true when posture change occurs. For example, upon standing, CVP decreases. Right ventricular stroke volume decreases in agreement with Starling's law. The output of the right heart decreases and left heart output temporarily exceeds right heart output. The imbalance causes blood to briefly accumulate in the systemic circulation, thereby increasing blood flow back to the right heart. Increased right atrial pressure increases right output via Starling's law. In this way, CVP is continually corrected towards a value that keeps Q equal to venous return (i.e. keeping left heart output equal to right heart output).

In response to orthostasis, one of the first compensations is heart rate (HR) acceleration. In healthy adults, this response is immediate, and well-established (Ewing et al., 1978; Borst et al., 1982). The initial HR increase is caused by a rapid withdrawal of the parasympathetic nervous system (PNS), while further increases in HR are due to sympathetic and vagal components (Borst *et al.*, 1982; Wieling *et al.*, 1983; Wieling *et al.*, 1985; Wieling *et al.*, 1991). This has been demonstrated through autonomic blocking experiments (Ewing et al., 1980; Wieling et al., 1991; Wieling et al., 1985; Wieling et al., 1983). Together with the HR response, total peripheral resistance (TPR) also increases; both in an attempt to maintain

mean arterial pressure (Matsuda *et al.,* 1984) and Q (Ziegler *et al*., 1977). TPR is sum of all the resistance in the peripheral vasculature of the systemic circulation (Equation 1.5.1). The instant that the body transitions from supine to upright the transmural pressure in the lower limb arteries becomes more positive because of gravity. VR subsequently drops, decreasing CVP, and naturally, the right ventricular output through the pulmonary artery.

$$TPR = MAP\text{-}CVP/Q \qquad \text{Equation 1.5.1}$$

The influence of sympathetic vasoconstriction in preserving BP during orthostasis has been shown through experimentation involving individuals with autonomic failure or other sympathetic outflow impairments causing severe orthostatic hypotension (Jacobs *et al.,* 1995; Jardine *et al.,* 1998; Mosqueda-Garcia *et al.,* 1997; Robertson *et al.,* 1994).

The principal mechanism by which humans quickly regulate blood pressure (e.g. by increasing HR and TPR) is through the baroreflexes (Eckberg & Sleight, 1992). The baroreceptors are mechanoreceptors that detect changes in the stretch of the vessel walls. Arterial baroreceptors (ABR) are located in the carotid sinuses, which monitor the pressure of the blood flowing to the brain, and in the aortic arch, which monitor blood being delivered to the systemic circulation. There are also baroreceptors found in the large systemic veins, in the pulmonary vessels, and in the walls of the right atrium and ventricles of the heart, termed the cardiopulmonary baroreceptors (CPBR).

Blood pressure is incessantly fluctuating. With each respiratory cycle, changes in intrathoracic pressure alter preload and afterload, thereby affecting SV and BP. During normal spontaneous ventilation, inspiration is initiated by contraction of the diaphragm and external intercostal muscles, which decreases intrathoracic pressure (i.e. pleural pressure), and thus draws air into the lungs. Because the pressure in the pleural space is negative, the lungs follow the expansion of the chest wall during inflation. As the intrathoracic pressure becomes more negative during inspiration, venous return is enhanced, increasing right

ventricular volume, and causing the intraventricular septum to deviate slightly towards the left ventricle. This action transiently decreases stroke volume from the left ventricle during spontaneous inspiration by decreasing left ventricular end-diastolic volume and also compliance. Both sets of baroreceptors work concurrently to sense these internal oscillations and can elicit reflex responses to counter them. The expected swings in BP that accompany posture change are corrected in the same manner. The decrease in BP reduces the firing rate of the baroreceptors in the carotid sinus and the aortic arch, which in turn increases efferent neural outflow to the heart and peripheral vasculature. The carotid and aortic baroreceptors tonically inactivate the vasomotor centers in the brain stem. The decreased firing rate removes the inhibitory stimulus, leading to an increase in sympathetic and decrease in parasympathetic activity. The immediate resulting consequences are increased systemic vasoconstriction, increases in inotropic and chronotropic cardiac responses, and activation of other neurohormonal systems that will help protect cardiovascular stability. Peripherally, in addition to arterial constriction and increase vascular resistance, the effects of the increased SNS activity stimulate the release of renin from the kidney, activating the renin-angiotensin-aldosterone system (RAAS). When angiotensin II production rises, the tone of the arterial vessels will further increase (Gordon *et al.*, 1967; Oparil & Habar, 1974).

To date, during low levels of LBNP, the relative contribution of the CPBR vs. ABR toward the restoration of BP has not been clearly established. Recently, it has been revealed that ABR are indeed unloaded at -10 and -20 mmHg LBNP (Fu *et al.*, 2009). For many years, it had been thought that LBNP levels of ≤20 mmHg solely targeted unloading of cardiopulmonary baroreceptors, thereby isolating the role of the cardiopulmonary baroreflexes from the arterial baroreflexes (Abboud & Mark, 1979; Zoller *et al*., 1972). This is no longer conventional opinion. In one of the earliest studies suggesting selective baroreflex responses, Zoller *et al*. (1972) found no transient changes in MAP with mild

LBNP, which was thought to demonstrate that the ABR were triggered. One of the first studies to refute this idea and report evidence that ABR are unloaded at small LBNP levels was that of Hakumaki and colleagues (1985), who measured aortic nerve activity in anesthetized dogs with diminishing aortic baroreceptor discharge despite stable MAP during hemorrhage. Since then, in addition to some animal studies (Hakumäki *et al*., 1985; Cornish *et al*., 1988), multiple human studies have shown evidence that cardiopulmonary baroreflexes are not independently unloaded (Aksamit *et al*., 1987; Taylor *et al*., 1995; Floras *et al*., 2001; Hisdal *et al*., 2002; Hisdal *et al*., 2001; Fu *et al*., 2009). This means that even low levels of LBNP (e.g. -10 or -15 mmHg), can produce a measurable transitory reduction in BP, with subsequent restoration.

When the innate cardiovascular responses work concurrently, the ultimate result is that blood pressure barely changes. The physiological responses to an orthostatic challenge differ greatly between individuals. For example, in some individuals, MAP may be maintained via large increases in HR, with a lesser increase in peripheral resistance, while another individual may respond to the same orthostatic stress with substantial vasoconstriction and only slight increases in HR. Detailed accounts for the timing for hemodynamic changes in response to LBNP have been recently reported (Hisdal *et al*., 2001; Fu *et al*., 2009; Cooke *et al*., 2004; Grasser *et al*., 2009; Smith *et al*., 1994; Thomas *et al*., 2009; Wieling *et al*., 2007). From these sources, general responses to incremental increases in LBNP are seen in Table 1.5.1.

Table 1.5.1 General responses to lower body negative pressure

LBNP	10-20 mmHg	20-40 mmHg	>40 mmHg	Syncope
Variable				
HR	↔	↑	↑	↓
MAP	↔	↔	↔↓	↓
PP	↔↑	↔	↓	↓
Q	↓	↓	↓	↓
CVP	↓	↓	↓	↔↓
SV	↓	↓	↓	↓
TPR	↑	↑	↑	↓

HR – heart rate; MAP – mean arterial pressure; PP – pulse pressure; Q – cardiac output; CVP – central venous pressure; SV – stroke volume; TPR – total peripheral resistance

If adequate cardiovascular adjustments are not made to maintain blood pressure, cerebral perfusion may drop to the point where compensatory syncope results. The ultimate physiologic goal is to always maintain blood flow to the brain.

1.6 Cerebral Autoregulation

1.6.1 The cerebral circulation

Most of the blood going to oxygenate the brain arrives through the two internal carotid and two vertebral arteries (Goadsby & Edvinnson, 2002a). The left and right vertebral arteries converge to form the basilar artery, which connects to branches of the internal arteries to form the circle of Willis. Cerebral arteries and arterioles are formed by layers of endothelium, smooth muscle cells, and adventitia. The endothelium is an intricate organ involved in various essential processes for local circulatory control (Andresen *et al*., 2006).

The brain requires the oxidation of glucose for its function, which is only possible with adequate oxygen and glucose delivery. Blood flow to the brain must be at least 50 mL/min/100 g of brain tissue in order to satisfy cerebral demand. The brain utilizes a quarter of the body's oxygen consumption, which is about 70 ml/min and up to 20% of total Q. This

is a large proportion of total Q, based on the relative size of the brain. Preservation of CBF is governed by the balance between intracranial pressure (ICP) and MAP. The difference between MAP and ICP is cerebral perfusion pressure (CPP; Equation 1.6.1). In healthy individuals, one of the most important determinants of CBF is cerebral perfusion pressure CPP. CBF is directly proportional to CPP and inversely proportional to cerebral vascular resistance (CVR; Equation 1.6.2). An interruption in CBF for about 8-10 seconds tends to cause a loss of consciousness, or syncope (Madsen *et al*., 1998; Njemanze, 1992).

$$CPP = MAP-ICP \quad \text{Equation 1.6.1}$$

$$CBF = (MAP-ICP)\bullet CVR \quad \text{Equation 1.6.2}$$

An increase in ICP or a decrease in MAP will reduce CPP and consequently, CBF. Normal CBF for an adult human is 45-60 mL/100g/min. The lowest flow rate at which the brain can avoid ischemia is termed the critical CBF, and is approximately 16-17 mL/100g/min.This flow rate may be reached if the CPP is reduced to 30-40 mmHg. If MAP decreases to where it equals ICP, CPP will equal zero, and CBF will terminate (Walters, 1998).

Tight junctions between endothelial cells in the vessels of the central nervous system (CNS) control the crossing of solutes from the blood into the cerebral spinal fluid (CSF). Carbon dioxide (CO_2) diffuses easily into the CSF producing immediate changes of pH in the blood. Acute changes in the arterial carbon dioxide tension ($PaCO_2$) between 20 and 60 mmHg have been shown to change CBF 1 to 2 ml/per minute per 100g of brain tissue for every 1 mmHg change in PCO_2 (Reivich, 1964). Between this $PaCO_2$ range, the relationship to CBF is fairly linear; though, beyond these upper and lower limits, the responsiveness of the cerebral vasculature is reduced because the vessels have reached their maximal dilating and constricting capacity (Grubb *et al*., 1974). In fact, in 2003, Ide and colleagues examined the relationship between end tidal CO_2 ($ETCO_2$) and CBF with TCD over a wide range of $ETCO_2$ values. CBF tended to follow changes in $ETCO_2$ very closely between 20-50 mmHg,

with slightly greater sensitivity at higher CO_2 values (Ide *et al.*, 2003). In general, in studies where participants breathed high CO_2 concentrations or hyperventilated while arterial oxygen was held constant, MCA BFV changed 2-5% per mmHg change in CO_2 (Ide *et al*., 2003; Poulin *et al.*, 1996). If arterial CO_2 increases without a concomitant compensatory increase in ventilation, the cerebral vasculature is exposed to high CO_2; this puts the CSF pH at risk of becoming overly acidic, potentially compromising neuronal signaling. In addition to tightly regulating the pH surrounding the brain, it is equally as important that the brain receive a continual supply of fuel. Glucose oxidation is thought to supply almost all of the energy required by neurons to sustain brain activity. This occurs at a rate of approximately 110-145 g/day (Reinmuth *et al*., 1965), and accounts for about 10-12% of the glucose used in the entire human body (Raichle *et al*., 1970; Boyle *et al*., 1994; Powers *et al*., 2007). Precisely controlling the internal environment of the brain is accomplished through intrinsic regulation of cerebral vascular tone, which modifies the incoming CBF carrying the essentials required for adequate brain function (Kety & Schmidt, 1948).

1.6.2 Autoregulation

Systemic blood pressure is prone to fluctuation. The cerebral autoregulatory system functions to preserve sufficient CBF during periods of blood pressure variation between 60-140 mmHg (Paulson *et al*., 1990). It is within this systemic BP range that the cerebral vasculature—with greatest involvement from the arterioles less than 100-250 μm—is able to maintain CPP through vasodilation and vasoconstriction. An increase in CVR occurs if BP is elevated, and a decrease in CVR occurs when systemic BP drops (Equation 1.6.2).

There are three mechanisms by which the autoregulatory system maintains CBF (Paulson *et al*., 1990; Aaslid *et al*., 1989; Strandgaard & Paulson, 1984). The first involves

the capacity of the smooth muscle cells in the arterioles to vasoconstrict under conditions of increased cerebral BP (Bayliss, 1902; Fog, 1937; Folkow, 1964; MacKenzie *et al*., 1979). Second, modifications in CVR can be accomplished through release of elements such as oxygen and adenosine (Paulson *et al.*, 1990), and by the presence on carbon dioxide (CO_2) (Ursino & Lodi, 1998) and NO (White *et al*., 1988; Joshi et al., 2000). An increase in CO_2 concentration induces vasodilation, while decreased CO_2 causes vasoconstriction (Harper & Glass, 1965; Lassen, 1964; Poulin *et al*., 1996; Lodi *et al*., 1998; Shapiro *et al*., 1970). There are numerous accounts suggesting that dilation of cerebral arterioles is dependent on NO formation (Moncada *et al*., 1991), in the mouse (Rosenblum *et al*., 1990; Irikura *et al*., 1995; Ma *et al*., 1996; Meng *et al*., 1996) and in some other species (Faraci, 1991; Wei *et al*., 1992; Faraci *et al*., 1993; Colonna *et al*., 1994). Human studies have also shown NO mediating endothelial-dependent relaxation in cerebral arteries (Onoue *et al*., 1994; Petersson *et al*., 1995). Third, there is a neurogenic mechanism contributing to cerebral regulation of blood flow, which is dependent on the innervation of cerebral vessels by sympathetic and parasympathetic nerves (Goadsby & Edvinnson, 2002b). The sympathetic system can generate vasoconstriction in the cerebral vessels (Gulbenkian *et al*., 2001); however, its primary role is likely involved in protecting cerebral vessels by moving the upper boundary of the cerebral autoregulatory curve to a higher pressure in response to sympathetic-derived increases in BP (Chillon & Baumbach, 2002; Goadsby & Edvinnson, 2002b). The precise influence of sympathetic stimulation on cerebral vessels during orthostatic stress has not yet been unequivocally determined. Upon stimulation, the parasympathetic system can induce vasodilation of cerebral vessels, yet does not seem to serve a significant purpose in cerebral autoregulation (Goadsby & Edvinnson, 2002a; Hamel, 2006).

In hypertensive individuals, BP has risen slowly over time resulting in a rightward shift of the pressure-flow curve. This is a compensatory mechanism that protects the cerebral

vasculature from further increases in BP (Strandgaard, 1976; Strandgaard & Paulson, 1995; Serrador *et al*., 2005). It is probable that shifting of the autoregulatory limits involves structural and functional changes in the cerebral vasculature owing to alterations in autonomic activity (Heagerty et al., 1993). These changes may include thickened arteriole walls, and narrowed vessel lumina due to hypertrophy and remodeling. In disease states such as diabetes and hypertension, there is endothelial dysfunction, which may impair CO_2 vasoreactivity (Lavi *et al.*, 2006). However, patients with treated hypertension can demonstrate normal cerebral autoregulation (Kastrup *et al.*, 1998; Eames *et al.*, 2003). In fact, after 6 months of successful treatment with antihypertensives, patients demonstrated increases in CBF velocity (CBFV) and carotid distensebility (Lipsitz *et al.*, 2005). The treatment did not impair cerebral autoregulation, establishing that treating systolic hypertension does not cause cerebral hypoperfusion, which is in agreement with other studies (Olsen *et al.*, 1995; Pandita-Guawardena & Clarke, 1999; Fu *et al.*, 2005), but had not always been the view in the past (Turner *et al*., 1977; Joshi *et al*., 2004) .

The number of studies examining autoregulation of CBF during orthostatic challenge is not abundant. To date, data show that CBFV in the MCA consistently decreases during orthostatic stresses, such as LBNP (Levine *et al.*, 1994; Bondar *et al.*, 1995) and upright tilt (Grubb *et al.*, 1991; Hughson *et al.*, 2001; Novak *et al.*, 1998; Serrador *et al.*, 2000b). Hams *et al*. (2000) confirmed that when MAP is measured at the level of the heart during a supine-to-standing transition, the derived BP at the level of the MCA drops by an average of 19 mmHg after 1 minute and 14 mmHg after 5 minutes. The decrease in MCA BP will happen despite MAP at the heart remaining constant or even slightly increasing. With evidence from Alperin and colleagues (2005a), the general consequence of a supine-to-standing posture change is a comparatively smaller drop in ICP (relative to MAP) so that CPP decreases sufficiently to result in a drop in CBF during posture change (Equation 1.6.1). The

underlying mechanisms for why CBF drops during orthostasis are not clear. The reduction in CBFV may be indicative of a paradoxical vasoconstriction in the cerebral vessels (Levine *et al*., 1994; Bondar *et al*., 1995; Joyner *et al.*, 1990). In 2007, Zhang *et al.* measured CBFV during LBNP, before and after autonomic ganglionic blockade, which precludes vasoconstriction in the cerebral vasculature and subsequent decreases in CBFV. Mean arterial pressure was maintained by infusion of the sympathomimetic agent, phenylephrine. Contrary to the initial hypothesis, CBFV dropped 14%, indicating that CBFV decreases through mechanisms other than cerebral vasoconstriction *per se*. The authors speculated that reduced pulse pressure or pulsatile flow may lower shear stress on the cerebral vessel walls, resulting in a drop in CBFV caused by flow-mediated constriction. In addition to inhibiting sympathetic activity, ganglionic blockade also blocks parasympathetic neural activity. Thus, reductions in CBFV during orthostasis due to inhibition of parasympathetic cerebral vasodilation cannot be ruled out (Zhang *et al.*, 2002).

The typical decrease in CBFV during orthostasis has also been connected to decreases in ET_{CO_2}. It is well recognized that cerebral vessels are strongly affected by partial pressure of arterial CO_2 (Immink *et al*., 2006; Brian *et al.*, 1996), and that postural hypocapnia is a common occurrence (Serrador *et al.*, 2006), particularly during head-up tilt (Cencetti *et al.*, 1997), and this occurs without changes in respiratory rate. However, reductions in CBFV during orthostasis cannot be exclusively a result of hypocapnia. Observations suggest that reductions in CBF and $PaCO_2$ during orthostasis may not be directly related because it has been doubtful as to whether changes in ET_{CO_2} during orthostasis accurately represent changes in $PaCO_2$ (Immink *et al*., 2006; Serrador *et al*., 2006). In these studies, the extent of the reductions in $PaCO_2$ during orthostasis were proportionally smaller than those detected in ET_{CO_2} (Immink *et al.*, 2006; Serrador *et al.*, 2006). Also, even when ET_{CO_2} was held at constant baseline values, this did not prevent the

decrease in CBFV during HUT (Blaber *et al*., 2001). Others have also demonstrated a lack of connection between decreases in $PaCO_2$ and CBFV (Serrador *et al.*, 2006), signifying that in addition to the reduction in $PaCO_2$, there must be other explanations for the reduction in CBFV during orthostatic challenges.

Technological developments such as transcranial Doppler ultrasound (TCD) and continuous photoplethysmographic blood pressure monitoring have made quantification of CBFV during dynamic conditions possible. The beat-by-beat output from TCD can be analyzed using a number of techniques depending on the details of the desired end product. Indices of cerebral autoregulation tend to involve calculations incorporating CBFV values. The cerebrovascular resistance index (CVRi) is commonly used as an indicator of cerebral autoregulation, and is defined by the equation below.

$$CVRi = \text{Mean BP} / CBFV \qquad \text{Equation 1.6.3}$$

Because measurements of MCA diameter are often unfeasible in the research setting, CBFV is used rather than CBF. This substitution relies on the assumption that CBFV in the MCA is representative of CBF. It is possible that changes in the diameter of the MCA due to altered distending pressure, sympathetic stimulation, PCO_2 or others factors may alter velocity independently of flow. However, the MCA is a large conduit vessel. It is thought that sympathetic activation does not alter the diameter of the conduit arteries, but instead, changes the patency of the small resistance vasculature. The interchangeability of CBFV for CBF has been demonstrated on a number of occasions (Valdueza *et al.*, 1997; Serrador *et al.*, 2000a; Panerai *et al.*, 2001). Valdueza *et al*. (1997) were the first to measure MCA diameter changes in conscious humans under hypocapnic conditions and found no change in diameter during normal versus hyperventilation. The majority of studies have found that diameter changes in the cerebral vasculature in response to arterial CO_2 occur in vessels that are downstream to the MCA, although some controversy exists about whether this is true (Valdueza *et al.*,

1999). Serrador's group (2000a) examined MCA diameter under hypercapnic conditions and found consistency between CBFV and CBF. MCA diameter changes are examined using magnetic resonance imaging (MRI), and although its precision is impressive, it is possible that smaller MCA diameter changes go undetected. Indeed, there could be a CO_2 effect on the size of the MCA that have not been identified, meaning that CBF rates may not be perfectly reflected by CBFV. However, to date, the supposition that CBFV closely follows CBF appears to be the most accepted resolution to the difficulty in directly measuring CBF.

In addition to the CVRi, there are additional methods to quantify cerebral autoregulation, such as the pulsatility index (PI).

PI = systolic CBFV – diastolic CBFV / mean CBFV Equation 1.6.4

This index is defined as the difference between systolic and diastolic CBFV limits divided by the mean CBFV value. PI is similar to CVRi in that both are inversely correlated to CBFV in steady state. Using PI as an independent indicator of dynamic cerebral autoregulation is flawed; however, because in the case of neurogenic syncope, PI will increase and CVRi will decrease (Schondorf *et al.*, 1997). An additional estimate for vasomotor resistance is the critical closing pressure, which describes the BP value at which CBF is expected to drop to a state of zero flow (Panerai, 2003). The rate of recovery is expressed as the changes in CVRi per second after a decrease in BP.

Rate of recovery = (ΔCVRi / ΔT) / ΔBP Equation 1.6.5

This quantification tends to be a representation of the efficiency of dynamic cerebral autoregulation (Aaslid *et al.*, 1989). Although CVRi is included in the calculation, the cross-sectional area element separating CBFV from CBF is still not accounted for. The rate of recovery expression has yet to be validated in all populations, particularly, in the elderly population (van Beek *et al.*, 2008). In 1995, Tiecks and colleagues introduced the Autoregulatory Index, when examining CVRi in response to changes in BP using thigh cuff

release (Tiecks *et al.*, 1995). For every change in BP, a hypothetical CBFV response is modeled, with autoregulatory index values ranging from 0 to 9. A value of zero indicates that CBFV passively follows BP, and a value of 9 representing a state wherein the CBFV recovers more quickly than BP.

In the frequency domain (i.e. physical signals that are described with respect to frequency rather than time), oscillatory patterns between BP and CBFV can be compared, as first described by Giller (1990). Using spectral analysis, a frequency domain graph can be created demonstrating the frequency spectrum of hidden oscillations found within the signal. Cerebral autoregulation can be investigated using this technique to look at the transfer of oscillations from BP to the CBFV. The gain or magnitude of the signal represents the dampening effect of cerebral autoregulation on the magnitude of the BP oscillations. A low gain is indicative of increased dynamic cerebral autoregulatory efficiency, while a high gain represents a less efficient autoregulatory process. Oscillations in CBFV and BP do not occur simultaneously. CBFV recovers more quickly than in the systemic circulation, and for that reason, an intact dynamic cerebral autoregulatory process is one where the oscillations in BP almost appear to *follow* those occurring in the cerebral vasculature (Kuo *et al.*, 2003). This reverse lag is termed a phase shift. Phase shifts can be thought of as a surrogate measure to the time delay in the autoregulatory process.

Changes in systemic BP may occur rapidly or gradually. Cerebral autoregulation must be proficient in accommodating both dynamic and static conditions. These two components of autoregulation may function using different control mechanisms, and dynamic regulation may be more vulnerable to pathological conditions, such as ischemic stroke (Dawson *et al*., 2000; Dawson *et al*., 2003). Regardless of the modality and type of analyses used for investigating cerebral autoregulation, all population types need be measured, contribute data, and include various disease states and the entire age spectrum.

1.7 Aging

1.7.1 General physiological changes

Age-related physiologic changes differ greatly between individuals, and are conditional on genetics, physical fitness, nutrition, socioeconomics, and environment, among others. Accordingly, the rate of age progression is also broad. Often, the term *biological age* is used to distinguish it from chronologic age. Regardless of what factors are propelling biological aging, there are several archetypical transformations that take place.

Aging has a tremendous effect on the structure and function of blood vessels. The greatest impact is seen in the mechanical properties of vessel walls. In order for the work of the heart to be as low as possible, the aorta needs to be easily distensible and offer little impedance. Impedance, or resistance, can be described as the ratio of the change in pressure inside the vessel to the change in volume flowing into it. In the young, arteries are elastic and absorb the energy associated with cardiac ejection of the stroke volume. However, the aorta and the major arterial segments (carotid, iliac, femoral and brachial) stiffen with age (Franklin *et al.*, 1997). The loss of compliance in these conduit arteries is a dominant risk factor for cardiovascular disease (Blacher *et al*., 1999a; Blacher *et al*., 1999b). The term *compliance* describes a change in the volume of arteries following a change in blood pressure (Simon & Levenson, 1991). Coronary artery perfusion is highest during the elastic recoil of the arteries in diastole. When elasticity is lost, coronary blood flow may be compromised, contributing to coronary artery disease (Dart & Kingwell, 2001; Madhavan *et al.*, 1994; Ouchi *et al.*, 1991).

The walls of the large arteries are normally full of elastin and collagen. Arterial remodeling occurs when elastin fractures and collagen is deposited excessively. As the heart pumps against a more rigid tube, the arteries are less able to accommodate the bolus of blood,

causing the generated pulse wave to travel faster through the arterial tree. The particular shape and amplitude of the pulse waveform ejected from the heart are highly dependent on the stiffness and thickness of the vessel wall, the timing of the returning wave reflection, and to a smaller degree, the inertia of the blood through contact with the vessel wall (Greenwald, 2007). With stiff arterial walls, blood travels more quickly through the vessels causing *early* return of the pulse wave to augment systolic pressure. This is in contrast to the compliant artery of the younger individuals. Early wave reflection exacerbates damage to arterial walls and perpetuates the destructive cycle of increased wall stress and structural changes. The Augmentation Index (AI) is the early wave reflection-induced increase in systolic pressure as a percentage of pulse pressure. In youth, AI is 0%, but increases toward 50% in older individuals (Laurent *et al.*, 2006; Lakatta & Levy, 2003; O'Rouke & Hashimoto, 2007; Agabiti-Rosei *et al.*, 2007; Vaitkevicius *et al.*, 1993). Increased proximal systemic resistance is detrimental to left ventricular heart function, and in organs with high resting flow rates (e.g. brain and kidney), pulsatile flow penetrates further into the vasculature to damage the microcirculatory arterioles (O'Rouke, 2007).

Up until the age of about 50 years, MAP, SBP, and DBP rise secondary to increasing peripheral resistance (Franklin *et al.*, 1997). After age 50 to 60, there is a decrease in DBP, with a concomitant increase in pulse pressure (PP), likely due to increase large artery stiffness (Franklin *et al.*, 1997). From Franklin and colleagues (Franklin *et al.*, 1997), it was also reported that if hypertension is left untreated, the rate at which large arteries stiffen becomes higher, proliferating the cycle of advancing hypertension and arterial stiffness. Studies from autopsies have shown that with age, thickening of the aortic wall entails increased intima media distance (Lakatta & Levy, 2003a; Lakatta & Levy, 2003b; Allan *et al.*, 1997; Nagai *et al.*, 1998; Gariepy *et al.*,

1998), regardless of prevalence within a population (Virmani *et al.*, 1991). Albeit there are increases in intima-media thickness (Rosvall *et al*., 2005a) with age, the overall diameter of the large arteries also increases (Virmani *et al.*, 1991). Larger IMT is associated with vascular risk factors (Salonen & Salonen, 1993; Bots *et al*., 1997; Chambless *et al*., 1997; O'Leary *et al*., 1999; Chambless *et al*., 2000; Iglesias del Sol *et al*., 2002; Hollander *et al*., 2003; Kitamura *et al*., 2004; Rosvall *et al*., 2005a; Rosvall *et al*., 2005b; Lorenz *et al*., 2006; Lorenz *et al*., 2007).

1.7.2 Aging and the baroreflex

It has been well established that the adjustments in R-R intervals modulated by the baroreflexes are weakened with age, and this has been recently reviewed (Monahan, 2007). However, some debate still exists about whether aging is associated with an alteration in baroreflex modulation of sympathetic outflow (Monahan, 2007). Studinger and others (Studinger *et al*., 2009) demonstrated for the first time that baroreflex regulation of vascular sympathetic involvement shows lesser activation and greater inhibition with age. Most research until then had shown no difference in arterial baroreflex control of sympathetic activity with age, or had ambiguous conclusions (Ebert *et al*., 1992; Matsukawa *et al*., 1994; Matsukawa *et al*., 1996; Davy *et al*., 1998; Rudas *et al*., 1999). The recent results of Studinger and colleagues (Studinger *et al.*, 2009) have also revealed that aging causes the sympathetic responses to decreases in BP to differ from those in response to BP increases. With age, when BP rapidly decreases, sympathetic activation declines, possibly because of baroreceptors vessel stiffening. During an increase in BP, sympathoinhibition is augmented, because of increased sensitivity in neural control. These data are based on cardiovascular responses to vasoactive drugs, which could have age-related changes on vascular smooth

muscle. Further elucidation of the age-dependent changes in baroreceptor compensation is needed. Particularly, the use of different methods to induce BP fluctuations (e.g., tilt; LBNP) is of great importance.

The effect of age on an exact segment of the baroreflex is unknown. In current research that focuses on baroreceptors, it is quite well accepted that cardiovagal baroreceptors sensitivity is inversely correlated with age ($r = -0.65–0.69$; Monahan *et al.*, 2001a; Laitinen *et al.*, 1998). Cardiovagal desensitivity may develop from changes in the afferent arm, central integration, or efferent arm of the baroreflex. It is not known whether a certain baroreceptor stimulus causes a similar change in afferent activity over all age ranges. Additionally, it could be possible that age-dependent arterial stiffening may contribute to baroreceptor dysfunction. The less compliant walls may reduce the stretch (stimulus for baroreceptors) during a given BP change. The sympathetic section of the baroreflex involves variation in sympathetic outflow in response to changes in baroreceptors input. Individuals with existing reduced baroreflex capacity exhibit a smaller change in sympathetic outflow in response to a particular change in BP. Direct measurements of sympathetic outflow are often substituted by measures of blood flow or vascular resistance. Some early studies reported that forearm vasoconstrictor responses to LBNP were attenuated in older individuals, relative to younger persons (Jingu *et al.*, 1989; Cléroux *et al.*, 1989). These findings served as preliminary evidence to support an age-related baroreflex dysfunction, but did not definitively reveal the effects of aging on sympathetic baroreflex function, specifically. In contrast to reported cardiovagal baroreceptors desensitivity with age, sympathetic baroreceptors function appears to remain intact with age (Monahan, 2007).

1.7.3 Aging and cerebral autoregulation

Because aging is associated with a diminished baroreflex (Gribbin *et al*., 1971) and a lower baseline CBFV (Krejza *et al.*, 1999; Matsuda *et al.*, 1984), the elderly are at increased risk and have a higher incidence of orthostatic hypotension (Shibao *et al*., 2007). Dynamic cerebral autoregulation research in the elderly has really only begun over the last ten years. There are some pathological conditions that are associated with impaired dynamic cerebral autoregulation, such as ischemic stroke (Eames *et al.*, 2002), which tends to be highly prevalent in the elderly population. In a group of middle-aged and older individuals with sustained untreated hypertension (mean 67 years), dynamic and static cerebral autoregulation were maintained (Eames *et al*., 2003). Conflicting evidence does exist, and meta-analyses of age-related changes in cerebral autoregulation are difficult because of important methodological discrepancies between experiments. In order to resolve the uncertainty, an understanding of the differences between methodologies is necessary. In a recent review (van Beek *et al.*, 2008), eight studies from 2000 to 2006 were examined. In all studies, authors generally reported aged individuals showing no changes in cerebral autoregulation during decreases in BP (Carey *et al.*, 2000; Lipsitz *et al.*, 2000; Narayanan *et al.*, 2001; Carey *et al.*, 2003; Heckmann *et al.*, 2003; Sorond *et al.*, 2005; Franke *et al.*, 2006; Yam *et al.*, 2005; van Beek *et al.*, 2010), and most recently, work from van Beek and colleagues (2010) and Hernandez and colleagues (2010) are also in agreement. The greatest inconsistency between these studies is the method used to assess dynamic cerebral autoregulation. Transfer function analysis, autoregulatory index, pulsatility index, rate of recovery, correlation index, and cerebrovascular resistance represent the range of methodologies that were used. However, despite these important differences, the overall inferences about dynamic cerebral autoregulation in elders are consistent.

Cerebral autoregulatory capacity also tends to be preserved in elderly individuals despite higher fitness levels (Heckmann *et al*., 2003; Hernandez & Franke, 2005), which was investigated because of an earlier hypothesis that suggested endurance athletes show tendency for lower orthostatic tolerance than untrained people (Geelen & Greenleaf, 1993; Raven & Pawelczyk, 1993; Levine *et al*., 1991). In general, the studies investigating fitness-related associations in autoregulation have been carried out in older participants between the ages of 50 to 75 years. Exploring cerebral autoregulatory changes during decreases in BP in the very elderly (>75 years) has yet to be documented.

An important limitation to the measurement of cerebral autoregulation is that TCD is indicative of *large* vessel CBFV only. When near-infrared spectroscopy (NIRS) has been used (which can measure *micro*vascular oxygenation), there have been discrepancies in assessment of autoregulatory function (Mehagnoul-Schipper *et al*., 2000; Mehagnoul-Schipper *et al*., 2001; Hunt *et al*., 2006). The research by Mahagnoul-Schipper and colleagues (2000) revealed that elderly participants (mean 74 years) exhibited reductions in frontal cortical blood oxygenation and blood volume during supine-to-standing compared to their younger counterparts. The same authors reproduced this finding a year later. In 2006, Hunt and others examined aged individuals (mean 60 years) and showed a postural decrease in the ratio between oxygenated hemoglobin (HbO_2) and total hemoglobin concentration (Hb). The use of various modalities has led to inconsistencies in results about age-related changes in cerebral autoregulation. Interpretation of results form study need to be made with caution. Most recently, Edlow and others (2010) used a new technique to evaluate cerebral hemodynamic responses to posture change across a large age range. The authors used a combination of NIRS and diffuse correlation spectroscopy to detect relative cerebral blood flow changes, and noted that with normal aging, there is a decrease in the magnitude of change in frontal cortex HbO_2, but no change in relative CBFV during a sit-to-stand

movement. Since there were no changes in relative CBFV with posture change, it was suggested that aging may not be associated with a decrease in overall cerebral autoregulatory function. However, the alterations in frontal cortical HbO_2 with posture change is consistent with the work of Kim and colleagues (2009) using NIRS. It seems, therefore, that disagreement in methods creates differing interpretations of age-linked physiological changes. Keeping in mind the earlier studies documenting age-associated declines in baseline CBF (Matsuda *et al*., 1984; Krejza *et al*., 1999), it is not surprising that younger individuals have a larger drop in frontal HbO_2 because their baseline CBF is higher and it is expected that their comparative decrease in CBF may be greater than the older individuals. Alternatively, or perhaps additionally, the incongruity between age-related postural changes in relative CBF and HbO_2 is explained by alterations in the distribution of blood flow through the Circle of Willis with age during posture change. In fact, aged individuals may be less able to redirect CBF from the anterior to the posterior region of the cerebrum during posture change and there is support for this impairment of posterior autoregulatory function in the elderly (Haubrich *et al.*, 2004; Sorond *et al*., 2005).

Static cerebral autoregulation can be measured by comparing CBF between steady states in response to a change in MAP. Dynamic cerebral autoregulation can be processed in a number of ways using various methodologies that either measure the amount of change, or the speed of change of CBF that occurs with manipulation of MAP (van Beek *et al*., 2008). In essence, uncertainty continues regarding clarification of age-dependent changes in CBF during orthostatic challenge. Fully exposing these mechanisms requires a broad knowledge base of the current limitations in this area. Regardless of the measurement type that is used, dynamic cerebral autoregulation is preserved in healthy elderly persons less than 75 years, although symptoms of cerebral hypoperfusion tend to be common among elderly persons.

1.7.4 Aging and cardiovascular responses to orthostasis

An assemblage of research has described the prevalence of orthostatic hypotension in the elderly population to be approximately 6% to 33% (Graagmans *et al*., 1996; Jonsson *et al*., 1990; Mader, 1989; Ooi *et al*., 1997; Rutan *et al*., 1992; Tilvis *et al*., 1996; Vogt, 1995). In general, a drop in systolic pressure of 20 mmHg or more upon changing towards an upright posture is considered orthostatic hypotension, and of noteworthy clinical importance (Kapoor *et al*., 1983). Because orthostatic hypotension tends to be an important risk factor for falls in the elderly population, investigating methods to improve tolerance is of interest to many. Research in younger individuals has shown some enhancement in tolerance with endurance training (Convertino, 1993), while others have not (Raven *et al*., 1984; Raven & Pawelczyk, 1993; Hernandez *et al*., 2005). In the same way, exercise training in elderly persons has also yielded conflicting results with some (Fortney *et al*., 1992; Gabbett *et al*., 2001; Hernandez & Franke, 2005; Hernandez *et al*., 2005; Hernandez *et al*., 2010; Panton *et al*., 2001; Formes *et al*., 2010; Shi *et al*., 2008) finding that orthostatic tolerance is unchanged by improved fitness. However, categorizing these studies is problematic because there are substantial differences between them. For example, Fortney and others (1992) examined one group of endurance-trained older adults and one group of age-matched controls. Aerobic conditioning was assessed by maximal oxygen uptake and orthostatic response was tested with LBNP levels up to -50 mmHg. Similarly, Formes *et al*. (2010) studied active and sedentary elderly participants, which was defined by oxygen uptake to graded cycling. LBNP was used to elicit a central hypovolemic response and cerebral auoregulation was measured. Peak oxygen consumption was also examined by Shi and colleagues (2008) in a group of physically active and sedentary elderly men in an attempt to assess carotid baroreceptor function and make inferences about carotid baroreceptor HR reflex sensitivity. They found that active lifestyle enhances carotid baroreceptor sensitivity. In 2005, Hernandez and colleagues tested fit and

unfit eldery, and fit and unfit young. Estimated peak oxygen uptake was used to assess the pre-existing fitness level of each participant, designating them as either fit or unfit. Tolerance to LBNP did not differ between groups. Perhaps most convincingly, Hernandez & Franke (2005) carried out an interventional study to determine the influence of a 6-month endurance training program on calf venous compliance and maximal LBNP tolerance in elderly individuals. The data indicated that endurance training did not alter LBNP tolerance despite improved venous compliance. Likewise, Panton *et al*. evaluated whether a 12-week resistance training program would alter cardiovascular response to LBNP in elderly. Muscle strength and size increase; however, responses to LBNP did not. Most recently, Hernandez and others (2010) studied active older and endurance-trained younger individuals. Because these participants were not contrasted aginst unfit counterparts, inferences about exercise effects cannot be made. However, these authors showed that in these fit individuals, despite their differering heart rate responses, MAP responses were not different between age categories, suggesting that aging alone does not predispose individuals to orthostatic intolerance.

Despite these inconsistencies, it is undeniable that the cardiovascular responses to orthostatic challenges differ between the elderly and the young. It seems as though aged persons are similarly able to maintain MAP during orthostatic stress, yet the mechanism by which this is achieved is different than in younger individuals. Researchers have repeatedly found a diminished tachycardia response to orthostatic stress in the elderly (Guo *et al*., 2005; Frey & Hoffler, 1988; Gabbett *et al*., 2001; Hernandez et al., 2005; Shi *et al*., 2000; Tsutsui *et al*., 2002). It is probable that the underlying mechanism for this age-related blunting of HR response is typical vagal dysfunction seen with age (Ebert *et al*., 1992; Hunt *et al*., 2001; Laitinen *et al*., 1998; Monahan, 2007; Monahan *et al*., 2001a; Tank *et al*., 2000; Smit *et al*., 1999).

Along with the attenuated HR response, maintaining MAP in elderly tends to be accomplished through a concomitant increase in TPR and/or differences in redistribution of blood flow (Laitinen *et al*., 2004; Shannon *et al*., 1991; Youde *et al*., 2003; Minson *et al*., 1999). However, some studies have shown that elderly persons have a reduced vasoconstrictor response to central hypovolemia (Groothuis *et al*., 2008; Länne & Olsen, 1997; Olsen & Länne, 1998; Olsen *et al*., 2000; Fu *et al*., 2002; Groothuis *et al*., 2008), while others have shown the opposite (Allan *et al.*, 1997; Shi *et al.*, 2000). Additionally, venous compliance has been reported to be unchanged with age (Olsen *et al*., 2000; Frey & Hoffler, 1988; van Hoeyweghen *et al*., 2001; Lindengerger & Länne, 2007), or it has been found that, at any rate, venous compliance has no significant impact on LBNP tolerance (Hernandez & Franke, 2004). Altogether, the exact mechanism by which the elderly regulate BP and CBF during orthostatic stress is still unclear.

2.0 Thesis Experiment

2.1 Purpose & Hypotheses

Objectives

The primary aim of this project was to contrast physiologic responses during *simulated* orthostasis (lower body negative pressure) and during *true* orthostasis (posture change). The secondary objectives were to investigate the within-condition responses to incremental lower body negative pressure and to posture change, and to contrast related variables in the two conditions.

Hypotheses

The primary hypothesis was that the two conditions would produce very similar physiologic responses, validating the use of incremental lower body negative pressure as a surrogate for posture change.

We expected the following directional changes for lower body negative pressure and posture change: increases in heart rate, total peripheral resistance, and brachial vascular resistance, and decreases in stroke volume, cardiac output, and exhaled carbon dioxide. In addition, central venous pressure, inferior vena cava diameter, and portal vein diameter were projected to decrease during lower body negative pressure (variables could not be measured during standing).

Additionally, we predicted relationships between stroke volume and pulse pressure; stroke volume and central venous pressure; pulse pressure and heart rate; and cerebral blood flow velocity and end tidal carbon dioxide.

2.2 Methods

The Office of Research Ethics at the University of Waterloo reviewed and approved all components of this study (ORE #16059). Two experimental conditions were tested and contrasted. Each participant underwent an incremental protocol of lower body negative pressure (LBNP) followed by a supine-to-standing posture change. The order in which the two conditions were tested was not altered between individuals; the posture change segment always took place after LBNP. This arrangement was maintained primarily to facilitate catheter set-up for the central venous pressure measurement. At the outset of the experiment, it was important to ensure that the venous pressure measurement was successful in each participant during LBNP before proceeding to the posture change maneuver. The time required to organize participants and equipment for the posture change was reasoned to have been sufficient to return physiological variables to baseline. Moreover, the two baselines were statistically compared for correspondence.

2.2.1 Population description

Ten independently-living individuals over the age of 70 years participated in this thesis project. Physical characteristics are listed in Table 2.3.1. All participants were recruited viva voce from nearby municipalities (Region of Waterloo and North Perth). Testing was carried out at the University of Waterloo in the Cardiorespiratory and Vascular Dynamics Laboratory. A pre-testing information session took place at a time and location that was convenient for the participant. Written, informed consent was obtained from each volunteering individual. Participants had no uncontrolled medical issues, based on a self-reported medical history (see Appendix for sample form), including current medications, past health issues, and presence of cardiovascular disease or related conditions was

obtained from each individual. Medical issues and medications and are shown in Table 2.3.2 and 2.3.3. Key Inclusion/Exclusion criteria are listed in Table 2.3.4. Participants were asked to refrain from consuming alcohol (Regan, 1990), caffeine (Whitsett et al., 1984), and nicotine (Benowitz et al., 1984) for at least 12 hours prior to testing, and avoid exercise and food for 3 hours prior to testing. In order to prevent skin blood flow from affecting brachial velocity measurements, the temperature of the room was maintained in range of 21-23ºC.

2.2.2 Experimental Protocol

Lower body negative pressure

Lower body negative pressure (LBNP) was used as a means to induce orthostatic stress while participants remained in the supine position. Keeping motionless during the protocol facilitates several measurements that are not possible when the participant is in motion (e.g. during active posture change or tilting). The LBNP apparatus consists of a tightly sealed wooden rectangular box that encloses the lower half of the participant's body. Neoprene material connects the box to the participant at the level of the iliac crest, and is secured with a belt to ensure an air-tight seal. A bicycle seat is situated inside the box to oppose the tendency for the participant to be pulled in the direction of the negative pressure, which is generated from a standard vacuum cleaner (Beaumark 99056) connected to the box. The intensity of negative pressure is adjusted using a voltage regulator (Staco Inc, Dayton, Ohio), and read using a pressure manometer (Traceable®).

The neoprene is a very stretch-resistant material, and fit tightly for each participant resembling a "skirt". Participants were assisted onto the table and into the LBNP box. Once comfortable, the neoprene was adhered to the box via fabric hook and loop fastener (i.e.

Velcro®), and secured around the participant with a belt. Care was taken not to compress abdominal contents. The integrity of the seal was quickly tested by starting the vacuum and observing the pressure manometer values decrease. The remainder of the monitoring equipment was then connected to the participant and verified for functionality. Participants were reminded about the symptoms they may experience and to notify the researchers if they feel dizziness, light headedness, or nausea. Test termination criteria are listed in Table 2.3.5.

The protocol is illustrated in Fig 2.3.1. Data collection was started after at least ten minutes of baseline quiet resting. The LBNP was then adjusted to -10 mmHg for 3 minutes. Subsequent to that, the negative pressure was increased to -20 mmHg for 3 minutes, -30 mmHg for 3 minutes, and -40 mmHg for 3 minutes. At that point, the negative pressure was removed and there was ten minutes of quiet rest. Selected equipment was disconnected and removed from the participant for ease of transfer and preparation for the posture change component of the testing session.

Supine-to-stand posture change

With assistance, participants moved to the bed located beside the LBNP box. They began in the supine position on the bed. After an acclimatization period of approximately ten minutes, participants were assisted, as needed, to a sitting position on the edge of the bed. As swiftly as possible, participants rose to a standing position on the floor and stood for 2 minutes. No data were used during the transition or transient sitting. The movement was meant to be fluid. This concluded data collection.

2.2.3 Data Acquisition

The analog signals (described below) were received by our data acquisition device (Powerlab, ADInstruments, Colorado Springs, CO, USA) and recorded continuously into the

corresponding software (Chart v5.5 ADInstruments, Colorado Springs, CO, USA). Data were prepared for analysis in a beat-by-beat format by creating a macro that captures data between each R-wave of the cardiac cycle.

Heart Rate

Electrocardiography (ECG) was captured continuously through testing. R-R intervals were measured by the Colin (Colin Pilot 9200, Colin Medical Instruments, San Antonio, Texas, USA), using a three electrode placement technique. Heart rate (HR) was later derived from the R-R intervals.

Blood pressure

A continuous approximation of brachial artery blood pressure was generated through measurement of finger arterial pressure (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). The finger is wrapped with an inflatable cuff that uses infrared plethysmography to measure the pulsation of arterial diameter during each heart beat. Internal components correct the shape and amplitude of the finger pressure waveform to that of the brachial artery waveform. Vertical height difference between the finger cuff and the heart is accounted for by fluid-filled tubing connected from the finger cuff to a pressure transducer and used to adjust the waveform. An additional calibration (*return-to-flow*) involving brief inflation of a brachial cuff further validates the height-corrected pressure before use during testing.

Stroke Volume

Stroke volume (SV) was calculated as

$$SV = \text{aortic velocity} \bullet (\pi \bullet \text{radius}^2) \bullet \text{R-R interval} \qquad \text{Equation 2.0}$$

where *aortic velocity* is the blood flow velocity of the ascending aorta and *radius* is half the diameter of the aortic root. Aortic velocity was evaluated transcutaneously at the suprasternal notch with a 2MHz probe using a Doppler ultrasound device (Compumedics Ltd., The DWL® Doppler Company, Singen, Germany). The diameter of the aortic root was imaged once during the testing session with a 1-5 MHz electronic micro-convex array transducer (Diagnostic Ultrasound system, Mindray, Model M5, Shenzen Mindray Bio-medical Electronics Co., Ltd., Shenzen, China). The area of collagenous density at the proximal aspect of the aortic root served as an easily visualized landmark on ultrasound. Aortic ring diameter does not change during orthostatic stress (Livingstone *et al*., 2010). Stroke volume was also estimated by the Finometer® device (Finapres Medical Systems, Amsterdam, The Netherlands). An internal algorithm generates an aortic flow waveform using the participant's age, sex, height, and weight. The output is an estimation of Q, which was used to calculate the Finometer SV (Equation 2.1).

Cardiac Output

Cardiac output (Q) is calculated as

$$Q = SV \bullet HR \qquad \text{Equation 2.1}$$

where *SV* was either Doppler SV or Finometer SV, as described above, and *HR*, in beats/min, was determined as described above.

Exhaled Carbon Dioxide

Exhaled carbon dioxide (CO_2) was collected continuously through nasal canulae. The Colin Pilot System (Colin Pilot 9200, Colin Medical Instruments, San Antonio, Texas, USA) measures % CO_2 concentration by infrared absorption. End-tidal CO_2 ($ETCO_2$) was identified and the maximum concentration of CO_2 reached on the capnographic output. $ETCO_2$ was converted to end tidal partial pressure of carbon dioxide ($PETCO_2$), with units of mmHg, by taking the difference between atmospheric pressure and the water vapour pressure.

Central Venous Pressure

Measuring central venous pressure (CVP) was accomplished by insertion of a 20-gauge catheter (BD Instyle, BD Medical Systems, Sandy, UT, U.S.A.) into the antecubital vein of the right arm. The catheter was connected to firm intravenous tubing filled with sterile saline and contained a pressure transducer (TranStar, Mediex Inc., Carlsbad, CA, U.S.A.). The transducer was kept at the level of the right heart with the use of a laser light for verification. CVP was collected continuously through one of the channels on the Colin Pilot System (Colin Pilot 9200, Colin Medical Instruments, San Antonio, Texas, USA).

Brachial Blood Flow Velocity

Brachial blood flow velocity was determined using Doppler ultrasound (Neurovision Transcranial Doppler System Model 500M, Multigon Industries Inc., Yonker, NY, USA). A 4MHz probe was used to measure mean blood flow velocity in the brachial artery proximal to the elbow. The site location on the skin of the participant was marked with ink for consistency throughout the testing session. The transducer was appropriately affixed with

tape to the participant's skin and its orientation was altered as needed for an optimal velocity signal.

Portal Vein Diameter

The liver was imaged while participants were supine with the probe oriented transversely in the coronal plane, midline, and slightly below the xyphoid process. The portal vein can be identified sonographically by its densely demarcated walls and its direct course into liver tissue. Its diameter was visualized using a 1-5 MHz convex-array transducer (Diagnostic Ultrasound system, Mindray, Model M5, Shenzen Mindray Bio-medical Electronics Co., Ltd., Shenzen, China) and subsequently measured offline. Several still frame images were taken from each video, which were generally up to 30 seconds in length for each level of LBNP. Because portal vein flow tends to change relative to portal venous diameter (Arbeille *et al*., 2003), portal velocity was not collected. Instead, portal diameter was used as a substitute measure for portal vein flow, which was used to represent changes in splanchnic vascular resistance. Because portal vein diameter tends to change during respiration (Sadek *et al*., 1996), calculations were made at end-exhalation whenever possible. When the phase of respiration was not apparent offline from the video, all visible diameter measurements were recorded, and then identified as either an inspiratory or expiratory diameter based on its size. An average of the "smaller" diameters was used in analysis. Figure 2.3.1 is an example of a portal vein image with ultrasound.

Inferior Vena Cava Diameter

Using a 1-5 MHz ultrasound probe (Diagnostic Ultrasound system, Mindray, Model M5, Shenzen Mindray Bio-medical Electronics Co., Ltd., Shenzen, China), the inferior vena cava

(IVC) was identified, video was recorded, and diameter measurements were analyzed offline. The IVC was visualized longitudinally through a subcostal coronal view with the probe directed rostrally and slightly toward the left clavicle. Under ultrasound examination, diameter measurements were taken just proximal to the entry of the hepatic veins into the IVC. The diameter of the IVC is subject to fluctuations in diameter throughout the respiratory cycle. For this reason, video was captured over multiple respiratory cycles whenever possible, diameter measurements were taken at end-exhalation. Figure 2.3.2 is an example of an inferior vena cava image with ultrasound.

Cerebral Blood Flow Velocity

Middle cerebral artery (MCA) blood flow velocity (BFV) was collected with transcranial Doppler ultrasonography (TCD; Compumedics Ltd., The DWL® Doppler Company, Singen, Germany). The flow velocity of the left middle cerebral artery was examined using a 2 MHz transducer placed on the left temporal region (Aaslid *et al*., 1982). The optimal location and orientation of the transducer, and the depth of insonation was achieved when the MCA BFV signal was at its strongest; this differed between individuals, but was consistent within individuals throughout testing. In general, the insonation site was on the squama of the temporal bone, superior to the zygomatic arch and anterior to the superficial temporal artery, as suggested (Ringelstein *et al*., 1990).

The path of the MCA from the internal carotid artery through to the outer cortex passes through more than one anatomical plane. For this reason, insonation angle is directed at the sphenoidal segment (M1) which runs in the axial plane (horizontally) and in the approximate direction of the nose to the ear. An insonation depth between 49 and 58 mm was sued for all individuals. Ideally, the sound waves emitted from the transducer

will be directed through the vessel longitudinally. From the power spectrum of the MCA BFV, a mean of the outer envelope was used for analysis.

2.2.4 Data organization before analysis

Beat-by-beat data including heart rate, blood pressure, cerebral blood flow velocity, exhaled carbon dioxide, central venous pressure, and calculated stroke volume, cardiac output, brachial vascular resistance index, cerebral vascular resistance index, were saved as Microsoft Excel files. Each measured variable required individual examination per cardiac contraction. All data corresponding to pre-ventricular contractions (PVCs) were omitted from analyses. The first minute of each stage of lower body negative pressure was not included in analyses. Ten second averages were calculated for each variable. When variables did not reach "steady-state" (i.e. consecutive 10 second averages differing by greater than 10%), changes were determined to be either cyclical (due to natural periodic variation) or as change towards a new steady state value. In instances where longer than one minute was require for the variable to reach steady state, 10 second averages were drawn from a smaller block of time. Periodic variations were treated as a steady state *range*, with inclusion of all 10 second averages in the calculation of the LBNP stage average.

Where automatic calibrations took place within the signals from the Finometer, those data were not included in analyses. Ultimately, one value was derived for each variable per stage of LBNP (baseline, -10 mmHg, -20 mmHg, -30 mmHg and -40 mmHg) and posture change (baseline and standing).

2.2.5 Statistical Analysis

All statistical analyses were performed using Statistical Analysis Software (SAS Institute, Cary NC, USA). A repeated measures analysis of variance (RM-ANOVA), with 5 factors for LBNP and 2 factors for posture change, was used to compare the percent change from baseline in each measured physiologic variable during LBNP, to every level of LBNP (i.e. -10, -20, -30, -40 mmHg), and also from supine baseline to stand during posture change. Percent changes during LBNP were also contrasted against percent changes during posture change with RM-ANOVA.

The nonparametric Spearman's rank test, which does not assume linearity, was used to evaluate relationships between variables. To examine differences between the two methods of stroke volume assessment (Finometer and Doppler), a one-way ANOVA (Tukey's test) was used. When $p<0.05$, differences were considered statistically significant.

2.3 Methodological Considerations

The use of Doppler ultrasound for the measurement of transcranial arterial vessels has limitations. The skull of the healthy adult is a significant barrier to ultrasound wave penetration. Sound waves emitted from conventionally operating Doppler transducers in the frequency range of 5 to 10 MHz are ineffective at penetrating the skull because their sound waves are readily attenuated. With lower operating frequencies, 1 to 2 MHz, waves are more capable of passing through the skull at its thinnest part—the temporal region. Notwithstanding, there is a proportion of individuals in whom this measurement is unsuccessful, because of anatomical limitations. This percentage increases with age, primarily because the bone of the temporal region becomes more porous with age, scattering the ultrasound waves so that they cannot return to the transducer. Additionally, we have used the average instantaneous outer envelop of the velocity tracing to represent blood flow

velocity. This assumption is only acceptable if the diameter of the MCA does not change. Evidence from Serrador and colleagues (2000a) supports the current belief that the MCA acts as a conduit vessel rather than a resistance vessel in the cerebral circulation during changes in blood pressure and arterial CO_2.

The technical limitations of our measurement of cardiac preload, central venous pressure should be also recognized. As the veins are quite distensible, they are prone to collapse. Adherence of the catheter to the inner wall of the vein can cause erroneous measurement. Even slight participant movements can falsely increase or decrease the reading. The aforementioned concerns were recognized and addressed as best as possible before proceeding with analysis.

Table 2.3.1 Participant characteristics

Variable	Value
Age (years)	74 ± 2
Sex (males/females)	4/6
Height (cm)	168 ± 7
Weight (kg)	72 ± 9
HR (beats/min)	69 ± 8
MAP (mmHg)	98 ± 12
SBP (mmHg)	146 ± 20
DBP (mmHg)	68 ± 8
PP (mmHg)	77 ± 17
Qfin ($L \cdot min^{-1}$)	5.41 ± 1.49
Qdop ($L \cdot min^{-1}$)	8.27 ± 1.96
SCBFV ($cm \cdot s^{-1}$)	73 ± 22
DCBFV ($cm \cdot s^{-1}$)	31 ± 8
MCBFV ($cm \cdot s^{-1}$)	48 ± 13
PI	0.87 ± 0.10
CVRi ($mmHg \cdot cm^{-1} \cdot s^{-2}$)	2.18 ±0.66
CVP (mmHg)	9.07 ± 5.22
SVfin (mL)	79 ± 18
SVdop (mL)	120 ±19
BV ($cm \cdot s^{-1}$)	3.22 ± 1.48
BVRi ($mmHg \cdot cm^{-1} \cdot s^{-2}$)	35.70±13.60
TPRfin ($mmHg \cdot L^{-1} \cdot min^{-2}$)	16.74 ± 4.57
TPRdop ($mmHg \cdot L^{-1} \cdot min^{-2}$)	10.84 ± 3.10
PVD (cm)	0.84±0.36
IVCD (cm)	1.80±0.47
PI/PP	0.0115±0.0019
CPP (mmHg)	88.3±10.0
ET_{CO_2} (mmHg)	40.07±3.01
RR ($breaths \cdot min^{-1}$)	18±5

n=10; all values are mean ± SD from baseline of lower body negative pressure condition; HR – heart rate; MAP – mean blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; Qd – cardiac output calculated using Doppler ultrasonography; Qf – cardiac output calculated from Finometer; SVd – stroke volume calculated using Doppler ultrasonography; SVf – stroke volume calculated from Finometer; TPRd – total peripheral resistance calculated using Doppler ultrasonography; TPRf – total peripheral resistance calculated from Finometer; CVP – central venous pressure; SCBFV – systolic blood flow velocity; DCBFV – diastolic cerebral blood flow velocity; MCBFV – mean cerebral blood flow velocity; PI – pulsatility index; RI – resistance index; CVRi – cerebrovascular resistance index; PI/PP - pulsatility ratio; CPP – cerebral perfusion pressure; BV – brachial velocity; BVRi – brachial vascular resistance index; PVD – portal vein diameter; IVCD – inferior vena cava diameter; ET_{CO_2} – end-tidal carbon dioxide; RR – respiratory rate

Table 2.3.2 Participant medical issues and history

Medical history/current issues	Frequency
diabetes mellitus type 2	1[b]
enteritis/colitis	1[a]
gastroesophageal reflux disease	1[b]
hypercholesterolemia	2[bc]
hypertension	3[abb]
osteoporosis	1[c]
varicose veins	1[c]

At time of testing: [a]not active problem; [b]well-controlled with medication; [c]current issue

Table 2.3.3 Participant medications at time of testing

Medications	Frequency
antihypergylcemic	1
bisphosphonate	1
calcium channel blocker	1
diuretic-thiazide	2
HMG-CoA reductase inhibitor (Statin)	1
proton pump inhibitor	1
thyroxine	1

Table 2.3.4 Inclusion/exclusion criteria

Inclusion Criteria
Men and post-menopausal women
Age >70 years
Functional independence
BP<140/90 mmHg (naturally or pharmaceutically-controlled)
Exclusion Criteria
History of CVD
Previous cardiac arrest or stroke
Major congenital anomalies (e.g. cardiac, thoracic)
Women taking hormone-replacement therapy
Inability to give informed, written consent

CVD-Cardiovascular disease

Table 2.3.5 Test termination criteria

Test termination criteria
Decrease in systolic blood pressure to <70 mmHg
Decrease in systolic blood pressure of >25 mmHg from baseline
Sudden drop in heart rate or cerebral blood flow velocity
Presyncopal symptoms: nausea, perspiration, pallor, visual disturbances, dizziness
Participant request

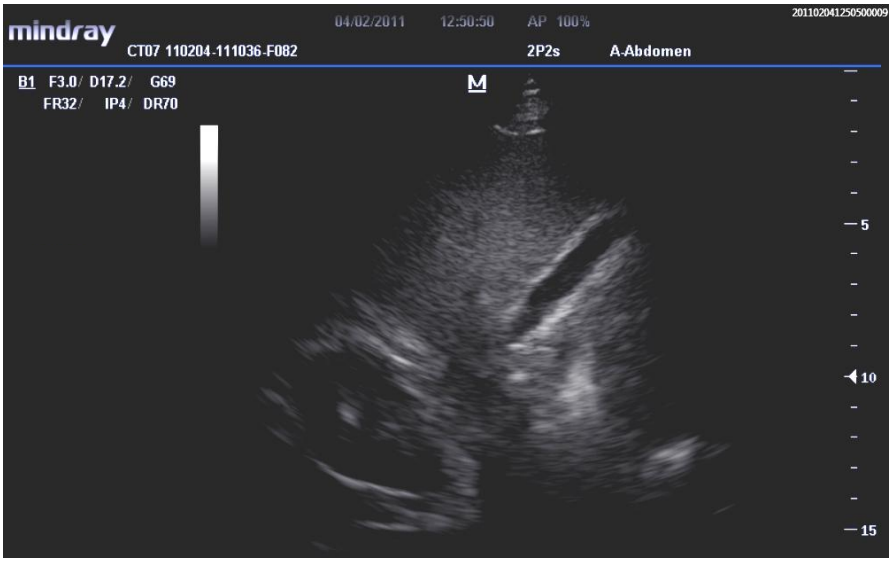


Fig 2.3.1 Ultrasound view of the portal vein
The portal vein was imaged while participants were supine with the probe oriented transversely in the coronal plane, midline, and slightly below the xyphoid process. Diameter was measured offline at a later time.

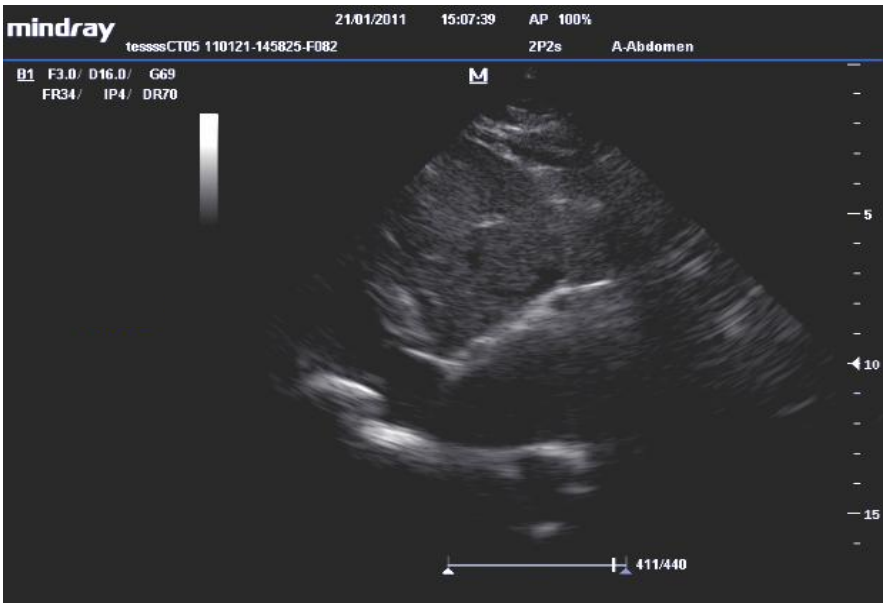


Fig 2.3.2 Ultrasound view of the inferior vena cava
The inferior vena cava was visualized longitudinally through a subcostal coronal view with the probe directed rostrally and slightly toward the left clavicle. Under ultrasound examination, diameter measurements were taken just proximal to the entry of the hepatic veins into the inferior vena cava.

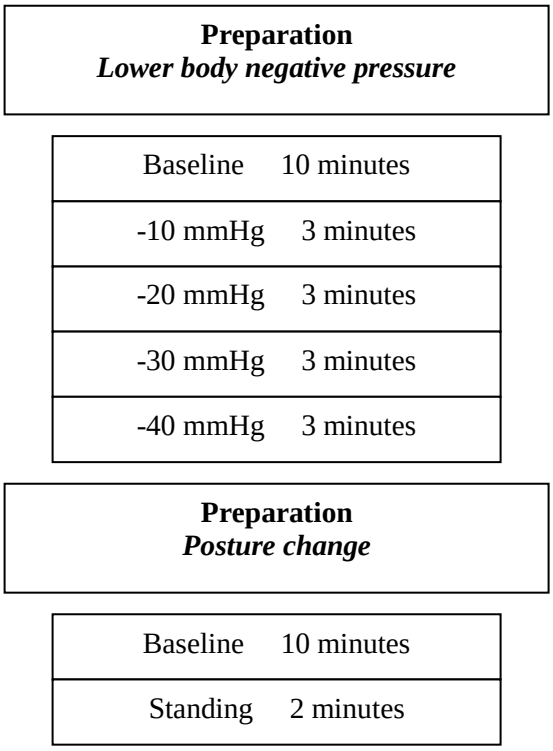


Fig 2.3.3 Experimental protocol timeline
The lower body negative pressure protocol consisted of approximately 10 minutes of supine, baseline measurements, followed by 3 minute stages of negative pressure in increments of 10 mmHg, beginning at -10 mmHg. Data were used in the final 3 minutes of each stage. The posture change protocol also included 10 minutes of supine baseline measurements, followed by 2 minutes of standing. Data were used from the final minute of standing.

Variables measured during lower body negative pressure only	**Variables measured during lower body negative pressure and posture change**
Central venous pressure Inferior vena cava diameter Portal vein diameter	Aortic velocity Blood pressure Brachial velocity Cardiac output - Finometer Cerebral blood flow velocity End-tidal carbon dioxide Heart rate Respiratory rate

Fig 2.3.4 Measured variables
List of variables appearing on the right were not measured during posture change due to technical limitations. Column on the right displays all other measured variables, which were used to calculate other variables of interest. See text.

2.4 Results

Participants

A description of the study sample has been depicted in Figure 2.4.0. Twenty-six individuals over the age of 70 years were contacted and introduced to the possibility of participating in this thesis research. Six people were not interested in partaking, and six others were unable to come into the university due to scheduling difficulties. A brief meeting was scheduled for fourteen potential participants. Three were unable to participate due to previous cardiac arrest or stroke. Eleven individuals met inclusion/exclusion criteria (Table 2.3.4) and came to the University of Waterloo for testing. Data were collected from the 11 participants, although crucial variables were unsuccessfully collected in one participant. Ten datasets (4 males) were included in the analysis for this project.

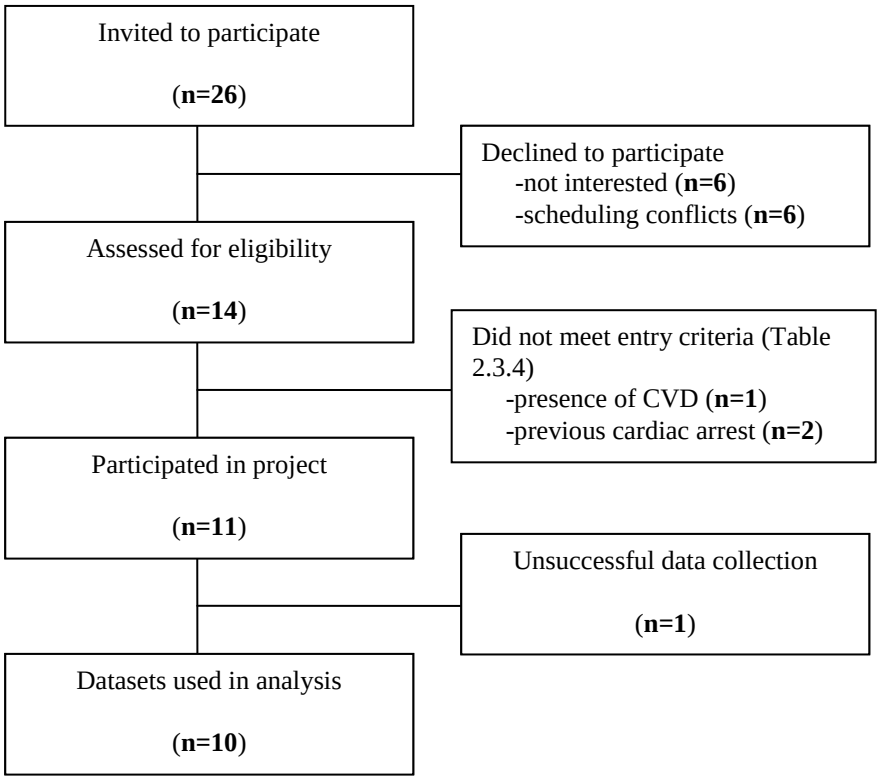


Figure 2.4.0 Enrolment flowchart
CVD-cardiovascular disease; Unsuccessful data collection: no transcranial Doppler signal found and no supine-to-stand maneuver

Lower Body Negative Pressure

All participants completed the lower body negative pressure (LBNP) protocol asymptomatically. The criteria for test termination are listed in Table 2.3.5. Baseline values for all tested physiologic variables are listed in Table 2.3.1, and responses to each level of LBNP are listed in Table 2.4.1. The group mean heart rate (HR) response rose significantly at all levels of lower body negative pressure (LBNP) compared to baseline (-10 mmHg, $p=0.0324$; -20 mmHg, $p=0.0023$; -30 mmHg, $p=0.0007$; -40 mmHg, $p<0.0001$), totalling a 9% increase (Figure 2.4.1). Systolic blood pressure (SBP) did not change over the course of the LBNP protocol, nor did MAP (Figure 2.4.2). Diastolic blood pressure (DBP) remained relatively constant during all levels of LBNP, barring the last stage of LBNP (-40 mmHg, $p=0.0114$), increasing 6% from baseline (Figure 2.4.3). The mean pulse pressure (PP) response was not significantly altered with LBNP. Stroke volume (SV), as measured using Doppler ultrasound, decreased significantly at all LBNP stages ($p=0.0399$, 0.0013, 0.0002, and 0.0011), resulting in a 25% decrease by the final stage (Figure 2.4.5). Similarly, cardiac output (Q), calculated from Doppler SV, decreased significantly at every level of LBNP ($p=0.0366$, 0.0019, 0.0010, and 0.0097), decreasing 19% from baseline at -40 mmHg (Figure 2.4.6). Total peripheral resistance (TPR), calculated with Doppler Q, rose significantly at each stage of negative pressure ($p=0.0115$, 0.0138, 0.0022, and 0.0214), amounting to an increase of 35% by the final stage (Figure 2.4.7). An additional measure of peripheral resistance, brachial vascular resistance index (BVRi), increased significantly, although, only in second and fourth stages of LBNP ($p=0.0098$, 0.0410). At -40 mmHg, BVRi had increased 40% from baseline (Figure 2.4.8). Our measure of preload, central venous pressure (CVP), dropped at all levels of LBNP ($p=0.0078$, 0.0013, 0.0160, 0.0254), with a maximum decrease of 27% (Figure 2.4.9).

Figure 2.4.10 depicts cerebral blood flow responses. In all four levels of LBNP, systolic cerebral blood flow velocity (SCBFV) was significantly reduced from baseline (p=0.0104, 0.0189, 0.0373, 0.0183), with the greatest decrease of 7% occurring in the final stage. Diastolic cerebral blood flow velocity (DCBFV) did not change significantly during LBNP, while mean cerebral blood flow velocity (MCBFV) significantly decreased in the first stage of negative pressure only (p=0.0361). Both pulsatility index (PI) and resistivity index (RI) decreased strongly at each stage of the protocol, reflecting the change in SCBFV. The cerebrovascular resistance index (CVRi) increased significantly in the second (p=0.0430) and fourth (p=0.0032) stages of LBNP, to 9% (Figure 2.4.11).

Splanchnic vascular resistance, as measured by the change in the diameter of the portal vein, increased in the final stage of LBNP (p=0.0398), with a total reduction of 28% (Figure 2.4.12). Diameters taken from the inferior vena cava (IVC) were variable. At -20 mmHg, IVC diameter reached its nadir (p=0.0239). The last two stages of LBNP were not significantly changed from baseline (Figure 2.4.13).

During the first, and last two levels of LBNP, end-tidal CO_2 (ET_{CO_2}) decreased significantly (p=0.0105, 0.0111, 0.0083). Interestingly, RR increased to significance by -30 mmHg (p=0.0375), but did not remain significant for the other LBNP stages of the protocol.

Inter-individual variability during LBNP

Some measured variables may not have reached significance at least, in part, due to the range of responses between individuals. Figure 2.4.14 depicts the between-subject variation in the pulse pressure response to LBNP with a plot of the responses from all ten participants. Systolic blood pressure was similarly diverse (data not shown).

Doppler versus Finometer® stroke volume measurements

Figure 2.4.14 illustrates the discrepancy in the measurement of SV with our two techniques. The change in Doppler SV was consistently greater than the SV predicted with the Finometer device. Doppler SV decreased significantly at each level of LBNP, while the Finometer SV did not change significantly until -40 mmHg. The SV values from the two techniques did not change in parallel. Doppler SV decreased 13, 23, 24, and 25% through the four levels of LBNP. Finometer SV decreased 3, 6, 8, and 15%. Pairwise multiple comparisons (Tukey's Test) were made between the methods at each level of LBNP and postures change. Absolute values were different at all stages of LBNP (0 mmHg, $p<0.001$; -10 mmHg, $p=0.018$; -40 mmHg, $p=0.044$) and posture change baseline ($p=0.034$), excluding negative pressures of 20 mmHg ($p=0.134$) and 30 mmHg ($p=0.066$), and during standing ($p=0.468$). The percent decrease in SV from 0 mmHg (baseline) was significantly different between methods at -10 mmHg ($p=0.030$), -20 mmHg ($p=0.018$), and -30 mmHg ($p=0.014$), but not at -40 mmHg ($p=0.153$). The percent delta decrease during posture change was also significantly different between methods ($p=0.011$).

Supine-to-Stand

Group mean responses for the supine-sit-stand test are described below and shown in Table 2.4.2. Upon standing, HR increased 14% from the supine position ($p=.0008$). Systolic, diastolic, and mean blood pressure were not significantly altered after the posture change. Despite the increase in HR, Q dropped significantly upon standing (24%; $p=0.0138$), owing to a 33% decrease in SV ($p=0.0010$; both Doppler measurements). Mean PP also showed no alteration with standing, and likewise to what was observed during the LBNP protocol, there was considerable variability. Total peripheral resistance increased 43% after standing

(p=0.0555). Our additional measure of peripheral resistance, BVRi, increased 111% from baseline in the upright position (p=0.0108).

In contrast to what was observed during LBNP, DCBFV and MCBFV decreased significantly in the standing position by 38% (p=0.0288) and 11% (p=0.0241), respectively. The SCBFV response represented another inconsistency between the two experimental conditions. Recall that SCBFV decreased significantly at every stage of negative pressure. No reduction was observed during standing (p=0.0941). Similarly, PI (p=0.3080) and RI (p=0.1261) did not change. However, the calculated CVRi did increase (12%; p=0.0292).

The small decrease in $ETCO_2$ observed in standing was not significant (p=0.1142), while the mean RR response did increase to a discernible extent (20%; p=0.0259).

Condition Comparisons – LBNP versus sit-stand

Repeated measures analyses of variance were used to evaluate how well responses to a supine-to-stand maneuver were represented by our measure of simulated orthostasis, LBNP. Tables 2.4.1 and 2.4.2 reveal variables that were significantly changed from baseline during LBNP and supine-stand, respectively. For all variables, excluding PP, baseline values of the LBNP and supine-stand conditions were similar. PP was significantly higher at rest preceding the supine-stand compared to resting pre-LBNP (p=0.0269), which was influenced by an elevated SBP approaching significance (p=0.0533). Standing values were significantly different than -40 mmHg values for HR and PP (p=0.0348 and p=0.0093, respectively).

Despite some variables not having their nadir or zenith values at the final increment of LBNP, the two conditions were compared using delta values between baseline and -40 mmHg for LBNP, and the delta response for the supine-to-stand maneuver. Delta LBNP (ΔLBNP: % change from 0 mmHg to -40 mmHg) was measured against delta supine-stand (%Δ in supine-stand). Delta values were significantly different between conditions for HR, DBP, PI, and DCBFV (p=0.0397; 0.0355; 0.0290, and 0.0355, respectively). Figure 2.4.16 reveals individual responses for the percent change in HR for LBNP and for the posture change. The mean increase in HR was greater during the posture change than during LBNP. The difference between the LBNP and posture change responses for DBP occurred because the mean DBP response increased during LBNP, and did not change during after standing. The changes in DCBFV were highly dissimilar between LBNP and supine-to-stand, changing in opposite directions (Figure 2.4.17). During LBNP, DCBFV remained relatively constant (and increased in some individuals), whereas it decreased in all ten participants during the posture change.

Relationships between variables

About twenty-five variables were measured or calculated during this project. The relationships between all of them were characterized with Spearman's rank correlation tests. Many of the variables were derived from one another, and so, intrinsically "unrelated" variables are reported only. Table 2.4.4 lists the notable correlated variables. Scatter plots were generated for selected relationships. During LBNP, the percent change (%Δ) in TPR (Doppler) was directly related to the %Δ in DCBFV (r = -0.733; Figure 2.4.18). The %Δ in TPR (calculated with Q from the Finometer) was directly related to DBP (r = 0.933; Figure 2.4.19). The scatter plot in Figure 2.4.20 reveals relationships between the %Δ in the

pulsatility ratio versus the %Δ in DCBFV and CVRi (r = 0.750). The pulsatility ratio was calculated as the cerebral PI divided by systemic PP. The %Δ pulsatility ratio was directly related to %ΔDCBFV (r = 0.750) and inversely related to %ΔCVRi during LBNP (r = -0.733). Finally, during LBNP, %ΔPP was directly related to %ΔCVRi (r = 0.717) and inversely related to %ΔDCBFV (r = -0.783), and %ΔCVP was directly related to %ΔDCBFV (r=0.683) and inversely related to %ΔPP (r = -0.783; Figure 2.4.21). Additionally, we did find that the change in PP was linearly related to the change in SV measured with the Finometer during LBNP (r = 0.6290; p=0.041), but not during stand (p=0.314). Doppler-derived SV was not related to PP during LBNP or during posture change.

During posture change, inverse relationships were found between %ΔSBP and %Δpulsatility ratio, %ΔRR and %Δ ET_{CO_2}, and %ΔHR and %ΔRR. The %Δ in TPR (Doppler) and %Δ in DCBFV were positively correlated during the supine-to-stand maneuver (Figure 2.4.22). Interestingly, BVRi was inversely related to TPR measured with the Finometer (r = -0.685, p=0.0252). All posture change correlation coefficients are listed in Table 2.4.4.

It is worth noting that there were a number of expected relationships that did not results in significant relations, including, Doppler SV and SVP, Finometer SV and CVP, TPR and BVRi, Doppler SV and PP, and CVP and BVRi.

Table 2.4.1 Mean values for cerebro- and cardiovascular variables during lower body negative pressure without statistical details. See Table 2.4.3 for statistically significant changes

	Baseline	*-10 mmHg*	*-20 mmHg*	*-30 mmHg*	*-40 mmHg*
HR beat/min	69±8	71±8	72±7	72±8	75±9
MAP mmHg	98±12	97±11	98±10	100±12	101±13
DBP mmHg	69±8	69±9	71±8	72±8	73±9
PP mmHg	77±17	74±14	74±15	76±18	74±17
Qd L/min	8.27±1.96	7.46±2.35	6.75±2.34	6.57±1.94	6.74±2.47
Qf L/min	5.41±1.49	5.43±1.72	5.29±1.73	5.15±1.34	4.93±1.35
SVd mL	120±19	104±23	92±26	91±22	90±29
SVf mL	79±18	76±21	74±24	72±20	66±18
TPRd mmHg/L/min	12.51±3.43	14.39±5.09	16.70±7.24	16.72±5.86	17.29±7.77
TPRf mmHg/L/min	19.48±5.87	19.81±6.98	20.38±6.70	20.84±6.11	22.06±6.85
CVP mmHg	9.70±5.22	8.50±4.74	7.94±4.17	7.14±3.14	7.05±3.18
SCBFV cm/s	73±22	70±23	68±24a	70±22	68±21
DCBFV cm/s	31±8	31±9	30±10	31±9	31±9
MCBFV cm/s	48±13	46±14a	46±15	47±14	46±13
PI	0.87±0.10	0.83±0.11	0.81±0.10	0.81±0.12	0.81±0.09
RI	0.57±0.04	0.56±0.04	0.55±0.04	0.55±0.05	0.54±0.05
CVRi mmHg/cm/s	2.18±0.66	2.27±0.75	2.36±0.83a	2.33±0.68	2.35±0.65
PI/PP	0.0115±0.0019	0.0114±0.0017	0.0113±0.0015	0.0110±0.0018	0.0113±0.0021
CPP mmHg	88.3±10.0	88.8±8.9b	90.4±8.5	93.4±11.7	94.0±13.4
BV cm/s	3.22±1.48	3.08±1.51	2.88±1.66	3.13±1.77	2.48±1.03
BVRi mmHg/cm/s	35.70±13.60	38.80±17.44	43.14±18.46	40.57±17.42	46.83±17.46
PVD cm	0.84±0.36	0.77±0.28	0.73±0.38	0.78±0.37	0.60±0.18
IVCD cm	1.80±0.47	1.91±0.47	1.60±0.48	1.86±0.52	1.73±0.67
ETCO_2 mmHg	40.07±3.01	39.27±2.93	39.41±3.41	38.90±3.32	38.68±3.27
RR breaths/min	18±5	19±6	16±4	18±5	18±5

All values are mean ± SD; HR – heart rate; MAP – mean blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; Qd – cardiac output calculated using Doppler ultrasonography; Qf – cardiac output calculated from Finometer; SVd – stroke volume calculated using Doppler ultrasonography; SVf – stroke volume calculated from Finometer; TPRd – total peripheral resistance calculated using Doppler ultrasonography; TPRf – total peripheral resistance calculated from Finometer; CVP – central venous pressure; SCBFV – systolic blood flow velocity; DCBFV – diastolic cerebral blood flow velocity; MCBFV – mean cerebral blood flow velocity; PI – pulsatility index; RI – resistance index; CVRi – cerebrovascular resistance index; PI/PP - pulsatility ratio; CPP – cerebral perfusion pressure; BV – brachial velocity; BVRi – brachial vascular resistance index; PVD – portal vein diameter; IVCD – inferior vena cava diameter; $ETCO_2$ – end-tidal carbon dioxide; RR – respiratory rate

Table 2.4.2 Mean values for cerebro- and cardiovascular variables during posture change without statistical details. See Table 2.4.3 for statistically significant changes

Variable	*Supine*	*Stand*
HR beats/min	68±7	78±9
MAP mmHg	104±10	105±17
PP mmHg	86±17	86±18
Qd L/min	8.23±3.13	6.24±2.74
Qf L/min	5.69±1.75	5.69±2.03
SVd mL	120±43	80±31
SVf mL	83±35	73±9
TPRd mmHg/L/min	14.56±6.07	20.12±9.38
TPRf mmHg/L/min	19.97±6.36	21.30±9.48
SCBFV cm/s	76±18	71±16
DCBFV cm/s	31±7	27±4
MCBFV cm/s	50±11	45±8
PI	0.89±0.09	0.98±0.16
RI	0.58±0.04	0.61±0.05
CVRi mmHg/cm/s	2.18±0.52	2.44±0.68
PI/PP	0.0106±0.0019	0.0120±0.0043
BV cm/s	3.05±1.88	1.81±1.43
BVRi mmHg/cm/s	43.65±19.98	87.38±50.37
ET_{CO_2} mmHg	39.33±3.14	38.50±3.59
RR breaths/min	17±3	20±4

All values are mean ± SD; HR – heart rate; MAP – mean blood pressure; PP – pulse pressure; Qd – cardiac output calculated using Doppler ultrasonography; Qf – cardiac output calculated from Finometer; SVd – stroke volume calculated using Doppler ultrasonography; SVf – stroke volume calculated from Finometer; TPRd – total peripheral resistance calculated using Doppler ultrasonography; TPRf – total peripheral resistance calculated from Finometer; SCBFV – systolic blood flow velocity; DCBFV – diastolic cerebral blood flow velocity; MCBFV – mean cerebral blood flow velocity; PI – pulsatility index; RI – resistance index; CVRi – cerebrovascular resistance index; PI/PP - pulsatility ratio; BV – brachial velocity; BVRi – brachial vascular resistance index; ET_{CO_2} – end-tidal carbon dioxide; RR – respiratory rate

Table 2.4.3 Mean percent changes for cerebro- and cardiovascular variables during both conditions with statistical significance identified

%	*-10 mmHg*	*-20 mmHg*	*-30 mmHg*	*-40 mmHg*	*stand*
HR	**1.73±2.24a**	**4.04±2.90b**	**5.11±3.29c**	**8.38±3.15c**	**12.24±7.46c**
MAP	0.79±1.78	2.10±4.34	2.70±5.11	3.11±5.25	0.42±10.71
DBP	1.04±2.21	3.08±6.13	4.32±7.16	**6.28±6.57a**	0.85±12.64
PP	0.16±4.10	-1.24±6.66	-1.52±8.86	-3.98±11.32	-0.12±11.32
Qd	**-12.44±12.19a**	**-21.46±14.45b**	**-20.94±12.02c**	**-19.70±19.21b**	**-21.69±24.34a**
Qf	-0.85±7.23	-2.17±17.11	-3.45±14.73	-7.78±14.70	0.55±23.64
SVd	**-13.96±12.30a**	**-24.26±13.74b**	**-24.67±12.32c**	**-26.06±18.48b**	**-31.57±19.23b**
SVf	-2.61±7.29	-5.64±16.20	-8.01±14.98	**-15.22±13.66a**	-11.58±19.48
TPRd	**17.25±17.31a**	**33.94±25.23a**	**32.20±18.30b**	**34.98±32.79a**	43.12±57.83
TPRf	2.14±7.82	7.35±21.03	9.63±24.89	**15.03±23.64b**	5.89±30.57
CVP	**-15.75±13.09b**	**-20.855±9.12b**	**24.12±15.90a**	**24.92±20.34a**	**-**
SCBFV	**-3.30±2.48a**	**-6.21±7.42a**	**-5.18±5.54a**	**-6.38±6.31a**	-5.70±9.66
DCBFV	0.39±2.40	-1.11±8.36	0.43±7.15	0.32±6.64	**-12.43±12.38a**
MCBFV	**-2.49±2.71a**	-4.64±7.64	-3.75±6.24	-4.74±6.45	**-9.70±9.20a**
PI	**-3.64±2.11c**	**-5.73±1.97c**	**-5.96±4.11c**	**-6.96±4.80c**	10.55±19.72
RI	**-2.82±1.67c**	**-4.07±1.47c**	**-4.58±2.81c**	**-5.44±3.12c**	4.99±10.02
CVRi	3.45±3.75	**7.77±11.07a**	7.13±9.17	**8.80±10.43b**	**11.87±13.87a**
PI/PP	**-3.66±4.20a**	**-4.21±5.81a**	**-4.05±6.51a**	**-10.76±29.68a**	12.75±30.77
CPP	**2.70±2.37b**	**4.71±4.81a**	**5.79±6.25a**	**6.32±7.10a**	**-**
BV	-5.25±20.57	-12.94±20.19	-4.80±21.04	**-19.16±22.38a**	**-41.47±21.87b**
BVRi	11.00±24.42	**22.20±24.61b**	13.55±29.84	**39.57±50.58a**	**110.84±124.37a**
PVD	-2.86±28.11	-11.62±25.54	-8.08±31.38	**-22.07±24.85a**	**-**
IVCD	2.66 ±16.29	**-9.22±14.48a**	4.88±37.48	-4.04±41.19	-
ET_{CO_2}	**-1.36±1.36a**	-1.09±2.51	**-2.95±3.03a**	**-3.49±3.40b**	-2.15±4.04
RR	4.74±8.85	**-10.15±13.65a**	-0.84±12.49	-1.76±29.41	**21.95±29.41a**

All values are mean percent changes ± SD of percent changes; a=p<0.05; b=p<0.01; c=p<0.001. HR – heart rate; MAP – mean blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; Qd – cardiac output calculated using Doppler ultrasonography; Qf – cardiac output calculated from Finometer; SVd – stroke volume calculated using Doppler ultrasonography; SVf – stroke volume calculated from Finometer; TPRd – total peripheral resistance calculated using Doppler ultrasonography; TPRf – total peripheral resistance calculated from Finometer; CVP – central venous pressure; SCBFV – systolic blood flow velocity; DCBFV – diastolic cerebral blood flow velocity; MCBFV – mean cerebral blood flow velocity; PI – pulsatility index; RI – resistance index; CVRi – cerebrovascular resistance index; PI/PP - pulsatility ratio; CPP – cerebral perfusion pressure; BV – brachial velocity; BVRi – brachial vascular resistance index; PVD – portal vein diameter; IVCD – inferior vena cava diameter; ET_{CO_2} – end-tidal carbon dioxide; RR – respiratory rate

Table 2.4.4 Variables with different percent change values in lower body negative pressure versus posture change

Variable	ΔLBNP 0 → -40 mmHg	ΔPosture Supine → Stand	p value
HR (%)	8.38±3.15	12.24±7.46	0.0397
DBP (%)	6.28±6.57	0.85±12.64	0.0355
DCBFV (%)	0.32±6.64	-12.43±12.38	0.0355
PI (%)	-6.96±4.80	10.55±19.72	0.0290

The percent changes in all other variables were not significantly different between lower body negative pressure and posture change. HR – heart rate; DBP – diastolic blood pressure; DCBFV – diastolic blood flow velocity; PI – pulsatility index

Table 2.4.5 Spearman rank correlation matrix for lower body negative pressure and posture change

	HR	SBP	DBP	Qf	SV	SVf	TPR	TPRf	CVP	SCBFV	DCBFV	CVRi	PI/PP	PVD	ET_{CO2}
SBP											-0.767		-0.70		
DBP				-0.85				0.93							
PP									-0.72		-0.78	0.72			
Qf			**-0.71**												
SV														-0.83	
TPR										-0.67	-0.73			0.83	
TPRf			**0.91**												
CVP											0.68				
SCBFV					**-0.63**										
DCBFV			**0.64**		**-0.67**		**0.84**						0.75		
CVRi					**0.84**								-0.73		
PI/PP		**-0.68**													
RR	**-0.70**														**-0.62**
BVRi								**-0.69**							

Upper right section displays correlation coefficients for variables during lower body negative pressure. Values are based on % changes from 0 mmHg to -40 mmHg. Lower left triangular section (highlighted area and bolded numbers) displays correlation coefficients for variables during posture change. Values are based on % change from supine to standing. All values are $p<0.05$. n = 10. HR – heart rate; SBP-systolic blood pressure; DBP-diastolic blood pressure; PP-pulse pressure; Qf-cardiac output calculated from Finometer; SV – stroke volume calculated using Doppler ultrasonography; SVf – stroke volume calculated from Finometer; TPR – total peripheral resistance calculated using Doppler ultrasonography; TPRf – total peripheral resistance calculated from Finometer; CVP – central venous pressure; SCBFV – systolic blood flow velocity; DCBFV – diastolic cerebral blood flow velocity; CVRi – cerebrovascular resistance index; PI/PP - pulsatility ratio; PVD – portal vein diameter; ET_{CO_2}– end-tidal carbon dioxide; RR – respiratory rate; BVRi – brachial vascular resistance index;

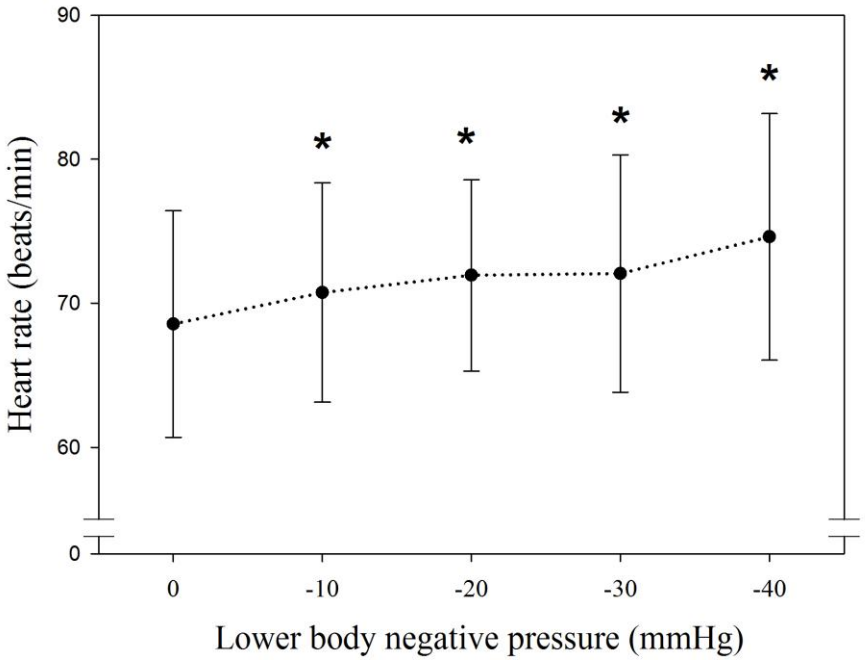


Figure 2.4.1 Heart rate during lower body negative pressure
Heart rate rose at each stage of lower body negative pressure. Values are expressed as mean ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

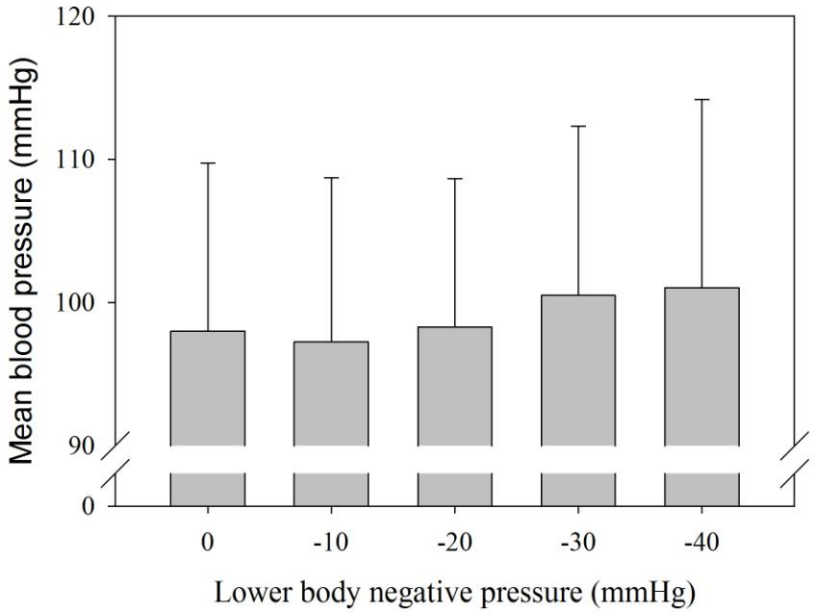


Figure 2.4.2 Mean arterial pressure during lower body negative pressure
Mean arterial pressure did not change during lower body negative pressure; n=10

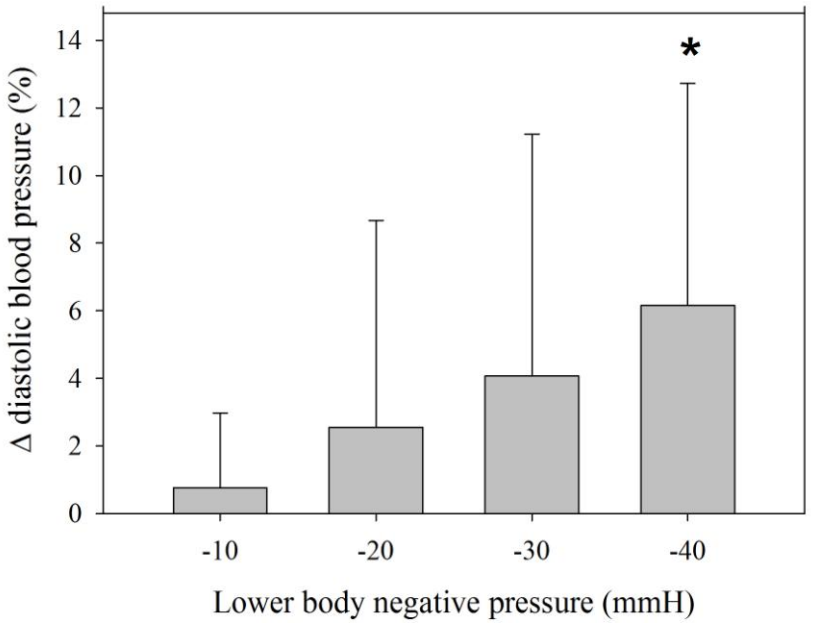


Figure 2.4.3 Change in diastolic blood pressure during lower body negative pressure
In the final stage of lower body negative pressure, the mean diastolic blood pressure response had increased significantly (6%); Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

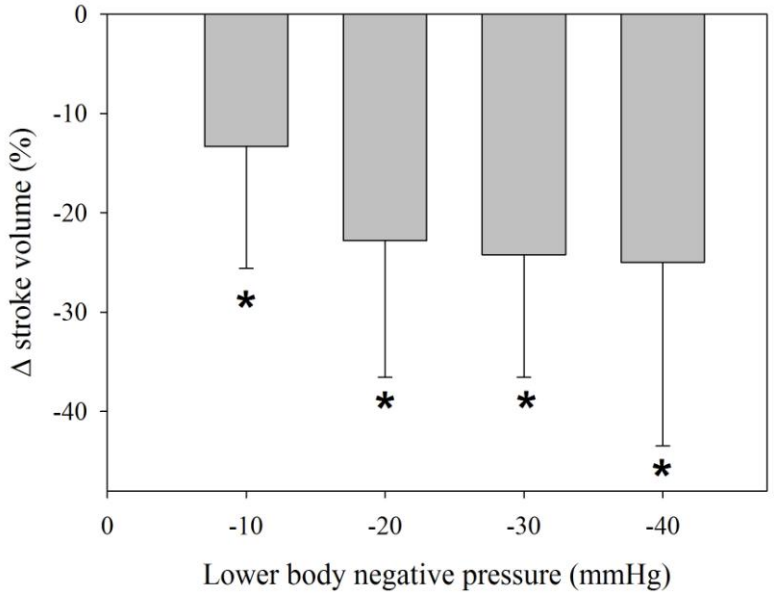


Figure 2.4.4 Change in stroke volume during lower body negative pressure
Stroke volume, measured using Doppler ultrasound, decreased significantly at each stage of lower body negative pressure. Values are expressed as mean percent change ± SD.
* indicates significantly different from baseline (0 mmHg); α=0.05; n=10

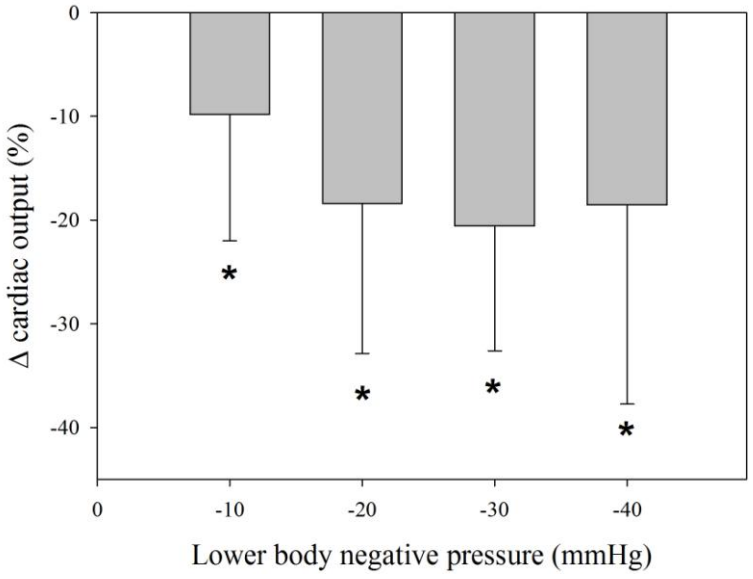


Figure 2.4.5 Change in cardiac output during lower body negative pressure
Cardiac output, measured using Doppler ultrasound, decreased significantly at each stage of lower body negative pressure. Values are expressed as mean percent change ± SD.
* indicates significantly different from baseline (0 mmHg); α=0.05; n=10

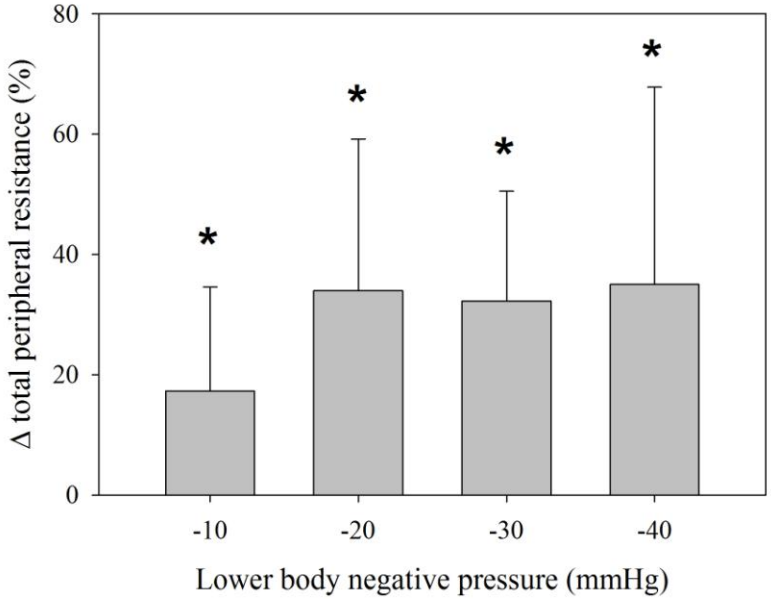


Figure 2.4.6 Change in total peripheral resistance during lower body negative pressure

Total peripheral resistance, measured using Doppler ultrasound, increased significantly at each stage of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

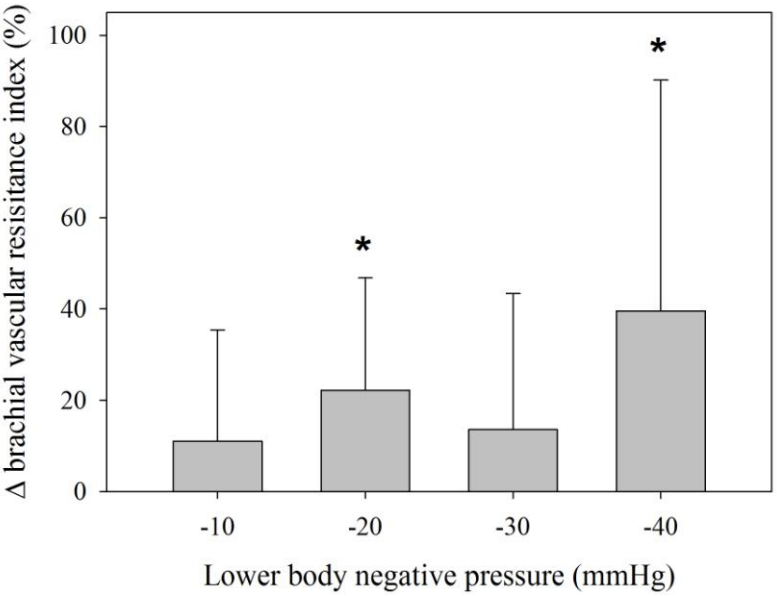


Figure 2.4.7 Change in brachial vascular resistance index during lower body negative pressure

Brachial vascular resistance index, calculated as mean arterial pressure divided by brachial velocity, increased significantly at the second and fourth stages of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

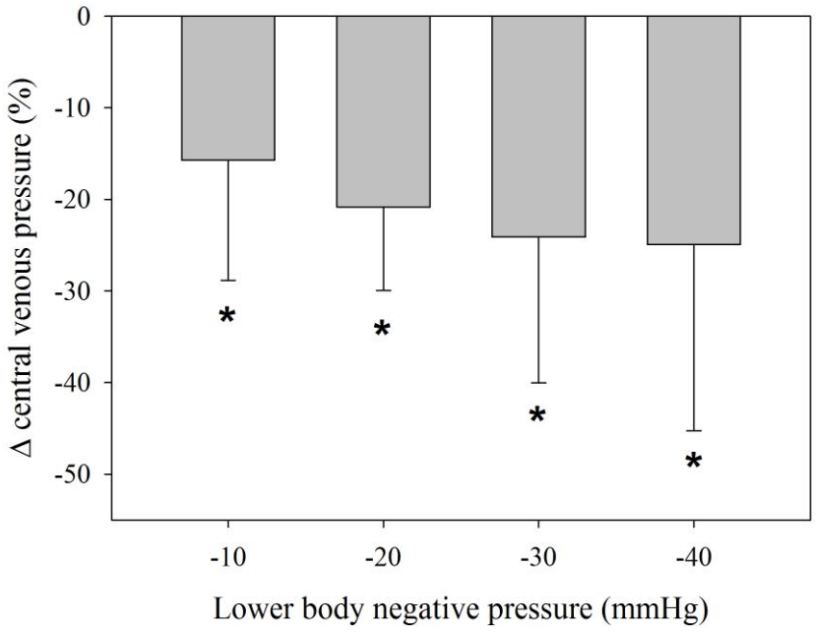


Figure 2.4.8 Change in central venous pressure during lower body negative pressure
Central venous pressure decreased significantly at every stage of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

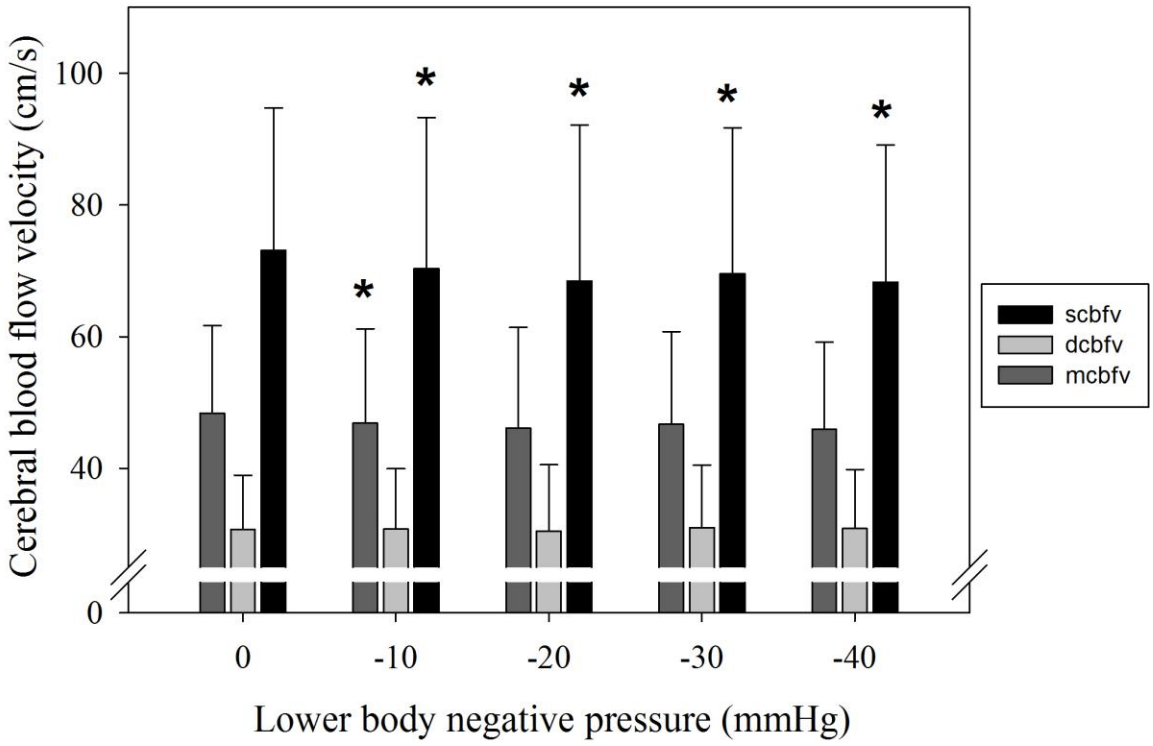


Figure 2.4.9 Cerebral blood flow velocity during lower body negative pressure
Systolic cerebral blood flow velocity (CBFV) decreased significantly at every stage of lower body negative pressure. Mean CBFV decreased in the first stage of negative pressure. Diastolic CBFV did not change significantly. Values are expressed as mean ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

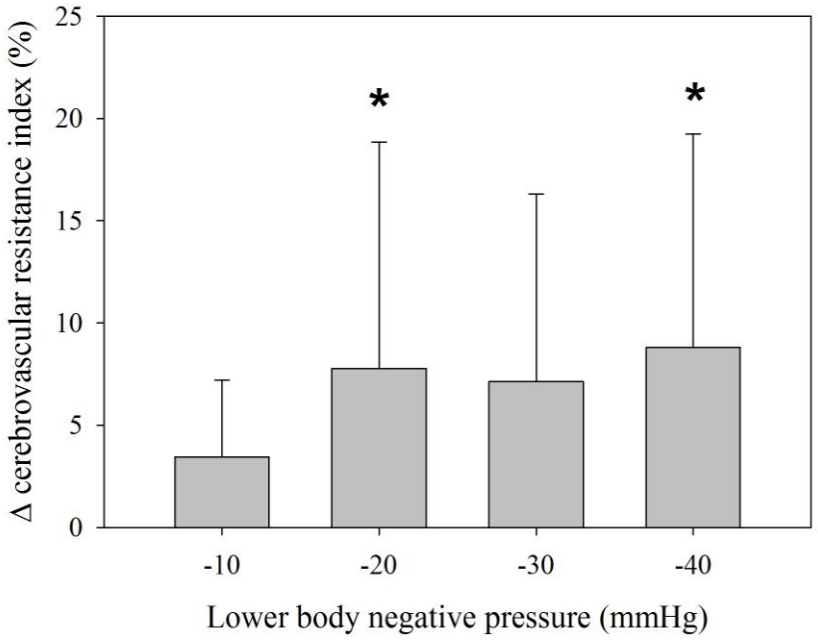


Figure 2.4.10 Change in cerebrovascular resistance index during lower body negative pressure
The cerebrovascular resistance index (CVRi), calculated as mean arterial pressure divided by mean CBFV, increased significantly in the second and fourth stages of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

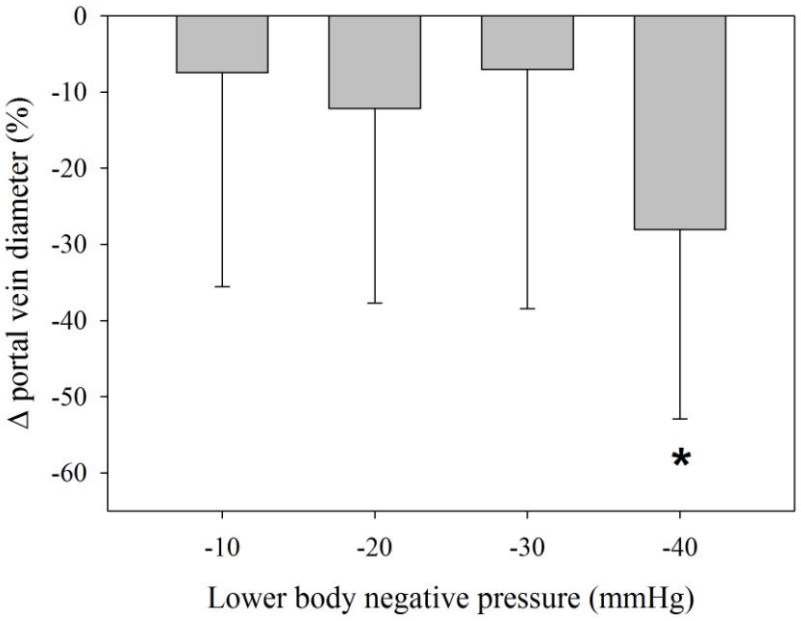


Figure 2.4.11 Change in portal vein diameter during lower body negative pressure
The diameter of the portal vein decreased in the final stage of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

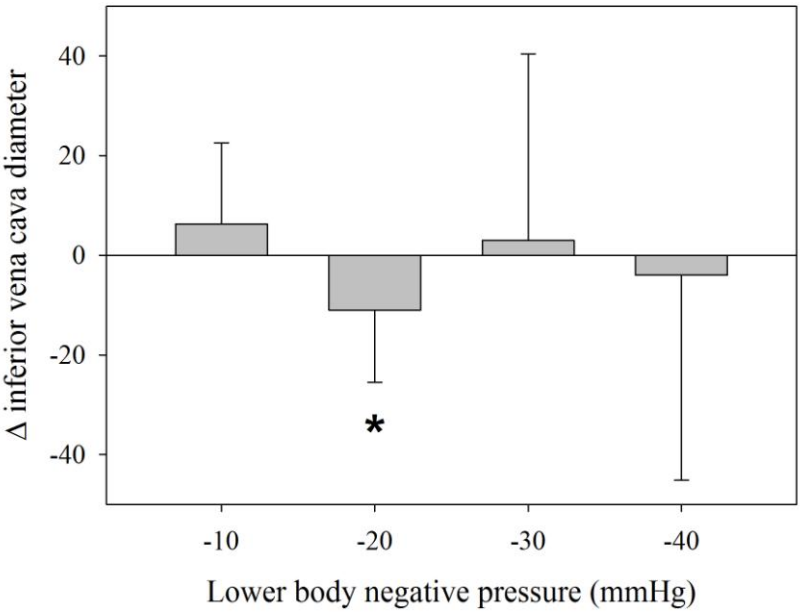


Figure 2.4.12 Change in inferior vena cava diameter during lower body negative pressure
The diameter of the inferior vena cava decreased in the second stage of lower body negative pressure. Values are expressed as mean percent change ± SD. * indicates significantly different from baseline (0 mmHg); α=0.05; n=10

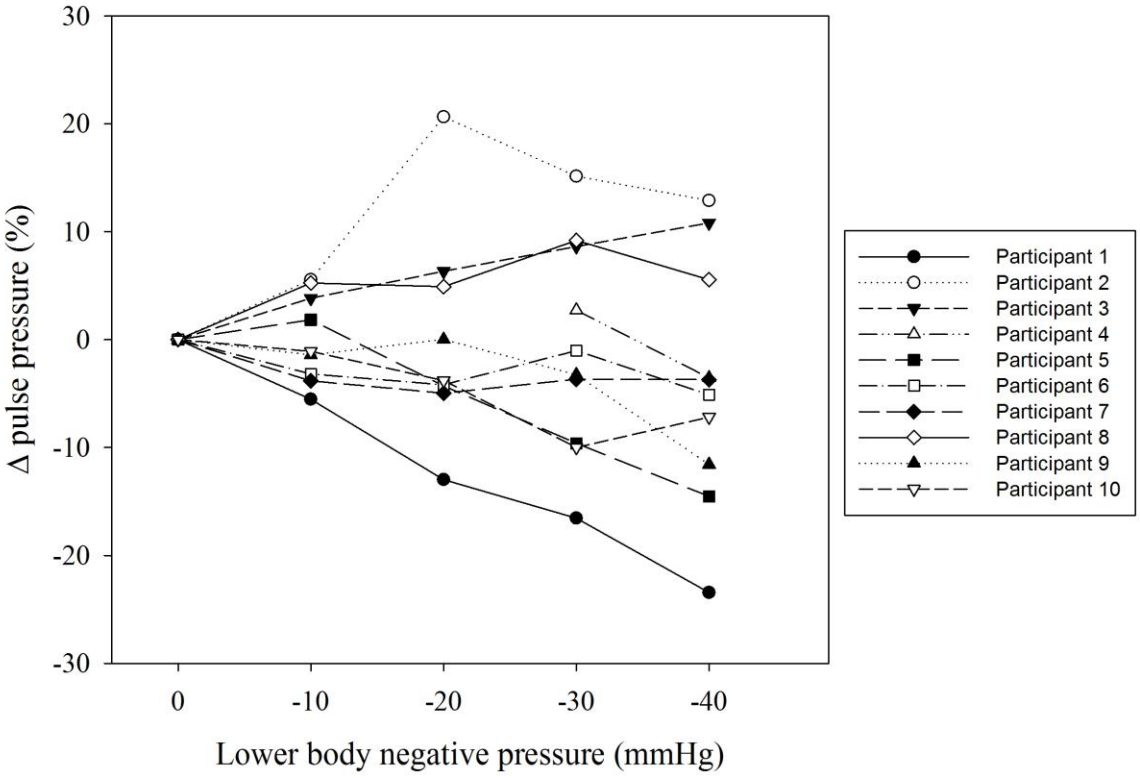


Figure 2.4.13 Change in systemic pulse pressure during lower body negative pressure

Individual responses for the percent change from baseline in pulse pressure, calculated as the difference between systolic and diastolic blood pressure, during lower body negative pressure. Diverse individual responses are displayed for all participants. Legend: participants 1-10.

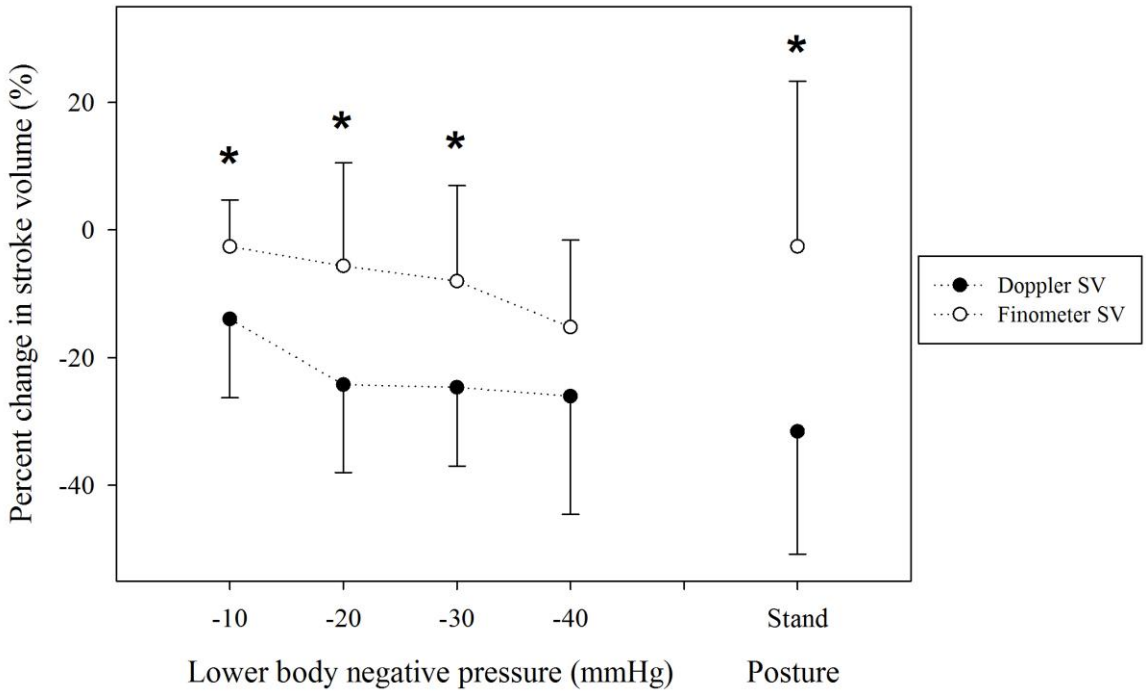


Figure 2.4.14 Change in stroke volume measured using the Doppler technique and measured from the Finometer® during lower body negative pressure
Stroke volume (SV) measured using Doppler ultrasound of aortic velocity was consistently lower than SV calculated with the Finometer during lower body negative pressure (LBNP). Doppler SV was significantly decreased at every stage of LBNP, while Finometer SV did not decrease significantly. Values are expressed as mean percent change ± SD; n=10

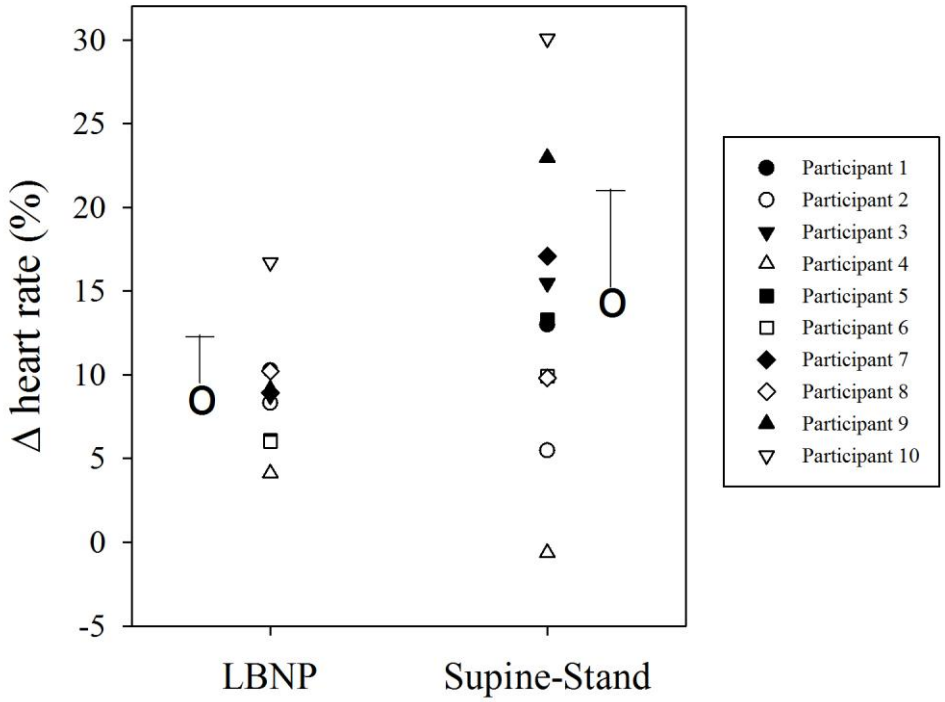


Figure 2.4.15 Individual responses for percent change in heart rate during lower body negative pressure and during supine-to-standing
Individual responses for the percent change in heart rate for lower body negative pressure (LBNP; left) and during supine-to-stand maneuver (right). Most individuals had greater heart rate increase during the supine-to-stand. Percent change during LBNP was defined as the difference between baseline and -40 mmHg. Mean percent change responses +SD are shown as large open circles to the left and right of individual values for LBNP and posture change, respectively. LBNP – lower body negative pressure. Legend: participants 1-10.

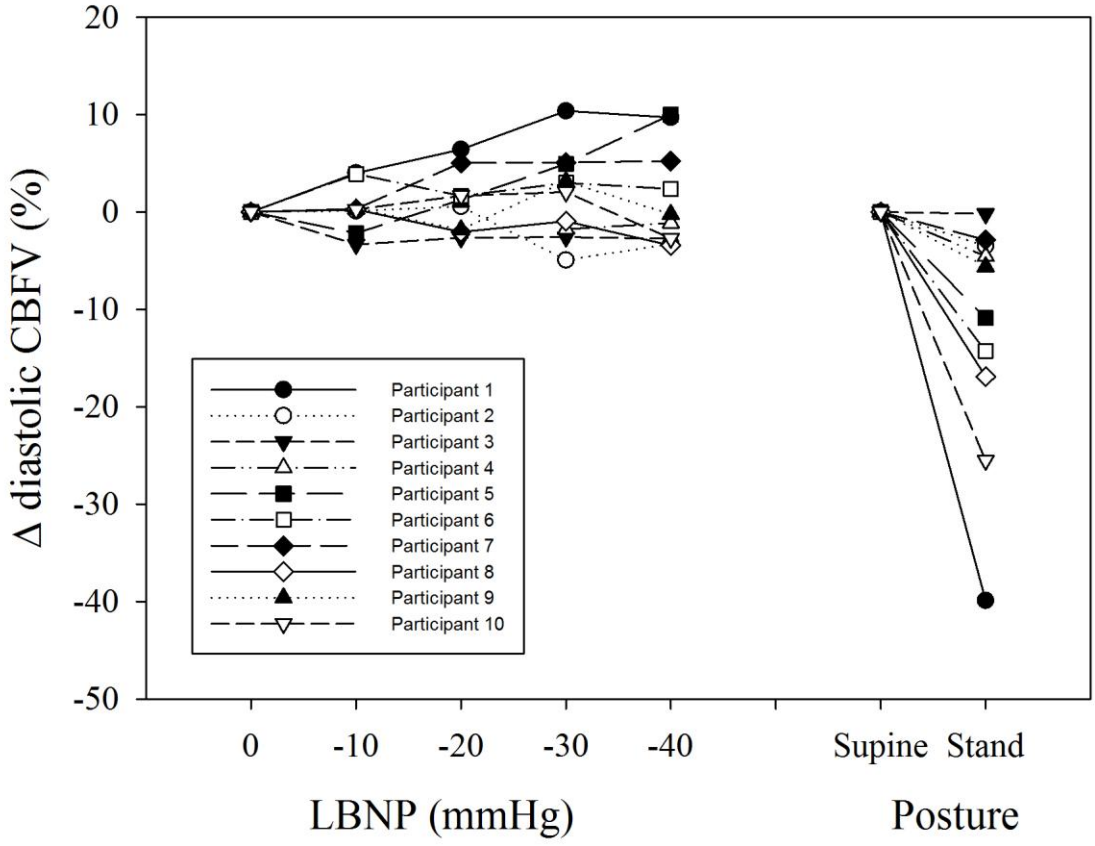
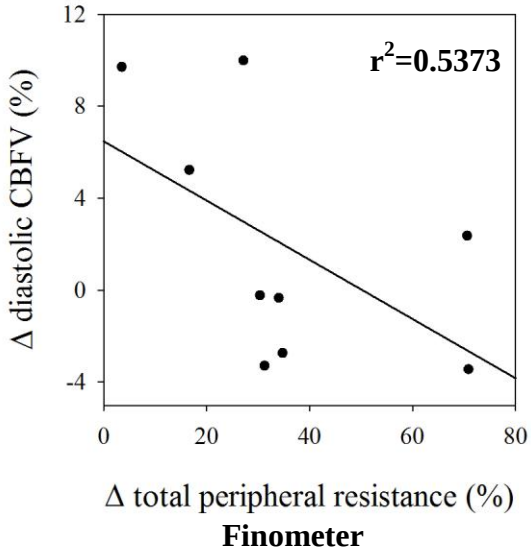
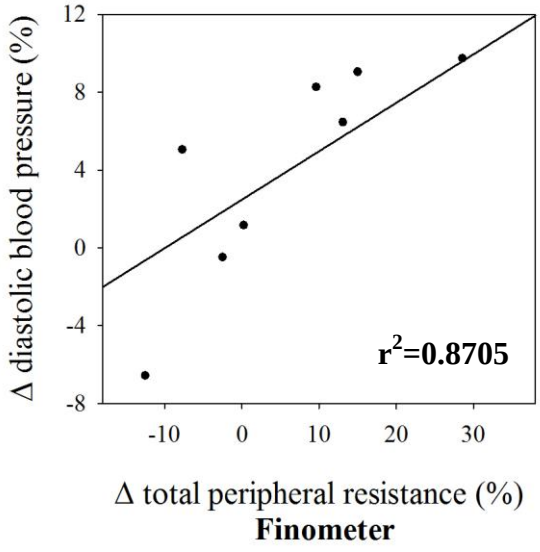


Figure 2.4.16 Individual responses for percent change in diastolic cerebral blood flow velocity during lower body negative pressure and supine-to-standing
Individual percent changes during all levels of lower body negative pressure (LBNP; left) and during supine-to-stand maneuver (right). During LBNP, diastolic cerebral blood flow velocity (DCBFV) tended to increase, while during standing, all participants experienced a decrease in DCBFV. LBNP – lower body negative pressure. Legend: participants 1-10.



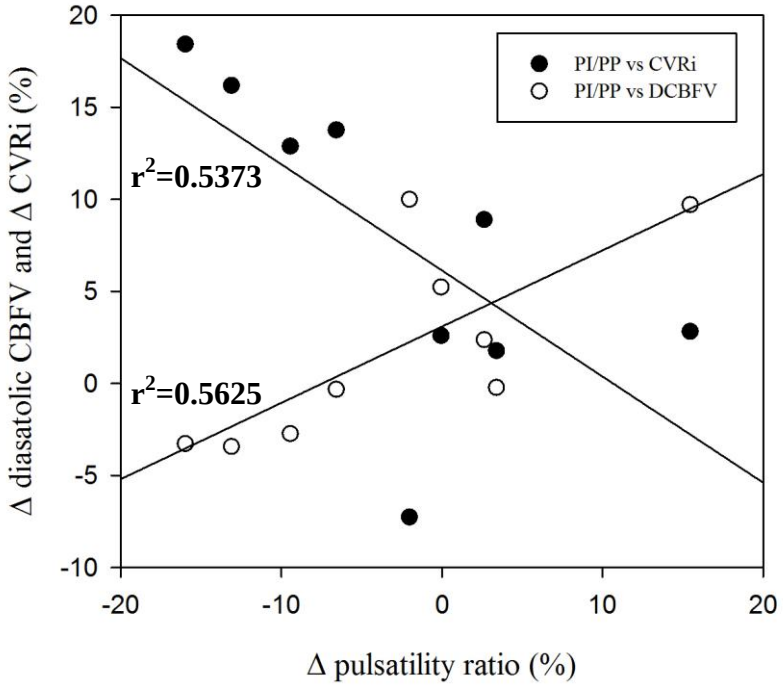
2.4.17 Relationship between change in total peripheral resistance and change in diastolic cerebral blood flow velocity

During lower body negative pressure, the percent increase in total peripheral resistance (measured with Doppler ultrasound) was inversely related to the percent change in diastolic cerebral blood flow velocity. $p=0.0200$; $r^2 = 0.5373$; $n=9$

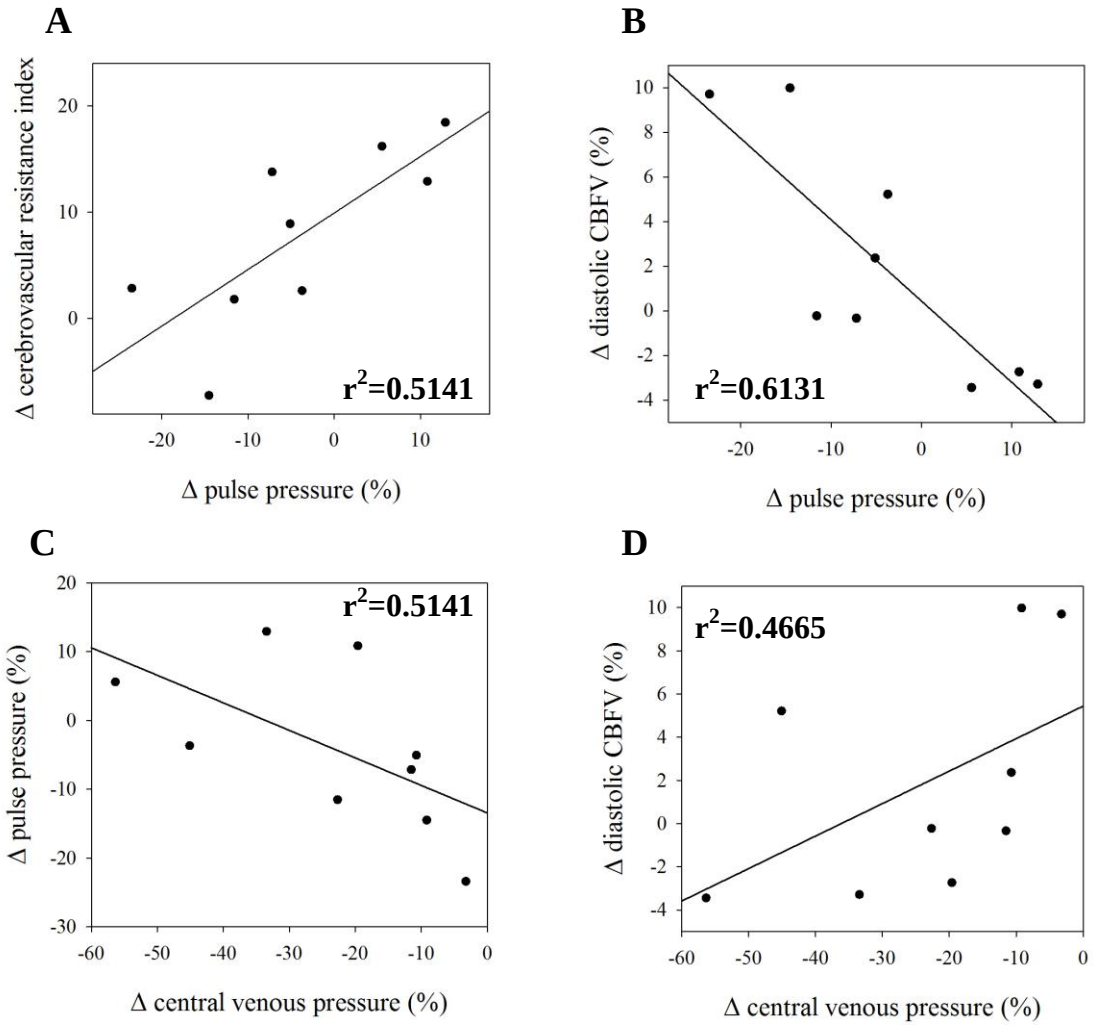


2.4.18 Relationship between change in total peripheral resistance and change in diastolic blood pressure

During lower body negative pressure, the percent change in total peripheral resistance (measured by the Finometer) was directly related to the percent change in diastolic blood pressure. $p<0.001$; $r^2 = 0.8705$; $n=9$

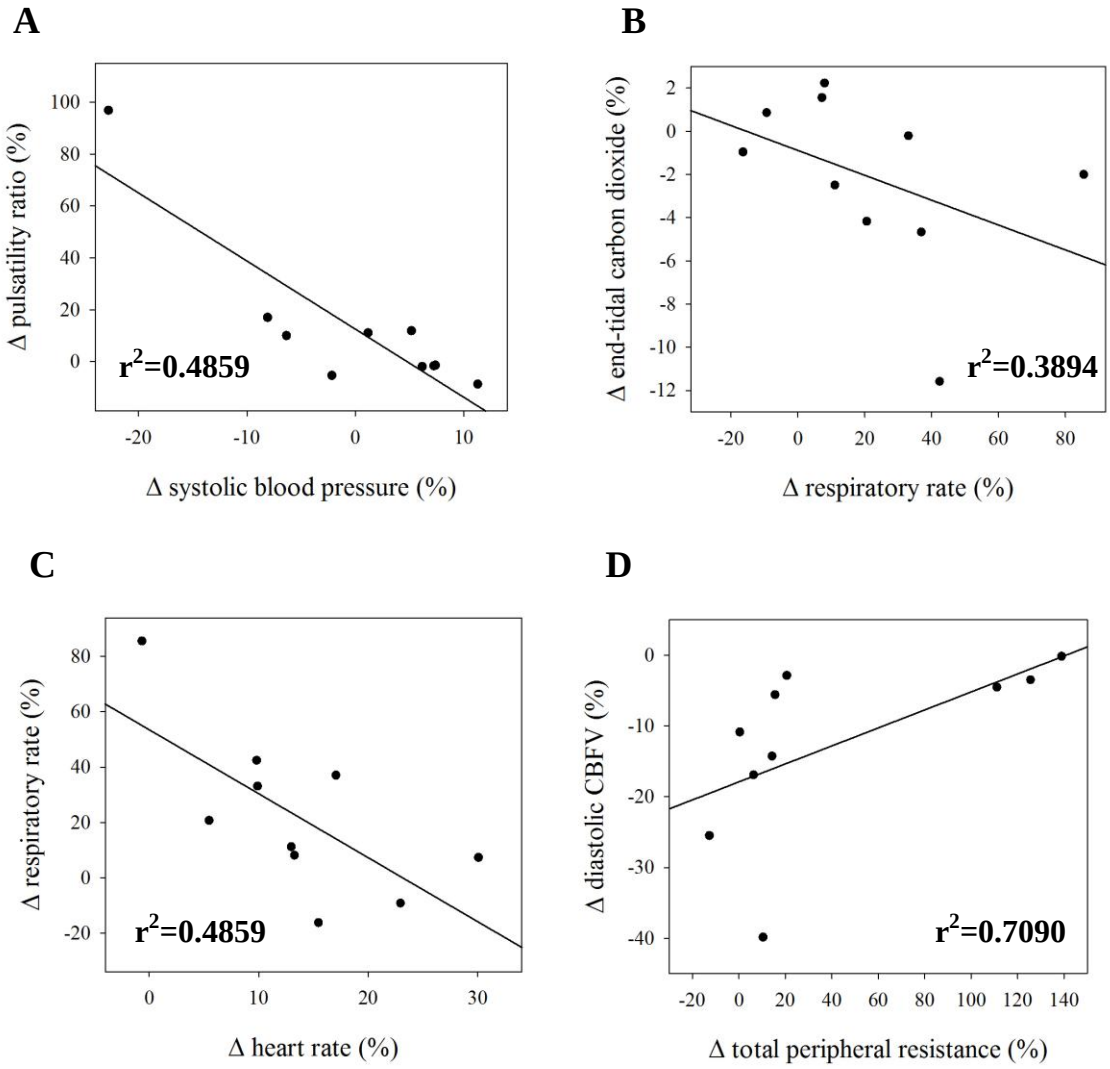


2.4.19 Relationship between change in cerebral pulsatility ratio and change in cerebrovascular resistance index and diastolic cerebral blood flow velocity
During lower body negative pressure, the percent change in the pulsatility ratio [pulsatility index (PI)/pulse pressure (PP)] was positively related to the percent change in diastolic cerebral blood flow velocity (CBFV; p=0.0158; r^2 = 0.5625; n=9) and negatively correlated to the percent change in cerebrovascular resistance index (CVRi; p=0.0200; r^2 = 0.5373; n=9). Pulsatility index (PI) = [systolic CBFV- diastolic CBFV] / mean CBFV.



2.4.20 Relationships between change in cerebrovascular resistance index, diastolic cerebral blood flow velocity, systemic pulse pressure, and central venous pressure during lower body negative pressure

During lower body negative pressure, the percent change in systemic pulse pressure (PP) was directly related to **A)** the change in cerebrovascular resistance index ($p=0.0301$; $r^2 = 0.6131$), and negatively related to **B)** the change in diastolic cerebral blood flow velocity (DCBFV; $p=0.0090$; $r^2 = 0.5141$). The percent change in central venous pressure (CVP) was negatively related to **C)** the change in PP ($p=0.0248$; $r^2 = 0.5141$) and positively related to **D)** the percent change in DCBFV ($p=0.0361$; $r^2 = 0.4665$); n=9 for all.



2.4.21 Relationships between percent change from supine to standing for selected variables

During the supine-to-stand maneuver, **A)** the percent change in systolic blood pressure was inversely related to the pulsatility ratio [PI/PP] ($p=0.0217$; $r^2 = 0.4859$), **B)** the percent change in respiratory rate (RR) was inversely related to the percent change in end-tidal carbon dioxide ($p=0.0480$; $r^2 = 0.3894$), **C)** the percent change in heart rate was inversely related to the percent change in RR ($p=0.0217$; $r^2 = 0.4859$), and **D)** the percent change in total peripheral resistance measured using Doppler ultrasound was positively correlated with the percent change in diastolic cerebral blood flow velocity ($p<0.0001$; $r^2 = 0.7090$); n=9 for all.

2.5 Discussion

The objectives of this project were successfully met by examining the cardio- and cerebrovascular compensatory responses to lower body negative pressure (LBNP) and to posture change in an elderly population. In contrast to our principal hypothesis, the two conditions differed in the responses of heart rate and diastolic cerebral blood flow velocity. The augmentation in heart rate and the decline in cerebral blood flow velocity were greater during standing. The latter finding led us to identify opposing cerebrovascular resistance responses in LBNP compared to active posture change (i.e. vasoconstriction and vasodilation, respectively). In young individuals, cerebrovascular resistance has been documented to increase during LBNP (Zhang & Levine, 2007) and decrease during stand (Alperin *et al*., 2005), although these concepts remains speculative, and have not been explicitly researched in the elderly. In examining steady state cardiovascular responses in elders to lower body negative pressure, we were able to determine that levels of up to -40 mmHg may not be sufficient to induce the same increase in heart rate seen in true orthostasis. With the host of variables that can be measured in the supine, stationary position, we were also able to gain insight into additional means through which elders respond to simulated orthostasis. In this elderly group, greater reductions in stroke volume (SV) during lower body negative pressure (LBNP) were associated with increased splanchnic vascular resistance, and reduced cardiac preload directly impacted diastolic cerebral blood flow velocity (CBFV). During standing, the maintenance of diastolic CBFV was more influenced by the reduction in cerebral pulse pressure, and protection of diastolic blood pressure (DBP). Importantly, when investigating cerebrovascular responses to orthostatic stresses, it is essential to recognize that cerebral autoregulation strategies are very different between standing and supine lower body negative pressure.

Heart rate

Postural change is a part of everyday lives of most humans. Gravity translocates blood out of the central thorax, decreasing blood pressure, activating the baroreflex-mediated heart rate (HR) response. This reflex is blunted in older adults (Kaplan *et al*., 1991; Taylor *et al*. 1992). That HR increases more in young versus old individuals in response to standing, is no longer a novel concept (Strandell, 1964). Lower body negative pressure (LBNP), used as an orthostatic challenge, similarly activates baroreflex mechanisms. The physiologic response to progressive LBNP in young healthy individuals has been closely examined and also reviewed (Blomqvist & Stone, 1983). At low levels of LBNP (i.e. up to -20 mmHg), HR is unchanged, with HR increases occurring at greater intensities of negative pressure. This pattern of increase results in a characteristic exponential-looking HR response to increasing LBNP. In the elderly, the HR response to LBNP is often described as attenuated (Frey & Hoffler, 1988; Gabbett *et al*., 2001; Hernandez *et al*., 2005; Shi *et al*., 2000; Tsutsui *et al*., 2002; Hernandez *et al*., 2010). It is not clearly acknowledged whether the HR response of elderly individuals also follows this delayed increase. In our study, there was no delayed HR increase with successive levels of LBNP, rising 9 percent by the final stage of LBNP, which is consistent with our initial hypotheses concerning directional changes in variables during LBNP.

In 1998, Fortney *et al*. examined trained and untrained elderly individuals during 3 minute increments of LBNP. HR in the control group (65.6±2.3 years), did not increase after the first stage of -8mmHg. At -16mmHg, -32mmHg, and -40 mmHg, HR increased 2, 6, and 4%, respectively. Despite the uneven LBNP increments, an overall linear increase was observed. However, in the comparison group of elderly athletes, a delayed HR response was observed. The protocol used by Tsutsui *et al* (2002) was 3 minute intervals of LBNP

beginning at -10 mmHg; identical to our protocol. The HR response in their elderly group (68.2±0.8 years) increased negligibly at low levels of LBNP and increased most rapidly toward the end of the protocol. However, by -40 mmHg, the overall HR increase was just 10% in the older group, which is significantly less than the young group and quite similar to our -40 mmHg value of 9%. Interestingly, no delayed increase was seen in the young group; the response was quite linear. Similarly, Hernandez *et al*. (2005) used 4 minute intervals of LBNP in 10 mmHg increments to evaluate effects of fitness and age on LBNP tolerance. Four groups of individuals were studied; young-fit, young-unfit, old-fit, and old-unfit. In the old, unfit group (70.9±1.0 years), HR rose inappreciably in the first two stages, increased by 1.5% at -30 mmHg, and then 4.3% by -40 mmHg. The greatest HR increase occurred in the final stage of LBNP and the lowest increase was seen during the first stage, and this trend was actually true of all four groups studied. The same authors (Hernandez & Franke, 2005) looked into endurance training effects in elders (68.1±3.1 years) with the same LBNP structure and found (pre-training) an overall mild linear climb in HR. There were minimal HR increases through the testing session (no change at -10 mmHg, 3% at -20 mmHg, no change at -30 mmHg, and another 3% at -40 mmHg), amounting to just over 6% at -40 mmHg. Most recently, the work done by Hernandez and colleagues in 2010 can be easily contrasted, as their LBNP protocol was again similar, and likewise, the investigators had participants straddle a padded seat rather than using a footplate, eliminating the element of lower limb muscular contractions during testing. The older participants (70.4±2.4 years) did not have an immediate HR response. In fact, no increase was seen until -40 mmHg, where HR rose 3%. Similarly, HR in the young group did not increase at the first level of LBNP, although subsequently, the rate of increase became higher with each stage. On the whole, younger individuals had an earlier and greater total increase in HR compared to their older counterparts in response to the same level of LBNP. Importantly, the authors identified their

elderly participants as “fit” and “physically active”. In the recruiting process, older individuals were considered if they reported regular aerobic exercise for more than 20-45 minutes on 3 or more days per week, for at least 2 years. Two of the elders were actually master athletes, accumulating 32-64 km per week of running. Because of the fitness dissimilarities between our elderly participants and those from the Hernandez *et al.* (2010) study, direct cardiovascular response comparisons are problematic.

A number of other LBNP studies conducted with elderly individuals use differing LBNP protocols, or focus on other variables without reporting HR. From the studies examined above, there were three accounts of a delayed HR response in the elderly (one in a group of *fit* elders), and two studies showing a relatively linear increase. Of the abovementioned studies that used a young comparison group, two showed no delayed HR increase in their young participants, while one did have participants displaying this pattern. Evidently, there are inconsistencies regarding the particular level of LBNP at which HR begins to increase in the elderly, and the rate of HR increase at subsequent levels. This may be a function of the amount of time participants were resting before the onset of LBNP, their relative comfort level, experience with laboratory testing, or the assortment of other variables being studied simultaneously. On the whole, the total HR response in elders is similar across studies, regardless of the LBNP level at which the increase begins. The HR increase in our elderly participants did not exceed 10% at a negative pressure level of approximately -40 mmHg, which was consistent with results from other investigators.

Blood pressure

Circulatory homeostasis is maintained through the function of neurovascular reflexes. Acute changes in blood pressure activate baroreceptors, which have stretch-sensitive elements within the arterial walls of the aorta, carotid artery and cardiopulmonary area that transmit afferent impulses to the central nervous system. This project was designed to study *steady-state* cardio- and cerebrovascular responses to orthostatic stress. Closely examining the dynamic beat-by-beat transitions between intensities of orthostatic stress is another focus, not included in this thesis. Under conditions of sustained, unchanging orthostatic stress, a new *steady-state* status should be attained within one minute (Sprangers *et al*., 1991; Hisdal *et al*., 2001). Because the focus of our analysis was after the dynamic accommodation period and at a sub-maximal LBNP level, BP was expected to remain quite stable. In projects examining *tolerance* to LBNP, a drop of SBP by >25mmHg is generally considered to be a presyncopal symptom, indicating possible imminent fainting, at which the test would be terminated. During LBNP levels up to and including -40 mmHg, Hernandez *et al.* (2005) did not observe a change in MAP; however, during final stages of the protocol the older unfit groups had significantly greater decreases in MAP than the older fit group and the young groups. Similarly, the elderly participants in the study from Fortney and colleagues (1992) did not experience a significant drop in MAP at -40 mmHg, nor did the unfit elderly in the study from Hernandez & Franke (2005) and from Franke *et al*. (2006). At sub-maximal levels of LBNP, there does not seem to be an effect of age or fitness on the MAP response (Hernandez *et al* 2005). However, there is an age-related increase in baseline MAP in elderly persons compared to their younger and their older fit counterparts.

The only authors to report separate systolic and diastolic BP changes with LBNP in the elderly were Hernandez *et al*. (2010). Again, these elderly participants were particularly fit; however, there were still no changes in systolic or diastolic blood pressure with LBNP up to -40 mmHg. Since most studies report MAP only, it is likely that changes in systolic (SBP) and diastolic (DBP) blood pressure were also negligible. In our study, DBP increased marginally, albeit significantly, in the final stage of LBNP. The mean group SBP response was unchanged, although the variability in the direction of the responses between individuals was high. The standard error of measurement was about 2.5 times greater in the final stage of LBNP for SBP compared to DBP, with the range of SBP values spread between Δ+10% and Δ-10% with LBNP. The homogeneous increase in DBP is worth exploring; there seems to have been less consistency in the systolic blood pressure.

The primary determinants of DBP at rest are Q and TPR (Rowell, 1993). Elevated diastolic blood pressure at rest has been found to have a strong correlation to arterial stiffness markers, augmentation index, pulse wave velocity (Nürnberger *et al*., 2003), and changes during exercise were found to be closely related to serum cholesterol levels (Brett *et al*., 2000). During exercise, total peripheral resistance (TPR) decreases as a result of vasodilation in the resistance vessels of working skeletal muscles. If there is an impaired vasodilatory response, one might expect DBP to rise inaptly. During orthostasis, peripheral resistance increases, decreasing total arterial compliance, and increasing DBP. When Q decreases as well, this counters the increase in TPR, and DBP may remain constant, which is likely the reason that DBP is rarely reported during LBNP research. In our experiment, the DBP of most participants increased less than 10% over the 4 levels of LBNP.

Sub-maximal LBNP has traditionally been considered to activate the cardiopulmonary baroreceptors only—without triggering the arterial baroreceptors. This notion was based on documentation that steady-state MAP did not change with low levels of LBNP. If MAP and PP remained constant, it was thought that the neural firing of the arterial baroreceptors had not been altered, and that decreases in CVP will solely activate the cardiopulmonary baroreceptors in order to keep MAP unaltered. More recently, however, depending on the way in which the mild LBNP is introduced (i.e. how quickly the targeted negative pressure is reached), MAP *was* transiently altered even though steady-state MAP remained unchanged, indicating that the arterial baroreceptors were unloaded at low levels of LBNP (Fu *et al*., 2009). Our LBNP protocol called for negative pressure levels of up to -40 mmHg, which likely unloaded arterial baroreceptors in all participants, and although this was not explicitly investigated, it is very probable that the group MAP response remained constant for reasons other than lack of arterial baroreceptor unloading.

Whether or not MAP is affected by greater levels of LBNP will vary inter-individually. MAP seems to be maintained so long as baroreceptor-mediated muscle sympathetic nerve activity action is not exhausted, or rather, does not begin to diminish (Sundlöf & Wallin, 1978; Convertino & Cooke, 2002). In other work from our lab, a similar LBNP protocol produced a significant decrease in SBP in young, healthy individuals, with no decrease in DBP (Grinberg, 2010). Logically, these same responses may not be expected in the elderly population because of vast differences in the relative intensity of the negative pressure on the two populations. This is primarily a result of age-related changes in leg vessel compliance; though, this was not measured in our population. Generally, accounts of maintained tolerance to orthostatic stress in this age group are attributed to reduced leg venous compliance.

Despite there being limited evidence of systolic and diastolic blood pressure changing with sub-maximal levels of LBNP, it is generally accepted that pulse pressure (PP) decreases with increasing LBNP (Stevens & Lamb, 1967). Systemic PP is dependent on SV and arterial tone (Burton, 1972; Sunagawa *et al*., 1983). This important relationship prompted the work of Michard and colleagues (1999; 2000), who examined PP as a predictor of changes in SV. If total arterial compliance were to remain constant during LBNP, PP and SV would have a linear association (Chelma *et al*., 1998). Convertino *et al.* (2006) investigated this relationship during LBNP and found that arterial distensebility does change throughout LBNP, revealing a SV-PP relationship that is best fit with a three-element regression model, such that as LBNP progresses, decreases in PP translate to exceptionally smaller reductions in SV. However, ninety-one percent of the variance in the SV-PP relationship was represented by a simple linear regression model. In our study, the change in SV estimated from the Finometer® was linearly related to the change in PP. It is not known whether this relationship becomes altered with age, although it seems to have been retained in our group of elders during LBNP.

Central venous pressure

The principal consequence to orthostatic stress that the body needs to counteract is the translocation of blood volume to dependent regions. This reduces the availability of blood to the right ventricle, which is difficult to measure. In this study, we utilized an invasive central venous pressure (CVP) measurement to evaluate changes in venous pressure in the right side of the heart, which more directly measures preload of the heart than other techniques (Convertino *et al*., 1988). The atria do not have valves at their opening from the venous circulation. At end-diastole, the catheter in the antecubial vein measures the pressure in the

vein of this "open" system. Unfortunately, this technique is fraught with limitations. In the context of this study, it is important to consider these factors in the interpretation of the CVP responses.

The mean CVP response decreased significantly at each stage of negative pressure, which is in concert with our hypotheses. The range of individual percent changes in CVP with LBNP was wide (-3.5% to -56%). It is possible that the same levels of LBNP did not unload central blood volume of all participants equally. A number of years ago, investigators noticed a smaller decrease in SV during LBNP in older individuals, when compared to younger individuals (Minson *et al*., 1999; Ebert *et al*., 1982). It was suggested that the lower venous compliance seen in the elderly contributes to a reduced peripheral fluid shift during LBNP, thereby conserving cardiac filling volume and maintaining SV (Lakatta, 1993; Olsen & Lanne, 1998; Olsen *et al*., 2000). Tsutsui et al (2002) used non-invasive mercury strain gauge plethysmography with venous occlusion to evaluate whether differences in venous compliance affected the rate at which the lower limbs filled with blood during LBNP. The authors related SV to changes in leg volume during LBNP, and found that a reduced leg compliance may result in a smaller increase in leg volume, consequently, lessening the drop in SV in elderly persons.

Veins stiffen as age advances (Fu *et al*., 2002; Hernandez & Franke, 2004; Monahan *et al*., 2001b; Olsen & Lanne, 1998; Tsutsui *et al*., 2002; Young *et al*., 2006), just as the arteries do (Greenwald, 2007; Seals *et al*., 2009; Zieman *et al*., 2005). Chronic exercise training has improved leg venous compliance in older individuals (Pawelczyk *et al*., 1988; Tanaka *et al*., 2000), although, this may not improve orthostatic *tolerance* to maximal LBNP (Hernandez & Franke, 2004), as was initially postulated by Tsutusui and colleagues (2002).

The debate about the influence of fitness on orthostatic tolerance remains unsettled. There are cross-sectional accounts of reduced tolerance to HUT or LBNP (Raven & Pawelczyk, 1993; Convertino, 1987), and others showing no effect of physical fitness on orthostatic tolerance (Franke *et al*., 2003; Hernandez *et al*., 2005). Even longitudinal studies have yielded mixed results; exercise training may reduce (Raven *et al*., 1984; Stevens *et al*., 1992), have no effect on (Convertino, 1993; Shvartz & Meyerstein, 1972), or improve tolerance (Convertino *et al*., 1984; Greenleaf *et al*., 1985).

Because the process of aging has been associated with a higher occurrence of orthostatic hypotension (Lipsitz, 1972), cross-sectional investigations do not suffice. In 2005, Hernandez & Franke conducted a six-month endurance training program with elderly men and women (Hernandez & Franke, 2005). Calf venous compliance increased 20-30%, with no changes in tolerance in LBNP. Post-intervention, fitness increased 14%, which may not have been sufficient to determine whether fitness level impacted LBNP tolerance. The standard deviation of intolerance scores did decrease after training, meaning less tolerant participants improved their tolerance index the most with training, while more tolerant individuals did not change. However, there was no relationship between calf venous compliance and LBNP tolerance index in the exercise group after training. The pertinent detail to consider here is that the age-related decrease in calf venous compliance influences the volume of central blood that is translocated to the lower limbs during orthostatic stress. Higher aerobic fitness levels are associated with greater venous compliance in older individuals (age 55-75years; Monahan *et al*., 2001b). The individuals participating in our project were unfit and lived sedentary lifestyles. While there was no aerobic fitness assessment as part of this thesis, all ten participants reported an "average" activity level (Self-report form in Appendix), and none had engaged in any type of advanced fitness training in

their past. Lower limb venous compliance was not assessed in this study, so it is not possible to determine whether the variety of CVP responses to LBNP were influenced by diverse compliance values between individuals. Because the participants were very close in age and perceived fitness level, it is less likely that there were considerable aerobic-related differences in leg venous compliance.

The overall mean CVP response to LBNP decreased significantly at each stage, with the largest magnitude drop seen after the first stage. The smallest magnitude drop occurred in the final stage, which was only 1% lower than the CVP value at -30 mmHg. In fact, seven of the ten participants displayed this non-linear pattern, resembling a very shallow "U" shape, or an exponential decay curve approaching an asymptote. It is possible that this observation is a result of the reduced venous capacitance response that is seen in elderly individuals (Olsen & Lanne, 1998). CVP has only ever been reported in the elderly on two other occasions (Fu *et al*., 2002; van Hoeyweghen *et al*., 2001). Fu *et al*. (2002) used an LBNP protocol with one mild level (-15 mmHg), and their measurement of CVP was merely an estimation based on changes observed in peripheral venous pressure. There was no calibration to the level of the heart. Pressure values in the elderly began at 1.2mmHg and dropped 1.5 mmHg to -0.3mmHg with 15mmHg of negative pressure. The results from Hoeyweghen and others (2001) can be more appropriately contrasted against our CVP responses. They used a peripherally-inserted catheter that was advanced so that its tip lay between the second and eighth thoracic vertebrae. Three levels of LBNP were applied (17.5, 35, and 50 mmHg) and maintained for 20 minutes each. The mean baseline CVP value was ~7 mmHg in nine healthy elderly volunteers (65-73 years)—details very similar to our project. After the first stage of LBNP, CVP dropped 37%. The decreases over the last two stages were less than half the magnitude of the initial drop, despite similar increases in the magnitude of lower body negative pressure.

Our results follow this trend, which is similar to what Fu *et al.* reported in 2004, and first by Murray *et al.* in 1968. In what was meant to be a comparison between sexes, the investigators (Fu *et al*., 2004) noticed that pulmonary capillary wedge pressure (PCWP) decreased rapidly with low levels of LBNP, and then more slowly reaching a plateau at higher levels of negative pressure toward presyncope. All of their participants (24-32 years) reached presyncope during the protocol. The authors described the PCWP plateau as "intriguing and unexpected" (Fu *et al*., 2004). Because calf volume increased linearly through progressive LBNP, it is unlikely that this PCWP pattern was due to accrued venous pooling in the lower limbs. Analogous to what was observed in some of the participants from the study herein, Fu and colleagues reported that when decreases in PCWP slowed, SV continued to drop, and HR continued to rise, suggesting that peripheral blood accumulation also continued as LBNP progressed. In making these comparisons, keeping in mind the functional differences between the techniques used to estimate preload, is essential. Fu and colleagues used a more invasive, and sophisticated approach involving catheterization of the pulmonary artery, and measured right atrial pressure (RAP) at the proximal end of the catheter. The similarities between the PCWP response (and RAP) to LBNP and our CVP response to LBNP is encouraging.

In our measure of CVP, the pressure transducer reading the fluid in the catheter and antecubital vein is extremely sensitive. Even slight right arm movements by the participant are visible on the pressure tracing. Care was taken during analysis to remove sections of erroneous readings and to determine whether pressure values had shifted physiologically, or due to technical inadequacies. It is a real possibility that the reason for the CVP plateau toward the end of LBNP may be a misreading in the equipment. It is easy for the catheter to become adhered to the side of the vein, thereby dampening further decreases in intraluminal

venous pressure. Venous pressure waveforms were inspected for dampening oscillations; however, this is not easily quantified. One way to investigate whether the CVP reading is reflecting true CVP values is by examining the corresponding stroke volume (SV) values at each level of LBNP.

The expected relationship between SV and CVP is roughly linear. According to the Frank-Starling principle, there is a plateau in SV where further increases in filling pressure do not increase SV. In the healthy resting human, this occurs in the supine position (Bundgaard *et al*., 2009). As LBNP increases, venous return decreases, dropping CVP, and consequently, SV. Although our cohort's mean plot of SV versus CVP was linear, the variability between the ten participants comprising each data point was extensive, with only about two of ten participants following a clear linear trend. Theoretically, if the CVP response is not linear because the participant was contracting leg muscles, SV should also follow this trend. Activating the muscles of the leg mechanically forces blood through the veins toward the heart, promoting venous return. Inspecting individual SV-CVP plots revealed that about four of ten participants had at least one occasion where they may have shifted the position of their lower body within the LBNP box (e.g. bending of knees; crossing of legs), which resisted decreases in venous return and SV. In instances where the SV response continued to decrease and the corresponding CVP did not change in concert, it is logical to suppose that this may have been a false CVP reading. The responses of three out of ten participants are consistent with this trend. In the case where SV increased and CVP decreased (occurred in one transition in two of ten participants), the most likely explanation is an unexpected improvement in the aortic velocity signal, resulting in a greater recorded velocity, and resultant SV. With the preceding considerations in mind, LBNP stages that appeared to have incongruous SV-CVP associations, like the ones mentioned above, were

removed for additional inspection for the sake of curiosity. In one individual, SV and CVP did not change appreciably (i.e. less than 10% in CVP and SV), and so the data were also omitted. With the remainder of the data points representing stages of LBNP with an appropriate SV-CVP relationship, patterns of responses were seen. In general, having a greater SV-CVP slope tended to correspond with a higher TPR response, and a lower TPR response was related to a flatter SV-CVP slope. In this group of elders, the CVP response was inversely related to pulse pressure (PP). Individuals with the largest reductions in CVP had greater percent delta PP changes, or rather, elders with the least reduction in CVP tended to have greater increases in DBP with LBNP. Moreover, greater decreases in CVP were met with greater decreases in diastolic CBFV. Further integrated responses are discussed below.

Stroke volume

SV is intimately related to the preload of the heart, and is also a function of the heart's contractility, and the downstream resistance. A reduction in SV is the expected response to orthostatic stress. In both conditions (LBNP and posture change), SV decreased in all participants, as was hypothesized at the outset of the experiment. The overall SV response followed an exponential decay pattern—even more dramatically than the CVP response. The first two stages of LBNP saw a much greater drop (13 and 10 percent) than the final two stages (both 1 percent). In the final stage of LBNP, the mean group response had decreased a total of 25%, and the residual standard deviation of the group was at its highest. Inter-individual variability was clearly an important factor requiring consideration.

In 1992, Fortney and colleagues enrolled men based on their age and fitness level. Athletes were elite long distance runners. For the purpose of comparison to our results, it is

most appropriate to consider the control group only. SV, measured with radionuclide angiography, decreased linearly to 35% by -40 mmHg. Tsutsui *et al*. (2002), using impedance cardiography, also saw a linear decrease in SV, although only to -20% at -40 mmHg in their healthy elderly population. Impedance cardiography seems to be accurate in monitoring trends in cardiovascular dynamics, but not necessarily in absolute values (Newman & Callister, 1999). Also using bioimpedance to measure SV, Formes and colleagues (2010) saw a 20% decrease in their sedentary elders, and a 30% SV decrease in their very active elders, but reported that the higher fitness was associated with improved intrinsic cerebral autoregulation and hypoperfusion. In 2005, Hernandez and Franke investigated responses of elderly individuals to LBNP before and after an endurance training program. The baseline SV response decreased linearly to about 30% by -40 mmHg of LBNP. Surprisingly, the baseline responses between the randomly allocated exercise and control groups were significantly different. Thus, the investigators also had a group of elderly individuals displaying an overall reduction in SV of just 10%. In a similar study (Hernandez *et al*., 2005), the same authors studied previously fit elderly individuals and compared them to other unfit elders and to age-matched young fit and unfit individuals. The LBNP-induced SV reduction in the old unfit group was a linear 33%.

Despite there not being an ostensible linear decrease in SV from our elders, the total reduction was very similar to what was reported in other elderly during comparable LBNP levels. While many reports do not explicitly detail percent changes in SV at each stage of LBNP, after observing the shape of many graphs, SV responses in some younger groups may follow the same trend as what was seen herein (Keller *et al*., 2009; Fischer *et al*., 2007). It is possible that the rapid decline in SV in the first stage of LBNP followed by a plateau in later stages may be due to the deterioration of the aortic velocity Doppler signal with LBNP. This

type of issue can usually be detected and resolved during analysis. Overall, the maximum SV reduction in our group was in accord with the results of others. The fact that the group SV-CVP plot was linear is encouraging, although individual participant plots were very inconsistent, highlighting the heterogeneity of responses. An important contribution to the shape of the SV and CVP curves is the resistance of the peripheral vessels.

Peripheral resistance

As an orthostatic challenge progresses the firing rate of arterial baroreceptors decreases, initiating a sympathetically-mediated vasoconstriction in the systemic vasculature. The mean TPR response of the participants in our study was fairly linear and at -40 mmHg, had increased 41% from baseline, and consistent with our hypotheses, was significantly elevated at each level of LBNP. This estimation of TPR was calculated using the Q that was measured from Doppler aortic velocity, and is therefore subject to any imprecision in the measurement of aortic velocity. Mathematically, TPR incorporates the flow through the vasculature (Q) and the pressure differential across the systemic circulation (MAP-CVP). The mean venous pressure component was omitted in our calculation in order that peripheral resistance measured during LBNP could be directly related to the supine-stand TPR values where CVP cannot be measured. Because venous pressure is proportionally much smaller than MAP, it is often excluded from TPR calculations.

Relative to younger individuals, healthy older adults have an elevated sympathetic nervous system tonic activity, as was originally suggested by total plasma norepinephrine levels (Hoeldtke & Cilmi, 1985; MacGilchrist *et al*., 1989; Rubin *et al*., 1982; Veith *et al*., 1986). Collective findings (Jones *et al*., 2003; Jones *et al*., 2001; Elliott *et al*., 1982) have

shown that the chronic age-related elevation in SNS activity causes impaired systemic α_1-adrenergic vasoconstrictor responsiveness, resulting in blunted baroreflex buffering. In the most recent review (Monahan, 2007), Monahan summarized that in response to decreases in BP, baroreflex control of vascular sympathetic outflow becomes impaired with age (Dinenno *et al*., 2001; Sugiyama *et al*., 1996), and that aging *per se* is associated with impaired limb vasoconstrictor responsiveness to SNS stimulation (Seals & Dinenno, 2004), which may be influenced by vascular stiffness in the baroreceptor-located vessels (Mattace-Raso *et al*., 2006). However, this does not counteract the ability of the SNS to activate greater vasoconstriction in the leg vasculature of elderly men (Smith *et al*., 2007).

If our study had a comparison group of young individuals, we might expect their peripheral vasoconstriction to be greater when faced with the same relative drop in central blood volume (Olsen *et al*., 2000). However, various areas of the arterial baroreflex may be altered differently, resulting in definite impairment in the control of HR, and perhaps less deterioration in the control of peripheral vascular resistance (Ferrari *et al*., 2003). A large part of the ambiguity around reports detailing age-related changes in baroreflex-mediated peripheral vasoconstrictor capacity and responsiveness comes from differences in how each population "physiologically perceives" the same intensity of LBNP. For example, at a LBNP level of -40 mmHg, the percent TPR response from a younger group may be twice that of an older group, but the relative drop in central blood volume was also halved in elders. In the past, such a discrepancy has been interpreted as an age-related reduction in baroreflex sensitivity.

Forearm vascular resistance

Fluid distribution is uneven throughout the body during LBNP. The forearm is accessible and simple to study for research purposes. Our measure of brachial velocity (BV) was considered to be an additional indication of resistance in the peripheral vasculature; however, changes in forearm vascular resistance do not necessarily mirror changes in TPR. In fact, cardiopulmonary baroreceptors seem to control forearm vascular resistance during orthostatic stress (Abboud *et al*., 1979). The ratio between the CVP-to-FVR response tends to be used as an indicator of cardiopulmonary baroreflex function (Hughson *et al*., 2004). There did not appear to be a relationship between brachial vascular resistance (BVRi) and CVP in our group, but this is consistent with the findings of others (Convertino *et al*., 1994). During posture change, BVRi was directly related to TPR measure with Q from the Finometer.

The cardiopulmonary baroreflexes have, in general, been given less concentration than the arterial baroreceptors. There is more debate about the possible age-related changes and their consequences. There seems to be evidence of cardiopulmonary reflex blunting with age (Cleroux *et al*., 1989; Jingu *et al*., 1989), yet it is unknown whether the issue is a receptor modulation of sympathetic activity or diminished responsiveness of the forearm vasculature to neural stimuli (Davy *et al*., 1998). However, there are accounts of preserved cardiopulmonary reflex control of forearm vascular resistance in the elderly (Shi *et al*., 1996; Lindenberger *et al*., 2007). The inconsistency in findings may be a result of differences in the extent to which the baroreceptors were unloaded in the elderly versus younger individuals. When investigators have reported a reduced ability of the elderly to increase forearm vascular resistance in response to orthostatic stress, this may stem from relative differences in the intensity of orthostatic stress.

In theory, a measure of resistance is a pressure differential divided by flow. We have used brachial velocity in lieu of flow because measuring brachial artery diameter and velocity simultaneously is somewhat unfeasible, and probably, superfluous. Instead, velocity was used in our estimation of vascular resistance in the forearm, generating BVRi, which is commonly used to track changes in forearm vascular resistance (Cooper & Hainsmorth, 2001; Brown & Hainsworth, 2000). The mean BVRi response in our study increased to 40% by the final stage of LBNP, which was almost identical to the TPR response. It has been demonstrated that individuals with better orthostatic tolerance have greater increases in forearm vascular resistance (El-Bedawi & Hainsworth, 2000). Tsutsui and colleagues (2002) found that during LBNP, the slope of the regression line between changes in forearm vascular resistance and changes in SV was the same in young and old participants.

Splanchnic region

Portal vein

The splanchnic circulation is comprised of the blood vessels of the intestines, spleen, pancreas, and liver. The vessel compliance in this area of the circulation is high, which creates an important reservoir of blood volume for the cardiovascular system (Rowell *et al*., 1972). For an experienced imager, the portal vein can be well-visualized with ultrasound. Blood flows primarily into the liver through the portal vein, which receives blood flow from all the other abdominal organs. Changes in portal vein cross-sectional area tend to change in proportion to portal vein flow volume changes, and as such, portal vein diameter is often used as an index of splanchnic flow (Arbeille *et al*., 2003), and this relationship has been demonstrated to hold true during LBNP (Hughson *et al*., 2004). Collectively, findings from research investigating splanchnic hemodynamic consequences of LBNP have revealed that

individuals that tend to better tolerate orthostatic stress, show a greater reduction in portal vein diameter with orthostatic stress than less-tolerant individuals (Arbeille *et al*., 2005; Arbeille *et al*., 2008).

The mean portal vein diameter response in our group decreased significantly to -28% in the final stage of LBNP; the overall decrease in diameter being consistent with our early predictions. At each level of LBNP, arterial splanchnic vasculature constricted to limit blood flow into this region of the circulation. The reduced incoming pressure caused the venous vessels to lose their distending pressure, slightly recoil, and expel blood volume from the very compliant splanchnic venous vasculature toward the right side of the heart. As we are reporting data from steady state conditions only, the progressive decrease in hepatic portal vein diameter signifies the extent to which blood flow is being limited into the splanchnic area (Rowell, 1965).

Not all of our participants responded to increasing LBNP with a decrease in portal vein diameter. This likely speaks to the heterogeneity with which these ten individuals responded to the same orthostatic stress. In fact, participants with an increase in portal vein diameter tended to have the greatest drop in stroke volume (SV) and cardiac output (Q). Interestingly, these individuals also seemed to have the greatest total peripheral restrictive response. It would seem that within our elderly population, elders with lesser mobilization of blood from the splanchnic region seem to compensate for this with greater peripheral vessel constriction. However, based on the vast pooling capacity of the splanchnic region, this technique for countering orthostatic stress seems to be inefficient as these individuals had the greatest reductions in SV.

Inferior vena cava

Measuring inferior vena cava (IVC) diameter at each stage of LBNP provided an additional variable with which to assess changes in venous return. With less distending pressure maintaining the patency of the large vessel, its diameter decreases. A reduction in venous return during LBNP should be mirrored by a decrease in the diameter of the IVC, as the IVC is a very compliant vessel. Contrary to our initial hypotheses, not all individuals displayed this relationship, despite experiencing a decrease in CVP. Given the inherent measurement error accompanying estimation of the IVC diameter, this may not be surprising. With each level of LBNP, the internal organs may shift, requiring a different angle on insonation with the ultrasound probe in order to adequately visualize the IVC. Additionally, most participants had extra abdominal adipose tissue that made sonographic visualization of the IVC from the sub-xyphoid view challenging. Ten percent of the diameter measurements were unsuccessful. That the IVC tends to collapse during inspiration added further difficulty to its measurement.

Cerebral blood flow velocity

During orthostatic stress, the fundamental role of the cardiovascular system is to maintain blood flow to the brain. Cerebral blood flow velocity (CBFV) is primarily determined by cerebral perfusion pressure (CPP) and cerebrovascular resistance (CVR). However, during orthostatic stress, such as LBNP, changes in CBFV seem to be primarily related to changes in local vascular tone (CVRi) rather than MAP (Franke *et al*., 2006). During progressive, *sub-maximal* degrees of LBNP, CBFV is expected to remain relatively constant under steady-state conditions. Because aging is linked to increased proportion of individuals with symptoms of dizziness and syncope, it has often been thought that cerebral autoregulation is impaired with aging. However, elders seem to maintain CBF just as well as young

individuals when subjected to severe orthostatic challenge progressing to syncope (Franke *et al*., 2006).

Resting CBF declines with age (Shaw *et al*., 1984; Meyer *et al*., 1993; Lossessner *et al*., 1995; Lipsitz *et al*., 2000; Scheel *et al*., 2000; Albayrak *et al*., 2007). Our group displayed a range of CBFV values at baseline (27cm/s to 68cm/s), mean 48±13cm/s, which is in agreement with transcranial Doppler results from others studying elders of equivalent age (Frank *et al*., 2006; van Beek *et al*., 2009). Our elderly group showed a significant decrease in systolic CBFV at all levels of LBNP, and mean CBFV decreased significantly in the first stage of LBNP only. These reductions were in spite of an unchanging MAP. Similar MCBFV and MAP responses were reported by Serrador and colleagues (2000b) and by Levine and colleagues (1994) with young healthy populations at LBNP levels of -40 mmHg. Decreased MCBFV amidst unchanging MAP during orthostatic stress has been reported before (Bondar *et al*., 1994; Larson *et al*., 1994; Giller *et al*., 1992; Cencetti *et al*., 1999; Carey *et al*., 2001b). The decrease in CBFV seems counterintuitive based on what is known about cerebral autoregulation—that CBFV should not change over a wide range of MAP. However, when compared to the results of others (Levine *et al*., 1994; Brown *et al*., 2003), there is consistency.

The precise impact of changes in systemic variables on cerebral blood flow is not known. Cardiac output (Q) and stroke volume (SV) decreased appreciably with LBNP herein. The percent decreases in MCBFV (10%) were less than the relative reductions in Q (24%), and the increase in systemic vascular resistance (TPR; 41%) was much greater than the estimated increase in CVR (9%). Some investigators have reasoned that this differential response may be the result of sensitivity differences to sympathetic influence on the systemic

versus cerebral vessels (Wei *et al*., 1975; Marcus & Heistad, 1973). Although recently, this proposition of cerebral vasoconstriction induced by sympathetic activation may not be probable (LeMarbre *et al*., 2003; Zhang *et al*., 2007). A number of older projects seem to support this idea (Nelson *et al*., 1970; Baumbach & Heistad, 1983; Brooks *et al*., 1989). In 2007, Zhang & Levine reported that the sympathetic influence on CBFV probably plays a role in cerebral autoregulation, but cannot be the sole cause for the decrease in CBF during orthostatic stress. The sympathetic system may be more important in cerebral blood flow regulation in terms of beat-to-beat dynamic control (Purkayastha & Raven, 2011).

Decreased arterial carbon dioxide (CO_2) causes cerebral vessel constriction as well, and therefore, may also contribute to reduce CBFV during orthostasis. Exhaled CO_2 (ET_{CO_2}) did decrease significantly with LBNP in our study, which is in agreement with what is known about ET_{CO_2} and orthostatic stress, and this has been observed in elderly individuals (Formes *et al*., 2010). Cencetti *et al*. (1997) demonstrated that CBFV and ET_{CO_2} decrease with head-up-tilt, despite an unchanged breathing rate. The reduction in CO_2 may be related ventilation-perfusion matching in the lung in the upright position. Mismatch of ventilation and perfusion may have occurred during LBNP, contributing to reduction in arterial CO_2. Since ET_{CO_2} was significantly reduced, its role in cerebral vasculature constriction cannot be overlooked. Contrary to our initial hypotheses, posturally-related hypocapnia was not observed. ET_{CO_2} was not decreased after one minute of standing. These ET_{CO_2} results cannot be confidently contrasted against the work of others because exhaled CO_2 has not often been reported in elderly populations during posture change. Edlow *et al*., (2010) reported that ET_{CO_2} decreased on standing "across the age continuum", although specific age ranges were not given. However, the authors did remark that their older individuals had smaller magnitudes of ET_{CO_2} reductions. Van Beek and colleagues (2010) used a protocol sith repeated posture

changes in their elders and found ET_{CO_2} to decrease, but only for sitting and standing using 10 sec intervals rather than 5 seconds. The posturally-related reduction in ET_{CO_2} is well documented in young (van Lieshout *et al*., 2001), and seems to be related to the shift in cardiac output distribution in the lung resulting in altered ventilation-perfusion matching. It may be possible that in elderly persons, upright posture does not result in disproportionately increased ventilation versus perfusion in the lung, thereby resulting in maintained ET_{CO_2} levels, which may have contributed to the lack of cerebrovascular constriction in standing. In a younger group, Immink and colleagues (2009) remarked that the contribution of $PaCO_2$ levels to a postural decline in CBFV is only transient, and is has no effect on cerebral blood flow after one minute of standing. Accordingly, in the group of elders from this thesis, the involvement of $PaCO_2$ in orthostasis-induced cerebral hypoperfusion seems unlikely; this is especially true because cerebral vessels were found to have dilated upon standing rather than constricted, which is the expected result with reduced ET_{CO_2}.

Cerebral perfusion pressure (CPP) is the pressure at which the brain is perfused, and represents the pressure gradient across the cerebral vascular bed. This is why central venous pressure (CVP) is sometimes used in the calculation of CPP, as it would be the differential between MAP and CVP that represents the driving pressure to the brain, and CVP is fairly simple to measure. In pathological conditions where intracranial pressure (ICP) exceeds cerebral venous pressure, ICP is added to the CVP value, and the sum of the two is subtracted from the MAP to the estimate perfusion pressure to the brain. In the upright position, the internal jugular veins collapse, and cerebral venous outflow occurs through the vertebral plexus, which may add complexity to the determination of CPP (Gisolf *et al*., 2004). While supine, the primary cerebral venous outflow is through the internal jugular veins, although there is some inter-individual variability (Doepp *et al*., 2004).

In the case of our protocol, participants were supine, with no head elevation. During LBNP, CVP decreased significantly even in the first stage of negative pressure. It is known that an increase in CVP, as would occur in venous congestion due to heart failure, decreases CPP. It may be plausible that the reduction in CVP with LBNP seen here, acted to increase CPP. If, with the introduction of LBNP, reductions in CVP are proportional to the reductions in cerebral inlet pressure (while MAP is constant), presumably, there would be no change in CPP. In this case, the slight reduction in SCBFV would be met with a slight increase in CVR. Indeed, our estimate of CVR (CVRi) increased with LBNP; however, an accurate estimation of cerebral vascular resistance (CVR) may not be given by the classical equation using MAP and MCBFV. Downstream resistance may be better represented by considering cerebral *and* systemic pulsatility (Carey *et al*., 2001a, 2001b; Czosnyka *et al*., 1996; Schondorf *et al*., 1997; Aaslid, 1992). By dividing the pulsatility index (PI) of the brain by the systemic arterial pressure pulsatility (PP), a pulsatility ratio is derived and has been proposed as an improved estimate of changes in downstream resistance in the brain (Giller *et al*., 1992; Levine *et al*., 1994). In both cases (response of PI alone or of PI/PP), the measure of pulsatility decreased with LBNP, signifying narrowing of the systolic-diastolic differential, and dilation of downstream vessels. Taken together, the cerebral pulsatility response and CVRi estimate are displaying conflicting results about what is happening with the cerebral vasculature during LBNP. Given this inconsistency, we might begin to consider that CBF was affected by variables outside Poiseuille's formula normally including perfusion pressure and vascular resistance dictating blood flow (Treib *et al*., 1996). Stroke volume decreased significantly and promptly with LBNP, indicating a reduction in pulsatile flow from the heart, and presumably, to the brain. The reduced shear stress associated with lower flow pulsatility may induce low-flow-mediated constriction in the small cerebral vessels through endothelial cell modulation of pressure-flow regulatory mechanisms (Zhang & Levine, 2007;

Treib *et al*., 1996; Rubanyi *et al*., 1986). Consequently, in the case of supine LBNP, instead of regarding the decrease in pulsatility to be due to a reduction in downstream resistance, it seems more appropriate to consider how reduced pulsatile flow from the heart may impact cerebral vessels. This explanation for why CVR increases during supine, simulated orthostatic stress despite an unchanging MAP seems to be speculative at this point, although the reasoning above is persuasive. In fact, if systemic PP had decreased significantly, as expected during LBNP (Stevens & Lamb, 1965), the pulsatility ratio may have actually shown an increase from baseline. Moreover, elderly individuals with the greatest reduction in PP during LBNP also had the greatest increase in CVRi.

Importantly, a reduction in CBFV with orthostatic stress is not evidence of autoregulatory failure. Brown and colleagues (2003) have proposed that the mechanism of cerebral autoregulation may function to keep cerebral blood flow within a physiological *range* that maintains sufficient perfusion, rather than at an inflexible value. Since all of our participants completed the protocol asymptomatically, adequate cerebral perfusion was assumed to have been preserved. The term "adequate cerebral perfusion" will need a conceptualized definition. For the purposes of this project, symptoms of presyncope were deemed termination points to the test. Whether or not there was adequate cerebral perfusion to perform cognitive tests, or other mental stimulating activities during LBNP is uncertain.

Comparison: Lower body negative pressure versus supine-to-standing

Studying responses to orthostasis is can be problematic because of the motion involved, the inability of particular populations to perform the task, and the associated-risk with posture change. Lower body negative pressure (LBNP) requires no motion on the part of the participant, and is easily reversible in impending syncope or other adverse events. The physiologic responses to LBNP depend both on the magnitude and duration of negative pressure applied (Wolthuis *et al*., 1974), and will vary between individuals. In general, the cardio- and cerebrovascular responses to LBNP are assumed to mimic responses in orthostasis, but the inter-individual variability complicates direct comparisons. In a review by Goswami *et al* (2008), a compilation of the existing literature revealed that at LBNP levels of -40 mmHg or -50 mmHg, responses seem to be similar to those in upright posture (Wolthuis *et al*., 1974; Dikshit, 1990; Watenpaugh *et al*., 1994). Of note, these generalizations were made in young, healthy populations, and it is known that the relative intensity of LBNP varies between individuals and, especially, between the young and old (Tsutsui *et al*., 2002). Such a generalization about what level of LBNP best elicits responses associated with actual orthostasis in the elderly has not been determined. This is the first study to examine the cardio- and cerebrovascular responses to both LBNP and a supine-to-stand maneuver in the same group of elderly individuals. If LBNP is going to be used as a model for orthostasis, it is essential to highlight and to take into consideration dissimilarities in responses. The heart rate (HR) increase of 14% with standing in our group is similar to the 12% increase that was reported by Mehagnoul-Schipper and colleagues (2000), with a similarly aged population. Protocol inconsistencies, including repeated sit-stand maneuvers (van Beek *et al*., 2010), sampling after 5 minutes of standing (Kim *et al*., 2011), and prolonged sitting before standing (Sorond *et al*., 2005) precluded direct comparisons to a few other studies, while others with

similar protocols to ours used aged participants that were younger (40-60, mean 48 years; Gatto *et al*., 2007), or did not report actual response differences between age categories (Edlow *et al*., 2010). We found that the HR response was significantly higher during the stand than during LBNP. This was in contrast to our primary hypothesis, and was in spite of very similar reductions in stroke volume (SV) between the two conditions. We had initially projected that the two conditions would produce very similar responses in all measured variables. Goswami and colleagues (2008) remarked that the HR increase in orthostasis tends to be similar during LBNP when the negative pressure reaches -50 mmHg. As mentioned above, it is uncertain whether this generality applies directly to the elderly population, although, based on our HR response *per se*, it would seem that a negative pressure of -40 mmHg is insufficient to increase HR to the magnitude that true orthostasis does in the elderly.

In addition to HR, the delta diastolic blood pressure (DBP) response was also different in standing than during LBNP. With LBNP, DBP increased significantly in the final stage of negative pressure, while the mean response during standing did not change. In the majority of individuals, DBP increased with standing, although, there were two moderately decreased responses and one extremely negative response. Augmented DBP with LBNP represents the increase in total peripheral resistance (TPR). The standing posture is generally associated with a maintained systolic blood pressure (SBP) and slightly elevated DBP because vessels in the lower leg constrict to limit blood pooling. Diastolic orthostatic hypotension measured one minute after standing is associated with mortality related to vascular causes in elderly persons (Luukinen *et al*., 1999; Luukinen *et al*., 2004). Perfusion in the left coronary artery takes place during diastole (Hoffman & Spaan, 1990). Diastolic hypotension may, therefore, impair coronary filling, contributing to poor survival prognosis.

Interestingly, the one participant that experienced a reduction in DBP during both LBNP and posture change underwent emergent multiple coronary artery bypass graft surgery a couple of months after testing. The related health complications were entirely unbeknownst to the participant at the time of testing. In all participants, their DBP response during LBNP matched what was observed in standing, aside from one individual who was speculated to have had an artificially elevated DBP response due to muscle tension in the lower limbs during LBNP. During both the supine-to-standing condition and during LBNP, DBP was positively correlated with total peripheral resistance (TPR), estimated with Q from the Finometer®. Based on the spread of individual responses, it is probable that the difference in delta DBP between LBNP and posture change was due to the impact of muscle contraction on DBP during LBNP.

The response of diastolic cerebral blood flow velocity (DCBFV) was also different with standing compared to with LBNP—additional evidence that LBNP and posture change do not produce identical responses, thereby contradicting our main hypothesis. As detailed above in the Discussion section regarding cerebrovascular responses to LBNP, DCBFV did not change during the *simulated* orthostasis, although, most participants showed a slight increase. Overall, the cerebral pulsatility was found to be decreased, most likely as a result of a reduced SV, which ended up corresponding to downstream constriction of cerebral circulation. In contrast to the stable DCBFV during LBNP, during standing, all individuals experienced a decrease in DCBFV. The relative decreases in systolic and diastolic CBFV were such that cerebral pulsatility index and the pulsatility ratio did not change significantly, although, the mean responses were +11% and +12%, respectively. These pulsatility results may suggest an increase in downstream resistance in the upright posture.

During standing, the internal jugular veins are positioned above the heart, causing them to collapse due to the gravitational effect on intravascular pressure, which becomes less than the tissue pressure in the neck (Cirovic *et al*., 2003). The alternate route of cerebral venous outflow during orthostasis is through the higher resistance vertebral venous plexus (Chaynes *et al*., 1998), which is considered to remain patent due to its attachments to rigid structures. Approximately 50% of the venous blood flow through the internal jugular veins shifts to the vertebral venous plexus system once upright, reducing the overall venous outflow by only 12% (Alperin *et al*., 2005a). A previous report described an internal jugular vein flow reduction of 90% and a decrease in total venous outflow of 75% (Valdueza *et al*., 2000), which would have represented a greatly increased resistance to venous outflow during orthostasis. In the past, the circulation from the thoracic aorta, through the cerebral vasculature to the veins leaving the skull into the right atrium was considered to be representative of a vascular siphon. This concept implies that because the pressure gradients are equal and opposite on both limbs of the loop, the heart need not produce additional force to increase the gravitational potential energy of the blood in the ascending loop—no extra work is done to overcome gravity (Burton, 1972). Today, this view is met with debate because the differences in compliance between the arterial inflow and venous outflow negate the presence of an actual descending limb and instead, blood flow leaving the brain acts in a "waterfall" effect, which does not assist ascending arterial blood in any way (Seymour & Johansen, 1987). Some simple hypothetical calculations performed by Gisolf *et al.* (2005) showed the resistance to venous outflow in the upright position to be 0.055 mmHg•sec•mL^{-1}, while the total resistance through the brain being 4.7 mmHg•sec•mL^{-1}, more than 85 times greater than the estimated outflow pathway. Considering this, there must be a discontinuance of the pressure transmission between the internal carotid arteries (inflow) and the cerebral venous plexus (outflow), preventing any siphoning effect, and removing the possibility that

venous outflow resistance might influence blood flow through the brain. It seems likely that in spite of internal jugular vein collapse upon standing (forcing an alternate route for venous outflow), there is no added resistance that effectively reduces CPP. Hence, if there is higher downstream cerebrovascular resistance while upright (slightly increased pulsatility), it is not likely to be because of venous outflow limitations.

With these details in mind, as our participants transitioned to upright posture from supine, unlike simulated orthostasis, pressure at the level of the brain decreased by a measure of **ρgh**, where ρ is a volumetric density of blood, g is the force of gravity, and h is the vertical distance between the heart and the brain. Without considering this gravitational decrease in blood pressure (BP) at the level of the middle cerebral artery (MCA) our calculation of cerebrovascular resistance (CVRi) increased significantly, which is in accordance with the observations of Murrell *et al.* (2011), but without having made the adjustment to CPP. Taking into account the proper upright CPP, the proportional decrease in BP at the MCA is far greater than the reduction in CBFV, resulting in an overall decline in CVR—contrary to our calculation. A reduction in CVRi with standing is consistent with the results from others (Kim *et al*., 2011; Edgell *et al*., 2012), and has been rationalized by Alperin and colleagues (2005a; 2005b) using magnetic resonance imaging (MRI) measurements. These authors found that when compared to the supine position, sitting upright was associated with a 2.8-fold increase in intracranial compliance index, with a corresponding decrease in intracranial pressure (ICP). These changes are consistent with an overall more compliant intracranial compartment. The most probable reason for this elevated cerebrovascular compliance is the gravity-induced reduction in cerebrovascular blood volume that occurs upon standing. Many years ago, ICP and intracranial elastance (inverse of compliance) were found to be exponential functions of intracranial volume (Marmarou *et al*., 1975), meaning, when the

volume of intracranial blood decreases, the compliance of the cerebral circulation increases. This concept has relevance to our results, which point to a reduction in CVR with standing to counter the dramatically decreasing CPP.

Cerebral pulsatility indices from the older individuals in the project from Edgell and colleagues (2012) increased after transition to sitting, and further increased after the transition to standing. The largest change took place after the first transition, which was also true of the decrease in CVRi. This seemed to have been due to a greater percent decline in diastolic CBFV after moving to the sitting position compared to after transitioning to stand. This is in contrast to systolic CBFV which decreased most from sitting to standing. During the posture change in our elderly group, systolic and diastolic CBFV were both inversely related to SV and Q, but only diastolic CBFV was related to TPR and DBP (both positively). This distinction provides evidence that during active posture change, systemic vascular resistance contributes to maintaining diastolic CBFV, and therefore, mean CBFV. Participants with greater TPR responses (and higher DBP during standing) maintained higher levels of diastolic CBFV.

Unlike the protocol of Edgell *et al*. (2012), ours did not include a steady-state sitting position, so it is unknown whether even greater changes would have been observed if variables in a seated condition were measured before standing. The purpose of our study was not to differentiate seated and standing postures, but rather to contrast changes during LBNP to changes in standing. With the seated values omitted, the percent increase in pulsatility from supine to standing was only 9% (Edgell *et al*., 2012), and ours was comparable at 10%, although our delta PI was not significant. The results from Kim and others (2011) did not include values for systolic and diastolic CBFV, and pulsatility was not reported. While the

populations in the studies from Alperin *et al.* (2005a) were young and healthy, like our results, reported no change in cerebral pulsatility with posture change. Even though their posture change included the supine-to-seated transition only, if pulsatility did change, it would seem that the larger delta value would have been observed during the measured phase. Examining changes in cerebral pulsatility may not provide the greatest utility for the interpretation of cerebral compliance/resistance, even when taking systemic pulse pressure into consideration. In our elderly group, there was tremendous variability in the systolic and diastolic BP responses to standing, which may not be surprising considering the variation that was observed even within familial groups (Harrap *et al*., 2004). As a result, the pulsatility ratio (PI/PP) might be useful only with very consistent and projected systemic BP responses, which may limit its utility in elders because of diversity in arteriolar remodeling affecting systemic compliance.

In evaluating the basis behind the opposing diastolic CBFV responses in LBNP versus stand, using standard estimation of cerebral pulsatility may not be an effective adjunct, at least in our project. Its utility has been questioned in other conditions as well (Schondorf *et al*., 1997; Czosnyka *et al*., 1996; Low *et al*., 1999). Regardless of whether the calculation of cerebral pulsatility changed after standing, the overall significant decline in MCBFV (-10%), and likely reduction in BP at the level of the MCA, estimated to be approximately 27% (see sample calculation in Appendix), CVRi must have decreased in standing in order to satisfy the equation (CBFV=CPP/CVRi). The main distinction between the two conditions (despite CBFV decreasing in both) was the cerebrovascular resistance response. During LBNP, CVRi increased, and in standing, cerebral vessels opened up. Because in general, a drop in diastolic velocity would suggest an increase in downstream resistance, other factors besides downstream cerebral vasculature must come into play in terms of the influence on diastolic

CBFV. Stroke volume was reduced considerably with standing, but instead of the low-pulsatile-flow-meditated contraction that was seen with LBNP, there was an increase in vascular compliance. This occurrence seems to have been a result of the profound reduction in CPP once upright. In the presence of deteriorating CPP, the compensatory reduction in cerebral resistance must supersede any effect of incoming reduced pulsatile flow. Given that SCBFV decreased concomitantly with diastolic CBFV during standing, the drop in diastolic velocity must have been due to the diminished SV. When the volume of blood leaving the heart decreases, so too does the volume of blood reaching the brain. In the case of supine LBNP, the constriction of downstream cerebral vessels seems to have maintained diastolic CBFV. In fact, the pulsatility ratio (PI/PP) was directly related to diastolic CBFV, indicating that individuals with the greatest reduction in cerebral pulsatile flow experienced the lowest diastolic CBFV responses during LBNP.

Doppler – Finometer® dissimilarities

Direct contrasts between Finometer- and Doppler-derived stroke volume (SV) measurements during orthostatic stress in the elderly have not been made to date. It is not known if this estimate of SV using the Modelflow method (Finometer) can be accurately predicted in an elderly population during LBNP. The shape of the aortic waveform generated by the Finometer is based on an algorithm that uses participant details including height, weight, age, and sex. The three-element model derives aortic characteristic impedance, arterial compliance, and systemic vascular resistance (Wesseling *et al*., 1993). The overall method has been evaluated against other SV measurements in a number of settings (Jansen *et al*., 2001; Rang *et al*., 2007; Sugawara *et al*., 2003; Senay *et al*., 2009; Harms *et al*., 1999; Dyson *et al*., 2010; Matsukawa *et al*., 2004), with some revealing inconsistencies, but generally

concluding that the Modelflow method provides fairly reliable estimations of changes in cardiac output (Q) to a range of stresses. During heat stress however, Modelflow Q is underestimated (Shibasaki *et al*., 2010) by thermodilution methods, and imprecision has been noted in younger groups at high levels of LBNP when compared to the Doppler ultrasound method (Gagné, 2009). Dyson and colleagues have also reported discrepancy between Modelflow and Doppler-derived SV measurements during dynamic changes in TPR independent of posture (Dyson *et al*., 2010). The authors noticed that even in system perturbations where TPR did not change, discrepancies between SV methods were still observed. During our LBNP protocol, the percent changes from baseline to -40 mmHg in the two methods of SV measurement were not different, while the percent change in the two methods at all other LBNP stages and during supine to standing, were different. Interestingly, our Finometer-derived TPR value increased significantly only at LBNP of -40 mmHg, and was not significantly increased during posture change. Thus, when the Modelflow calculation of TPR was unchanged, SV estimates seemed to be significantly different between methods. In fact, the only instance where the percent delta Modelflow SV exceeded the percent delta Doppler SV was during the final stage of LBNP. This observation is consistent with those of Dyson and colleagues (2010) who noted that factors causing increases in TPR tended to results in overestimates of Modelflow SV compared with Doppler SV estimates. However, it is likely that it is not solely the change in TPR that has bearing on the consistency between SV methods (Dyson *et al*., 2010).

In nine of ten participants from our project, the *absolute* SV estimate from the Finometer was less than the SV estimated from the Doppler method. The mean Finometer SV response in our population did not change significantly during LBNP, while Doppler SV decreased at each stage of negative pressure. Because CVP also decreased significantly at

each stage of LBNP, the Doppler estimates of SV were used when reporting outcomes. The mean values between the methods were significantly different at baseline, -10 mmHg, and -40 mmHg, converging slightly during mid-LBNP, and then separating in the final stage. During posture change, the two methods were different in supine rest, but became statistically similar during standing. Conceivably, because the Finometer SV tended to be an underestimate relative to SV calculated by the Doppler method, it is possible that the Modelflow algorithm miscalculates aortic compliance and/or downstream vessel resistance in this group of individuals, and perhaps in others.

Since Gagné and colleagues (2007) found linear relations between Q measured with the Finometer and Q using Doppler ultrasound, they were able to predict CVP from SV estimated by the Finometer. Based on our results with elderly persons, discordance between Finometer and Doppler estimations precluded us from making similar generalizations. In estimating SV from the Doppler method, a diameter measurement of the aorta needs to be made. Although this does not affect the *change* in SV over time during LBNP, it does affect the overall absolute discrepancy between the two SV measurement techniques. Our diameter measurements are in accordance with what has been measured previously in this age group (Vasan *et al*., 1995).

Naturally, validation studies need to be carried out in this population during LBNP and posture change to verify SV responses. The use of the Modelflow method in individuals >70 years of age during LBNP may not provide accurate estimates of absolute SV values and also changes in SV. Importantly, the limitations of Doppler-derived SV estimates need to be recognized as well, namely, the shift in heart position with orthostatic stress altering the measurement of aortic velocity. The expected linear relationship between pulse pressure and

SV was only found in Finometer-derived SV, and not with Doppler SV. That forearm vascular resistance was directly related to TPR from the Finometer, provides some evidence that the Modelflow estimate of Q may be superior to the estimate of from Doppler ultrasound during posture change.

Considerations and future directions

Studying steady-state changes to orthostatic stress has many advantages, though, determining the precise chronological sequence of compensatory changes is not possible. In the past, some seemingly paradoxical responses have led to inexact explanations of which variable induced what change. We have determined potential explanations for some of the typical changes seen during orthostatic stress, and have identified mechanistic differences in cerebral blood flow regulation between lower body negative pressure and true posture change. To date, a genuinely comparable study to ours with younger individuals is not available. Keeping in mind the established age-related changes during orthostatic stress sheds some light on how our elderly population may have compared to a younger cohort.

Given that there is an age-related mitigation of the SV reduction in response to orthostatic stress, we might expect that during LBNP, younger individuals with a greater reduction in pulsatile flow from the heart may experience an overall greater decrease in cerebral blood flow, with an associated greater magnitude constriction of the cerebral vessels. During active posture change, the primary influence on CBFV was the drop in CPP upon standing. The extent of this decrease should not be markedly altered with age, even supposing there is an age-related decline height because the compression of intervertebral discs in the cervical spine region is probably negligible compared to the thoracic and lumbar

sections. However, the peripheral vasculature does change with age. In contrast to the inverse relationship observed during LBNP, diastolic CBFV and TPR were positively correlated during posture change. While greater reductions in diastolic CBFV seemed to be ineffectively countered with greater increases in systemic resistance during LBNP, a weak TPR response upon standing seems to have contributed, at least in part, to greater drops in diastolic DBFV in the upright position. This relationship was complemented by evidence of inverse relations between diastolic CBFV and both SV and Q in standing. During LBNP, CBFV seemed to have been primarily influenced by the reduced incoming pulsatile flow. This is evidenced by the inverse relation between diastolic CBFV and PP, and by the direct relationship between diastolic CBFV and CVP. When CVP decreased during LBNP, individuals with greater drops in CVP had higher percent changes in PP relative to the rest of the participants. Of note, "*higher percent change in PP*" could still represent a decrease in PP; the magnitude decrease would merely be less than their peers. The inverse relationship between PP and CVP seems illogical because a reduced preload results in a reduced SV, yielding a decrease in PP. Since neither of our methods of measuring of SV was linearly related to CVP, the contradictory CVP-PP relation may not be genuine. However, systemic PP was inversely related to diastolic CBFV. This means that individuals with decreases in PP had greatest maintenance of diastolic CBFV, probably because they had great increases in DBP, which was directly related to TPR from the Finometer and inversely related to Q from the Finometer. Individuals with increases in PP during LBNP showed either slight decreases or no change in diastolic CBFV because these elders had increases in SBP, which was inversely correlated to diastolic CBFV and the pulsatility ratio. This latter relationship suggests that as the pulsatility in the cerebral vasculature decreases, there is an associated increase in SBP. The direct relation between diastolic CBFV and the pulsatility ratio

facilitates understanding of this concept; an increase in the cerebrovascular pulsatility (implied downstream vasoconstriction), results in an increase in diastolic CBFV.

As mentioned above, CBFV seems to have been principally impacted by incoming flow from the heart, while during standing, CBFV was affected more so by the diminished perfusion pressure. During posture change, diastolic CBFV was directly related to DBP. This was not the case for LBNP; no relationship was found. In individuals where DBP was better maintained, so was diastolic CBFV. Similar to during LBNP, DBP was related to both Q and TPR from the Finometer. It seems that these DBP relationships become increasingly important for posture change over LBNP.

In making inferences about changes in cerebral blood flow with orthostatic stress, an important assumption needs to be made about the diameter of the vessel. Without advanced equipment with the capacity to monitor middle cerebral artery (MCA) diameter, the velocity through the artery is used to represent changes in flow, as velocity is simple to measure. For the purposes of our study, the soundness of this assumption seems reasonable because even during manipulation of CO_2 levels and LBNP, no changes were detected in the diameter of the MCA (Serrador *et al*., 2000a). Similar conclusions have been drawn in older studies as well (Huber & Handa, 1967; Lindegaard *et al*., 1987; Giller *et al*., 1993; Newman *et al*., 1994; Poulin & Robbins, 1996). What appears to be missing from the literature is evidence of MCA diameter consistency during active posture change, and, of course, confirmation in the elderly, as there is none to date in that age category. Despite the great deal of convincing material about constancy in the diameter of the MCA, the possibility that this conduit vessel's diameter does impact cerebral blood flow in elders during various orthostatic stresses cannot be overlooked, and must be acknowledged.

We have found valuable information about differences between LBNP and true posture change in this elderly group. Application of these results to a larger elderly population needs to be made with caution. The small sample size included in this project needs to be acknowledged. However, the repeated measures element strengthens the overall study design. It was not feasible to exclude every person with health issues. Some of the participants were hypertensive, or diabetic, or had high cholesterol levels. Diabetes is known to be associated with autonomic impairment. Blood pressure regulation studies have been carried out in diabetics revealing impaired peripheral vasculature responses (Marthol *et al*., 2007). The majority of the participants of the current study had premature ventricular contractions (PVC) throughout the testing session, although, only two individuals required considerable attention removing the PVC's from their datasets. One participant required open-heart surgery within a couple of months of testing. Had the participant been aware of this underlying condition at the time of testing, he would have been excluded. Sex-related differences have been reported regarding resting cerebral blood flow and also in responses to posture change (Edgell *et al*., 2012). No such discrepancies were noted in our study. This is probably a result of our female participants being older, having had a greater number of years being post-menopausal. None of the participants had been involved in a research study before. Ideally, participants are comfortable and relaxed, with minimal psychological impact on physiologic variables. Care was taken to make the experience simple and easy for the participants, but it is not certain that these actions lead to optimal comfort levels in all participants.

A very early objective of this thesis focused on predicting central venous pressure (CVP) during posture change based on its relationship to stroke volume (SV) during LBNP. The measurement of CVP was not consistent enough to develop these linear relationships in

each participant. The fundamental direct link between CVP and SV was not evident in most cases. This is can be attributed to inconsistency in the measurement of CVP combined with some variability within the measures of SV. Overall changes and trends in these two variables were successfully tracked during orthostatic stress; however, the precision required in both measured at each stage of negative pressure was not great enough to consistently model predictive equations. It is possible that this elderly population presented additional difficulty influencing the CVP measurement on account of potential age-related changes in the venous vessels. In order for more advanced predictive relationships to be developed in this population during orthostatic stress, it is possible that a superior means to measure right atrial pressure (e.g. central venous access catheter directly into right atrium) may need to be employed.

There are additional considerations that need brief mentioning. Firstly, our aortic velocity signal was measured form the ascending aorta and our aortic diameter was measured once, at the aortic ring. It is possible that during orthostatic stress, slight deformation of the ascending aorta may have contributed to erroneous estimation of SV. Secondly, it was not possible to have all participants to schedule testing at the same time of day. Diurnal variation in end-tidal CO_2 and cerebral blood flow have been reported (Lewis *et al*, 2010; 2011), and may have contributed to variation in between participant results, but should not have affected the comparison between LBNP and standing. Lastly, blood samples were taken from the venous catheter at baseline and at -40 mmHg for all participants and stored for later analysis. It would have been beneficial to examine changes in variables such as angiotensin II, plasma renin, atrial natriuretic peptide, antidiuretic hormone, aldosterone, and catecholamines, epinephrine, norepinephrine, and dopamine. A more comprehensive view of each participant's baseline values and responses could have been gained with the additional

knowledge about blood-markers of cardiovascular disease. Unquestionably, this research provides foundation for further work to be done.

2.6 Conclusions

In a group of ten elderly individuals ranging in age from 71-76 years, we compared the effects of graded lower body negative pressure to active posture change. Also, we investigated several additional variables during supine LBNP with the purpose of further understanding the compensatory responses of elderly individuals during simulated orthostasis. Contrary to our hypothesis regarding the likely similarities between LBNP and posture change, we found that the heart rate response between the two conditions was different. A greater increase was noted during standing, suggesting that a negative pressure of 40 mmHg may not be sufficient to elicit the same effects of true gravity on heart rate in elders. Cerebral blood flow velocity dropped in both conditions, although, the mechanisms through which compensation took place were dissimilar. This is an important concept to consider for future research and for the interpretation of existing research involving cerebrovascular responses to lower body negative pressure and to standing. Like others, we have found that orthostatic stress reduced blood flow to the brain in the elderly, although these reductions were tolerated without difficulty, further corroborating evidence that cerebral autoregulation operates to maintain flow within a *range* of values, rather than at a fixed level. Some level of decrease appears to be expected, which is true of younger individuals as well.

During simulated orthostasis, diastolic CBFV was stable, and during posture change, it was reduced, which was an unexpected deviation from our hypotheses. In this elderly

population during LBNP, CVP was directly related to diastolic CBFV, reflecting the impact reduced cardiac preload has on blood flow to the brain. During posture change, diastolic CBFV had a direct relationship to DBP, reflecting the importance of maintaining CPP through increased TPR when upright. Interestingly, greater drops in diastolic CBFV during LBNP were associated with greater increases in TPR, while during posture change, the compensatory increase in TPR seemed to have helped maintain diastolic CBFV.

Research using lower body negative pressure as a means to induce orthostatic stress is applicable to daily living only if the associated responses are comparable to actual posture change. Recognizing the differences between true and simulated orthostasis is essential, and in this elderly population, there are important differences to consider.

Appendix

Sample calculation for estimate of cerebral perfusion pressure (CPP) in upright posture:

Assuming:

-average mean arterial pressure of 90 mmHg;
-average vertical distance from heart to level of middle cerebral artery of 33 cm (Kim *et al*., 2011)

Conversion: 0.735 mmHg / cmH_2O

33 cm x 0.735 mmHg / cmH_2O = 24.3 mmHg

Subtract blood pressure between heart and brain:

90 mmHg – 24.3 mmHg = 65.7 mmHg

Percent decrease in mean arterial pressure with standing:

65.7 / 90 * 100 - 100 = -27%

Thus, in theory, the average decrease in mean arterial pressure for this population after transitioning from supine to upright posture was 27%

Health Status Form:

Participant Code:
Age:

SELF REPORT CHECK LIST

Past Health Problems:

Heart Murmur	( )	Heart Burn	( )
High Cholesterol	( )	Bleeding from Intestinal Tract	( )
High Blood Pressure	( )	Bleeding Disorders	( )
Low Blood Pressure	( )	Blood Clots	( )
Congenital Heart Disease	( )	Rheumatic Fever	( )
Heart Attack	( )	Enteritis/Colitis/Diverticulitis	( )
Heart Operation	( )	Emphysema, Pneumonia,	
Stroke	( )	Asthma, Bronchitis	( )
Transient Ischemic Attacks	( )	Back Injuries	( )
Peripheral Vascular Disease	( )	Parkinson's Disease	( )
Varicose Veins	( )	Tremors	( )
Kidney or Liver Disease	( )	Nervous System Disorders	( )
Diabetes (diet or insulin)	( )	Epilepsy	( )
Ulcers	( )	Atrial fibrillation	( )
Pacemaker	( )	Other (describe overleaf)	( )

Present Health:

List current problems:	List medications taken now or in last 3 months:	
1.	1.	5.
2.	2.	6.
3.	3.	7.
4.	4.	8.

Are you taking medication to thin your blood? Y or N

List Symptoms of Current Health Problems:

Irregular Heart Beat	( )	Fatigue	( )
Chest Pain	( )	Cough Up Blood	( )
Short of Breath	( )	Back Pain	( )
Persistent Cough	( )	Leg Pain	( )
Wheezing (asthma)	( )	Dizziness/Light-Headedness	( )

Habits: Smoking Never ( ) Ex-smoker ( ) Regular ( ) Average # cigarettes/day ( )

Physical Activity: I consider my activity level to be: High ( ) Average ( ) Low ( ).

List the types of physical activity that you do on a regular basis:

_____
Participant (signature)

_____ _____
Witness (print name) Witness (signature)

_____ _____
Date Location

References

Aaslid, R. (1992) Cerebral hemodynamics. In D. W. Newell & R. Aaslid (Eds.), *Transcranial Doppler* (pp. 49-55), New York: Raven Press

Aaslid, R., Lindegaard, K.F., Sorteberg, W., & Normes, H. (1989) Cerebral autoregulation dynamics in humans. *Stroke,* 20(1), 45-52.

Aaslid, R., Markwalder, T. M., & Nornes, H. (1982) Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg,* 57(6), 769-74.

Abboud, F. M., Eckberg, D. L., Johannsen, U. J., Mark, A. L. (1979) Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man. *J Physiol*, 286, 173-84.

Abboud, F.M. & Mark, A.L. (1979). Cardiac barorecpetors in circulatory control in humans. In R.Hainsworth, C. Kidd, & R. J. Linden (Eds.), *Cardiac Receptors* (pp. 437-462). Oxford, UK: Cambridge University Press.

Agabiti-Rosei, E., Mancia, G., O'Rouke, M. F., Roman, M. J., Safar, M. E., Smulyan, H., Wang, J. G., Wilkinson, I. B., Williams, B., & Vlachopoulos, C. (2007) Central blood pressure measurements and antihypertensive therapy: a consensus document. *Hypertension,* 50(1), 154-160.

Albayrak, R., Degirmenci, B., Acar, M., Haktanir, A., Colbay, M., & Yaman, M. (2007). Doppler sonography evaluation of flow velocity and volume of the extracranial internal carotid and vertebral arteries in healthy adults. *J Clin Ultrasound,* 35(1), 27-33.

Allan, P. L., Mowbray, P. I., Lee, A. J., & Fowkes, F. G. (1997) Relationship between carotid intima-media thickness and symptomatic and asymptomatic peripheral arterial disease. The Edinburgh Artery Study. *Stroke,* 28(2), 348-353.

Alperin, N., Lee, S.H., Sivaramakrishnan, A., & Hushek, S. G. (2005a) Quantifying the Effect of Posture on Intracranial Physiology in Humans by MRI Flow Studies. *J Magn Reson Imaging,* 22(5), 591–596.

Alperin, N., Hushek, S. G., Lee, S. H., Sivaramakrishnan, A. & Lichtor, T. (2005b) MRI study of cerebral blood flow and CSF flow dynamics in an upright posture: the effect of posture on the intracranial compliance and pressure. *Acta Neurochir Suppl*, (95)4, 177-181.

Andresen, J., Shafi, N. I., & Bryan, R. M. J. (2006) Endothelial influences on cerebrovascular tone. *J Appl Physiol,* 100(1), 318-327.

Arbeille, P., Ayoub, J., Besnard, S., & Pascaud, L. (2003) Portal vein flow volume and diameter changes during stand test in normal subject. *J Gravit Physiol,* 10, 75-76.

Arbeille. P., Besnard, S. S., Kerbeci, P. P., Pascaud, L. & Mohty, D. (2005) Portal vein cross-sectional area and flow and orthostatic tolerance: a 90-day bed rest study. *J Appl Physiol*, 99(5), 1853–1857.

Arbeille, P., Kerbeci, P., Mattar, L., Shoemaker, J. K. & Hughson, R. (2008) Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physio*l, 295(5), H1846-54

Asmussen, E., Christensen, E. H., & Nielsen, M. (1940) The regulation of circulation in different postures. *Surgery*, 8, 604-616.

Baumbach, G. L., Heistad, D. D. (1983) Effects of sympathetic stimulation and changes in arterial pressure on segmental resistance of cerebral vessels in rabbits and cats. *Circ Res*, 52(5), 527– 33.

Bayliss, W.M. (1902) On the local reaction of the arterial wall to changes of internal pressure. *J Physiol*, 28, 220-231.

Benditt, D. G., Ferguson, B. P., Grubb, B. P., Kapoor, W. N., Kugler, J., Leman, B. B., Maloney, J. D., Raviele, A., Ross, B., Sutton, R., Wolk, M. J., & Wood, D. L. (1996) Tilt table testing for assessing syncope. *J Am Coll Cardiol*, 28(1), 263-275.

Benowitz, N. L., Kuyt, F., Jacob, P. (1984) Influence of nicotine on cardiovascular and hormonal effects of cigarette smoking. *Clin Pharmacol Ther*, 36(1), 74–81.

Blaber, A. P., Bondar, R. L., Moradshahi, P., Serrador, J. M., & Hughson, R. L. (2001) Inspiratory CO_2 increases orthostatic tolerance during repeated tilt. *Aviat Space Environ Med*, 72(11), 985-991.

Blacher, J., Asmar, R., Djane, S., London, G. M., & Safar, M. E. (1999a) Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension*, 33(5), 1111-1117.

Blacher, J., London, G. M., Safar, M. E., & Mourad, J. J. (1999b) Influence of age and end-stage renal disease on the stiffness of carotid wall material in hypertension. *J Hypertens*, 17(2), 237-244.

Blomqvist, C. G. & Stone, H. L. (1984) Cardiovascular adjustments to gravitational stress. In J. T. Shepard & F. M. Abboud (Eds.), *Handbook of Physiology: The Cardiovascular System. Peripheral circulation and organ blood flow, Part 2* (pp.1025-1063). Bethesda, Maryland: American Physiological Society.

Bondar, R. L., Kassam, M. S., Stein, F., Dunphy, P. T., Fortney, S., & Riedesel, M. L. (1995) Simultaneous cerebrovascular and cardiovascular responses during presyncope. *Stroke*, 26(10), 1794-1800.

Bondar, R. L., Kassam, M. S., Stein, F., Dunphy, P. T. & Riedesel, M. L. (1994) Simultaneous transcranial Doppler and arterial blood pressure response to lower body negative pressure. *J Clin Pharmacol,* 34(6), 584–9.

Borst, C., Wieling, W., van Brederode, J. F., Hond, A., de Rijk, L. D., & Dunning, A. J. (1982) Mechanisms of initial heart rate response to postural change. *Am J Physiol,* 243(5), 676-681.

Bots, M. L., Hoes, A. W., Koudstaal, P. J., Hofman, A., & Grobbee, D. E. (1997) Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation,* 96(5), 1432-1437.

Bradshaw, M. J. & Edwards, R. T. (1986) Postural hypotension--pathophysiology and management. *Q J Med,* 60(231), 643-657.

Brett, S. E., James, B. N., Ritter, M., Philip J., Chowienczyk, P. J. (2000) Diastolic blood pressure changes during exercise positively correlate with serum cholesterol and insulin resistance. *Circulation,* 101(6), 611-615.

Brian, J. E. Jr., Faraci, F. M., & Heistad, D. D. (1996) Recent insights into the regulation of cerebral circulation. *Clin Exp Pharmacol Physiol,* 23(6-7), 449-457.

Brooks, D. J., Redmond, S., Mathias, C. J., Bannister, R., Symon, L. (1989) The effect of orthostatic hypotension on cerebral blood flow and middle cerebral artery velocity in autonomic failure, with observations on the action of ephedrine. *J Neurol Neurosurg Psychiatry,* 52(8), 962–6.

Brown, C. M. & Hainsworth, R. (1999) Assessment of capillary fluid shifts during orthostatic stress in normal subjects and subjects with orthostatic intolerance. *Clin Auton Res,* 9(2), 69-73.

Brown, C. M. & Hainsworth, R. (2000) Forearm vascular responses during orthostatic stress in control subjects and patients with posturally related syncope. *Clin Auton Res,* 10(2), 57-61.

Brown, C. M., Dütsch, M., Hecht, M. J., Neundörfer, B. & Hilz, M. J. (2003) Assessment of cerebrovascular and cardiovascular responses to lower body negative pressure as a test of cerebral autoregulation *J Neurol Sci,* 208(1-2), 71– 78.

Bungaard-Nielson, M., Sørensen, H., Dalsgaard, M., Rasmussen, P., & Secher, N. H. (2009) Relationship between stroke volume, cardiac output and filling of the heart during tilt. *Acta Anaesthesiol Scand,* 53(10), 1324-1328.

Burton, A. C. (1972) *Physiology and Biophysics of the Circulation* (2nd Ed.), Chicago, IL: Year Book Medical Publishers.

Butler, G. C., Yamamoto, Y., Xing, H. C., Northey, D. R., & Hughson, R. L. (1993) Heart rate variability and fractal dimension during orthostatic challenges. *J Appl Physiol,* 75(6), 2602-2612.

Carey, B. J., Eames, P. J., Blake, M. J., Panerai, R. B., Potter, J. F. (2000) Dynamic cerebral autoregulation is unaffected by aging. *Stroke*, 31(12), 2895-2900.

Carey, B. J., Eames, P. J., Panerai, R. B. & Potter, J. F. (2001a) Carbon dioxide, critical closing pressure and cerebral haemodynamics prior to vasovagal syncope in humans. *Clin Sci (Lond)*, 101(4), 351–358.

Carey, B. J., Manktelow, B. N., Panerai, R. B. & Potter, J. F. (2001b) Cerebral autoregulatory responses to head-up tilt in normal subjects and patients with recurrent vasovagal syncope. *Circulation*, 104(8), 898–902.

Carey, B. J., Panerai, R. B., Potter, J. F. (2003) Effect of aging on dynamic cerebral autoregulation during head-up tilt. *Stroke*, 34(8), 1871-1875.

Cencetti, S., Bandinelli, G., & Lagi, A. (1997) Effects of P_{CO_2} changes induced by head-upright tilt on transcranial Doppler recordings. *Stroke*, 28(6), 1195-1197.

Cencetti, S., Lagi, A., Cipriani, M., Fattorini, L., Bandinelli, G., Bernardi, L. (1999) Autonomic control of the cerebral circulation during normal and impaired peripheral circulatory control. *Heart*, 82(3), 365–372.

Chambless, L. E., Folsom, A. R., Clegg, L. X., Sharrett, A. R., Shahar, E., Nieto, F. J., Rosamond, W. D., & Evans, G. (2000) Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol*, 151(5), 478-487.

Chambless, L. E., Heiss, G., Folsom, A. R., Rosamond, W., Szklo, M., Sharrett, A. R., & Clegg, L. X. (1997) Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol*, 146(6), 483-494.

Charkoudiam, N. & Rabbitts, J. A. (2009) Sympathetic neural mechanisms in human cardiovascular health and disease. *Mayo Clin Proc*, 84(9), 822-830.

Charkoudian, N., Joyner, M. J., Barnes, S. A., Johnson, C. P., Eisenach, J. H., Dietz, N., & Wallin, B. G. (2005) Balance between cardiac output and sympathetic nerve activity in resting humans: role in arterial pressure regulation. *J Physiol*, 568(Pt 1), 315-321.

Chaynes, P., Verdie, J. C., Moscovici, J., Zadeh, J., Vaysse, P., Becue, J. (1998) Micosurgical anatomy of the internal vertebral venous plexuses. *Surg Radiol Anat*, 20(1), 47-51.

Chelma, D., Hérbert, J. L., Coirault, C., Zamani,K., Suard, I., Colin, P. & Lecarpentier, Y. (1998) Total arterial compliance estimated by stroke volume-to-aortic pulse pressure ratio in humans. *Am J Physiol*, 274(2 Pt 2), H500–H505.

Chillon, J. M. & Baumbach, G. L. (2002). Autoregulation: arterial and intracranial pressure. In L. Edvinsson & D. N. Krause (Eds.), *Cerebral blood flow and metabolism* (2nd Ed., pp. 395-412). Philadelphia, PA: Lippincott Williams & Wilkins.

Cirovic, S., Walsh, C., Fraser, W. D. & Guilino, A. (2003) The effect of posture and positive pressure breathing on the hemodynamics of the internal jugular vein. *Aviat Space Environ Med*, 74(2), 125-131.

Cléroux, J., Giannattasio, C., Bolla, G., Cuspidi, C., Grassi, G., Mazzola, C., Sampieri, L., Seravalle, G., Valsecchi, M., & Mancia, G. (1989) Decreased cardiopulmonary reflexes with aging in normotensive humans. *Am J Physiol*, 257(3 Pt 2), H961-H968.

Convertino, V. A. (1987) Aerobic fitness, endurance training, and orthostatic intolerance. *Exerc Sport Sci Rev*, 15, 223–259.

Convertino, V. A. (1993) Endurance exercise training: conditions of enhanced hemodynamic responses and tolerance to LBNP. *Med Sci Sports Exerc*, 25(6), 705-712.

Convertino, V. A. (2001) Lower body negative pressure as a tool for research in aerospace medicine and military medicine. *J Gravit Physiol*, 8(2), 1-14.

Convertino, V. A. & Cooke, W. H. (2002) Relationship between stroke volume and sympathetic nerve activity: new insights about autonomic mechanisms of syncope. *J Gravit Physiol*, 9(1), P63-P66.

Convertino, V. A., Cooke, W. H., Holcomb, J. B. (2006) Arterial pulse pressure and its association with reduced stroke volume during progressive central hypovolemia. *J Trauma*, 61(3), 629-634.

Convertino, V. A., Doerr, D. F., Flores, J. F., Hoffler, G. W. & Buchanan, P. (1988) Leg size and muscle functions associated with leg compliance. *J Appl Physiol*, 64(3), 1017–1021.

Convertino, V. A., Doerr, D. F., Ludwig, D. A. & Vernikos, J. (1994) Effect of simulated microgravity on cardiopulmonary baroreflex control of forearm vascular resistance. *Am J Physiol Regul Integr Comp Physiol*, 266(6 Pt 2), R1962-R1969.

Convertino, V. A., Montgomery, L. D. & Greenleaf, J. E. (1984) Cardiovascular responses during orthostasis: effect of an increase inVO_2 max. *Aviat Space Environ Med*, 55(8), 702–708.

Cooper, V. L. & Hainsworth, R. (2001) Carotid baroreceptor reflexes in humans during orthostatic stress. *Exp Physiol*, 86(5), 677-681.

Cornish, K. G., Gilmore, J. P., & McCulloch, T. (1988) Central blood volume and blood pressure in conscious primates. *Am J Physiol*, 254(4), 693-701.

Czosnyka, M., Richards, H. K., Whitehouse, H. E. & Pickard, J. D. (1996) Relationship between transcranial Doppler-determined pulsatility index and cerebrovascular resistance: an experimental study. *J Neurosurg*, 84(1), 79-84.

Davy, K. P., Seals, D. R., & Tanaka, H. (1998) Augmented cardiopulmonary and integrative sympathetic baroreflexes but attenuated peripheral vasoconstriction with age. *Hypertension*, 32(2), 298–304.

Dawson, S. L., Blake, M. J., Panerai, R. B., & Potter, J. F. (2000) Dynamic but not static cerebral sutoregulation is impaired in acute ischaemic stroke. *Cerebrovasc Dis,* 10(2), 126-132.

Dawson, S. L., Panerai, R. B., & Potter, R. B. (2003) Serial changes in static and dynamic cerebral autoregulation after acute ischaemic stroke. *Cerebrovasc Dis,* 16(1), 69-75.

Dikshit, M. B. (1990) Lower-body suction and cardiovascular reflexes: physiological and applied considerations. *Indian J Physiol Pharmacol,* 34(3), 12.

Dinenno, F. A., Tanaka, H., Stauffer, B. L., & Seals, D. R. (2001) Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented α-adrenergic vasoconstriction. *J Physiol,* 536(Pt 3), 977-983.

Doepp, F., Schreiber, S. J., von Münster, T., Rademacher, J., Klingebiel, R., Valdueza, J. M. (2004) How does the blood leave the brain? A systematic ultrasound analysis of cerebral venous drainage patterns. *Neuroradiology,* 46(7), 565-70.

Dyson, K. S., Shoemaker, J. K., Arbeille, P., Hughson, R. L. (2010) Modelflow estimates of cardiac output compared with Doppler ultrasound during acute changes in vascular resistance in women. *Exp Physiol*, 95(4), 561–568.

Eames, P. J., Blake, M. J., Dawson, S. L., Panerai, R. B., & Potter, J. F. (2002) Dynamic cerebral autoregulation and beat to beat blood pressure control are impaired in acute ischaemic stroke. *J Neurol Neurosurg Psychiatry,* 72(4), 467-472.

Eames, P. J., Blake, M. J., Panerai, R. B., & Potter, J. F. (2003) Cerebral autoregulation indices are unimpaired by hypertension in middle aged and older people. *Am J Hypertension,* 16(9), 746-753.

Ebert, T. J., Hughes, C. V., Tristani, F. E., Barney, J. A. & Smith. J. J. (1982) Effect of age and coronary heart disease on the circulatory responses to graded lower body negative pressure. *Cardiovasc Res,* 16(11), 663–669.

Eckberg, D. L. & Sleight, P. (1992). *Human baroreflexes in health and disease*. (1st Ed.) USA: Oxford University Press.

Edgell, H., Robertson, A. D., Hughson, R. L. (2011) Hemodynamics and brain blood flow during posture change in younger women and postmenopausal women compared with age-matched men. *J Appl Physiol*, 112(9), 1482-93.

Edlow, B. L., Kim, M. N., Durduran, T., Zhou, C., Putt, M. E., Yodh, A. G., Greenberg, J. H., & Detre, J. A. (2010) The effects of healhty aging on cerebral hemodynamic responses to posture change. *Physiol Meas,* 31(4), 477-495.

el-Bedawi, K. M. & Hainsworth, R. (1994) Combined head-up tilt and lower body suction: a test of orthostatic tolerance. *Clin Auton Res,* 4(1-2), 41-47.

Elliott, H. L., Sumner, D. J., McLean, K. & Reid, J. L. (1982) Effect of age on the responsiveness of vascular alpha-adrenoceptors in man. *J Cardiovasc Pharmacol,* 4(3), 388–392.

Ewing, D. J., Campbell, I. W., Murray, A., Neilson, J. M., & Clarke, B. F. (1978) Immediate heart-rate response to standing: simple test for autonomic neuropathy. *Br Med J,* 1(6106), 145-147.

Ferrari, A. U., Radaelli, A. & Centola, A. (2003) Invited Review: Aging and the cardiovascular system. *J Appl Physiol,* 95(6), 2591–2597.

Fink, F., Batzel, J. J., & Kappel, F. (2004) An optimal control approach to modeling the cardiovascular-respiratory system: an application to orthostatic stress. *Cardiovasc Eng,* 4(1), 27-38.

Fischer, D. M., Arbeille, P., Shoemaker, J. K., O'Leary, D. D., Hughson, R. L. (2007) Altered hormonal regulation and blood flow distribution with cardiovascular deconditioning after short-duration head down bed rest. *J Appl Physiol*, 103(6), 2018–2025.

Fog, M. (1937) Cerebral circulation: The reaction of the pial arteries to a fall in blood pressure. *Arch Neurol Psychiatry,* 37(2), 351-364.

Folkow, B. (1964) Description of the myogenic hypothesis. *Circ Res,* 15 suppl, 279-287.

Formes, K., Zhang, P., Tierney, N., Schaller, F., & Shi, X. (2010) Chronic physical activity mitigates cerebral hypoperfusion during central hypovolemia in elderly humans. *Am J Physiol Heart Circ Physiol,* 298(3), H1029-H1037.

Fortney, S., Tankersley, C., Lightfoot, J. Y., Drinkwater, D., Clulow, J., Gerstenblith, G., O'Connor, F., Becker, L., Lakatta, E., & Fleg, J. (1992) Cardiovascular responses to lower body negative pressure in trained and untrained older men. *J Appl Physiol,* 73(6), 2693-2700.

Franke, W. D., Allbee, K. A., Spencer, S. E. (2006) Cerebral blood flow responses to severe orthostatic stress in fit and unfit young and older adults. *Gerontology*, 52(5), 282–289.

Franke, W. D., Mills, K. K., Lee, K., & Hernandez, J. P. (2003) Training mode does not affect orthostatic tolerance in chronically exercising subjects. *Eur J Appl Physiol,* 89(3-4), 263–270.

Franklin, S. S., Gustin, W. 4th., Wong, N. D., Larson, M. G., Weber, M. A., Kannel, W. B., & Levy, D. (1997) Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation,* 96(1), 308-315.

Frey, M. A., & Hoffler, G. W. (1988). Association of sex and age with responses to lower body negative pressure. *J Appl Physiol*, 65(4), 1752–1756.

Fu, Q., Arbab-Zadeh, A., Perhonen, M. A., Zhang, R., Zuckerman, J. H. & Levine, B. D. (2004) Hemodynamics of orthostatic intolerance: implications for gender differences. *Am J Physiol Heart Circ Physiol*, 286(1), H449–H457.

Fu, Q., Iwase. S., Niimi, Y., Kamiya, A., Michikami, D., Mano, T., Suzumura, A. (2002) Age-related changes in vasomotor reflex control of calf venous capacitance response to lower body negative pressure in humans. *Jpn J Physiol*, 52(1), 69–76.

Fu, Q., Shibata, S., Hastings, J. L., Prasad, A., Palmer, M. D., & Levine, B. D. (2009) Evidence for unloading arterial baroreceptors during low levels of lower body negative pressure in humans. *Am J Physiol Heart Circ Physiol*, 296(2), H480-H488.

Gabbett, T. J., Weston, S., Barrett, R. S., & Gass, G. C. (2001) Cardiovascular regulation during head-up tilt in healhty 20-30-year-old and 70-75-year-old men. *Clin Sci Lond*, 100(2), 199-206.

Gagné, N. (2009) *Developing a Non-Invasive Method to Monitor Cardiovascular Control during Orthostatic Challenge Considering the Limitation of the Finometer*™. Unpublished master's thesis. University of Waterloo, Waterloo, ON.

Gagné, N., Fischer, D., Greaves, D. K. & Hughson, R. L. (2007) WISE-2005: developing a non-invasive method to monitor cardiovascular deconditioning. *J Gravit Physiol.* 14(1), P137-8.

Gatto, R., Hoffman, W., Paisansathan, C., Mantulin, W., Gratton, E. & Charbel, F. T. (2007) Effect of age on brain oxygenation regulation during changes in position. *J Neurosci Methods*, 164(2), 308-311.

Gelman, S. (2008) Venous function and central venous pressure: a physiologic story. *Anesthesiology*, 108(4), 735-748.

Giller, C. A. (1990) The frequency-dependent behaviour of cerebral autoregulation. *Neurosurgery*, 27(3), 362-368.

Giller, C. A., Bowman, G., Dyer, H., Mootz, L., Krippner, W. (1993) Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery*, 32(5), 737–742.

Giller, C. A., Levine, B. D., Meyer, Y., Buckey, J. C., Lane, L. D., & Borchers, D. J. (1992) The cerebral hemodynamics of normotensive hypovolemia during lower-body negative pressure. *J Neurosury*, 76(6), 961-6.

Gisolf, J. A., van Lieshout, J. J. & Karemaker, J. M. (2005) The siphon controversy: an integration of concepts and the brain as baffle. *Am J Physiol Regul Integr Comp Physiol*, 289(2), R627-629.

Gisolf, J. A., van Lieshout, J. J., van Heusden, K., Pott, F., Stok, W. J., Karemeker, J. M. (2004) Human cerebral venous outflow pathway depends on posture and central venous pressure. *J Physiol,* 560(1), 317-327.

Goadsby, P. J. & Edvinnson, L. (2002a). *Cerebral blood flow and metabolism*. (2nd Ed.) New York, NY: Lippincott Williams & Wilkins.

Goadsby, P.J. & Edvinnson, L. (2002b). Neurovascular control of the cerebral circulation. In L. Edvinsson & D. N. Krause (Eds.), *Cerebral blood flow and metabolism* (2nd Ed., pp. 172-188). Philadelphia, PA: Lippincott Williams & Wilkins.

Gordon, R. D., Küchel, O., Liddle, G. W., & Island, D. P. (1967) Role of the sympathetic nervous system in regulating renin and aldosterone production in man. *J Clin Invest,* 46(4), 599-605.

Goswami, N., Loeppky, J. A., & Hinghoffer-Szalkay, H. (2008) LBNP: past protocols and technical considerations for experimental design. *Aviat Space Environ Med,* 79(5), 459-71.

Gould, L., Venkataraman, K., Goswami, M., Gomprecht, R. F. (1973) The cardiac effects of coffee. *Angiology,* 24(8), 455-463.

Grasser, E. K., Goswami, N., Rössler, A., Vrecko, K., & Hinghofer-Szalkay, H. (2009) Hemodynamic and neurohormonal responses to extreme orthostatic stress in physically fit young adults. *Acta Astronaut,* 64, 688-696.

Greenleaf, J. E., Brock, P. J., Sciaraffa, D., Polese, A., & Elizondo, R. (1985) Effects of exercise-heat acclimation on fluid, electrolyte and endocrine responses during tilt and +Gz acceleration in women and men. *Aviat Space Environ Med,* 56(7), 683–689.

Greenwald, S. E. (2007) Ageing of the conduit arteries. *J Pathol,* 211(2), 157-172.

Gribbin, B., Pickering, T. G., Sleight, P., & Peto, R. (1971) Effect of age and high blood pressure on baroreflex sensitivity in man. *Circ Res,* 29(4), 424-431.

Grinberg, A. (2010). *Cardiovascular Deconditioning Resulting from 28-hour Bed-rest and the Efficacy of the Fluid Loading Countermeasure*. Unpublished master's thesis. University of Waterloo, Waterloo, ON

Grubb, B. P., Gerrard, G., Roush, K., Temesy-Armos, P., Montford, P., Elliot, L., Hahn, H., & Brewster, P. (1991) Cerebral vasoconstriction during head-upright tilt-induced vasovagal syncope. A paradoxic and unexpected response. *Circulation,* 84(3), 1157-1164.

Gulbenkian, S., Uddman, R., & Edvinnson, L. (2001) Neuronal messengers int eh human cerebral circulation. *Peptides,* 22(6), 995-1007.

Hagan, R. D., Diaz, F. J., & Horvath, S. M. (1978) Plasma volume changes with movement to upright position. *J Appl Physiol,* 45(3), 414-417.

Hainsworth, R. (1986) Vascular capacitance: its control and importance. *Rev Physiol Biochem Pharmacol*, 105, 101-173.

Hainsworth, R. (1990) The importance of vascular capacitance in cardiovascular control. *News Physiol Sci*, 5(6), 250-254.

Hakumäki, M. O., Wang, B. C., Sundet, W. D., & Goetz, K. L. (1985) Aortic baroreceptor discharge during nonhypotensive hemorrhage in anesthetized dogs. *Am J Physiol*, 249(2), 393-403.

Hamel, E. (2006) Perivascular nerves and the regulation of cerebrovascular tone. *J Appl Physiol*, 100(3), 1059-1064.

Harms, M. P., Wesseling, K. H., Pott, F., Jenstrup, M., van Goudoever, J., Secher, N. H., van Lieshout, J. J. (1999) Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)*, 97(3), 291–301.

Harrap, S. B., Cui, J. S., Wong, Z. Y., Hopper, J. L. (2004) Familial and Genomic Analyses of Postural Changes in Systolic and Diastolic Blood pressure. *Hypertension*, 43(3), 586-591.

Haubrich, C., Wendt, A., Diehl, R. R., & Klötzsch, C. (2004) Dynamic autoregulation testing in the posterior cerebral artery. *Stroke*, 35(4), 848-852.

Heagerty, A. M., Aalkjaer, C., Bund, S. J., Korsgaard, N., & Mulvany, M. J. (1993) Small artery structure in hypertension. Dual processes of remodeling and growth. *Hypertension*, 21(4), 391-397.

Heckmann, J. G., Brown, C. M., Cheregi, M., Hilz, M. J., & Neundörfer, B. (2003) Delayed cerebrovascular autoregulaatory response to ergometer exercise in normotensive elderly humans. *Cerebrovasc Dis*, 16(4), 423-429.

Heitterachi, E., Lord, S. R., Meyerkort, P., McCloskey, I., & Fitzpatrick, R. (2002) Blood pressure changes on upright tilting predict falls in older people. *Age Ageing*, 31(3), 181-186.

Hernandez, J. P. & Franke, W. D. (2004) Age- and fitness-related differences in limb venous compliance do not affect tolerance to maximal lower body negative pressure in men and women. *J Appl Physiol*, 97(3), 925-929.

Hernandez, J. P. & Franke, W. D. (2005) Effects of a 6-mo endurance-training program on venous compliance and maximal lower body negative perssure in older men and women. *J Appl Physiol*, 99(3), 1070-1077.

Hernandez, J. P., Karandikar, A., & Franke, W. D. (2005) Effects of age and fitness on tolerance to lower body negative pressure. *J Gerontol A Biol Sci Med Sci*, 60(6), 782-786.

Hernandez, J. P., Roever, K., & Seed, T. (2010) Cardiovascular responses to maximal graded and acute lower body negative pressure. *J Aging Phys Act*, 18(1), 27-42.

Hinghofer-Szalkay, H., Haditsch, B., Loder, I., Pilz, K., Rössler, A., & Jezovà, D. (2004) Head down tilt at -6 degrees to -24 degrees can neutralize the cardiovascular effects of LBNP at -15 or -35 mmHg. *Aviat Space Environ Med*, 75(11), 947-951.

Hinghofer-Szalkay, H. & Moser, M. (1986) Fluid and protein shifts after posture changes in humans. *Am J Physiol*, 250(1), 68-75.

Hinghofer-Szalkay, H. G., Vigas, M., Sauseng-Fellegger, G., König, E. M., Lichardus, B. & Jezovà, D. (1996) Head-up tilt and lower body suction: comparison of hormone responses in healthy men. *Physiol Res*, 45(5), 369-378.

Hisdal, J., Toska, K., & Walløe, L. (2001) Beat-to-beat cardiovascular responses to rapid, low-level LBNP in humans. *Am J Physiol Regul Integr Comp Physiol*, 281(1), R213-R221.

Hoeldtke, R. D. & Cilmi, K. M. (1985) Effects of aging on catecholamine metabolism. *J Clin Endocrinol Metab*, 60(3), 479–484.

Hoffman, J. I. & Spaan, J. A. (1990) Pressure-flow relations in coronary circulation. *Physiol Rev*, 70(2), 331- 390.

Hollander, M., Hak, A. E., Koudstaal, P. J., Bots, M. L., Grobbee, D. E., Hofman, A., Witteman, J. C., & Breteler, M. M. (2003) Comparison between measures of atherosclerosis and risk of stroke: the Rotterdam Study. *Stroke*, 34(10), 2367-2372.

Howard, P. (1977) Gravity and the circulation. *Proc R Soc Lond B Biol Sci*, 199(1137), 485-491.

Huber, P. & Handa, J. (1967) Effect of contrast material, hypercapnia, hyperventilation, hypertonic glucose and papaverine on the diameter of the cerebral arteries: Angiographic determination in man. *Invest Radiol*, 2(1), 17–32.

Hughson, R. L., Edwards, M. R., O'Leary, D. D., & Shoemaker, J. K. (2001) Critical analysis of cerebrovascular autoregulation during repeated head-up tilt. *Stroke*, 32(10), 2403-2408.

Hughson, R. L., Shoemaker, J. K., Arbeille, P., O'Leary, D.D., Pizzolitto, K. S. & Hughes, M. D. (2004) Splanchnic and peripheral vascular resistance during lower body negative pressure (LBNP) and tilt. *J Gravit Physiol*, 11(2), P95-6.

Ide, K., Eliasziw, M., & Poulin, M. J. (2003) Relationship between middle cerebral artery blood velocity and end-tidal P_{CO_2} in the hypocapnic-hypercapnic range in humans. *J Appl Physiol*, 95(1), 129-137.

Iglesias del Sol, A., Bots, M. L., Grobbee, D. E., Hofman, A., & Witteman, J. C. (2002) Carotid intima-media thickness at different sites: relation to incident myocardial infarction; The Rotterdam Study. *Eur Heart J*, 23(12), 934-940.

Immink, R.V., Secher, N. H., Roos, C. M., Pott, F., Madsen, P. L., & Lieshout, J. J. (2006) The postural reduction in middle cereral artery blood velocity is not explained by $PaCO_2$. *Eur J Appl Physiol*, 96(5), 609-614.

Jansen, J. R., Schreuder, J. J., Mulier, J. P., Smith, N. T., Settels, J. J. & Wesseling, K.H. (2001) A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *Br J Anaesth*, 87(2), 212–222.

Jingu, S., Takeshita, A., Imaizumi, T., Sakai, K., & Nakamura, M. (1989) Age related decreases in cardiac receptor control of forearm vascular resistance in humans. *Clin Exp Hypertens A*, 11(Suppl 1), 211-216.

Jones, P. P., Christou, D. D., Jordan, J., & Seals, D. R. (2003) Baroreflex buffering is reduced with age in healthy men. *Circulation*, 107(13), 1770–1774.

Jones, P. P., Shapiro, L. F., Keisling, G. A., Jordan, J., Shannon, J. R., Quaife, R. A., & Seals, D. R. (2001) Altered autonomic support of arterial blood pressure with age in healthy men. *Circulation*, 104(20), 2424–2429.

Joshi, S., Meyers, P. M., Pile-Spellman, J., Wang, M., & Sahlein, D. H. (2004) Intracarotid verapamil decreases both proximal and distal human cerebrovascular resistance. *Anesthesiology*, 100(4), 774-781.

Joshi, S., Young, W. L., Hoang, D. D., Ostapkovich, N. D., Aagaard, B. D., Hashimoto, T., & Pile-Spellman, J. (2000) Intracarotid infusion of the nitric oxide synthase inhibitir, L-NMMA, modestly decreases cerebral blood flow in human subjects. *Anesthesiology*, 93(3), 699-707.

Kaplan, D. T., Furman, M. I., Pincus, S. M., Ryan, S. M., Lipsitz, L. A., Goldberger, A. L. (1991) Aging and the complexity of cardiovascular dynamics. *Biophys J*, 59(4), 945–949.

Kapoor, W. N., Karpf, M., Wieand, S., Peterson, J. R., & Levey, G. S. (1983) A prospective evaluation and follow-up of patients with syncope. *N Engl J Med*, 309(4), 197-204.

Kastrup, A., Dichgans, J., Niemeier, M., & Schabet, M. (1998) Changes of cerebrovascular CO_2 reactivity during normal aging. *Stroke*, 29(7), 1311-1314.

Keller, D. M., Low, D. A., Wingo, J. E., Brothers, R. M., Hastings, J., Davis, S. L. & Crandall, C. G. (2009) Acute volume expansion preserves orthostatic tolerance during whole-body heat stress in humans. *J Physiol*, 587(5), 1131–1139.

Kim, Y. S., Bogert, L. W., Immink, R. V., Harms, M. P., Colier, W. N., van Lieshout, J. J. (2011) Effects of aging on the cerebrovascular orthostatic response. *Neurobiol Aging*, 32(2), 344-53.

Kitamura, A., Iso, H., Imano, H.O., Sato, S., Kiyama, M., Tanigawa, T., Yamagishi, K., & Shimamoto, T. (2004) Carotid intima-media thickness and plaque characteristics as a risk factor for stroke in Japanese elderly men. *Stroke,* 35(12), 2788-2794.

Krejza, J., Krejza, J., Mariak, Z., Walecki, J., Szydlik, P., Lewko, J., & Ustymowics, A. (1999) Transcranial color Doppler sonography of basal cerebral arteries in 182 healthy subjects: age and sex variability and normal reference values for blood flow parameters. *Am J Roentgenol*, 172(1), 213-218.

Kuo, T. B., Chern, C. M., Yang, C. C., Hsu, H. Y., Wong, W. J., Sheng, W. Y., & Hu, H. H. (2003) Mechanisms underlying phase lag between systemic arterial blood pressure and cerebral blood velocity. *Cerebrovasc Dis*, 16(4), 402-409.

Laitinen, T., Hartikainen, J., Vanninen, E., Niskanen, L., Geelen, G., & Länsimies, E. (1998) Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol*, 84(2), 576-583.

Laitinen, T., Niskanen, L., Geelen, G., Länsimies, E., & Hartikainen, J. (2004) Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. *J Appl Physiol*, 96(6), 2333-2340.

Lakatta, E. G. (1992). *Length modulation of muscle performance: Frank-Starling Law of the Heart*. New York, NY: Raven Press.

Lakatta, E. G. (1993) Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev,* 73(2), 413–467.

Lakatta, E. G. & Levy, D. (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circulation*, 107(1), 139-146.

Larsen, F. S., Olsen, K. S., Hansen, B. A., Paulson, O. B. & Knudsen, G. M (1994). Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. *Stroke,* 25(10), 1985–1988.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannallasio, C., Hayaz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I., Strujiker-Boudier, H., & European Network for Non-invasive Investigation of Large Arteries (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*, 27(21), 2588-2605.

Lavi, S., Gaitini, D., Milloul, V., & Jacob, G. (2006) Impaired cerebral CO_2 vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol*, 291(4), H1856-H1861.

LeMarbre, G., Stauber, S., Khayat, R. N., Puleo, D. S., Skatrud, J. B., & Morgan, B. J. (2003) Baroreflex-induced sympathetic activation does not alter cerebrovascular CO_2 responsiveness in humans. *J Physiol*, 551(2), 609–616.

Levine, B. D., Giller, C. A., Lane, L. D., Buckey, J. C., & Blomqvist, C. G. (1994) Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. *Circulation*, 90(1), 298-306.

Levinson, G. E., Frank, M. J., Nadimi, M. & Braunstein, M. (1967) Studies of cardiopulmonary blood volume: measurement of left ventricular volume with dye dilution. *Circulation,* 35(6), 1038-1048.

Lewis, N. C., Atkinson, G., Lucas, S. J., Grant, E. J., Jones, H., Tzeng, Y. C., Horsman, H., & Ainslie, P.N. (2010) Diurnal variation in time to presyncope and associated circulatory changes during a controlled orthostatic challenge. *Am J Physiol Regul Integr Comp Physiol*, 299(1), R55–R61.

Lewis, N. C., Atkinson, G., Lucas, S. J., Grant, E. J., Jones, H., Tzeng, Y. C., Horsman, H. & Ainslie, P. N. (2011) Is there diurnal variation in initial and delayed orthostatic hypotension during standing and head-up tilt? *Chronobiol Int*, 28(2), 135-145

Lindegaard, K.F., Lundar, T., Wiberg, J., Sjøberg, D., Aaslid, R. & Nornes, H. (1987) Variations in middle cerebral artery blood flow investigated with noninvasive transcranial blood velocity measurements. *Stroke,* 18(6), 1025–1030.

Lindenberger, M. & Länne, T. (2007) Decreased capillary filtration but maintained venous compliance in the lower limb of aging women. *Am J Physiol Heart Circ Physiol*, 293(6), H3568-74.

Lipsitz, L. A. (1989) Orthostatic hypotension in the elderly. *N Engl J Med*, 321(14), 952–957.

Lipsitz, L. A., Gagnon, M., Vyas, M., Iloputaife, I., Kiely, D. K., Sorond, F., Serrador, J., Cheng, D. M., Babikian, V., & Cupples, L. A. (2005) Antihypertensive therapy increases cerebral blood flow and carotid distensibility in hypertensive elderly subjects. *Hypertension*, 45(2), 216-221.

Lipsitz, L. A., Mukai, S., Hamner, J., Gagnon, M. & Babikian, V. (2000) Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke*, 31(8), 1897-1903.

Livingstone, K., Peralta-Huertas, J., Phillips, A., Klentrou, P. & O'Leary, D. D. (2010) Hemodynamic response to lower body negative pressure in children: a pilot study. *Auton Neurosci*, 155(1-2), 115–120.

Loessner, A., Alavi, A., Lewandrowski, K. U., Mozley, D., Souder, E., Gur, R. E. (1995) Regional cerebral function determined by FDG-PET in healthy volunteers: normal patterns and changes with age. *J Nucl Med,* 36(7), 1141–1149.

Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M., & Sitzer, M. (2007) Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. *Circulation,* 115(4), 459-467.

Lorenz, M. W., von Kegler, S., Steinmetz, H., Markus, H., & Sitzer, M. (2006) Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke*, 37(1), 87-92.

Low, P. A. (2008) Prevalence of orthostatic hypotension. *Clin Auton Res*, 18(Suppl 1), 8-13.

Low, P. A., Novak, V., Spies, J. M., Novak, P. & Petty, G. W. (1999) Cerebrovascular regulation in the postural orthostatic tachycardia syndrome (POTS). *Am J Med Sci*, 317(2), 124–133.

Low, P. A., Opfer-Gehrking, T. L., McPhee, B. R., Fealey, R. D., Benarroch, E. E., Willner, C. L., Suarez, G. A., Proper, C. J., Felten, J. A., Huck, C. A., & Corfits, J. L. (1995) Prospective evaluation of clinical characteristics of orthostatic hypotension. *Mayo Clin Proc*, 70(7), 617-622.

Ludbrook, J. (1966) The musculovenous pumps of the human lower limb. *Am Heart J*, 71(5), 635-641.

Luukinen, H., Koski, K., Laippala, P. & Airaksinen, K. E. (2004) Orthostatic hypotension and the risk of myocardial infarction in the home-dwelling elderly. *J Intern Med*, 255(4), 486–493.

Luukinen, H., Koski, K., Laippala, P. & Kivelä, S. L. (1999) Prognosis of diastolic and systolic orthostatic hypotension in older persons. *Arch Intern Med*, 159(3), 273-280.

MacGilchrist, A. J., Hawksby, C., Howes, L. G., & Reid, J. L. (1989) Rise in plasma noradrenaline with age results from an increase in spillover rate. *Gerontology*, 35(1), 7–13.

MacKenzie, E. T., Farrar, J. K., Fitch, W., Graham, D. I., Gregory, P. C., & Harper, A. M. (1979) Effects of hemorrhagic hypotension on the cerebral circulation. I. Cerebral blood flow and pial arteriolar caliber. *Stroke*, 10(6), 711-718.

Madsen, P., Potts, F., Olsen, S. B., Nielson, H. B., Burev, I., & Secher, N. H. (1998) Near-infrared spectrophotometry determined brain oxygenation during fainting. *Acta Physiol Scand*, 162(4), 501-507.

Marcus, M. L. & Heistad, D. D. (1973) Effects of sympathetic nerves on cerebral blood flow in awake dogs. *Am J Physiol*, 236(4), H549-H553.

Marmarou, A., Shulman, K., & La Mores, J. (1975) Compartmental analysis of compliance and outflow resistance of the CSF system. *J Neurosurg*, 43(5), 523–534.

Marthol, H., Zikeli, U., Brown, C. M. Tutaj, M. & Hilz, M. J. (2007) Cardiovascular and cerebrovascular responses to lower body negativev pressure in type 2 diabetic patients. *J Neurol Sci*, 252(2), 99-105.

Mathias, C. J. (2002), To stand on one's own legs. *Clin Med*, 2(3), 237-245.

Matsuda, H., Maeda, T., Yamada, M., Gui, L. X., Tonami, N., & Hisada, K. (1984) Age-matched normal values and topographic maps for regional cerebral blood flow measurements by Xe-133 inhalation. *Stroke,* 15(2), 336-342.

Matsukawa, K., Kobayashi, T., Nakamoto, T., Murata, J., Komine, H., & Noso, M. (2004) Noninvasive evaluation of cardiac output during postural change and exercise in humans: comparison between the modelflow and pulse dyedensitometry. *Jpn J Physiol,* 54(2), 153–160.

Mattace-Raso, F. U., van der Cammen, T. J., Knetsch, A. M., van den Meiracker, A. H., Schalekamp, M. A., Hofman, A. & Witteman, J. C. (2006) Arterial stiffness as the candidate underlying mechanism for postural blood pressure changes and orthostatic hypotension in older adults: the Rotterdam Study. *J Hypertens,* 24(2), 339–344.

Mehagnoul-Schipper, D. J., Vloet, L. C., Colier, W. N., Hoefnagels, W. H. & Jansen, R. W. (2000) Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke*, 31(7), 1615-1620.

Meyer, J. S., Terayama, Y. & Takashima, S. (1993) Cerebral circulation in the elderly. *Cerebrovasc Brain Metab Rev,* 5(2), 122–146.

Michard, F., Boussat, S., Chemla, D., Anguel, N., Mercat, A., Lecarpentier, Y., Richard, C., Pinsky, M. R. & Teboul, J. L. (2000) Relation between respiratory changes in arterial pulse pressure and fluid responsiveness in septic patients with acute circulatory failure. *Am J Respir Crit Care Med*, 162(1), 134–138.

Michard, F., Chemla, D., Richard, C., Wysocki, M., Pinsky, M. R., Lecarpentier, Y. & Teboul, J.L. (1999) Clinical use of respiratory changes in arterial pulse pressure to monitor the hemodynamic effects of PEEP. *Am J Respir Crit Care Med*, 159(3), 935–939.

Minson, C. T., Halliwill, J. R., Young, T. M., & Joyner, M. J. (2000) Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transductions in young women. *Circulation,* 101(8), 862-868.

Minson, C. T., Wladkowski, S. L., Pawelczyk, J. A., & Kenney, W. L. (1999) Age, splanchnic vasoconstriction, and heat stress during tilting. *Am J Physiol*, 276(1), R203-R212.

Monahan, K. D. (2007) Effect of aging on baroreflex function in humans. *Am J Physiol Regul Integr Comp Physiol*, 293(1), R3-R12.

Monahan, K. D. (2009) A new answer to an old question: does ageing modify baroreflex control of vascular sympathetic outflow in humans*? J Physiol,* 587(Pt 9), 1857.

Monahan, K. D., Dinenno, F. A., Seals, D. R., Clevenger, C. M., Desouza, C. A., & Tanaka, H. (2001a) Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. *Am J Physiol Heart Circ Physiol*, 281(1), H284-H289.

Monahan, K. D., Dinenno, F. A., Seals, D. R., & Halliwill, J. R. (2001b) Smaller age-associated reductions in leg venous compliance in endurance exercise trained men. *Am J Physiol Heart Circ Physiol*, 281(3), H1267–H1273.

Moncada, S., Palmer, R. M., & Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*, 43(2), 109-142.

Murray, R. H, Thompson, L. J., Bowers, J. A., & Albright, C. D. (1968) Hemodynamic effects of graded hypovolemia and vasodepressor syncope induced by lower body negative pressure. *Am Heart J*, 76(6), 799–811

Murrell, C. J., Cotter, J. D., George, K., Shave, R., Wilson, L., Thomas, K., Williams, M. J., & Ainslie, P. N. (2011) Cardiorespiratory and cerebrovascular responses to head-up tilt I: influence of age and training status. *Exp Gerontol*, 46(1), 9-17.

Nelson, E. & Rennels, M. (1970) Innervation of intracranial arteries. *Brain*, 93(3), 475– 90.

Newell, D. W., Aaslid, R., Lam, A., Mayberg, T. S., Winn, H. R. (1994) Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke*, 25(4), 793–797.

Newman, D. G. & Callister, R. (1999) The non-invasive assessment of stroke volume and cardiac output by impedance cardiography: a review. *Aviat Space Environ Med*, 70(8), 780-9.

Njemanze, P. C. (1992) Critical limits of pressure-flow relation in the human brain. *Stroke*, 23(12), 1743-1747.

Novak, V., Spies, J. M., Novak, P., McPhee, B. R., Rummans, T. A., & Low, P. A. (1998) Hypocapnia and cerebral hypoperfusion in orthostatic intolerance. *Stroke*, 29(9), 1876-1881.

Nurmi, L. S. & Luthje, P. M. (2002) Incidence and costs of falls and fall injuries among elderly in institutional care. *Scand J Prim Health Care*, 20(2), 118-122.

Nurmi, L. S., Luthje, P. M., & Kataja, J. M. (2004) Long-term survival after falls among the elderly in institutional care. *Arch Gerontol Geriatr*, 38(1), 1-10.

Nürnberger, J., Dammer, S., Opazo Saez, A., T Philipp T. & Schäfers R.F. (2003) Diastolic blood pressure is an important determinant of augmentation index and pulse wave velocity in young, healthy males**.** *J Hum Hypertens*, 17(3), 153–158.

O'Leary, D.H., Polak, J.F., Kronmal, R.A., Manolio, T.A., Burke, G.L., & Wolfson, S.K., Jr. (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*, 340(1), 14-22.

O'Rourke, M. F. (2007) Arterial aging: pathophysiological principles. *Vasc Med,* 12(4), 329-341.

O'Rourke, M. F. & Hashimoto, J. (2007) Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol,* 50(1), 1-13.

Olsen, H. & Länne, T. (1998) Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *Am J Physiol Heart Circ Physiol,* 275(3 Pt 2), H878–H886.

Olsen, H, Vernersson, E., & Lanne, T. (2000) Cardiovascular response to acute hypovolemia in relation to age. Implications for orthostasis and hemorrhage.*Am J Physiol Heart Circ Physiol,* 278(1), H222–H232.

Onoue, H., Kaito, N., Tomii, M., Tokudome, S., Nakajima, M., & Abe, T. (1994) Human basilar and middle cerebral arteries exhibit endothelium-dependent responses to peptides. *Am J Physiol,* 267(3), H880-H886.

Ooi, W. L., Barrett, S., Hossain, M., Kelley-Gagnon, M., & Lipsitz, L. A. (1997) Patterns of orthostatic blood pressure change and their clinical correlates in a frail, elderly population. *JAMA,* 277(16), 1299-1304.

Oparil, S. & Habar, E. (1974) The renin-angiotensin system. *N Engl J Med,* 291(8), 389-401.

Panerai, R. B. (2003) The critical closing pressure of the cerebral circulation. *Med Eng Phys,* 25(8), 621-632.

Panton, L. B., Franke, W. D., Bleil, D. A., Baier, S. M., & King, D. S. (2001) Effects of resistance training on cardiovascular responses to lower body negative pressure in the elderly. *Clin Physiol,* 21(5), 605-611.

Paulson, O. B., Strandgaard, S., & Edvinson, L. (1990) Cerebral autoregulation. *Cerebrovasc Brain Metab Rev,* 2(2), 161-192.

Pawelczyk, J. A., Kenney, W. L., & Kenney, P. (1988) Cardiovascular responses to head-up tilt after an endurance training program. *Aviat Space Environ Med,* 59(2), 107–112.

Petersson, J., Zygmunt, P. M., Brandt, L., & Högestätt, E. D. (1995) Substance P-induced relaxation and hyperpolarization inhuman cerebral arteries. *Br J Pharmacol,* 115(6), 889-894.

Poon, I. O. & Braun, U. (2005) High prevalence of orthostatic hypotension and its correlation with potentially causative medications among elderly veterans. *J Clin Pharm Ther,* 30(2), 173-178.

Poulin, M. J. & Robbins, P. A. (1996) Indexes of flow and cross-sectional area of the middle cerebral artery using doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke,* 27(12), 2244-2250.

Poulin, M. J., Liang, P. J., & Robbins, P. A. (1996) Dynamics of the cerebral blood flow response to step changes in end-tidal PCO_2 and PO_2 in humans. *J Appl Physiol*, 81(3), 1084-1095.

Purkayastha, S. & Raven, P. B. (2011) The functional role of the alpha-1 adrenergic receptors in cerebral blood flow regulation. *Indian J Pharmacol*, 43(5), 502–506.

Rang. S., de Pablo Lapiedra, B., van Montfrans, G. A., Bouma, B. J., Wesseling, K. H., Wolf, H. (2007) Modelflow: a new method for noninvasive assessment of cardiac output in pregnant women. *Am J Obstet Gynecol*, 196(3), e231–e238.

Raven, P. & Pawelczyk, J. (1993) Chronic endurance exercise training: a condition of inadequate blood pressure regulation and reduced tolerance to LBNP. *Med Sci Sports Exerc*, 25(6), 713–721.

Raven, R. B., Rohm-Young, D., & Blomqvist, C. G. (1984) Physical fitness and cardiovascular response to lower body negative pressure. *J Appl Physiol*, 56(1), 138–144.

Regan, T. J. (1990) Alcohol and the Cardiovascular System. *JAMA*, 264(3), 377-381.

Reinmuth, O. M., Schneinberg, P., & Bourne, B. (1965) Total cerebral blood flow and metabolism. *Arch Neurol*, 12(1), 49-66.

Reivich, M. (1964) Arterial PCO_2 and cerebral hemodynamics. *Amer J Physiol*, 206(1), 25-35.

Ringelstein, E. B., Kahlscheuer, B., Niggemeyer, E., Otis, S. M. (1990) Transcranial doppler sonography: Anatomical landmarks and normal velocity values**.** *Ultrasound Med Biol*, 16(8), 745-761.

Robbins, A. S. & Rubbenstein, L. Z. (1972) Postural hypotension in the elderly. *J Am Geriatr Soc*, 32(10), 769–774.

Rosvall, M., Janzon, L., Berglund, G., Engström, G., & Hedblad, B. (2005a) Incidence of stroke is related to carotid IMT even in the absence of plaque. *Atherosclerosis*, 179(2), 325-331.

Rosvall, M., Janzon, L., Berglund, G., Engström, G., & Hedblad, B. (2005b) Incident coronary events and case fatality in relation to common carotid intima-media thickness. *J Intern Med*, 257(5), 430-437.

Rothe, C. F. (1983) Reflex control of veins and vascular capacitance. *Physiol Rev*, 63(4), 1281-1342.

Rowell, L. B. (1993). *Human Cardiovascular Control*. New York: Oxford University Press.

Rowell, L. B., Detry, J. M., Blackmon, J. R., Wyss, C. (1972) Importance of the splanchnic vascular bed in human blood pressure regulation. *J Appl Physiol*, 32(2), 213-20.

Rubanyi, G. M., Romero, J. C., Vanhoutte, P. M. (1986) Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol*, 250(6 Pt 2), H1145–H1149.

Rubin, P. C., Scott, P. J. W., McLean, K., & Reid, J. L. (1982) Noradrenaline release and clearance in relation to age blood pressure in man. *Eur J Clin Invest,* 12(2), 121–125.

Rutan, G. H., Hermanson, B., Bild, D. E., Kittner, S. J., LaBaw, F., & Tell, G. S. (1992) Orthostatic hypotension in older adults. The Cardiovascular Health Study. CHS Collaborative Research Group. *Hypertension*, 19(6), 508-519.

Sadek, A. G., Mohamed, F. B., Outwater, E. K., El-Essawy, S. S., Mitchell, D. G. (1996) Respiratory and postprandial changes in portal flow rate: assessment by phase contrast MR imaging. *J Magn Reson Imaging*, 6(1), 90-3.

Salonen, J. T. & Salonen, R. (1993) Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation*, 87(3 Suppl), II56-II65.

Scheel, P., Ruge, C., Petruch, U. R., Schöning, M. (2000) Color duplex measurement of cerebral blood flow volume in healthy adults. *Stroke,* 31(1), 147-150.

Schondorf, R., Benoit, J., & Wein, T. (1997) Cerebrovascular and cardiovascular measurements during neurally mediated syncope induced by head-up tilt. *Stroke,* 28(8), 1564-1568.

Seals, D. R. & Dinenno, F. A. (2004) Collateral damage: cardiovascular consequences of chronic sympathetic activation with human aging. *AJP – Heart,* 287(5), H1895-H1905.

Seals, D. R., Walker, A. E, Pierce, G. L & Lesniewski, L. A. (2009) Habitual exercise and vascular ageing. *J Physiol,* 587(Pt 23), 5541–5549.

Segal, N., Dougherty, R., & Sackner, M. A. (1973) Effects of tilting on pulmonary capillaary blood flow in normal man. *J Appl Physiol*. 35(2), 244-249.

Senay. S., Toraman, F., Gelmez, S., Dagdelen, S., Karabulut, H., Alhan, C. (2009) Continuous arterial pressure waveform analysis accurately detects cardiac output in cardiac surgery: a prospective comparison with thermodilution, echocardiography, and magnetic resonance techniques. *Heart Surg Forum,* 12(2), E75–E78.

Serrador, J. M., Hughson, R. L., Kowalchuck, J. M., Bondar, R. L., & Gelb, A. W. (2006) Cerebral blood flow during orthostasis: role of arterial CO_2. *Am J Physiol Regul Integr Comp Physiol*, 290(4), R1087-R1093.

Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, J. K., & Bondar, R. L. (2000a) MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke*, 31(7), 1672-1678.

Serrador, J. M., Shoemaker, J. K., Brown, T. E., Kassam, M. S., Bondar, R. L., & Schlegel, T. T. (2000b) Cerebral vasoconstriction precedes orthostatic intolerance after parabolic flight. *Brain Res Bull*, 53(1), 113-120.

Seymour, R. S. & Johansen, K. (1987) Blood flow uphill and downhill: does a siphon facilitate circulation above the heart? *Comp Biochem Physiol A*, 88(2), 167–170.

Shannon, R. P., Maher, K. A., Santinga, J. T., Royal, H. D., & Wei, J. Y. (1991) Comparison of differences in the hemodynamic response to passive postural stress in healthy subjects greater than 70 years years and less than 30 years of age. *Am J Cardiol*, 67(13), 1110-1116.

Shaw, T. G., Mortel, K. F., Meyer, J. S., Rogers, R. L., Hardenberg, J., Cutaia, M. M. (1984) Cerebral blood flow changes in benign aging and cerebrovascular disease. *Neurology*, 34(7), 855–862.

Shi, X., Gallagher, K. M., Welch-O'Connor, R. M., & Foresman, B. H. (1996) Arterial and cardiopulmonary baroreflexes in 60- to 69- vs. 18- to 36-yr old humans. *J Appl Physiol*, 80(6), 1903– 1910.

Shi, X., Schaller, F. A., Tierney, N., Chanthavong, P., Chen, S., Raven, P. B., & Smith, M. L. (2008) Physically active lifestyle enhances vagal-cardiac function but not central autonomic neural interaction in elderly humans. *Exp Biol Med*, 233(2), 209-218.

Shi, X., Wray, D. W., Formes, K. J., Wang, H. W., Hayes, P. M., O-Yurvati, A. H., Weiss, M. S., & Reese, I. P. (2000) Orthostatic hypotension in aging humans. *Am J Physiol Heart Circ Physiol*, 279(4), H1548-H1554.

Shibao, C., Grijalva, C. G., Raj, S. R., Biaggiono, I., & Griffin, M. R. (2007) Orthostatic hypotension-related hospitalizations in the United States. *Am J Med*, 120(11), 975-980.

Shibasaki, M., Wilson, T. E., Bundgaard-Nelsen, M., Seifert, T., Secher, N. H. & Crandall, C. G. (2011) Modelfow underestimates cardiac output in heat-stressed individuals. *Am J Physiol Regul Integr Comp Physiol*, 300(2), R486-R491.

Shvartz E & Meyerstein N. (1972) Relation of tilt tolerance to aerobic capacity and physical characteristics. *Aerospace Med*, 43(3), 278–280.

Simon, A. & Levenson, J. (1991) Use of arterial compliance for evaluation of hypertension. *Am J Hypertens*, 4(1 Pt 1), 97-105.

Sjostrand, T. (1953) Volume and distribution of blood and their significance in regulating the circulation. *Physiol Rev*, 33(2), 202-228.

Smit, A. A., Halliwill, J. R., Low, P. A., & Wieling, W. (1999) Pathophysiological basis of orthostatic hypotension in autonomic failure. *J Physiol*, 519(1), 1-10.

Smith, E. G., Voyles, W. F., Kirby, B. S., Markwald, R. R., Dinenno, F. A. (2007) Ageing and leg postjunctional α-adrenergic vasoconstrictor responsiveness in healthy men. *J Physiol,* 582(Pt 1), 63-71.

Smith J. J. & Ebert, T. S. (1990). General response to orthostatic stress. In *Circulatory response to upright posture* (pp. 1-46). Boca Raton, FL: CRC Press.

Smith, J. J. & Porth, C. M. (1991) Posture and the circulation: the age effect. *Exp Gerontol,* 26(2-3), 141-162.

Sorond, F. A., Khavari, R., Serrador, J. M., & Lipsitz, L. A. (2005) Regional cerebral autoregulation during orthostatic stress: age-realted differences. *J Gerontol A Biol Sci Med Sci,* 60(11), 1484-1487.

Sprangers, R. L., Wesseling, K. H., Imholz, A. L., Imholz, B. P., Wieling, W. (1991) Initial blood pressure fall on stand up and exercise explained by changes in total peripheral resistance. *J Appl Physiol,* 70(2), 523–530.

Strandell, T., (1964) Circulatory studies in healthy old men. *Acta Med Scand,* 414(Suppl. 414), 1-44.

Stevens, G. H., Foresman, B. H., Shi, X., Stern, S. A., & Raven, P. B. (1992) Reduction in LBNP tolerance following prolonged endurance exercise training. *Med Sci Sports Exerc,* 24(11), 1235–1244.

Stevens, P. M. & Lamb, L. E. (1965) Effects of lower body negative pressure on the cardiovascular system. *Am J Cardiol,* 16(4), 506-515.

Studinger, P., Goldstein, R., & Taylor, J. A. (2009) Age- and fitness-related alterations in vascular sympathetic control. *J Physiol,* 587(9), 2049-2057.

Sugawara, J., Tanabe, T., Miyachi, M., Yamamoto, K., Takahashi, K., Iemitsu, M., Otsuki, T., Homma, S., Maeda, S., Ajisaka, R. & Matsuda, M. (2003) Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiol Scand,* 179(4), 361–366.

Sugiyama, Y., Matsukawa, T., Shamsuzzaman, A. S., Okada, H., Watanabe, T., & Mano, T. (1996) Delayed and diminished pressor response to muscle sympathetic nerve activity in elderly. *J Appl Physiol,* 80(3), 869–875.

Sunagawa, K., Maughan, W. L., Burkhoff, D. & Sagawa, K. (1983) Left ventricular interaction with arterial load studied in the isolated canine ventricle. *Am J Physiol,* 245(5 Pt 1), H773–H780.

Sundlöf, G. & Wallin, B. G. (1978) Effect of lower body negative pressure on human muscle sympathetic activity. *J Physiol,* 278, 525-532.

Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A., & Seals, D. R. (2000) Aging, habitual exercise, and dynamic arterial compliance. *Circulation*, 102(11), 1270–1275.

Taneja, I., Moran, C., Medow, M. S., Glover, J. L., Montgomery, L. D., & Stewart, J. M. (2007) Differential effects of lower body negative pressure and upright tilt on splanchnic blood volume. *Am J Physiol Heart Circ Physiol*, 292(3), 1420-1426.

Taylor, J. A., Hand, G. A., Johnson, D. G., Seals, D. R. (1992) Sympathoadrenal-circulatory regulation of arterial pressure during orthostatic stress in young and older men. *Am J Physiol*, 263(5 Pt 2), R1147–R1155.

Thijs, R. D., Bruijnzeels, M., Kamper, A. M., & van Dijk, J. G. (2007) Assessment of orthostatic fluid shifts with strain gauge plethysmography. *Clin Sci (Lond)*, 113(9), 369-374.

Thompson, W. O., Thompson, P. K., & Dailey, M. E. (1928) Effect of posture on the composition of blood in man. *J Clin Invest*, 5(4), 573-604.

Tiecks, F. P., Lam, A. M., Matta, B. F., Strebel, S., Douville, C., & Newell, D. W. (1995) Effects of the valsalva maneuver on cerebral circulation in healthy adults. A transcranial Doppler Study. *Stroke*, 26(8), 1386-1392.

Tinetti, M. E., Mendes de Leon, C. F., Doucette, J. T., & Baker, D. I. (1994) Fear of falling and fall related efficacy in relationship to functioning among community-living elders. *J Gerontol*, 49(3), M140-M147.

Tordi, N., Perry, S., Harvey, A., & Hughson, R. L. (2003) Oxygen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans. *J Appl Physiol*, 94(2), 533-541.

Treib, J., Haass, A., Koch, D., Grauer, M. T. & Schimrigk, K. (1996) Influence of blood pressure and cardiac output on cerebral blood flow and autoregulation in acute stroke measured by TCD. *Eur J Neurol*, 3(6), 539 –543.

Tsutsui, Y., Sagawa, S., Yamauchi, K., Endo, Y., Yamazaki, F., & Shiraki, K. (2002) Cardiovascular responses to lower body negative pressure in the elderly: role of reduced leg compliance. *Gerontology*, 48(3), 133–139.

Turner, J. M., Powell, D., Gibson, R. M., & McDowall, D. G. (1977) Intracranial pressure changes in neurosurgical patients during hypotension induced with sodium nitroprusside or trimetaphan. *Br J Anaesth*, 49(5), 419-425.

Ursino, M. & Lodi, C. A. (1998) Interaction among autoregulation, CO_2 reactivity, and intracranial pressure: a mathematical model. *Am J Physiol*, 274(5), H1715-H1728.

Vaitkevicius, P. V., Fleg, J. L., Engel, J. H., O'Connor, F. C., Wright, J. G., Lakatta, L. E., Yin, F. C., & Lakatta, E. G. (1993) Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation*, 88(4 Pt 1), 1456-1462.

Valdueza, J. M., Balzer, J. O., Villringer, A., Vogl, T. J., Kutter, R., & Einhäupl, K. M. (1997) Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *AJNR Am J Neuroradiol*, 18(10), 1929-1934.

Valdueza, J. M., Draganski, B., Hoffmann, O., Dirnagl, U., & Einhäupl, K. M. (1999) Analysis of CO_2 vasomotor reactivity and vessel diameter changes by simultaneous venous and arterial Doppler recordings. *Stroke*, 30(1), 81-86.

Valdueza, J. M., von Munster, T., Hoffman, O., Schreiber, S. J., Einhaupl, K. M. (2000) Postural dependency of the cerebral venous outflow. *Lancet*, 355(9199), 200–201.

van Beek, A. H., Claassen, J. A., Rikkert, M. G., & Jansen, R. W. (2008) Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab*, 28(6), 1071-1085.

van Beek, A. H., Olde Rikkert, M. G., Pasman, J. W., Hopman, M. T., Claassen, J. A. (2010) Dynamic cerebral autoregulation in the old using a repeated sit-stand maneuver. *Ultrasound Med Biol*, 36(2), 192-201.

van Hoeyweghen, R., Hanson, J., Stewart, M. J., Dethune, L., Davies, I., Little, R. A., Horan, M. A., Kirkman, E. (2001) Cardiovascular response to graded lower body negative pressure in young and elderly man. *Exp Physiol*, 86(3), 427-35.

van Lieshout, J. J., Wieling, W., Karemaker, J. M., & Secher, N. H. (2003) Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol*, 94(3), 833-848.

Vasan, R. S., Martin, G., Larson, M. G. & Levy, D. (1995) Determinants of Echocardiographic Aortic Root Size - The Framingham Heart Study. *Circulation*, 91(3), 734-740.

Veith, R. C., Featherstone, J. A., Linares, O. A., & Halter, J. B. (1986) Age differences in plasma norepinephrine kinetics in humans. *J Gerontol*, 41(3), 319–324.

Virmani, R., Avolio, A. P., Mergner, W. J., Robinowitz, M., Herderick, E. E., Cornhill, J. F., Guo, S. Y., Liu, T. H., Ou, D. Y., & O'Rouke, M. (1991) Effect of aging on aortic morhphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. *Am J Pathol*, 139(5), 1119-1129.

Walters, F. J. M. (1998) Intracranial pressure and cerebral blood flow. *Update in Anaesthesia*, 8(4), 1-4.

Watenpaugh, D. E., Ballard, R. E., Schneider, S. M., Lee, S. M., Ertl, A. C., William, J. M., Boda, W. L., Hutchinson, K. J., & Hargens, A. R. (2000) Supine lower body negative pressure exercise during bed rest maintains upright exercise capacity. *J Appl Physiol*, 89(1), 218-227.

Watenpaugh, D. E., Ballard, R. E., Stout, M. S., Murthy, G., Whalen, R. T., & Hargens, A. R. (1994) Dynamic leg exercise improves tolerance to lower body negative pressure. *Aviat Space Environ Med*, 65(5), 412–8.

Watenpaugh, D. E., Breit, G. A., Buckley, T. M., Ballard, R. E., Murthy, G., & Hargens, A. R. (2004) Human cutaneous vascular responses to whole-body tilting, Gz centrifugation, and LBNP. *J Appl Physiol*, 96(6), 2153-2160.

Wei, E. P., Raper, A. J., Kontos, H. A & Patterson, J. L. (1975) Determinants of response of pial arteries to norepinephrine and sympathetic nerve stimulation. *Stroke,* 6(6), 654-658.

Wesseling, K. H., Jansen, J. R., Settels, J. J. & Schreuder, J. J. (1993) Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*, 74(5), 2566–2573.

White, R. P., Deane, C., Vallance, P., & Markus, H. S. (1988) Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. *Stroke,* 29(2), 467-472.

Whitsett, T. L., Manion, C. V. & Christensen, H. D. (1984) Cardiovascular effects of coffee and caffeine. *Am J Cardiol*, 53(7), 9l8-22.

Wolthuis, R. A., Bergman, S. A., & Nicogossian, A. E. (1974) Physiological effects of locally applied reduced pressure in man. *Physiol Rev,* 54(3), 566-595.

Youde, J., Panerai, R., Gillies, C., & Potter, J. (2003) Reproducibility of circulatory changes to head-up tilt in healthy elderly subjects. *Age Ageing*, 32(4), 375-381.

Young, C. N., Stillabower, M. E., DiSabatino, A. & Farquhar, W. B. (2006) Venous smooth muscle tone and responsiveness in older adults. *J Appl Physiol*, 101(5), 1362–1367.

Zhang, R. & Levine, B. D. (2007) Autonomic ganglionic blockade does not prevent reduction in cerebral blood flow velocity during orthostasis in humans. *Stroke,* 38(4), 1238-1244.

Zhang, R., Witkowski, S., Fu, Q., Claassen, J. A., & Levine, B. D. (2007) Cerebral hemodynamics after short- and longterm reduction in blood pressure in mild and moderate hypertension. *Hypertension*, 49(5), 1149–1155.

Zhang, R., Zuckerman, J. H., Iwasaki, K., Wilson, T. E., Crandall, C. G., & Levine, B. D. (2002) Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation,* 106(14), 1814-1920.

Ziegler, M. G., Lake, C. R., & Kopin, I. J. (1977) The sympathetic-nervous-system defect in primary orthostatic hypotension. *N Engl J Med*, 296(6), 293-297.

Zieman, S. J., Melenovsky, V. & Kass, D. A. (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol*, 25(5), 932–943.

Zoller, R. P., Mark, A. L., Abboud, F. M., Schmid, P. G., & Heistad, D. D. (1972) The role of low pressure baroreceptos in reflex vasoconstrictor responses in man. *J Clin Invest,* 51(11), 2967-2972.