The Effect of Spironolactone on Cerebral Blood Flow and Cognition

by

Jason Xeni

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of

Doctor of Philosophy
in
Kinesiology

Waterloo, Ontario, Canada, 2018

Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

External Examiner	Kevin S. Heffernan Dean's Associate Professor Department of Exercise Science Syracuse University
Supervisor	Richard L. Hughson Professor Department of Kinesiology University of Waterloo
Internal Member	Laura E. Middleton Assistant Professor Department of Kinesiology University of Waterloo
Internal-External Member	George A. Heckman Associate Professor School of Public Health and Health Systems University of Waterloo
Internal Member	Robert S. McKelvie Adjunct Professor Department of Kinesiology University of Waterloo

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.

I understand that my thesis may be made electronically available to the public.

Abstract

Hypertension and arterial stiffness are associated with structural and functional changes in cerebral circulation and cognitive function, but based on existing evidence the effects are potentially reversible by spironolactone (Spiro) reducing blood pressure (BP) and arterial stiffness. This thesis consisted of a double-blind, controlled trial with older hypertensive adults (OA) who were receiving stable treatment with centrally acting angiotensin converting enzyme inhibitors randomly assigned to Spiro or placebo to test the hypothesis that reductions in BP and arterial stiffness would be associated with improved anterior cerebral blood flow (aCBF) and cognitive function. Secondary objectives were to investigate how Spiro would affect cerebrovascular autoregulation and to conduct a supplementary experimental study in younger adults (YA) comparing blood pressure and arterial stiffness to OA; and to determine how induced acute small changes in BP could impact interpretation of the chronic changes in BP and arterial stiffness associated with Spiro therapy.

The pooled data from the randomized, controlled trial (RCT) in OA (n=18) showed that age, mean arterial pressure (MAP), systolic (SBP), diastolic (DBP) and pulse pressure (PP) were 65±3 years, 97±9 mmHg, 142±16 mmHg, 75±8 mmHg, and 66±13 mmHg respectively. There was a significant association between age and carotid distensibility coefficient ($r = -0.51$, $P<0.05$). Six months of Spiro significantly reduced SBP and PP by 14±14 mmHg and 12±14 mmHg respectively. However arterial stiffness estimated by regional indicator carotid-femoral pulse wave velocity (cfPWV), or local indicators carotid distensibility coefficient and β-stiffness index, remained unchanged. Spiro did not significantly improve aCBF or cognitive function scores.

Cerebrovascular autoregulation response to standing upright remained unchanged after Spiro compared to placebo. Mean adherence to study-drug was at least 95% for both groups. The calculated Cohen's *d* effect size for Spiro was 0.3 from this thesis RCT data; much smaller than the desired Cohen's *d* effect size of 1.0 that was derived from observational data used to calculate sample size for the RCT. The supplementary experimental study in YA (n=14) demonstrated that OA in the RCT had greater MAP (97±9 mmHg vs 86±9 mmHg, P<0.01), SBP (142±16 mmHg vs 124±12 mmHg, P<0.01), DBP (75±8 mmHg vs 67±9 mmHg, P<0.05), PP (66±13 mmHg vs 57±7 mmHg, P<0.05), and arterial stiffness was greater as indicated by faster cfPWV (7.22±1.09 m/s vs 5.43±1.13 m/s, P<0.001), smaller carotid distensibility coefficient (0.0014±0.0006 $mmHg^{-1}$ vs 0.0031±0.0008, P<0.001), and greater β-stiffness index (8.78±3.53 a.u. vs 3.74±0.91, P<0.001). Application of lower body negative pressure to induce acute hemodynamic changes in these YA reduced stroke volume (P<0.001) and cardiac output (P<0.001); and increased total peripheral resistance (P<0.001) while MAP remained unchanged. There were also small acute decreases in both SBP (P<0.05) and PP (P<0.001) in these YA that were concurrent with a non-significant increase in arterial stiffness (cfPWV increase, carotid distensibility coefficient decrease, β-stiffness index increase). Transit times from R-peak of QRS complex to foot of aortic velocity pulse, carotid artery, or finger artery significantly increased with progressively increasing LBNP as a consequence of longer pre-ejection period (P<0.001).

In conclusion for the main thesis objective, Spiro safely and effectively reduced BP while arterial stiffness, aCBF, cognitive function, and cerebrovascular autoregulation remained unchanged. OA, compared to YA, had greater blood pressure and arterial stiffness for all measures. In YA,

acute reductions in systolic and pulse pressure affected stiffness indicators, in contrast to unchanged stiffness indicators observed in OA. These limited data should be interpreted with caution given the small sample size in the RCT, small effect size of Spiro and that acute reductions in SBP and PP may affect arterial stiffness.

Acknowledgements

This thesis would not have been possible without support from very important people. I am eternally grateful to my advisor, Professor Richard Hughson, for his critical guidance along this journey, and how easily he provided opportunity for academic and personal growth.

It is a pleasure to thank my committee, George Heckman, Bob McKelvie, and Laura Middleton. I appreciated your unique insights into my research and encouragement on how, and especially why, to finish. I wish to also thank Kevin Heffernan for serving as external examiner.

I wish to express sincere gratitude to all the research team in the Hughson Lab that contributed to this thesis in some way. To Danielle, Andrew, Laura, Katelyn, Dianne, Kathryn, Tom, Chantel and even Rodrigo, thank you.

To my wife Paula, and children Jack and Julia, thank you for patiently allowing me to show you I could do it.

Dedication

To Jack.

He was a man. Take him for all in all.

I shall not look upon his like again.

Hamlet, Act 1, Scene 2

Table of Contents

List of Equations

List of Figures

List of Tables

List of Abbreviations

ACE	Angiotensin converting enzyme
ATII	Angiotensin II
aCBF	anterior Cerebral Blood Flow
adTT	aorta-digit Transit Time
acTT	aorta-carotid Transit Time
BBB	Blood brain barrier
BP	Blood Pressure
CCA	Common Carotid Artery
CO	Cardiac Output
CO_2	Carbon dioxide
CSA	Cross-Sectional Area
CVA	Cerebrovascular autoregulation
CVC	Cerebrovascular Conductance
CVRi	Cerebrovascular Resistance Index
DBP	Diastolic Blood Pressure (c-carotid)
Dia	Diameter (s-systolic and d-diastolic)
eGFR	Estimated Glomerular Filtration Rate (MDRD)
$ETCO_2$	End-tidal carbon dioxide
K^+	Serum potassium
hcTT	heart-carotid Transit Time (i-index, v-velocity)

hdTT	heart-digit Transit Time (i-index)
LBNP	Lower Body Negative Pressure
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCI	Mild cognitive impairment
MoCA	Montreal Cognitive Assessment
MMP	Matrix metalloproteinase
MMSE	Mini-Mental State Exam
PEP	Pre-Ejection Phase
PP	Pulse Pressure (c-carotid)
PWV	Pulse Wave Velocity (cf-carotid-femoral)
RAAS	Renin-angiotensin aldosterone system
SV	Stroke Volume
SBP	Systolic Blood Pressure (c-carotid)
TCD	Transcranial Doppler
TD	Transit Distance
TPR	Total peripheral resistance
TT	Transit Time

CHAPTER 1 Effect of Spironolactone on Cerebral Blood Flow and Cognition

The purpose of this chapter is to provide supportive background information on arterial aging, hypertension, arterial stiffness, cerebral blood flow and cognitive function for subsequent thesis chapters examining the effect of spironolactone on blood pressure, arterial stiffness, cerebral blood flow and cognition. Another important purpose is to outline thesis objectives, research questions, and hypotheses.

1.1 An Aging Canada

Canadians are aging. The median age of the Canadian population in 2011 was 39.9 years, up from 26.2 years in 1971 (Milan, 2010). The fastest growing age group is 65 years and older, likely reflecting an increase in life expectancy and the aging of the baby boomer generation. Five million Canadians were at least 65 years old in 2011, and this number is expected to double by 2035, so that by 2051 about a quarter of Canadians is expected to be over 65 (StatisticsCanada, 2011).

1.1.1 Age is an Important Risk Factor for Hypertension

Hypertension, defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, affects about 1 in 5 of the Canadian adult population (Daskalopoulou et al., 2015; Robitaille et al., 2012). Age is an important risk factor for developing hypertension due to

changes in artery wall structure, endothelial dysfunction, up-regulation of renin-angiotensin-aldosterone system (RAAS) activity and rise in pulse pressure. The structural changes that occur within large elastic arteries include intimal wall thickening with increased matrix proteins such as collagen, fragmentation of elastic lamellae possibly due to repetitive strain injury, and consequently dilatation that is accompanied by a reduction in compliance and increase in vascular stiffness (Fukutomi & Kario, 2010; Nilsson, 2008). Other key features of early vascular aging include chronic vascular inflammation, capillary rarefaction and dysfunctional regulation, oxidative stress, impairment of nitric oxide pathway, and arterial calcification (Nilsson, 2008). These age-related pathophysiological changes contribute to the development of hypertension by increasing central pulse pressure from increased arterial stiffness, exacerbating systolic hypertension, and arterial pressure wave reflection from an increase in pulse wave velocity, and narrowing of resistance arteries through inward eutrophic remodeling raising peripheral resistance (Buus et al., 2013; Mitchell, 2008).

1.1.2 Aldosterone is Associated with Reversible Vascular Changes

Angiotensin II (AT II) is an integral part of the RAAS designed to regulate blood pressure. Briefly, the proteolytic enzyme renin converts angiotensinogen to angiotensin I, which is converted to AT II by the angiotensin converting enzyme (ACE) in the vascular endothelium. AT II stimulates aldosterone secretion from the adrenal cortex leading to salt and water retention maintaining plasma volume, acts as a direct vasoconstrictor and stimulates sympathetic activity raising peripheral resistance, and plays an integral role in the development of vascular disease partly

through activation of matrix metalloproteinase (MMP) (M. Wang, Kim, Monticone, & Lakatta, 2015). MMPs are enzymes involved in arterial wall extracellular matrix degradation and remodelling. Independent of the effects of AT II on activation of MMPs, aldosterone induces reversible functional and structural vascular changes that increase systemic vascular resistance and arterial stiffness (Davies, Gavin, Band, Morris, & Struthers, 2005; Farquharson & Struthers, 2000, 2002; Kithas & Supiano, 2010; Mahmud & Feely, 2005). Functional changes related to aldosterone include endothelial dysfunction, increased sympathetic tone, sodium retention, vascular inflammation and oxidative stress leading to reduced nitric oxide and endothelial-derived hyperpolarizing factor bioavailability (Barrett, McCurley, & Jaffe, 2013; Bellien et al., 2010; Duprez, 2007; Farquharson & Struthers, 2002; Zieman, Melenovsky, & Kass, 2005). Structural changes include elevated deposition of collagen, up-regulation of angiotensin II receptors, smooth muscle hypertrophy, and elevated vasoconstrictive and fibrotic effects linked with endothelin-1 (Bernini et al., 2008; Zieman et al., 2005). These detrimental, yet potentially reversible, changes have led to key findings that high plasma aldosterone is also associated with reduced aortic compliance and mild cognitive impairment (Scuteri, Brancati, Gianni, Assisi, & Volpe, 2005; Yagi et al., 2011). From the existing literature, it appears that aldosterone activates pathways responsible for functional and structural changes in cerebral blood vessels that could affect cerebral blood flow; consequently the aldosterone antagonist spironolactone was chosen for this thesis research.

1.2 Local and Regional Arterial Stiffness Indicators

Stiffness is the resistance offered by an elastic body to deformation. All measures of stiffness ultimately represent relations between forces applied (e.g., blood pressure) to an elastic body (e.g., arterial wall) which result in mechanical stress and deformation (strain). This circumferential wall stress in arteries can be estimated by the direct relationship with the vessel radius and transluminal pressure, and inverse relationship to wall thickness. For arteries, the pressure-strain relationship is not linear: at higher applied forces vessels become stiffer due to the curvilinear nature of arterial compliance. Acute changes in blood pressure shift the operational point of the arterial compliance curve to the steeper, flatter portion, resulting in functionally stiffer arteries on the same arterial compliance curve (Chirinos, 2012; Shibata & Levine, 2011). Therefore, assessment of arterial stiffness will depend on the ambient blood pressure, and introduces an important confounding variable by changing operational point on the same compliance curve, versus mechanically stiffer arteries that operate on a different compliance curve. CHAPTER 7 explores how these indicators of arterial stiffness are affected by acute small changes in blood pressure. The mechanical properties of arteries can be expressed using indicators that relate local changes in pressure and changes in volume, diameter, or area. Pressure-volume relations are influenced by stiffness of the arterial wall. Examples of indicators of local arterial stiffness include the carotid distensibility coefficient and β-stiffness index. Distensibility (arterial compliance) is the fractional change in arterial volume relative to the change in arterial pressure. The β-stiffness index is the natural logarithm of the ratio of maximum-to-minimum pressure relative to a fractional change in diameter. Due to the mathematical correction of the logarithm of maximum-to-minimum pressure, values within individuals may be less sensitive to distending blood pressure (Chirinos, 2012; Shibata & Levine,

2011). These arterial stiffness indicators are derived from local pressure-volume measurements. There are, however, regional indicators of arterial stiffness not derived from pressure-volume or pressure-diameter relationships; such as transit time (TT) and pulse wave velocity (PWV).

TT is a useful and robust index of arterial stiffness that represents a functional parameter directly affected by arterial wall stiffness. TT is calculated as the time delay between the R-peak of the QRS complex and the time at which the pulse wave arrives at the arterial site within the same cardiac cycle. There are two distinct components of TT: Pre-ejection period (PEP), which is the time from the start of ventricular depolarization to the onset of ventricular ejection, and the time delay for the arterial pulse wave to travel from the aortic valve to a point in the peripheral arteries. PWV is simply speed the pulse wave travels between two arterial sites (such as the carotid and femoral arteries) that is calculated as the ratio of transit distance to TT.

Carotid-femoral PWV (cfPWV) is the gold standard index of arterial stiffness due to the relative ease in determination and growing evidence demonstrating its association with incident cardiovascular disease and all-cause mortality (Laurent et al., 2001; Laurent et al., 2006; Meaume et al., 2001; Mitchell et al., 2010; O'Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002; Sutton-Tyrrell et al., 2005). Normal aging, which may be amplified byhypertension, is accompanied by increased arterial stiffness, pulse pressure, and PWV, leading to a decrease in pulse wave transit time (Franklin et al., 1997; Hasegawa, Nagao, Kinoshita, Rodbard, & Asahina, 1997; Koivistoinen et al., 2007; Mitchell et al., 2004; Schiffrin, 2004). PWV is directly related to

arterial distensibility (Young's elastic modulus expressed as the ratio of tensile stress to tensile strain), wall thickness, interior diameter and density of blood that is estimated with the Moens-Korteweg equation for wave propagation within a vessel of uniform geometry and properties: $PWV^2 = Eh/2R\rho$. Where PWV denotes the speed at which the pressure wave propagates, *E* is the Young's modulus of elasticity (material stiffness), *h* is the vessel wall thickness, *R* is the internal vessel radius, and ρ is the density of blood (Hughes, Babbs, Geddes, & Bourland, 1979; Nichols, 2011). With *Eh* the structural stiffness, this equation shows that increases in either material stiffness or wall thickness can impact hemodynamics equally. The actual arterial system is obviously not a simple cylindrical vessel but branches, tapers, and terminates as microcirculation. These geometric changes along the arterial system path toward the microcirculation are sites of changing resistance to flow (impedance) that cause pressure wave reflections back towards the heart (Mitchell, 2009). Therefore arterial pressure is the sum of forward and backward travelling waves at that site. When wave reflection occurs at the microcirculation, there is minimal time delay between forward and backward waves leading to optimal overlap with wave reflection. Consequently, pressure pulse waves are amplified with increasing distance from the heart (Nichols, 2011).

Existing studies have shown a significant correlation between cerebral blood flow (total cerebral blood flow measured using MRI) and cfPWV (Tarumi et al., 2014; Xing et al., 2017). There is also evidence of higher cfPWV being independently associated with manifestations of brain small-vessel disease (i.e., greater volume of white matter hyperintensities) in hypertensive patients (Henskens et al., 2008). Cerebral circulation is a high flow, low impedance,

system particularly susceptible to hemodynamic pulsatility in the rigid structure of the skull. Loss of stiffness gradient between aorta and muscular arteries leading to impedance matching may enhance transmission of pulsatile hemodynamic energy into fine penetrating cerebral circulation. Deleterious hemodynamic pulsatility may contribute to damage of deep microcirculation manifesting as white matter hyperintensities and cognitive dysfunction. Cerebrovascular structure and function are reviewed in the following section 1.3 to provide background to the subsequent thesis chapters on cerebral blood flow.

1.3 Cerebral Blood Flow

The brain is one of the most highly perfused organs in the body receiving 14% of resting cardiac output and accounts for nearly 20% of resting oxygen consumption. The special arterial adaption in the brain called the Circle of Willis (Figure 1) helps safeguard this high demand of oxygen-rich blood by forming collateral circulation from vascular networks that allows for redistribution of blood flow when extracranial or large intracranial vessels are occluded. The arterial blood supply to the brain reaches the Circle of Willis through two pairs of large arteries, the right and left internal carotid and the right and left vertebral arteries which branch off of the subclavian arteries (A. A. Phillips, Chan, Zheng, Krassioukov, & Ainslie, 2016). The internal carotid arteries transmit approximately 70% of total brain blood flow, while the remainder is provided by the vertebral arteries. The two vertebral arteries join to form the basilar artery that anastomoses with internal carotid arteries at the Circle of Willis that gives rise to three pairs of main arteries, the anterior, middle, and posterior cerebral arteries. The vertebral arteries primarily provide blood flow to the brainstem, cerebellum, and occipital cortex.

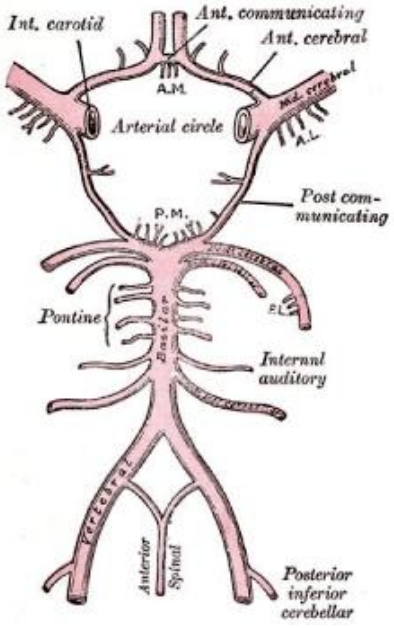


Figure 1 Circle of Willis

(Cipolla, 2009)

The anterior, middle, and posterior arteries divide into progressively smaller vasoactive pial arteries that run on the surface of the brain within the pia-arachnoid space, then branch into deep penetrating arterioles that become parenchymal arterioles within the brain. Pial arteries on the brain surface have perivascular nerves and as penetrating arterioles become parenchymal arterioles with the brain, they become associated with neurons and astrocytes (Cipolla, 2009). Extracranial arteries (internal carotid and vertebral arteries), large arteries of the brain (e.g., middle cerebral artery) and pial arteries are all innervated by sympathetic and parasympathetic neurons. Unlike parenchymal arterioles which are regulated by neuronal

activation and astrocytic modulation (A. A. Phillips et al., 2016). All arteries of the brain are lined with specialized endothelial cells that tightly regulate exchange between the blood supply and the brain, called the blood-brain barrier. Importantly, certain drugs like ACE inhibitors can cross the blood-brain barrier, thus affecting arterial structure and function.

1.3.1 Cerebrovascular Autoregulation is Critical to Maintain Cerebral Blood Flow

Derived from Ohm's law, cerebral blood flow (CBF) is directly proportional to cerebral perfusion pressure (CPP = MAP minus intra-cranial pressure) and inversely proportional to cerebrovascular vascular resistance (Willie, Tzeng, Fisher, & Ainslie, 2014). Under steady-state conditions, MAP is determined by cardiac output and total vascular resistance. Importantly, cerebrovascular resistance can have a significant effect on CBF by even small changes in lumen diameter (Ainslie & Tzeng, 2010). Autoregulation of CBF is the automated ability of the brain to maintain relatively constant blood flow despite changes in CPP (Aaslid, Markwalder, & Nornes, 1982). This special task to maintain O_2 supply (seemingly at all costs) with changing CPP has likely evolved to ensure the brain of 2% body mass, continues to get about 14% of resting cardiac output, representing 20% of resting O_2 consumption (Levick, 2010). In normotensive adults, CBF is maintained at ~50ml per 100g whole brain tissue, provided CPP is between 60-160 mmHg (S. J. Phillips & Whisnant, 1992). Above and below this wide CPP range, autoregulation is lost and CBF becomes dependent on MAP (van Beek, Claassen, Rikkert, & Jansen, 2008). To maintain stable CBF in response to changes in CPP, cerebral arteries adapt cerebrovascular resistance (CVR) by means of arterial blood gases (CO_2 and O_2), myogenic,

neurogenic, or metabolic mechanisms (Ainslie & Tzeng, 2010; A. M. Brown & Ransom, 2007; Willie et al., 2014).

Cerebrovascular reactivity reflects the compensatory dilatory capacity of cerebral arterioles to a dilatory stimulus and is an important mechanism for maintaining CBF by adapting CVR. Carbon dioxide (CO_2) has a profound and reversible effect on CBF, such that hypercapnia causes dilation of cerebral arteries and arterioles and increase blood flow, whereas hypocapnia causes vascular constriction and decreased blood flow (Battisti-Charbonney, Fisher, & Duffin, 2011; Skow et al., 2013; Willie et al., 2012). This sensitivity to CO_2 appears to be more uniform across hypercapnic range compared to responses during hypocapnia (Willie et al., 2012). Since alteration in alveolar gas exchange (either in alveolar ventilation or metabolic generation of CO_2) produces concomitant changes in both partial pressure of CO_2 and pH, it is likely that both are responsible for changes in CBF. Vasodilation is partly mediated through endothelial nitric oxide and partly by diffusion of non-polar CO_2 molecules across the blood-brain barrier that can induce a corresponding pH change in the extracellular space surrounding pial arteries, and thus alter smooth muscle tone (Willie et al., 2012). A normal cerebrovascular reactivity to CO_2 is very important for a physiologic blood supply to the brain and an impaired cerebrovascular reactivity to CO_2 has been associated with higher risk of stroke, vascular complications such as microalbuminuria and retinopathy in diabetes mellitus, described in non-controlled hypertension, and even under the condition of acute major depression (Fulesdi et al., 1997; Neu, Schlattmann, Schilling, & Hartmann, 2004; Settakis et al., 2003; Silvestrini, Troisi, Matteis, Cupini, & Bernardi, 1996; Yonas, Smith, Durham, Pentheny, & Johnson, 1993). Despite the drive

to maintain O_2 supply to the brain at all costs, vascular tone is less responsive to O_2 until a threshold of ~80% arterial saturation, or hypoxia reduces partial pressure of O_2 lower than ~50 mmHg, below which CBF increases substantially (Ainslie & Subudhi, 2014; Masamoto & Tanishita, 2009). This increase in CBF is accompanied by improved O_2 extraction from hemoglobin represented by a ~100% saturation at partial pressure of O_2 > 70 mmHg to about half saturation at partial pressure of O_2 < 50 mmHg (Johnston, Steiner, Gupta, & Menon, 2003). Hypoxia reduces cerebrovascular smooth muscle tone through a complex interplay of neurovascular coupling involving potassium and calcium channels, release of adenosine and production of nitric oxide (Ainslie & Tzeng, 2010).

Neurovascular coupling describes the tight relationship between local cerebral metabolism and local brain perfusion. Local metabolism can evoke functional hyperemia from vasoactive substances that include interstitial potassium ion, hydrogen ion, O_2 or adenosine from neural activity (Levick 2010). This coupling of an anatomic and metabolic relationship is between neurons, astrocyte glial cells and vascular smooth muscle cells of deep penetrating neurovascular unit (A. A. Phillips et al., 2016). Briefly, excitatory and inhibitory neurons synapse on both astrocytes and interneurons, where their interneurons are in close association with astrocytes which terminate and envelope cortex-penetrating arterioles (Iadecola & Nedergaard, 2007). Glutamate release by the synapse activates the release of nitric oxide from the interneuron and simultaneously activates epoxyeicosatrienoic acid and prostaglandin E2, which all serve to dilate cerebral arteries (A. A. Phillips et al., 2016). Autoregulation of CBF when pressure fluctuates at the high end of the autoregulatory curve is most likely due to the

myogenic behaviour of cerebral smooth muscle that constrict in response to elevated CPP and dilate in response to decrease pressure (Mellander, 1989; Osol, Brekke, McElroy-Yaggy, & Gokina, 2002).

Both extracranial and pial arteries are innervated by sympathetic neurons originating from the superior cervical ganglion which may serve to support cerebral blood flow through release of norepinephrine and neuropeptide Y (Hamel, 2006; Hamner, Tan, Lee, Cohen, & Taylor, 2010). Parasympathetic neurons from cranial nerves modulate cerebrovascular tone by releasing acetylcholine, vasoactive intestinal peptide, and nitric oxide although human data are lacking.

The response to a variation in CPP as an adaptation in CVR will adjust cerebral blood flow back to steady-state baseline. This hemodynamic process is called static cerebrovascular autoregulation (sCVA) when CPP changes gradually and dynamic CVA (dCVA) with more abrupt changes in CPP (Aaslid et al., 1982; Panerai, 1998; van Beek et al., 2008). However, the availability of transcranial Doppler (TCD) ultrasound and finger-cuff beat-to-beat monitoring of blood pressure have provided the opportunity to investigate the dynamic pressure-flow relationship with rapid changes in mean arterial pressure and cerebral blood flow velocity (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989; Sorond, Serrador, Jones, Shaffer, & Lipsitz, 2009; van Beek et al., 2008). These non-invasive tools will be used in this thesis to allow the high temporal resolution assessment of dynamic dCVA from changing posture from sitting to standing expressed as absolute and percent changes in CVR for the middle cerebral artery (MCA) blood velocity (BFV) and MAP: CVR = MAP/BFV(Lipsitz, Mukai, Hamner, Gagnon, &

Babikian, 2000; Sorond et al., 2009). This sit-to-stand posture change results in a transient fall in MAP resulting from several potential mechanisms that are associated with active rising: The rate of blood volume leaving the heart (cardiac output) into the arterial vasculature is temporarily less than the rate blood volume is leaving the arterial vasculature (vascular resistance) back to the heart; such that cardiac output is not matching peripheral resistance effects on arterial outflow (Wieling, Krediet, van Dijk, Linzer, & Tschakovsky, 2007). The reduction in vascular resistance has been attributed to rapid vasodilation in the working muscle through local intrinsic factors and possibly from an sudden rise in right atrial pressure from compression of intramuscular veins facilitating venous return that activates cardiopulmonary mechanoreceptors initiating an abrupt reflex withdrawal of sympathetic vasoconstrictor tone, both mechanisms leading to a subsequent transient fall in total systemic vascular resistance (Wieling et al., 2007). There is little consensus as to where cerebral resistance modulation actually takes place and how (myogenic or autonomic, or both) it is achieved (Willie et al., 2014).

1.3.2 Orthostatic Challenge Triggers Compensatory Hemodynamic Responses

As already reviewed in previous Section 1.3.1., the availability of transcranial Doppler ultrasound to measure CBF velocity and monitor beat-to-beat changes in blood pressure have allowed the opportunity to measure how older adults in this thesis respond to acute hypotension from standing before and after spironolactone. The immediate responses to standing from seated position are typically an increase in heart rate, an increase in cardiac

output, and a decrease in systemic vascular resistance with corresponding decrease in mean arterial blood pressure (Sorond et al., 2009; Wieling et al., 2007).

Orthostasis (maintaining upright posture) presents a serious challenge to the human circulation, because gravity causes redistribution of venous blood away from the heart. This is potentially similar to the orthostatic effects of progressively increasing lower body negative pressure as described in Section 1.6. Gravity increases the hydrostatic pressure component of transmural pressure in compliant veins, and the resulting distension increases the volume of blood by 500-800 mL in the lower body pulled from the thoracic vessels (Rowell, 1993). This reduces intrathoracic blood volume by 20% over ~15 seconds, lowering central venous pressure from 5 mmHg to ~0 mmHg and consequently reducing myocyte length-dependent force generation (through the Frank-Starling mechanism), lowering stroke volume by 30-40% prior to compensatory neuroendocrine responses needed to regain MAP (Levick, 2010). Consequently, the unloading of arterial baroreceptors (from reduced carotid pulse pressure and mean sinus pressure) and cardiopulmonary mechanoreceptors (from fall in central venous pressure) during orthostatic challenge triggers the following responses mediated through cardiac vagal withdrawal and increase sympathetic outflow (Breeuwsma et al., 2017; Levick, 2010; Rowell, 1993) after the first 60 seconds of quiet standing upright:

- Heart rate increases
- Right atrial mean pressure decreases
- Stroke volume decreases (despite increase in myocardial contractility and splanchnic venoconstriction)

- Cardiac output decreases (due to reduced stroke volume)
- Central blood volume decreases
- Peripheral vascular resistance increases in skeletal muscle, splanchnic and renal vascular beds
- MAP essentially unchanged (pulse pressure decreases due to a decrease in systolic pressure and increase in diastolic pressure)

1.4 Validated Cognitive Function Assessment Tools

Executive cognitive function is defined by cognitive skills that are responsible for complex decision making, goal-directed behaviour, initiation, sequencing, monitoring of complex behaviour, problem solving and delayed recall (Roman, 2003). A validated cognitive assessment tool such as The Montreal Cognitive Assessment (MoCA) can provide a brief measure of global cognitive function although originally developed to detect mild cognitive impairment (MCI) (Nasreddine et al., 2005). The MoCA is a single page 30-point cognitive test that takes about 15 minutes to complete. It reliably assesses several cognitive domains with an 87% specificity to exclude elderly normal controls and 90% sensitivity to detect MCI described as the transitional intermediate clinical state between normal cognitive aging and dementia. Cognitive domains include visuospatial abilities using a clock-drawing task and three-dimensional cube copy. A short-term memory recall task that involves learning five nouns and delayed recall after 5 minutes. Trail-Making B test assessing task switching by connecting progressively increasing numbers with alternating letters as quickly as possible. Attention, concentration, and working

memory are evaluated using a sustained attention task of tapping when a particular letter is called in a long sequence of random verbalized letters, serial subtraction by 7 starting at 100, and reciting back from memory progressively longer number sequences in forward or back order. Language is assessed by repetition of two syntactically complex sentences and reciting as many words as possible that begin with the letter "f" within 60 seconds. Finally, orientation in time and place are evaluated by asking the participant the day, month, year, and location the test is being conducted.

1.5 Hypertension and Arterial Stiffness are Risk Factors for Cognitive Impairment

Hypertension and central arterial stiffness are risk factors for cognitive impairment (Elias & Davey, 2009; Hanon et al., 2005; Paran, Anson, & Reuveni, 2003; Scuteri et al., 2005; Triantafyllidi et al., 2009; Waldstein et al., 2008; Waldstein et al., 2003). Arterial stiffness can occur with increasing age, and has been associated with worse cognitive performance (Elias & Davey, 2009). Of all the cognitive domains, executive function is particularly vulnerable to the pathological changes associated with hypertension and vascular stiffness (Kuo et al., 2004; Saxby, Harrington, McKeith, Wesnes, & Ford, 2003; Waldstein et al., 2008). It is estimated that close to 1 in 3 persons over the age of 60 may suffer from executive dysfunction based on the San Luis Valley Health and Aging Study (1313 individuals over 60 years old) as assessed by voluntary goal-oriented behaviour and self-regulation, and consequently may have significant difficulty following medical advice (D. A. Cahn-Weiner, P. F. Malloy, P. A. Boyle, M. Marran, & S. Salloway, 2000; J. Grigsby et al., 2002; D. R. Royall, D. V. Espino, M. J. Polk, R. F. Palmer, & K. S.

Markides, 2004; D. R. Royall, R. Palmer, L. K. Chiodo, & M. J. Polk, 2004). The incidence of more global cognitive impairment was observed in an epidemiological study of cognition in 1107 older patients in the primary care setting; they found 31% scored <25 on MMSE (Ganguli et al., 2004). Several cross-sectional studies in elderly, or patients with subjective memory complaints, have shown that central arterial stiffness was significantly and inversely related to executive function (as measured by the Cognitive Efficiency Profile) and difficulties in other cognitive domains (MMSE score and memory), as well as white matter hyperintensities and endothelial dysfunction (Elias & Davey, 2009; Hanon et al., 2005; Kearney-Schwartz et al., 2009; Scuteri et al., 2005; Triantafyllidi et al., 2009).

The exact mechanism by which hypertension and arterial stiffness affect cognitive function is unclear, although it likely involves cerebral hypoperfusion and pressure pulse wave amplification (de la Torre, 2000; Ruitenberg et al., 2005). CBF velocity (an estimate of brain blood flow) was assessed using transcranial Doppler in 1730 patients with dementia in the Rotterdam Study, to show that greater CBF velocity (and correspondingly brain blood flow) was related to significantly less cognitive decline over 6.5 years (Ruitenberg et al., 2005). Several small studies using MRI have shown lower cerebral blood flow associated with processing speed, executive function, and dementia (Alosco, Gunstad, et al., 2013; Alosco, Spitznagel, et al., 2013; Poels et al., 2008; Rabbitt et al., 2006). Hypertension has been associated with a decrease in CBF and the treatment of uncontrolled hypertensive patients showed significant increases in CBF velocity (Ameriso, Paganini-Hill, Meiselman, & Fisher, 1990; Lipsitz et al., 2005). Several longitudinal trials have suggested that antihypertensive treatments may be beneficial at

preventing cognitive decline (Duron & Hanon, 2010). The first randomized controlled trial to show reduction in incidence of predefined secondary outcome of dementia (-50% over 2 years, p=0.05, calcium channel blocker vs placebo) was the SYST-EUR trial in isolated systolic hypertensive patients (Forette et al., 1998; Staessen, Birkenhager, Fagard, & Forette, 1998).

1.5.1 Potentially Reversible Effects of Aldosterone Antagonism on Arterial Stiffness

Drugs that inhibit the RAAS have demonstrated reductions in arterial stiffness beyond blood pressure reduction in hypertensive older adults, as well as in patients with diabetes and heart failure (Mahmud & Feely, 2004). Aldosterone antagonists in particular, have improved endothelial function, induced left ventricular reverse remodelling in heart failure patients, and reduced both sympathetic activity and arterial stiffness in patients with essential hypertension, independent of BP lowering effects (Davies et al., 2005; Macdonald, Kennedy, & Struthers, 2004; Mahmud & Feely, 2005; Pitt, 2003; Pitt et al., 2003; Savoia, Touyz, Amiri, & Schiffrin, 2008). The aldosterone antagonist spironolactone, that participants were randomized to in this thesis RCT, has been shown in existing evidence to decrease cfPWV and blood pressure in geriatric hypertensive patients, with or without diabetes, and in patients with chronic kidney disease (Edwards, Steeds, Stewart, Ferro, & Townend, 2009; Kithas & Supiano, 2010). Kithas et al (2010) evaluated 24 older hypertensive patients (mean age 70 years) taking spironolactone for six months to show a significant decrease in systolic, diastolic, and pulse pressure, as well as PWV ($P<0.001$). Specifically, 24-hour systolic pressure was reduced from 142 mmHg to 126

mmHg and cfPWV was reduced from 9.6 m/s to 8.6 m/s independent of the percent change in systolic blood pressure. Also in hypertensive patients, the aldosterone antagonist eplerenone has been shown to significantly reduce the stiffness of small resistance arteries structurally by decreasing the collagen/elastin ratio and decreasing circulating inflammatory mediators (Savoia et al., 2008). These structural changes are anticipated from the use of spironolactone in this thesis RCT. These data of decreasing the collagen/elastin ratio and decreasing circulating inflammatory mediators are supported by the observation that aldosterone levels are associated with hypertension and central vascular stiffness (Mahmud & Feely, 2005; Scuteri et al., 2005; Tzamou, Kyvelou, Karpanou, Petras, & Vyssoulis, 2015). Arterial stiffness is an important determinant of systolic hypertension an independent predictor of mortality in hypertensive patients (Laurent et al., 2001; Tzamou et al., 2015). Tzamou et al. (2015) recently studied 1330 untreated hypertensive patients to evaluate the relation between aldosterone levels and central vascular stiffness estimated as cfPWV. They found that patients with high aldosterone levels have higher cfPWV compared to patients with low aldosterone levels ($P<0.001$) with a significant correlation between cfPWV and aldosterone levels (Tzamou et al. 2015). This relationship between increased levels of aldosterone and decreased systemic compliance in patients with long-standing hypertension is supported by other existing evidence (Blacher et al., 1997). In addition, the presence of aldosterone receptors in the vasculature has led to the important observation that aldosterone may promote vascular stiffness induced by inflammation, oxidative stress, vascular remodelling, and endothelial dysfunction (Briet & Schiffrin, 2013; N. J. Brown, 2008; Schiffrin, 2006). Animal models have shown that vascular

fibrosis and hypertrophic remodeling are induced by the presence of aldosterone particularly in a high-salt diet (Briet & Schiffrin, 2013).

Previous investigations have shown possible benefits for cognitive function from ACE inhibitors in heart failure patients (Zuccala et al., 2005). Furthermore, subgroup analyses of large prospective trials (HOPE, SCOPE, PROGRESS, HYVET-COG trials) suggest that treatment with ACE inhibitors or angiotensin receptor blockers may reduce the risk of cognitive impairment or lower the rate of cognitive decline in patients with a history of vascular disease or hypertension (Duron & Hanon, 2010; Skoog et al., 2005; Tzourio et al., 2003). As a part of the HOPE trial for example, 9297 high risk vascular patients that were followed for 4.5 years to prospectively observe outcomes of stroke and cognitive function, ACE inhibitor therapy significantly reduced cognitive decline associated with stroke by 41% ($p < 0.05$) compared to placebo (Bosch et al., 2002). In subgroup analyses of the SCOPE trial, treatment with the angiotensin receptor blocker candesartan was associated with less decline in attention and episodic memory ($p=0.04$) and significantly less cognitive decline was observed in patients on candesartan with a lower baseline cognitive function ($p=0.04$, MMSE 24-28, $n=2070$) compared to a higher baseline cognitive function (MMSE 29-30, $n=2867$) (Saxby et al., 2003; Skoog et al., 2005). In patients with cerebrovascular disease, treatment with perindopril with/without indapamide resulted in a significant reduction in the risk of cognitive decline by 19% ($p=0.01$), reductions in risk of cognitive decline with recurrent stroke by 45% ($p < 0.001$) and reduction in dementia associated with recurrent stroke by 34% ($p=.03$) (Tzourio et al., 2003). In the HYVET-COG trial of diuretics with/without an ACE inhibitor in elderly non-demented hypertensive patients showed a trend

for less incident dementia by 14% (95% CI 0.67-1.09), likely due to lack of power as the trial was terminated early because of stroke and mortality reduction with treatment compared to placebo (Peters et al., 2008). However, a meta-analysis of four randomized trials (PROGRESS, SYST-EUR, SHEP, HYVET-COG) of antihypertensive therapy did suggest that blood pressure lowering may reduce the risk of dementia (relative risk 0.087, p=0.045) (Peters et al., 2008). It is important to note that none of these trials included cognitive function as the primary outcome. Another potentially important consideration, and applicable to this thesis, is that blood-brain-barrier (BBB) crossing ACE inhibitors (e.g. perindopril and ramipril are centrally-active) have been shown to reduce cognitive decline in hypertensive seniors with Alzheimer's disease compared to non-BBB-crossing ACE inhibitors (e.g. enalapril, p=0.0023), possibly through structural or functional changes in cerebral circulation associated with inhibition of angiotensin II (Ohrui et al., 2004). The Cognition Substudy of the Cardiovascular Health Study showed that in treated hypertensive patients, centrally-active ACE inhibitors were associated with 65% less decline in modified mini-mental state examination scores (1054 participants, over 6 years, p=0.01) and non-BBB-crossing ACE inhibitors were associated with 20% greater risk of dementia compared to non-ACE inhibitors (Sink et al., 2009). Small randomized trials with angiotensin receptor blockers (ARB) have shown significant improvement in memory tests ($p < 0.05$) among elderly hypertensives compared with atenolol, or improved cognitive function compared with hydrochlorothiazide (HCT, MMSE 22 increase to 26 after 24 months, $p < 0.001$) (Fogari et al., 2003; Tedesco et al., 1999). Another small prospective ARB trial with telmisartan-HCT combination therapy in elderly hypertensives was shown to improve cognitive function test scores in word-list recall ($p < 0.05$) and executive function test Trails B ($p < 0.05$) compared to

lisinopril-HCT after 6 months of therapy (Fogari et al., 2006). It's fairly clear that RAAS inhibition plays a part in the risk of cognitive function and aging.

There are currently no curative medications available for cognitive decline by increasing cerebral blood flow. Factors related to vascular aging, such as hypertension and arterial stiffness, are identifiable risk factors for reduced brain blood flow and cognition function. Studies with older hypertensive adults taking RAAS inhibitors have suggested reduction in cognitive decline with corresponding improvements in cerebral perfusion, although the ability of the RAAS inhibitor to cross the blood-brain barrier may have been a confounding variable. Aldosterone antagonists have the potential to protect cognitive function through their actions on improving vascular stiffness and cerebral blood flow. However, to date, very limited data are available investigating aldosterone antagonism effects on cognitive function (Duron & Hanon, 2010). Recently, a small non-randomized prospective trial of 7 patients matched for age, sex, and cognitive function showed that 6 months of aldosterone antagonism (5 patients taking eplerenone, and 2 taking spironolactone) can significantly improve cognitive function (MMSE 23.7 increased to 25.4, $p < 0.05$) and decrease systolic blood pressure (-14 mmHg, $p < 0.05$) without a change in mean blood pressure (Yagi et al., 2011). This preliminary study supports the need for further investigation, such as this thesis randomized controlled trial, as to whether treatment with an aldosterone antagonist has clinical benefits on cognitive function with associated changes in brain blood flow in older adults with controlled hypertension.

The inhibition of the RAAS system has been associated with improvements in cerebral blood flow velocity and executive cognitive function in older hypertensive patients (Ihab Hajjar et al., 2013; Lipsitz et al., 2005). Magnetic resonance imaging has securely shown in these older adults that there is a link between reduced cerebral perfusion and poorer performance on executive function cognitive assessments (Alosco et al., 2013). ACE inhibitors (e.g., lisinopril) and angiotensin receptor blockers (e.g., candesartan) have been associated with a significant increase in cerebral blood flow velocity (MCA velocity, $P<0.03$) with lowering of blood pressure, especially in patients with lower baseline cerebral blood flow velocity (Hajjar et al., 2013; Lipsitz et al., 2005). The ACE inhibitors that cross the blood-brain barrier in particular (e.g., ramipril, lisinopril, perindopril) have been associated with less cognitive decline versus other antihypertensive medications, and non-centrally active ACE inhibitors (e.g., enalapril) were associated with a greater risk of dementia compared to centrally-active ACE inhibitors (Sink et al., 2009). Some interesting evidence has pointed to high baseline aldosterone levels as being associated with poorer MCA blood flow velocity, less CO_2 vasoreactivity, and poorer cognitive function (Hajjar, Hart, Mack, & Lipsitz, 2015; Yagi et al., 2011). High baseline aldosterone may be a predictor of potential improvements in cognitive function and CO_2 vasoreactivity following RAAS inhibition with an ACE inhibitor or angiotensin receptor blocker (I. Hajjar, Hart, Mack, & Lipsitz, 2015; Yagi et al., 2011). Yagi et al. (2011) evaluated 68 hypertensive patients (mean age 63 years) to find an association between plasma aldosterone concentration and global cognitive function (using MMSE scores, coefficient - 0.51, $P<0.01$); to then show in 7 matched pairs of patients an improvement in cognitive function (MMSE score, $P<0.05$) after 6 months of an MR antagonist significantly lowered systolic blood pressure. Likely supported by the abundant

expression of aldosterone receptors (mineralocorticoid receptors, MRs) in the brain, and especially the hippocampus, which plays a role in cognitive function (Reul et al., 2000). Animal models have shown brain MRs also appear to play a major role in progression to hypertension through an MR-angiotensin II type 1 receptor pathway (H. W. Wang et al., 2016). Preclinical studies have also shown how spironolactone increases middle cerebral artery diameter and reduces wall/lumen ratio, and reduces expression of intracellular adhesion molecule (ICAM-1) that is a marker of inflammation (Rigsby, Ergul, Portik Dobos, Pollock, & Dorrance, 2011). Currently, there are no published human studies evaluating the effect of spironolactone on cerebrovascular hemodynamics with associated changes in executive cognitive function.

1.6 Acute Changes in Blood Pressure from Progressively Increasing Lower Body Negative Pressure (LBNP)

Spironolactone, as used in the current study with older, hypertensive individuals, is expected to chronically lower arterial blood pressure and to affect arterial stiffness indicators. Because of the potential for acute changes in blood pressure to directly affect arterial stiffness, the current study included a method to acutely change arterial blood pressure in younger adults to examine whether the results of the main study could be influenced by factors independent of chronic changes in stiffness indicators. Lower body negative pressure (LBNP) is a well-established technique in research settings to study the cardiovascular effects of central hypovolemia, blood loss, and manipulation of baroreceptors (Butler, Yamamoto, Xing, Northey, & Hughson, 1992; Convertino, 2001; Convertino, Cooke, & Holcomb, 2006; Johnson et al., 2014; Johnston et al., 2003). The LBNP chamber is a tightly sealed rectangular box that encloses the

lower body of the participant. Air pressure inside the chamber is reduced by a vacuum pump, making pressure inside the chamber less than atmospheric pressure. Application of sub-atmospheric pressure to the lower body redistributes fluid from the upper body to the lower extremities, thus reducing venous return and central venous pressure (Convertino, Ludwig, & Cooke, 2004; W. H. Cooke, C. A. Rickards, K. L. Ryan, T. A. Kuusela, & V. A. Convertino, 2009; Goswami, Grasser, Roessler, Schneditz, & Hinghofer-Szalkay, 2009). At low levels of LBNP, the initial fall in CVP stimulates the cardiopulmonary baroreflex causing an increase in sympathetic vasoconstrictor tone, and a small reduction in stroke volume with no change in heart rate (Convertino, 2001; C. Lydakis et al., 2008; Rowell, 1993). In addition to an arterial baroreflex mediated withdrawal of parasympathetic activity to increase heart rate, increases in sympathetic nerve activity augment the increase in heart rate, myocardial contractility and total peripheral vascular resistance affecting mean, systolic, and diastolic pressures (Crystal & Salem, 2015; Fu et al., 2009; Hisdal, Toska, Flatebo, & Walloe, 2002; Kay & Rickards, 2015; Ryan, Rickards, Hinojosa-Laborde, Cooke, & Convertino, 2012).

1.6.1 Acute Changes in Blood Pressure Can Affect Arterial Stiffness Indicators

The use of LBNP allows the unique opportunity to investigate how arterial stiffness indicators are affected by increases in sympathetic vasomotor tone and distending arterial pressure. It is important to note that elastic properties of central arteries can be affected by the state of vascular smooth muscle tone through activation of the sympathetic nervous system induced by LBNP. Severe LBNP (> - 50 mmHg) elicits sympathetic nervous system activation that has been

shown to increase arterial stiffness (i.e., increase cfPWV) and decrease arterial compliance (stroke volume/pulse pressure) (A. A. Phillips, S. S. Bredin, A. T. Cote, C. T. Drury, & D. E. Warburton, 2013). Milder LBNP (< - 50 mmHg) however was not shown to have this effect on arterial stiffness.

Lydakis et al. (2008) used LBNP to explore how arterial stiffness (T_i = transit time of the pressure pulse wave to return to the heart from peripheral sites) was dependent on blood pressure and sympathetic tone. LBNP increased sympathetic tone and heart rate, but reduced systolic and pulse blood pressure to show how arterial stiffness was essentially unchanged at moderate LBNP; only to increase arterial stiffness at higher LBNP (Lydakis et al., 2008). These results imply that the isolated sympathetic engagement during a volume-shift beyond moderate LBNP may affect the elastic properties and smooth muscle cell tone of the central arteries. This factor is important for this thesis research whereby progressively increasing LBNP to only moderate levels were applied to observe the effect of acute small blood pressure changes on arterial stiffness indicators. Importantly, this thesis will expand on existing evidence by examining the effect of progressively increasing LBNP on carotid stiffness, cfPWV, and PEP.

In summary, aging elevates the risk of hypertension and arterial stiffness through potentially reversible structural and functional arterial changes likely related to the RAAS system. Importantly, hypertension and arterial stiffness are risk factors for major adverse cardiovascular events and cognitive impairment. It is possible that cognitive impairment may be from cerebral hypoperfusion linked to central arterial stiffness related damage to microcirculation in the

brain. Antagonism of the RAAS system with spironolactone has been shown in small trials, and supported by evidence with other RAAS inhibitors, to have the potential to reduce blood pressure and arterial stiffness, that may improve cerebral blood flow and cognition.

1.7 Thesis Objectives

1.7.1 Main Objective

The main objective of this thesis was to conduct a randomized, double-blind, placebo-controlled trial with the aldosterone antagonist spironolactone in older hypertensive adults stably treated by centrally acting ACE inhibitors. The purpose was to determine if spironolactone through its influence on blood pressure and arterial stiffness could improve cerebral blood flow and cognition. The thesis flow diagram is provided as Figure 2.

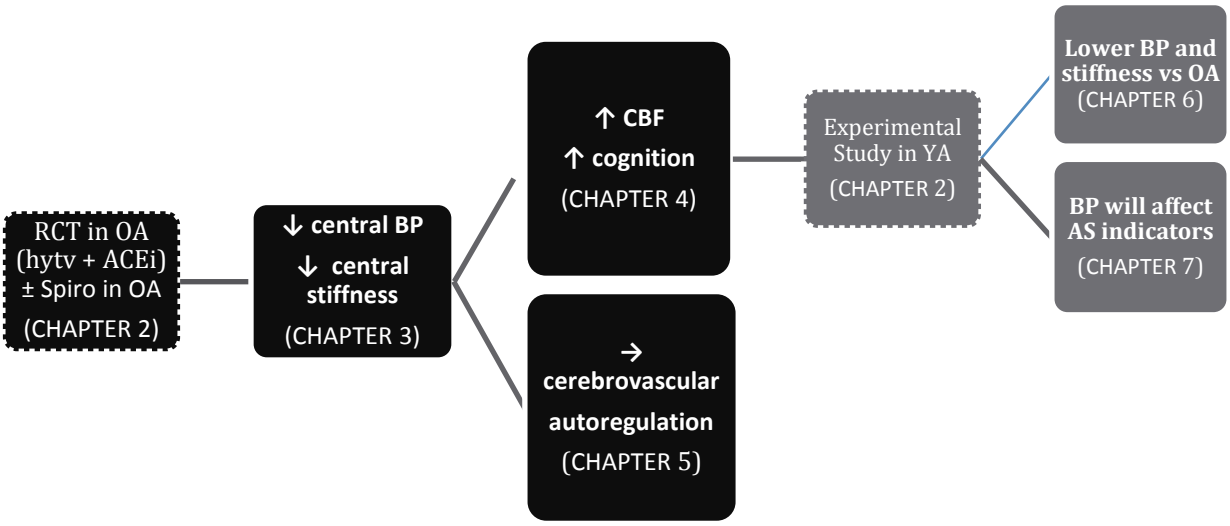


Figure 2. Flow diagram of two thesis studies and five hypotheses

Thesis flow diagram showing the main study (dark solid boxes) and supplementary study (light solid boxes) with corresponding hypotheses and chapter numbers. Boxes with dotted outline include study design. RCT= randomized, double-blind, placebo-controlled trial, hytv = hypertensive, ACEi = angiotensin converting enzyme inhibitor, +Spiro = spironolactone, -Spiro = placebo, BP = blood pressure, ↓ = reduction, ↑= increase, → = maintain, AS = arterial stiffness, OA = older adults, YA = younger adults.

1.7.2 Secondary Objectives

Important secondary objectives were to investigate cerebrovascular autoregulation changes with spironolactone, and to conduct a supplementary experimental study in younger adults. The important purpose of the experimental study in younger adults was to demonstrate expected blood pressure and arterial stiffness differences in older adults in the RCT, and to investigate the potential for acute changes in blood pressure to affect indicators of arterial stiffness that were applied to older adults in the RCT independent of chronic blood pressure changes. The former is intended to demonstrate how even small, brief, changes in blood pressure can affect arterial stiffness indicators, potentially limiting interpretation of the impact of spironolactone on arterial stiffness.

1.8 Specific Research Questions

To address these objectives, there were 5 main research questions:

1. In older hypertensive adults stably treated by centrally acting ACE inhibitors, will spironolactone decrease blood pressure and arterial stiffness compared to placebo? (CHAPTER 3).

2. In older hypertensive adults stably treated by centrally acting ACE inhibitors, does spironolactone improve cerebral blood flow and cognition compared to placebo? (CHAPTER 4).

3. In older hypertensive adults stably treated by centrally acting ACE inhibitors, does spironolactone affect cerebrovascular autoregulation compared to placebo? (CHAPTER 5)

4. How does blood pressure and arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compare to healthy younger adults? (CHAPTER 6).

5. Are indicators of arterial stiffness unaffected by acute changes in blood pressure? (CHAPTER 7)

1.9 Hypotheses

1. It was hypothesized that in older hypertensive adults stably treated by centrally acting ACE inhibitors, spironolactone will decrease blood pressure and arterial stiffness compared to placebo. (CHAPTER 3).

2. It was hypothesized that in older hypertensive adults stably treated by centrally acting ACE inhibitors, spironolactone through effects on blood pressure and arterial stiffness, will improve cerebral blood flow and cognition compared to placebo. (CHAPTER 4).

3. It was hypothesized that in older hypertensive adults stably treated by centrally acting ACE inhibitors, spironolactone will not affect cerebrovascular autoregulation compared to placebo. (CHAPTER 5)

4. It was hypothesized older hypertensive adults stably treated by centrally acting ACE inhibitors would have higher blood pressure and greater arterial stiffness compared to healthy younger adults. (CHAPTER 6).

5. It was hypothesized that acute changes in blood pressure would affect arterial stiffness indicators. (CHAPTER 7)

CHAPTER 2 General Methods and Materials

2.1 Overview of Studies

This chapter outlines all the methods and materials for this thesis research. There was a main study and a supplementary study conducted as follows:

1) A double-blind study in generally healthy older adults on stable centrally acting ACE inhibitor therapy for hypertension were randomly assigned to aldosterone antagonist spironolactone or placebo for 6 months.
2) An experimental study in younger adults.

2.2 Description of Participants

2.2.1 Younger Adults

Younger adult participants were recruited, using inclusion and exclusion criteria in Table 17 in APPENDIX 2.IE, from the University of Waterloo. Assessments were carried out at the University of Waterloo in the Cardiorespiratory and Vascular Dynamics Laboratory. Written and informed consent was obtained from each volunteer. Based on a self-reported medical history (APPENDIX 3.HIF), participants were generally healthy. Participants were asked to refrain from alcohol, nicotine, and caffeine the day of the assessments, avoid strenuous exercise 24 hours before, and have only a light meal 2 hours prior to assessments. Room temperature was maintained from 21-23°C. Participant's height and weight were recorded at each visit.

2.2.2 Older Adults

Generally healthy older hypertensive participants on stable ACE inhibitor therapy were recruited from Family Physician offices at New Vision Family Health Team and The Centre for Family Medicine Family Health Team in Kitchener, Ontario, Canada. Screening for potential participants applied specific inclusion (APPENDIX 2.IE, Table 18) and exclusion criteria (APPENDIX 2.IE, Table 19) to over 20,000 electronic medical records. Medical staff at these physician offices contacted eligible participants and asked their permission for a student investigator to contact them about the study. Sixty-three candidates were interviewed for 20-30 minutes by phone to ensure inclusion and exclusion criteria were met and to explain study activity if they were still eligible. Most declined to participate due to multiple blood work requirements, modification of daily medication regime close to planned travel, and perception of risk to their current subjective healthy lifestyle. Eventually twenty-three participants were enrolled into the study. Participants arrived at the University of Waterloo and completed a comprehensive health screen, a battery of cognitive tests, and a familiarization with the study protocol prior to vascular assessments. The first three participants were assessed at the New Vision Family Health Team medical office before assessments were transitioned to University of Waterloo in the Cardiorespiratory and Vascular Dynamics Laboratory. Participants were requested to avoid exercise, caffeine, nicotine and alcohol on both days of their arterial and cognitive assessments. All participants provided written, informed consent to the procedures outlined in this study, following approval from the Office of Research Ethics at the University of

Waterloo (#18636). Each participant was informed about voluntary withdrawal at any time and was provided a wallet sized contact information card for emergency access to Investigators.

2.3 Measurement of Blood Pressure, Heart Rate and End-Tidal Carbon Dioxide

Supine brachial blood pressure was measured 3 times manually by the same investigator after 5 minutes of resting according to American Heart Association guidelines (Pickering et al., 2005). Then brachial pressure was continuously collected non-invasively by finger-cuff photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands). Brachial artery pressure data was reconstructed from finger blood pressure waveform filtering and calibrated using a return-to-flow technique (Guelen et al., 2003). The finger-cuff was placed on the right middle finger between the proximal inter-phalangeal and the distal inter-phalangeal joints and maintained at heart level during the entire assessment, including seated and standing postures. Heart rate was measured over an average of 60-90 consecutive cardiac cycles using a 3-electrode electrocardiogram (ECG, Finapres Medical Systems, Amsterdam, The Netherlands) to collect electrocardiographic data (QRS complex) by placing the white electrode just below the clavicle of the right shoulder, red electrode at the left eighth intercostal space, and the black electrode just below the clavicle of the left shoulder forming the standard Einthoven's triangle. Exhaled carbon dioxide (CO_2) collected through a nasal cannula was measured with an infrared CO_2 analyser (Pilot 9200, Colin Medical Instruments, San Antonio, TX, USA or Datex-Ohmeda 5200 CO_2 Monitor, Madison, WI, USA).

Data were continuously recorded at 1 kHz using a data acquisition system (PowerLab, ADInstruments, Colorada Springs, CO, USA) and software (LabChart 5 or 7, ADInstruments). Analysis of hemodynamics offline involved beat-by-beat averaging gated to the ECG R-peak of the QRS complex. MAP was corrected to brain level while participant was seated or standing upright to account for orthostatic pressure gradient between the brain and heart (distance x 0.78 mmHg/cm).

Carotid pressure at the common carotid artery was measured over 15-20 cardiac cycles using applanation tonometry (SPT-301, Millar Instruments, TX, USA). Optimal pulse wave contour was determined visually by maximizing the amplitude and minimizing the diastolic pressure variation (Robertson, Tessmer, & Hughson, 2010). To correct for probe contact pressure, the mean and diastolic tonometric signals were calibrated to the mean and diastolic pressure measured by the Finometer Pro (Finapres Medical Systems, Amsterdam, The Netherlands).

2.4 The Randomized, Double-Blinded, Placebo-Controlled Study in Older Adults

2.4.1 The Intervention

Participants were randomized in the ratio of 1:1 using specialized software in a double-blinded manner to receive spironolactone or placebo for 6 months as shown in the flow diagram (Figure 3) below. This study was not registered in *clinicaltrials.gov*; however results will be made available to the public. The dispensing pharmacist (Section 2.4.2) kept a master list of study-drug allocation and was not part of data collection or analysis. Study-drug was delivered in a

sealed bag directly to the medical clinic with the participant's name for pickup by the participant. Study-drug was started at 25 mg daily, and then titrated up to 50 mg daily at one month for the remainder of study (Y. Yano et al., 2011; Zannad et al., 2011). Adherence to study-drug was estimated by the dispensing pharmacist counting the number of pills remaining on the day of their 2nd assessment---no investigators were in contact with the study-drug. This number was subtracted from the total number of pills dispensed to estimate how many pills they had taken and divided by the number of pills they should have taken between first day of dispensing and their second assessment to calculate percent adherence.

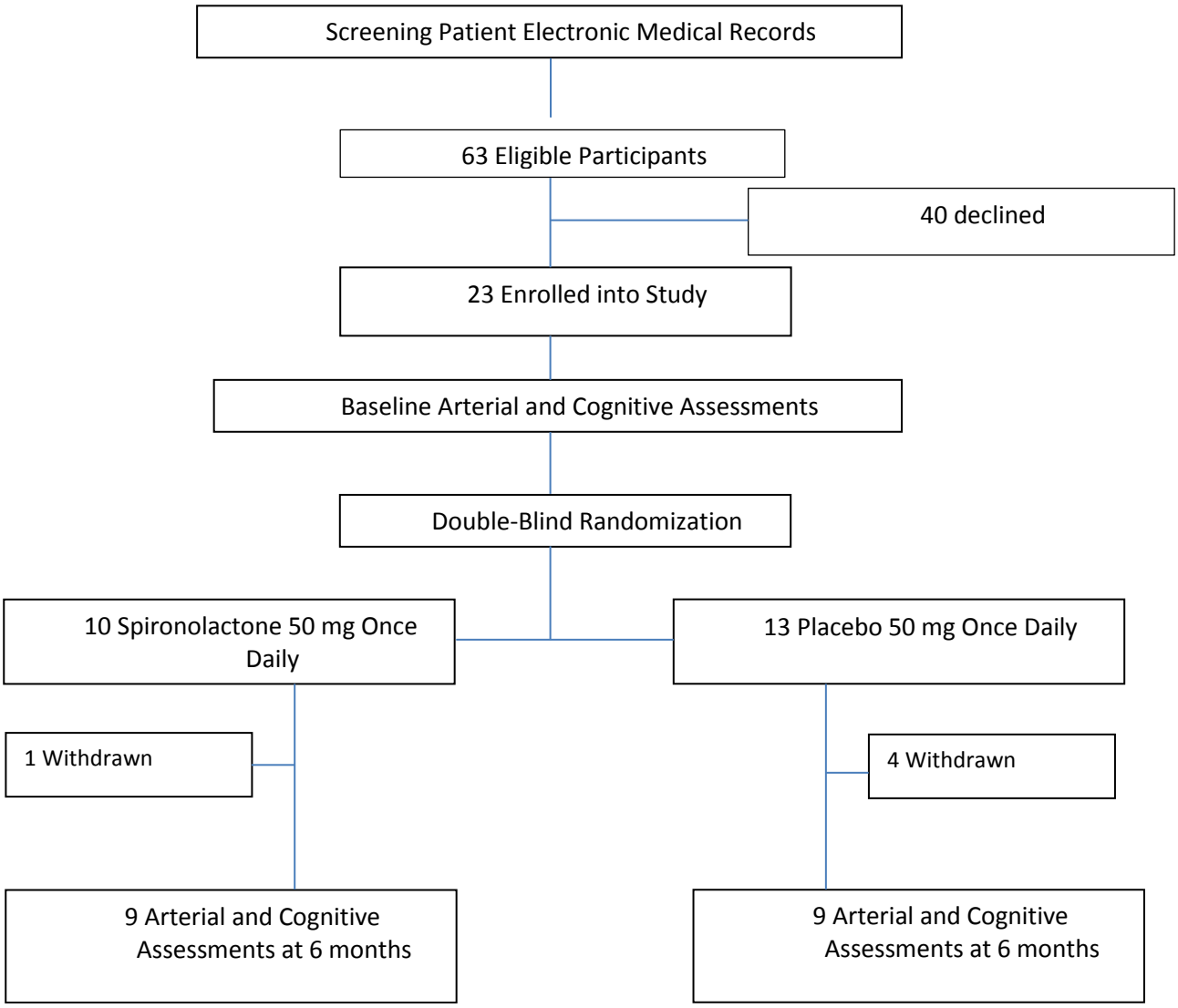


Figure 3. Flow diagram of RCT study activities in older adults

Participants from New Vision FHT and The Centre for Family Medicine FHT electronic medical records were screened using inclusion and exclusion criteria in APPENDIX 2.IE. Eligible participants that were enrolled had baseline cognitive and vascular assessments then were randomized in a double-blinded manner, to either spironolactone (50mg once daily) or placebo. Estimated glomerular filtration rate (eGFR) and serum potassium (K+) were checked prior to start, after first week, then at weeks 5, 12 and 18 after first dose. Arterial and cognitive assessments were repeated after 6 months to end the study. Four participants in the Placebo group withdrew due to mild diarrhea, hyperkalemia prior to first dose of study-drug, brief symptom of rapid heart rate, and voluntarily. The one participant in the spironolactone group voluntarily withdrew.

Participants had safety blood work prior to taking the study-drug, then at week-1, week-5, week-12, and week-18 at a local certified medical laboratory (CML Medical Lab, Greenbrook Drive, Kitchener, ON) to monitor serum potassium, sodium, creatinine and estimated glomerular filtration rate (eGFR). All participants had their blood work done at the same laboratory; however the timing for each sample varied 3-5 days around the predefined weeks. Instruction was given to each participant during the reminder call, or email, to have the blood sample taken by a specific date; and to eat and drink as per their normal routine.

2.4.2 Randomization and Study-Drug Dispensing

Randomization using software, and double-blinding, was conducted by the University of Waterloo School of Pharmacy, and retail, pharmacist Shanthi Sampath at *The Pharma Shoppe (Joseph Street Site of The Centre For Family Medicine Family Health Team)*. The School of Pharmacy used *The Pharma Shoppe* as their dispensing and medication consultation service. Treatment Code allocations were kept confidential until data analysis was complete.

2.4.3 Study Procedures

A comprehensive Health History Form was completed with each participant at baseline (APPENDIX 3.HIF). Participant's contact information, physician's name, date of birth, sex, years of formal education, history of vascular disease (eg., angina, stroke, myocardial infarction),

medication list, smoking status, and self-reported tendency to be adherent to medication was included. Participants were asked to bring their medication with them to their first visit (only) to authenticate the type and dosing. Participants were indicated as smokers if they reported smoking cigars, pipes, or cigarettes during the past 60 days. Room temperature was maintained between 21-23°C.

2.4.4 Measurement of Cerebrovascular and Cardiac Hemodynamics

Cerebrovascular Hemodynamics

Anterior cerebral blood flow (aCBF) was measured by combined brightness-mode imaging and Doppler ultrasound (Linear array transducer 5-10 MHz, Mindray 5, Mindray Medical Limited, BC, Canada) investigation of the right and left internal carotid arteries. aCBF was calculated as the product of the internal carotid artery cross-sectional area ($CSA=\pi*(diameter/2)^2$) and mean spectral blood velocity (Equation 1) and measured 1-2 cm distal to the carotid bifurcation with the participants chin slightly elevated and turned to the contralateral side for greater exposure of the artery.

aCBF = (CSA x internal carotid artery blood velocity)

Equation 1 Anterior cerebral blood flow (aCBF)

Anterior cerebral blood flow (aCBF) calculated as the product of the internal carotid cross-sectional area (CSA) and mean spectral blood velocity (Newell, Aaslid, Lam, Mayberg, & Winn, 1994).

Diameters were measured in triplicate at diastole over two beats and maximum spectral velocities were averaged over two cycles of four beats. Only well-defined maximum frequency shift envelopes were used to quantify mean velocities overestimating true mean blood cell velocity. The true mean velocity is derived from average weighting of all the spectral signals from across the artery being recorded; including high-velocity blood cells in the centre and low-velocity cells near the walls. However, mean spectral outline velocity reflects maximum velocities occurring at the central portion of the artery. A change in flow will produce proportional changes in true mean, and spectral outline, velocity profiles. Spectral outline velocity profiles were chosen to minimize error introduced by small movements in sample volume between assessments that can affect reflected power erroneously interpreted as change in flow, and useful measurements could be made with a lower signal-to-noise ratio (Newell et al., 1994). Bilateral internal carotid artery flows were summed to quantify the measure of total aCBF.

Cerebrovascular conductance index (CVCi) was calculated as the ratio of aCBF to MAP.

CVCi = aCBF / MAP.

Equation 2 Cerebrovascular conductance index (CVCi)

Cerebrovascular conductance index (CVCi) was calculated as the anterior cerebral blood flow divided by MAP.

Cardiac Hemodynamics

Ultrasound brightness-mode imaging (Linear array transducer 1-5 MHz, Mindray 5, Mindray Medical Limited, BC, Canada) was used to assess the cross-sectional area (CSA=

$\pi*(diameter/2)^2$) of the aortic root which was then combined with aortic velocity measured with Doppler ultrasound (2MHz, Multigon, New York, NY) and R-R interval to determine stroke volume (SV).

SV = aortic velocity x CSA x R-R interval

Equation 3 Stroke volume (SV)

Stroke volume (SV) calculated as the product of aortic velocity, cross-sectional area (CSA) and R-R interval

Aortic velocity is the blood velocity of the ascending aorta and radius is half the diameter of the aortic root. 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 (Alessandri et al., 2010). Cardiac output (Q) was determined as the product of SV and heart rate.

2.4.5 Transcranial Doppler

The mean velocity of red blood cells moving through the left middle cerebral artery (MCA) was measured with a transcranial Doppler (TCD) with a 2MHz ultrasound probe (Multigon, New York, NY) only in older adults. The transducer was placed against the transtemporal acoustic window, with a slightly upward and forward orientation (Aaslid et al., 1989). The insonation depth was placed between 45 mm and 55 mm, consistent with the M1 segment of the artery (Gillard, Kirkham, Levin, Neville, & Gosling, 1986; Purkayastha & Sorond, 2012). Auditory pitch, spectral velocity trace, signal strength and TCD probe angle were consistently used to confirm

optimal MCA insonation (Gillard et al., 1986). Photos of the TCD probe placement, documentation of probe position and angle, sample volume depth and width, were used to ensure repeatability of sample volume between assessments for each participant.

TCD ultrasonography uses the Doppler shift frequency, the difference in the frequency between the emitted and reflected ultrasound waves, which is directly proportional to the speed of moving red blood cells (blood flow velocity) as described in Equation 4.

$$\textbf{Flow Velocity (cm/s)} = \frac{\textbf{(Doppler Shift) x propagation speed}}{\textbf{2 x Incident frequency x cos } (\Theta)}$$

Equation 4 Flow velocity

Propagation speed (1541 m/s for soft tissue), Θ is angle of insonation (angle of the emitted wave relative to the direction of the vessel blood flow), incident frequency is of the emitted wave from the probe.

The laminar blood flow through the MCA gives a mixture of different Doppler frequency shifts forming a spectral display of the distribution of the velocities of individual red blood cells (Purkayastha & Sorond, 2012).

Cerebrovascular resistance index (CVRi) was calculated as the ratio of mean arterial pressure to MCA blood flow velocity (MCAv) shown here in equation 5.

$$\textbf{CVRi} = \textbf{MAP/MCAv.}$$

Equation 5 Cerebrovascular resistance index (CVRi)

Cerebrovascular resistance index (CVRi).

It should be noted that the MCAv has been shown to be a marker of changes in cerebral blood flow despite the assumption that the diameter remains constant (Serrador, Picot, Rutt, Shoemaker, & Bondar, 2000). A slight change in arterial caliber could affect the association between MCA velocity and flow. Since conditions such as maximal hypercapnia may induce MCA diameter change, it is important to be cautious when interpreting MCA velocity measures from TCD (Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014; Coverdale, Lalande, Perrotta, & Shoemaker, 2015; Willie et al., 2012).

2.4.6 Cerebrovascular Autoregulation

The immediate responses to standing from seated position (called sit-to-stand technique) is typically an immediate increase in heart rate (within approximately 3 seconds), an increase in cardiac output (maximum about 7 seconds), and a decrease in systemic vascular resistance with corresponding decrease in mean arterial blood pressure (Sorond et al., 2009; Wieling et al., 2007). Changes in posture were used to assess dynamic cerebral autoregulation. During posture change from seated to standing, MCAv, MAP (calibrated to MCA level) and end-tidal carbon dioxide ($ETCO_2$) were measured. The validated sit-to-stand posture change was used to provide immediate orthostatic hypotension without altering the spatial or gravitational relation between the Doppler probe and the MCA (Lipsitz et al., 2005; Serrador et al., 2005; Sorond et al., 2009). After 5 minutes of resting in the seated position with feet on the floor, participants

casually stood upright for 2 minutes. Changes in MCAv and MAP were measured after sitting (average 2 minutes of data) and at MAP nadir immediately after standing (average of 5 values) as shown in Figure 4.

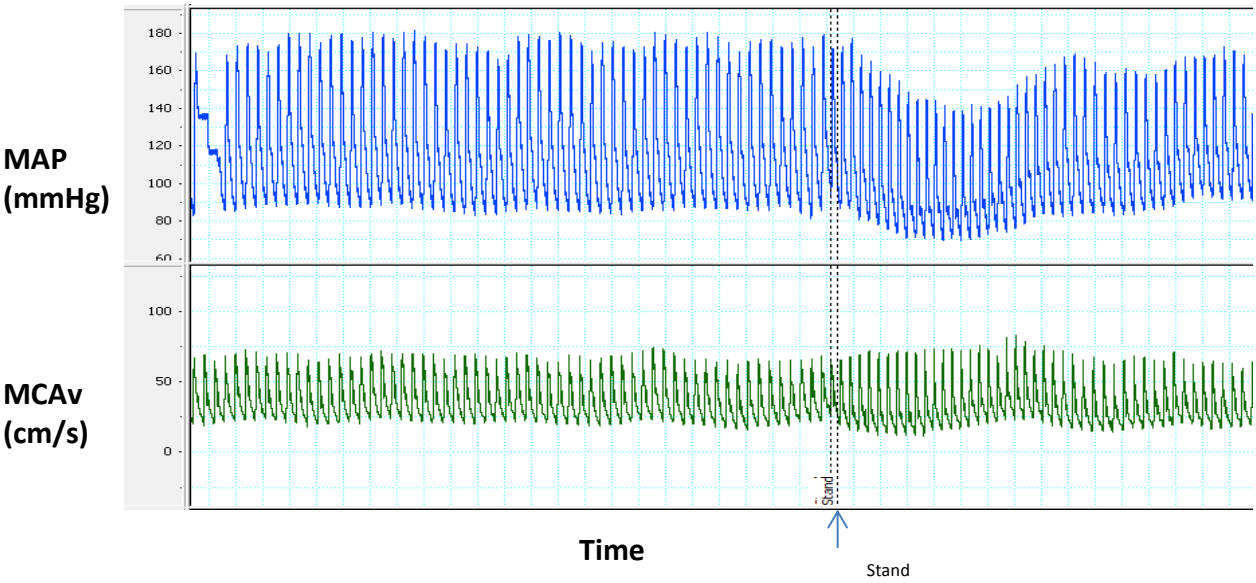


Figure 4. Representative data during sit-to-stand posture change

Representative data showing mean arterial pressure (MAP, top channel) and blood flow velocity through the left MCA (MCAv, bottom channel) responses before and after sit-to-stand posture change. The beginning of standing from seated posture is marked by upward arrow.

MCAv response= (MCAv stand – MCAv sit)

Equation 6 MCAv response from sit-to-stand posture change

MCAv response from sit-to-stand posture change at MCAv nadir (average of 5 values).

MAP response = (MAP stand – MAP sit)

Equation 7 MAP response from sit-to-stand posture change

MAP response from sit-to-stand posture change at MAP nadir (average of 5 values).

CVRi change (Equation 8) as absolute and percent values were measured as the difference between sitting CVRi and the CVRi from posture change.

CVRi response = (CVRi stand – CVRi sit)

Equation 8 CVRi response from sit-to-stand posture change

CVRi response from sit-to-stand posture change.

Absolute and percent changes in MAP, MCAv and CVRi after 6 months of study-drug were compared to establish ability to maintain cerebrovascular autoregulation.

2.4.7 Cerebrovascular Reactivity

Reactivity was measured as the absolute and percent change in MCAv induced by voluntary changes in breathing pattern while supine. $ETCO_2$ was measured through a nasal cannula comfortably placed in the nostrils and fastened to the TCD headset (APPENDIX 1.M.5). Breath-by-breath values for $ETCO_2$ concentration were obtained and time-matched to the beat-by-beat data for analysis. The partial pressure of CO_2 was calculated from the product of CO_2 concentration and barometric pressure. Following 5 minutes of rest, participants hyperventilated for 1 minute at a duty cycle of 2 seconds of maximal voluntary deep breaths, returned to spontaneous normal breathing for 2 minutes, then at the end of a normal breath voluntarily held their breath for at least 30 seconds. The mean $ETCO_2$ of the last three breaths

during hyperventilation estimated hypocapnia and the $ETCO_2$ of the first exhaled breath after optimal breath-hold represented hypercapnia. Participants were asked and coached to only breathe through their nose during assessments. The response in MCAv induced from the hypocapnic stimulus while hyperventilating was assessed as the difference between MCAv following 2 minutes of normal breathing and MCAv at nadir (10-15 beats).

MCAv change hypocapnia= (MCAv hyperventilation–MCAv normal breathing)

Equation 9 MCA velocity change to hypocapnia

MCA velocity change to hypocapnia during hyperventilation

The absolute MCAv change induced by the hypercapnic stimulus was measured as the difference in MCAv following 2 minutes of normal breathing and peak MCAv (10-15 beats) resulting from maximal voluntary breath-hold (Equation 10).

MCAv change hypercapnia = (MCAv breath-hold – MCAv normal breathing)

Equation 10 MCAv change from hypercapnia

MCAv change from hypercapnia during maximal voluntary breath-hold

Percent changes induced by ventilation modification were calculated as MCAv change divided by MCAv following 2 minutes of normal breathing prior to change in ventilation.

2.4.8 Central Arterial Stiffness Indicators

Central arterial stiffness was assessed as the gold standard cfPWV, β-stiffness index and carotid distensibility coefficient (Lipsitz et al., 2005; Y. Zhang et al., 2014). Blood flow velocity pulse waves were sequentially recorded at the common femoral artery and common carotid artery. cfPWV (Equation 11) was estimated with Doppler ultrasound (5 MHz transducer, Multigon Industries, NY, USA) as transit distance (TD = surface distance from suprasternal notch to femoral artery site *minus* surface distance from suprasternal notch to carotid site using a calibrated wooden caliper) divided by the carotid-femoral transit time (cfTT = time from R-peak of QRS complex to femoral velocity waveform to reach artery site *minus* time from R-peak to carotid velocity waveform to reach artery site).

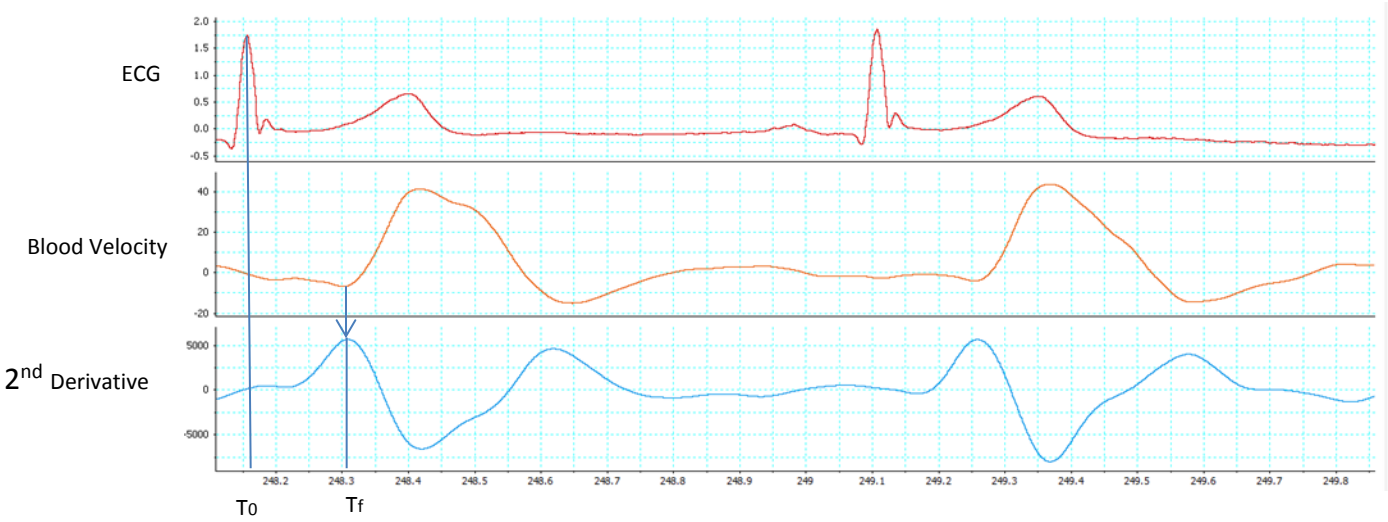
cfPWV = TD/cfTT

Equation 11 carotid-femoral pulse wave velocity (cfPWV)

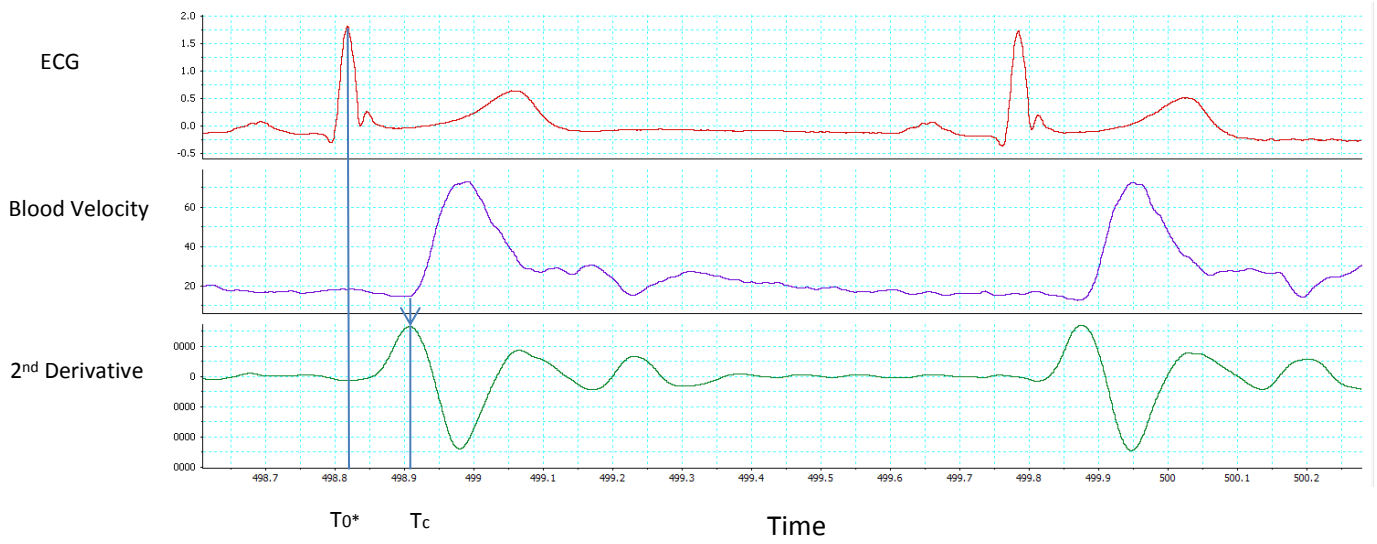
Transit distance (TD) is the surface distance from suprasternal notch to femoral artery site minus surface distance from suprasternal notch to carotid site. Carotid-femoral transit time (cfTT) represents time from R-peak of QRS complex to femoral artery site *minus* time from R-peak to carotid artery site.

Analysis of transit times were done offline involving beat-by-beat averaging of 15-20 beats using a Savitzky-Golay smoothing 3rd order polynomial. Transit time for the carotid velocity waveform, or femoral velocity waveform, was defined as the time lag between the R-peak of the QRS complex and the foot of the pulse wave velocity spectral trace within the same cardiac cycle. The foot of each pulse wave velocity spectral trace was identified as the time at which the 2nd derivative of the smoothed velocity waveform was maximum as shown in Figure 5 (Chiu, Arand, Shroff, Feldman, & Carroll, 1991). To ensure optimal identification of this waveform

foot, the 2nd derivative window size used during analysis was specifically selected for each participant at the start of the beat-by-beat averaging (Appendix 1.M.1).



(A) Femoral artery pulse wave velocity transit time



(B) Common carotid artery pulse wave velocity transit time

Figure 5. Calculation of transit times for carotid-femoral pulse wave velocity (cfPWV)

Representative data showing femoral artery pulse wave velocity transit time (A) with ECG as Channel 1, femoral blood flow velocity smoothed with Satvisky-Golay 3rd order polynomial window size 125 as Channel 2, and 2nd derivative (window size 201) pulse wave velocity waveform as Channel 3. Common carotid artery pulse wave velocity transit time (B) with ECG as Channel 1, common carotid blood flow velocity smoothed with Satvisky-Golay 3rd order

polynomial window size 51 as Channel 2, and 2nd derivative (window size 175) of pulse wave velocity waveform as Channel 3. The arrows indicate the foot of the pulse waves at the maximum of the 2nd derivative waveform. T_0, T_f, T_0*, and T_c represent times at R-peak of QRS complex proximal to femoral site, at femoral site, at R-peak of QRS complex proximal to carotid site, and at carotid site respectively. The transit time (TT) was calculated as $(T_f - T_0) - (T_c - T_0^*)$ used to calculate cfPWV.

The common carotid artery (CCA) diameter was measured from brightness-mode ultrasound images of the left and right CCA within 1-2 cm of the bifurcation. Three pairs of digital calipers were manually positioned along 1 cm segment of images captured at systole and diastole identified by the QRS complex over two beats to give an average of six values each.

Carotid artery stiffness was assessed as carotid distensibility coefficient and β-stiffness index. Carotid distensibility coefficient was calculated as the change in CCA diameter (systole diameter (sDia) minus diastole diameter (dDia) divided by diastole diameter) divided by the carotid pulse pressure (cPP = systole carotid pressure (sCP) minus diastole carotid pressure (dCP)) described in Equation 12.

carotid distensibility coefficient = [(sDia-dDia)/dDia]/(sCP-dCP)

Equation 12 Carotid distensibility coefficient

Carotid distensibility coefficient was calculated as the change in CCA diameter (systole diameter (sDia) minus diastole diameter (dDia) divided by diastole diameter) divided by the carotid pulse pressure (cPP = systole carotid pressure (sCP) minus diastole carotid pressure(dCP)).

β-stiffness index was calculated as the natural logarithm of the ratio of systolic carotid pressure (sCP) to diastolic carotid pressure (dCP) divided by the change in CCA diameter described in Equation 13.

β-stiffness index = ln(sCP/dCP)/[(sDia-dDia)/dDia]

Equation 13 β-stiffness index

β-stiffness index was calculated as the natural logarithm of the systolic carotid pressure (sCP) to diastolic carotid pressure (dCP) ratio divided by the change in CCA diameter (systole diameter (sDia) minus diastole diameter (dDia) divided by diastole diameter).

2.4.9 Assessment of Cognitive Function

Cognitive assessments were only performed in older adults. The battery of cognitive function assessments were done with the participant comfortably seated and relaxed at a table prior to arterial assessments. The Montreal Cognitive Assessment (MoCA) was used as a brief measure of global cognitive function with correction for years of education (APPENDIX 4.ECF) (Nasreddine, Phillips, & Chertkow, 2012; Nasreddine et al., 2005). The MoCA has been demonstrated to be more sensitive to mild cognitive impairment associated with vascular aging than the Mini-Mental State Exam (Ihara, Okamoto, & Takahashi, 2013; Nasreddine et al., 2005). The Trail Making Test part A (APPENDIX 5.T) and B (APPENDIX 5.T) were used to calculate the time for Trails B-A using a stop-watch (Tombaugh, 2004). There is an association between Trails B-A time and executive function such as attention, visual scanning and set-shifting (Ashendorf et al., 2008). Digit Span Forward, followed by Digit Span Backward, used to assess short-term memory testing was scored as the number of rows successfully completed (Richardson, 2007) (APPENDIX 6.DS). Caution should be noted when extrapolating changes in cognitive function

measures to the general population because of bias introduced from participant's familiarity with cognitive tests taken 6 months apart.

2.5 Pulse Wave Transit Time Calculation

Heart-digit transit time (hdTT) was calculated as the time interval between the R-peak of the QRS complex and foot of the smoothed middle finger artery (proper palmar digital artery) pressure wave form within the same cardiac cycle (Figure 6). A Savitzky-Golay smoothing 3rd order polynomial with sample window of 21 was applied to the finger arterial pressure waveform (Appendix 1.M.1). The foot was identified as the time at the peak of the 2nd derivative signal of the smoothed pressure wave form (Chiu et al., 1991). A particular 2nd derivative sample window chosen at the start of waveform beat-by-beat analysis as one of 175, 201, 225, or 251 points to optimize reproducibility of identification of peak value corresponding to foot of pressure wave form (Appendix 1.M.1). Selection of sample window was visually done at supine posture for older adults and at the start of each level of LBNP in younger adults while supine as well. Data were continuously recorded at 1 kHz using a data acquisition system (PowerLab, ADInstruments, Colorada Springs, CO, USA) and software (LabChart 5 or 7, ADInstruments). Analysis of transit times were done off-line involving beat-by-beat averaging of 30-60 consecutive cardiac cycles.

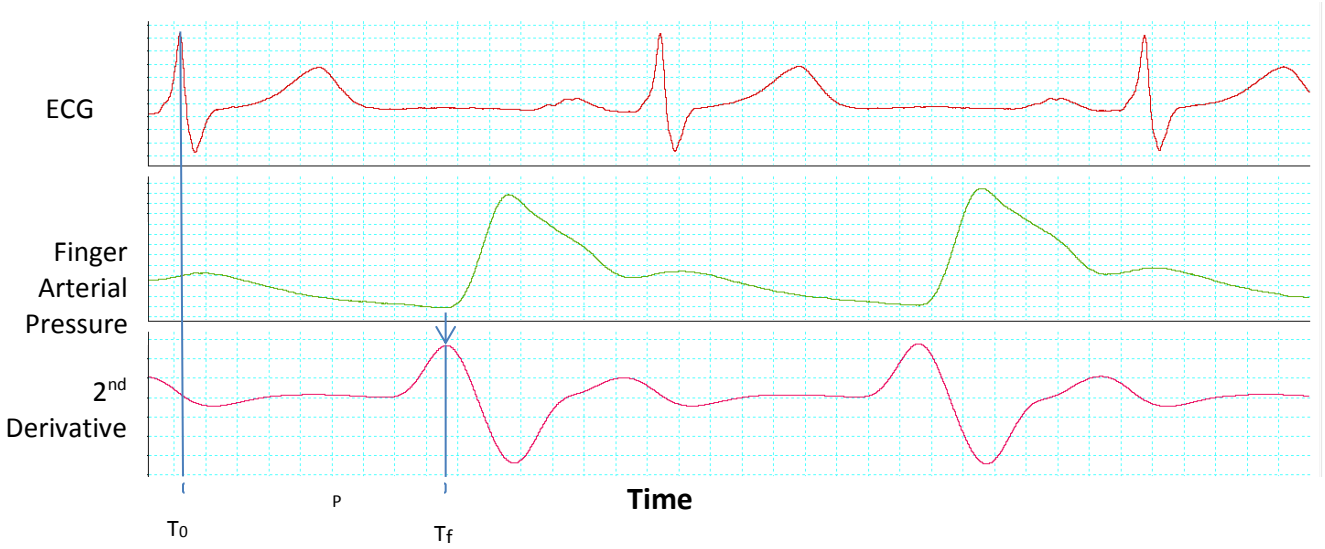


Figure 6. Estimation of heart-digit transit time (hdTT)

Sample data showing ECG, smoothed finger (digit) arterial pressure wave form, and 2nd derivative of finger arterial pressure waveform. The downward arrow indicates the foot of the finger pressure pulse waveform corresponding to the maximum of the 2nd derivative waveform. T_0 and T_f represent times at R-peak of QRS complex and time at foot of pressure waveform respectively. hdTT was calculated as T_f-T_0.

Since hdTT is highly correlated to systolic blood pressure (Gesche, Grosskurth, Kuchler, & Patzak, 2012; Payne, Symeonides, Webb, & Maxwell, 2006; Zheng, Yan, Zhang, & Poon, 2015), a $hdTT_i$ was calculated for each participant as the mean hdTT divided by mean systolic blood pressure during 30-60 cardiac cycles (Equation 14). Consequently, $hdTT_i$ is transit time normalized to systolic blood pressure. This index was not normalized to participant's height, or arm length, and there is no published data to exploring this stiffness index.

$$\mathbf{hdTT_i = hdTT / SBP}$$

Equation 14 heart-digit transit time index

$hdTT_i$ equals the normalization ratio of hdTT to systolic blood pressure (SBP).

Heart-carotid pressure transit time (hcTT) was calculated as the time interval between the R-peak of the QRS complex and foot of the smoothed carotid artery pressure wave form within the same cardiac cycle. A Savitzky-Golay smoothing 3rd order polynomial with sample window of 21 was applied to the carotid arterial pressure waveform. The foot was identified as time at the peak of the 2nd derivative signal of the smoothed pressure wave form (Chiu et al., 1991). A particular 2nd derivative sample window was chosen for each older adult before and after 6 months study-drug while supine, and for each younger adult while supine at each LBNP level, as one of 125, 151 or 175 points to optimize reproducibility of identification of peak value corresponding to foot of pressure wave form (APPENDIX 1.M.1). Selection of sample window was unique to supine posture for older adults and unique to each level of LBNP in younger adults while supine as well. Carotid pressure at the common carotid was measured over 15-20 consecutive beats using applanation tonometry (SPT-301, Millar Instruments, TX, USA). Optimal pulse wave contour was determined visually by maximizing the amplitude and minimizing the diastolic pressure variation (Robertson et al., 2010).

Heart-carotid velocity transit time (hcTTv) was calculated as the time interval between the R-peak of the QRS complex and foot of the smoothed carotid artery velocity wave form within the same cardiac cycle (APPENDIX 1.M.1). A Savitzky-Golay smoothing 3rd order polynomial with sample window of 51 was applied to the carotid arterial velocity waveform. The foot was identified as time at the peak of the 2nd derivative signal of the smoothed velocity wave form (Chiu et al., 1991). A particular 2nd derivative sample window was chosen as one of 125, 151, 175, 201, or 225 points to optimize reproducibility of identification of peak value corresponding

to foot of pressure wave form. Selection of sample window was unique to supine posture for older adults and unique to each level of LBNP in younger adults while supine as well. Carotid blood velocity was measured at the carotid artery site using Doppler ultrasound probe (4 MHz, Multigon, New York, NY). Data were continuously recorded at 1 kHz using a data acquisition system (PowerLab, ADInstruments, Colorada Springs, CO, USA) and software (LabChart 5 or 7, ADInstruments). The correlation between hcTT and hcTTv was examined in APPENDIX M.M2.

2.6 Lower Body Negative Pressure

The lower body negative pressure (LBNP) chamber provides a controlled stress, similar to upright posture, to the cardiovascular system while participants are supine. The LBNP chamber is a tightly sealed rectangular box that encloses the lower body of the participant. Air pressure inside the chamber is reduced by a vacuum pump, making pressure inside the chamber less than atmospheric pressure. Consequently, blood shifts from an area of relatively high pressure (i.e., the upper body, outside the chamber) toward an area of relatively low pressure (i.e., the legs inside the chamber) decreasing central blood volume to elicit compensatory hemodynamic responses in an effort to maintain circulation.

All assessments were performed with the younger adult participants supine and the lower body enclosed in a negative pressure chamber. Participants were asked to wear comfortable athletic t-shirts and shorts prior to putting on a neoprene “skirt” while standing. After they placed their lower body into the chamber, and positioned themselves comfortably on a bicycle seat (to

oppose the tendency to be pulled into the box) with straightened legs, an airtight seal between the skirt and the chamber was created. A belt is placed at the level of the participant's iliac crest to maintain this airtight seal while not mechanically compressing the intestinal region minimizing effect on splanchnic blood flow (Goswami et al., 2009). The air pressure inside the chamber was lowered by turning on a vacuum pump with adjustable suction capacity (Beaumark 99056). The vacuum was plugged into a commercially-available variable voltage transformer (Staco Inc, Dayton, Ohio) to allow for variable applied voltage that created a range of LBNP (0 mmHg to -30 mmHg) monitored in real-time by a hand-held manometer (VWR, Traceable®, Radnor, Pennsylvania).

The data collection was started after at least 5 minutes of baseline resting. Assessments were made after 3 minutes at each LBNP level. The LBNP was adjusted to -10 mmHg for 3 minutes, -20 mmHg for 3 minutes, and finally at -30 mmHg applied suction for 3 minutes before data collection. After data collection, the negative pressure was stopped and equipment was disconnected and removed from the participant. This equipment included a finger-cuff photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands) and a 3-electrode electrocardiogram (ECG, Finapres Medical Systems, Amsterdam, The Netherlands).

2.6.1 Pre-ejection Period

Pre-ejection period (PEP) was only assessed in younger adults. PEP was calculated as the time interval between the time at the R-peak of the QRS complex and the foot of the smoothed aortic velocity wave form within the same cardiac cycle (Figure 7). Aortic blood velocity was measured at the suprasternal notch with a cardiac Doppler ultrasound probe (2MHz) with sample volume placed at the aortic arch (Multigon, New York, NY). A Savitzky-Golay smoothing 3rd order polynomial with a sample window of 75 was applied to the aortic velocity waveform. The foot of each pulse wave was identified as the time at which the 2nd derivative of pulse wave was maximum using a 151, 175 or 201 point sample window selected to optimize reproducibility of identification of peak value corresponding to foot of velocity waveform (APPENDIX 1.M.1). Selection of 2nd derivative window size was made at each level of LBNP.

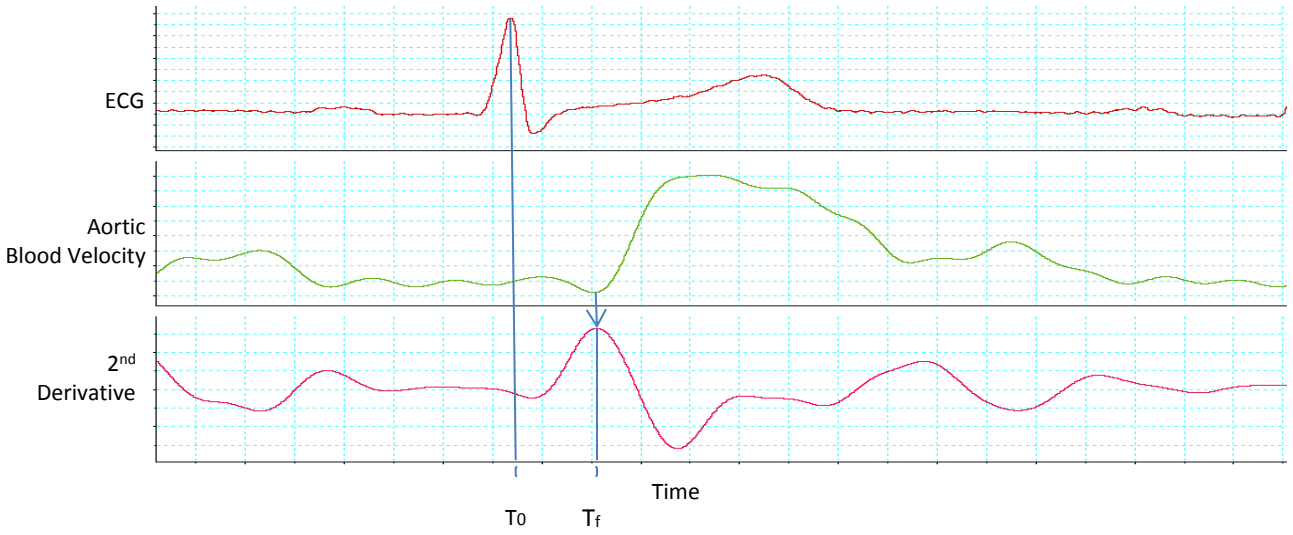


Figure 7. Calculation of pre-ejection period (PEP)

Sample data showing ECG, aortic blood velocity waveform, and 2nd derivative of aortic velocity waveform. The arrow indicates the foot of the aortic velocity waveform at the maximum of the 2nd derivative. T_0 and T_f represent time at R-peak of QRX complex and time at the foot of the aortic velocity waveform respectively. The PEP was calculated as $T_f - T_0$.

The proportion that PEP represented within hdTT was calculated as PEP divided by hdTT (Equation 16). Aorta-digit transit time (adTT) was calculated as the difference between hdTT and PEP as shown in Equation 17.

PEP %= PEP/hdTT x 100

Equation 15 Pre-ejection period (PEP) proportion of hdTT

Pre-ejection period (PEP) calculated as PEP divided by pulse wave transit time (hdTT) multiplied by 100.

adTT = hdTT - PEP

Equation 16 Aorta-digit transit time (adTT)

Aorta-digit transit time (adTT) calculated as heart-digit transit time (hdTT) minus pre-ejection period (PEP).

The aorta-carotid transit time (acTT) was calculated as the interval between the time at the R-peak of the QRS complex and foot of the carotid pressure wave form (hcTT) within the same cardiac cycle minus PEP (Equation 17). Analysis of PEP times were done off-line involving beat-by-beat averaging of 30 cardiac cycles.

acTT = hcTT - PEP

Equation 17 Aorta-carotid transit time (acTT)

Aorta-carotid transit time (acTT) calculated as the hcTT minus PEP. This is a measure of pressure waveform transit time.

2.7 Data preparation for analysis

Beat-by-beat data including blood pressure, heart rate, middle cerebral artery blood flow velocity, exhaled carbon dioxide, carotid artery blood flow velocity, femoral artery blood flow velocity, aortic artery blood flow velocity, finger arterial pressure, and carotid artery pressure, were saved as Microsoft Excel files. Each measured variable was examined per cardiac cycle. Signals from the Finometer when automatic calibrations for changes in finger artery contraction and dilatation took place using a built-in Physiocal algorithm were not included in the analysis. One mean value was derived for each variable per stage of LBNP (baseline, -10 mmHg, -20 mmHg, -30 mmHg), posture (supine and standing), and before or after study-drug in RCT. Table 1 below summarizes what was measured in OA and YA.

Table 1 Summary of measures

Measure	Older Adults	Younger Adults
Age	X	X
Aorta-carotid transit time		X
Aorta-digit transit time		X
Brachial Diastolic Pressure	X	X
Brachial Mean Arterial Pressure	X	X
Brachial Pulse Pressure	X	X
Brachial Systolic Pressure	X	X
Cardiac output		X
Carotid Diastolic Pressure	X	X
Carotid distensibility coefficient	X	X
Carotid Mean Arterial Pressure	X	X
Carotid Pulse Pressure	X	X
Carotid Systolic Pressure	X	X
Cerebral blood flow	X	
Cerebrovascular Conductance index	X	
Cerebrovascular Resistance Index	X	
cfPWV	X	X
cfPWV Index		X
Heart Rate	X	X
Heart-carotid transit time	X	X
Heart-digit transit time		X
Heart-digit transit time index	X	X
Middle cerebral artery velocity		X
Pre-ejection phase		X
Stroke volume		X
Total peripheral resistance		X
β-stiffness index	X	X

2.8 Statistical Analyses

The RCT design required 32 participants to be randomized 1:1 to detect a significant difference between the groups based on pilot data (Professor Richard Hughson) that estimated a desired Cohen's *d* effect size of 1.0. Targeted recruitment was 40 participants to account for some study discontinuation. Sample size was calculated for this trial using an unpaired t-test

comparing two groups with a desired power of 80% and α of 0.05. The anticipated effect size of main study outcome anterior cerebral blood flow on cognitive function was based on mean *poorest* quartile of *lowest* cerebral blood flow (479 mL/min) and *slowest* speeds on Trails B-A (for cognitive function measure) compared to mean cerebral blood flow (592 mL/min) of all other quartiles of speeds on Trails B-A. The corresponding mean difference and pooled standard deviation of cerebral blood flow were 113 mL/min and 110 mL/min respectively. Notably, there is no published data on effect size of spironolactone on cerebral blood flow *and* cognition from which to calculate sample size.

Testing for association was by Pearson product-moment correlation. To test for treatment effect after 6 months of spironolactone or placebo, a 2-way repeated measures ANOVA was used to test main effects (treatment group or time) and interactions (treatment group and time). A 2-way repeated measures ANCOVA was used to test treatment effects on carotid distensibility with age as a covariate. A 1-way repeated measures ANOVA was used to determine main effect for level of LBNP on dependent variables in younger adults. The post hoc Holm-Sidak Test was used to determine significance between groups. Treatment effect for all other comparisons between group differences was with an unpaired 2-tailed t-test. When assumption of normality, or equal variance failed, a Mann-Whitney Sum Rank Test was used. A Wilcoxon Signed Rank Test was used when normality test failed for paired t-testing. Data are expressed as mean ± standard deviation. Significance was set at $P<0.05$. All statistical analyses were completed using SigmaPlot Software 12.5 (Systat Software Inc, San Jose, California, USA).

CHAPTER 3 Effect of Spironolactone on Blood Pressure and Arterial Stiffness in Older Adults with Hypertension

3.1 Objectives

The objective of this chapter was to assess the effect of the aldosterone antagonist spironolactone administered for 6 months on blood pressure and arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compared to placebo in a RCT. Carotid blood pressure was assessed and arterial stiffness was estimated by cfPWV, carotid distensibility coefficient and β-stiffness index. Independent of the effects of angiotensin II, aldosterone induces reversible functional and structural vascular changes that increase systemic vascular resistance and arterial stiffness (Davies et al., 2005; Farquharson & Struthers, 2000, 2002; Kithas & Supiano, 2010; Mahmud & Feely, 2005). Therefore it was hypothesized that in older hypertensive adults stably treated by centrally acting ACE inhibitors, spironolactone would decrease blood pressure and arterial stiffness compared to placebo.

3.2 Methods

As described in CHAPTER 2 General Methods and Materials.

3.3 Results

Pooled baseline participant's age, mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) were 65±3 years, 97±9 mmHg, 142±16 mmHg, 75±8 mmHg, and 66±13 mmHg respectively. Arterial stiffness indicators cfPWV, carotid

distensibility coefficient and β-stiffness index were 7.22±1.09 m/s, 0.0014±0.0006 $^{-1}$mmHg and 8.78±3.53 au respectively. There was a significant negative linear correlation between age and carotid distensibility coefficient (Figure 8, r= - 0.51, P<0.05).

All baseline measures of carotid pressures and arterial stiffness indicators were similar between the two groups (Table 2). The Spironolactone (Spiro) group carotid systolic blood pressure (cSBP) and pulse pressure (cPP) were numerically greater than Placebo before 6 months of study-drug. The carotid MAP (cMAP), diastolic blood pressure (cDBP), cfPWV, carotid distensibility coefficient, and β-stiffness index were similar between groups at baseline. Randomization appeared to be effective.

The change in cMAP after 6 months of study-drug for the Spiro group was not significantly different than Placebo (Figure 9). However, the change in cSBP (P<0.05) and cPP (P<0.05) after 6 months of study-drug for Spiro was significantly different than Placebo (Figure 9). Figure 10 shows a non-significant difference in cfPWV for Spiro compared to Placebo after 6 months of study-drug.

The change in carotid distensibility coefficient (Table 3) and β-stiffness index (Table 3) was similar between the two groups after 6 months of study-drug.

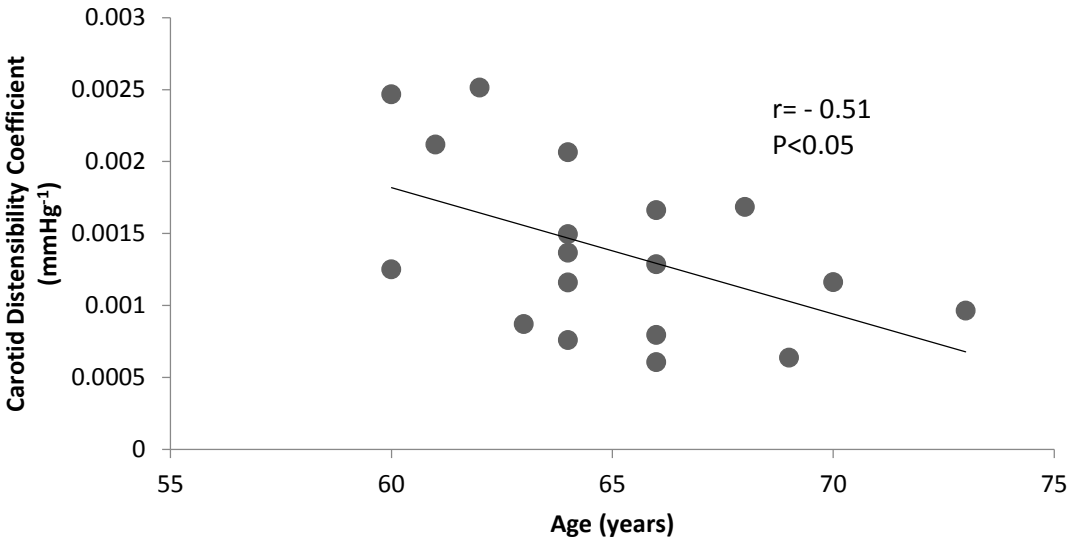


Figure 8. Scatterplot of carotid distensibility coefficient versus age

A scatterplot (black dots) of carotid distensibility coefficient ($mmHg^{-1}$) versus age (years) for pooled data (n=18). There was a significant linear negative correlation between carotid distensibility and age as represented by the trend line (r= - 0.51, P<0.05).

Table 2. Baseline carotid blood pressure and arterial stiffness

Measure	Spiro (n=9) mean ±SD	Placebo (n=9) mean ±SD
Carotid Pressure (mmHg)		
cMAP	96±16	91±7
cSBP	144±24	131±17
cDBP	73±13	71±5
cPP	71±14	60±17
Arterial Stiffness		
cfPWV (m/s)	7.42±1.37	6.99±0.68[€]
Carotid Distensibility Coefficient ($mmHg^{-1}$)	0.0013±0.0005	0.0015±0.0007
β-stiffness index, arbitrary units	8.46±2.22	9.15±4.75

Spiro=spironolactone group, Placebo=placebo group, cMAP=carotid mean arterial pressure, cSBP=carotid systolic blood pressure, cDBP=carotid diastolic blood pressure, cPP=carotid pulse pressure, cfPWV=carotid-femoral pulse wave velocity, [€]n=8.

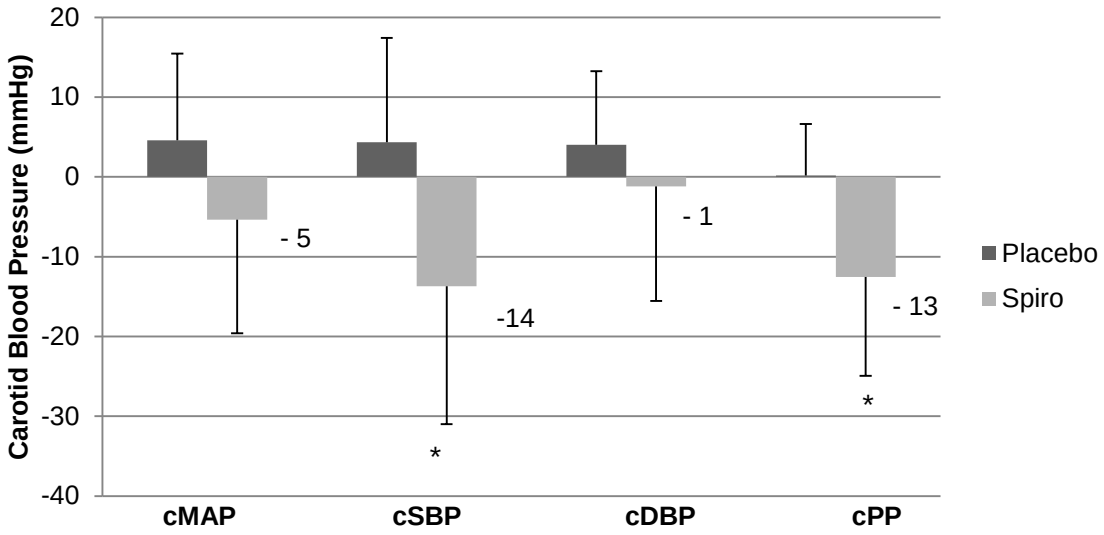


Figure 9. Changes in carotid pressures after 6 months of study-drug

Carotid MAP (cMAP, mmHg, left pair of bars), systolic blood pressure (cSBP, mmHg, second from left pair of bars), diastolic blood pressure (cDBP, mmHg, second from right pair of bars), and pulse pressure (cPP= cSBP – cDBP, mmHg, right pair of bars) differences are shown for placebo (dark bars) and spironolactone (Spiro, light bars) after 6 months. Two-way repeated measures ANOVA were performed to establish significant interactions between Spiro and Placebo after 6 months of study-drug for cSBP ($P<0.05$) and cPP ($P<0.05$). There were no significant interactions for cMAP ($P=0.13$) and cDBP ($P=0.40$) between Spiro and Placebo. Data are means with error bars as standard deviation.

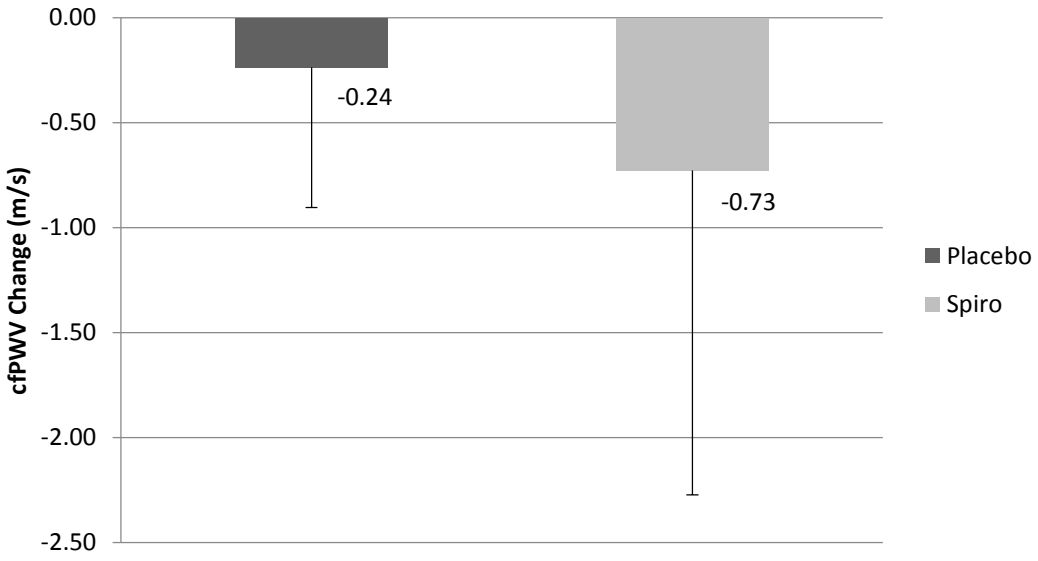


Figure 10. Change in carotid-femoral pulse wave velocity (cfPWV) after 6 months of study drug

cfPWV (m/s) differences after 6 months of study-drug are shown for placebo (Placebo, dark bar) and spironolactone (Spiro, light bar). A 2-way repeated measures ANOVA was performed to establish no significant interaction between Placebo and Spiro groups after 6 months of study-drug (P=0.42). Data are means with error bars as standard deviation.

Table 3. Changes in arterial stiffness after 6 months of study-drug

Measure	Spiro (n=9) mean ±SD	Placebo (n=9) mean ±SD	P-value[a]
Carotid Distensibility Coefficient ($^{-}$mmHg^{-1})	0.0002±0.0005	-0.0000±0.0006	0.47[b]
β-stiffness index (a.u.)	-0.54±2.86	-1.24±3.43	0.65

[a]2-way repeated measures ANOVA for treatment and time interaction, [b]2-way ANCOVA with age as covariate, Spiro=spironolactone group, Placebo=placebo group, a.u.=arbitrary units.

3.4 Discussion

The objective of this chapter was to test the hypothesis that spironolactone administered for 6 months would decrease blood pressure and arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compared to placebo. Important findings from this RCT were a significant reduction in cSBP and cPP; but cfPWV, carotid distensibility coefficient, and β-stiffness index, remained unchanged after 6 months of study-drug.

The first notable finding from the pooled baseline data was a significant association between carotid distensibility coefficient and age at baseline ($r = -0.51$, $P<0.05$); which is consistent with current studies (Mattace-Raso et al., 2006; Mitchell, 2008). These participants were then effectively randomized as supported by the similar central arterial measures and blood pressure at baseline. Six months of mineralocorticoid receptor (MR) antagonism with spironolactone significantly reduced both carotid systolic and pulse pressure; only numerically improving cfPWV and carotid distensibility coefficient. In agreement with these results, Hwang et al. (2013) found in response to MR antagonism that arterial stiffness numerically improved despite significant decrease in systolic and pulse pressure. This randomized, double-blind, crossover study using eplerenone (an MR antagonist) in 23 generally healthy older adults (mean age 64 years) for one month showed a numeric decrease in PWV (9.17±1.19 Placebo vs. 8.92±1.19 m/sec MR antagonist, P=0.5) or increase in carotid distensibility (23.1±1.8 Placebo vs. 23.3±1.7 10^{-3}/kPa MR antagonist, P=0.8). Although one month is notably less than the six months of treatment used in the current study, there was sufficient time in their study to significantly decrease systolic pressure by 7 mmHg and pulse pressure by 5 mmHg. It is possible

that the significant reduction in pulse pressure shown in this thesis RCT may have reduced the deleterious hemodynamic pulsatility experienced in the high flow, low impedance, cerebral circulation. This could correspondingly reduce the potential damage to deep microcirculation manifesting as white matter hyperintensities and cognitive dysfunction (Mitchell, 2008; Mitchell, 2011). In contrast, MacKenzie et al. (2009) observed a slight increase in PWV with a corresponding reduction in systolic and pulse pressures. These results should be compared to the current study with caution, because the MacKenzie et al. (2009) study involved ACE inhibition (perindopril) for 10 weeks, not MR antagonism (spironolactone) for 6 months, and only measured blood pressure in duplicate using a mercury sphygmomanometer instead of continuous beat-by-beat blood pressure monitoring. De Souza et al. (2010) investigated the effects of spironolactone (median dose 50 mg daily) in 173 hypertensive patients already taking antihypertensives (94% ACE inhibitors/AR blockers) finding that cfPWV numerically improved after 2-4 months of therapy from baseline 10.4 m/s to 10.0 m/s; consistent with this thesis RCT. Their study was not prospectively designed to evaluate cfPWV, therefore no statistical analysis was done to compare baseline to post-therapy values. The lack of a significant increase in carotid distensibility in the current study is consistent with Lipsitz et al. (2005) that showed carotid distensibility in controlled-hypertension and normotensive study arms remained unchanged with 6 months of RAAS inhibition. Only the aggressively managed uncontrolled-hypertensive arm (> 160 mmHg at entry into study) in their study showed an increase in carotid distensibility.

A potential mechanism to explain how arterial stiffness remained essentially unchanged (i.e., only numeric improvements in cfPWV and carotid distensibility coefficient) after MR

antagonism could be explained by recent evidence that acute inhibition (single dose) of MRs with eplerenone impairs vascular dilation by specifically impairing vascular endothelial function independent of blood pressure change or vascular smooth muscle responsiveness to exogenous vasodilator nitric oxide (Hwang et al., 2016). This is supported by evidence in older adults free from cardiovascular disease that one month of MR antagonism did not lead to reduced oxidative stress, or improved arterial stiffness, despite significant blood pressure reduction (Hwang et al., 2013). Hwang et al., 2016 noted that in 22 healthy older adults (mean 61 years) endothelium-dependent vasodilation following acute inhibition of MR activation with eplerenone was significantly reduced by 19% ($P \leq 0.03$). Endothelial nitric oxide synthase activity, that represents capacity to produce vasodilator nitric oxide in normal healthy aging, was correspondingly reduced ($P=0.02$) with the MR antagonism compared to placebo supporting that MR activation plays a role in regulating endothelial nitric oxide activity to promote endothelium-dependent dilation in healthy aging (Hwang et al., 2016). Aldosterone activation of MR on endothelial cells leads to production of nitric oxide that diffuses to vascular smooth muscle cells and counteracts the MR-dependent vasoconstriction (Galmiche et al., 2014; Skott et al., 2006). There is a dynamic balancing of affects from aldosterone MR activation between endothelial-dependent vasodilation and smooth muscle-dependent vasoconstriction. The effects of MR activation on vascular reactivity in healthy humans remains controversial however, because of conflicting results on improvement of endothelial-dependent relaxation (McCurley & Jaffe, 2012).

Since it is well established that ACE inhibitors have consistently shown to improve endothelial function in animals and coronary artery disease patients through up-regulation of endothelial nitric oxide expression, it is possible that stable ACE inhibition in the current study prior to baseline vascular assessments had already improved endothelial function enough to minimize further improvements (Ceconi et al., 2007; Su, 2015). If indeed, MR antagonism could provide improvement in endothelial function as addressed above. Perhaps data from a randomized, double-blind, placebo-controlled study of 115 patients, without the detrimental effects of hypertension or coronary artery disease, supports this explanation: the significant decrease in systolic blood pressure was independent of cfPWV after 3 months of spironolactone (Hare et al., 2013). Enrolling patients without the bias introduced from stable ACE inhibitor therapy could allow for a better estimate of the effects of spironolactone on central vascular stiffness. The study by Kithas et al. (2010) supports possible explanation since the beneficial effects of reduced cfPWV and blood pressure with 6 months of spironolactone in a geriatric hypertensive population was after antihypertensive medication withdrawal prior to baseline measures of cfPWV and blood pressure. Mahmud & Feely (2005) assessed 24 hypertensive older adults naïve to antihypertensive therapy in order to show a significant (1.54 m/s) reduction in cfPWV and blood pressure after one month of spironolactone.

Mean cfPWV from the current study (7.4 m/s spironolactone and 7.0 m/s placebo) was lower than suggested normative data for mean age 64-65 with hypertension of 10.2 m/s (Alecu et al., 2008). Alecu et al. (2008) proposed, using a multivariate analysis, that age, BMI, diabetes, heart rate, MAP and antihypertensive treatment were all determinants of cfPWV in hypertensive

patients that collectively affect cfPWV explaining 19.8% of the variance. Although, the current study baseline cfPWV is consistent with central PWV measured by Kroner et al (2014) that directly measured aortic PWV using MRI to be 7.4±1.4 m/s with velocity-encoded MRI in generally healthy older adults (mean age 56 years). cfPWV in this thesis RCT was also consistent with the large Framingham Heart Study Third Generation cohort that measured cfPWV in 3,205 mid-aged (mean 46) participants as 7.1±1.4 m/s (Torjesen et al., 2017).

3.4.1 Limitations

This thesis RCT has several limitations. First, study findings are only generalizable to hypertensive patients stably treated with centrally acting ACE inhibitors willing to take spironolactone for 6 months. The few participants that enrolled, then went on to complete the study, tended to be motivated male patients. Participants were not self-selected to participate in research studies ongoing at their physician's office. This could have minimized sample bias that may be introduced by participants actively seek opportunity to enrol in research studies that may be more sensitive to maintaining a healthy lifestyle. However, it is always important to be cautious if generalizing study results to a population other than the sample studied.

Stiffness of central elastic arteries like the aorta predicts cardiovascular risk and cfPWV is the accepted gold standard for central arterial stiffness assessment (Mitchell et al., 2010; Van Bortel et al., 2012; Vlachopoulos, Aznaouridis, & Stefanadis, 2010). However different

measurement techniques to generate cfPWV show a lack of standardization, reinforcing the need for caution when interpreting results across trials it is difficult to compare across studies due to the variability in methodologies (Townsend et al., 2015; Van Bortel et al., 2012). Notable different techniques include the use of transcutaneous infrared plethysmography or pulse tonometry to estimate pulse arrival time (Phillips et al., 2013). This thesis RCT used Doppler ultrasound to estimate pulse wave transit times based on blood velocity instead of a common mechanotransducer method (Complior®, ALAM Medical, Vincennes, France) or applanation tonometry method (SphygmoCor, AtCor Medical, West Ryde, Australia) to measure transit time, making the assumption that transduction of arterial pressure pulse wave velocity corresponds to the flow PWV of the spectral Doppler ultrasound. These common systems use mechanotransducers applied to the skin and measure real-time pressure pulse waves at the carotid and femoral sites. Notably, it has been demonstrated that there is a strong association between Complior method (i.e., pressure pulse wave propagation) and Doppler ultrasound (i.e., blood cell velocity pulse wave) with an intra-class correlation coefficient of 0.91 (Calabia et al., 2011). This thesis also demonstrated a strong correlation between pressure pulse wave velocity and blood cell pulse wave velocity at the carotid artery site ($r = 0.92$, $P<0.00001$, Figure 29, APPENDIX 1.M.2). Lastly, there is potential for acute changes in blood pressure to affect indicators of arterial stiffness; as explored in CHAPTER 7.

In conclusion, spironolactone significantly reduced blood pressure but had no effect on central arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compared to placebo in an RCT. These data do not support the hypothesis that spironolactone

will decrease blood pressure and decrease arterial stiffness in older adults on stable ACE inhibitor treatment for hypertension; likely due to mechanisms and limitations described.

CHAPTER 4 Effect of Spironolactone on Cerebral Blood Flow and Cognition in Older Adults with Hypertension

4.1 Objectives

The objective of this chapter was to assess the effect of spironolactone administered for 6 months on cerebral blood flow and cognitive function in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. Cerebral blood flow was estimated as anterior cerebral blood flow (aCBF) and cognitive function assessed using the Montreal Cognitive Assessment (MoCA), Trails B-A, and Digit Span Forward and Digit Span Backward tests. It was hypothesized that in older hypertensive adults stably treated by centrally acting ACE inhibitors that spironolactone will improve cerebral blood flow and cognition compared to placebo based on existing studies that have collectively shown that RAAS inhibition improves arterial stiffness, cerebral blood flow velocity, and cognition (Lipsitz et al., 2005; Tarumi et al., 2014; Yagi et al., 2011).

4.2 Methods

As described in CHAPTER 2 General Methods and Materials.

4.3 Results

There were eighteen participants (mean age 65±3 years) that completed the RCT (Table 4). All participants were taking a centrally acting ACE inhibitor; most of which were taking ramipril. Approximately half were taking a statin for lowering lipids, and half were taking a combination

ACE inhibitor with hydrochlorothiazide diuretic. Serum sodium and potassium were similar between Spiro and Placebo. Renal function, represented as serum creatinine or estimated glomerular filtration rate (eGFR), was also similar between the groups during the study. There were 3 participants in the Spiro group and 1 participant in the Placebo group that a self-proclaimed history of chest pain or heart attack; otherwise clinical characteristics were similar. The mean adherence to study-drug was excellent; 95% or greater for both groups.

There were no significant differences in any of the baseline measures of age, sex, BMI, brachial pressure, or cognitive function measures between the groups suggesting successful randomization (Table 5). The Spiro group had higher systolic blood pressure (SBP,) and pulse pressure (PP,) compared to the Placebo group. However, the Placebo group had a higher BMI and heart rate than Spiro group. It is unclear if these were relevant differences given the sample size. MAP and diastolic pressures (DBP) were similar between the two groups. MAP and DBP changes after 6 months of study-drug were not significantly different between Spiro and Placebo (Figure 11). In contrast, SBP ($P<0.05$) and PP ($P<0.05$) changes after 6 months of study-drug were significantly different between the groups (Figure 11). There was a trend toward an increase in heart rate for Spiro group ($P=0.06$, Table 6).

Baseline anterior cerebral blood flow (aCBF) was similar between the two groups and remained similar after 6 months of study-drug (Figure 12). Baseline CVCi (cerebrovascular conductance index=aCBF/MAP) was similar between the two groups and remained similar after 6 months of study-drug (Figure 13). Changes in Montreal Cognitive Assessment Test (MoCA) scores and

Trails B-A times after 6 months of study-drug for Spiro were similar to Placebo (Table 6); as were Digit Span Forward and Digit Span Backward changes after 6 months of study-drug (Table 5).

Table 4. Study medications, biochemistry, and renal function during study

Measure	Spiro (n= 9) mean ±SD	Placebo (n=9) mean ±SD
Medications (%)		
Statin	44	56
ASA	33	33
Ramipril (ACE inhibitor)	67	78
Lisinopril (ACE inhibitor)	0	11
Perindopril (ACE inhibitor)	33	11
Anticoagulant	0	11
HCTZ	44	56
Calcium Channel Blocker	11	11
Beta-Blocker	33	22
Adherence to Study Drug (%)	95±2	97±4
Biochemistry (mmol/L)		
Serum Sodium	138±2	139±1
Serum Potassium	4.4±0.2	4.2±0.3
Renal Function		
Serum Creatinine (µmol/L)	89±14	82±16
eGFR (mL/min/1.73m^2)	77±14	76±9

Spiro=spironolactone group, Placebo=placebo group, Statin= HMG-CoA reductase inhibitor, ASA= acetylsalicylic Acid, HCTZ=hydrochlorothiazide, eGFR= Estimated Glomerular Filtration Rate (Modification of Diet in Renal Disease).

Table 5. Baseline characteristics

Measure	Spiro (n=9) mean ±SD	Placebo (n=9) mean ±SD
Age (years)	66±3	64±4
Male (%)	100	67
BMI (kg/m^2)	29±3	33±6
Cognitive Function		
MoCA (score)	25±2	25±2
Trails B-A (seconds)	34.5±16.6	44.9±25.1
Digit Span Forward (row number)	5±1	4±1
Digit Span Backward (row number)	4±1	4±1
Heart Rate (beats/min)	64±9	72±14
Brachial Pressure (mmHg)		
MAP	98±12	93±7
SBP	145±17	132±14
DBP	75±12	73±5
PP	70±13	59±13
Cerebrovascular Hemodynamics		
aCBF (mL/min)	828.9±256.6	874.6±193.7
CVC_i (mL/min/mmHg)	9.5±2.2	8.6±2.8

Spiro=spironolactone group, Placebo=placebo group, BMI = Body Mass Index, MoCA=Montreal Cognitive Assessment, MAP=Mean Arterial Pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, PP=pulse pressure, aCBF = anterior cerebral blood flow, CVC_i= cerebrovascular conductance index (aCBF/MAP).

Table 6. Changes in cognitive function, blood pressure, and cerebral blood flow after 6 months of study-drug

Measure	Spiro Change (n= 9) mean ±SD	Placebo Change (n=9) mean ±SD	P-value[a]
Cognitive Function			
MoCA (score)	2±2	1±2	0.55
Trails B-A (seconds)	-4.0±13.5	-7.4±16.0	0.63
Digit Span Forward (row)	0±1	0±1	0.84
Digit Span Backward (row)	0±1	0±1	0.46
Heart Rate (beats/min)	4±9	-5±9	0.06
Brachial Blood Pressure (mmHg)			
MAP	-7±11	1±8	0.14
SBP	-14±14	0±11	<0.05
DBP	-3±12	-0±7	0.52
PP	-12±11	0±8	<0.05
Cerebral Blood Flow			
aCBF (mL/min)	4.0±136.6	-38.6±140.3	0.52
aCBF (%)	0.5±17.1	-6.2±18.0	0.55[b]

[a]Two-way repeated measures ANOVA for treatment and time interaction, [b]unpaired t-test, Spiro=spironolactone group, Placebo=placebo group, MoCA=Montreal Cognitive Assessment, MAP= Mean Arterial Pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, PP=pulse pressure, aCBF = anterior cerebral blood flow.

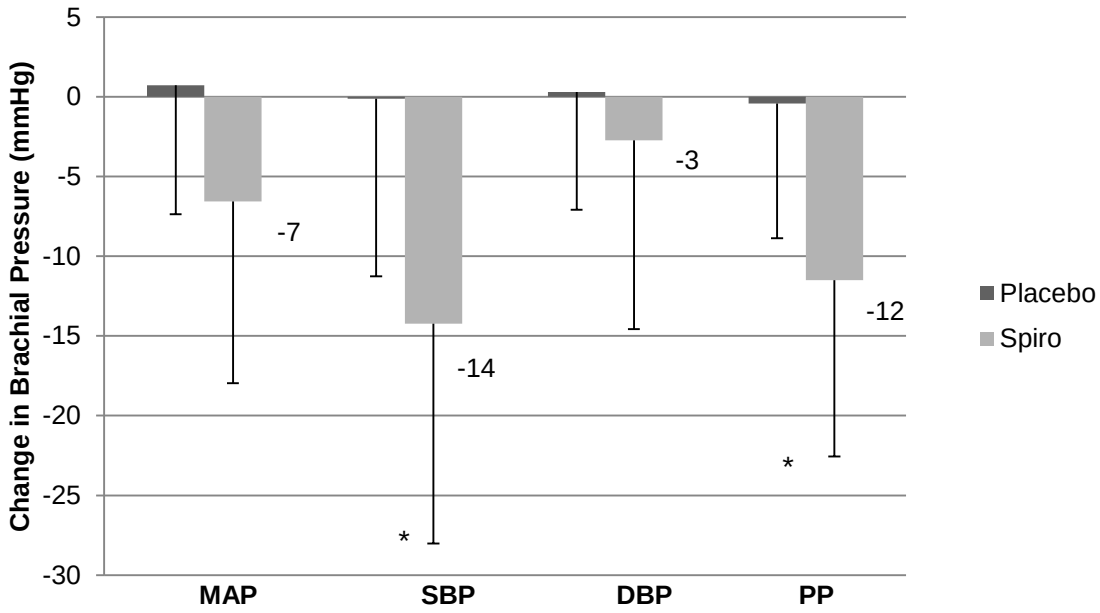


Figure 11. Change in brachial pressures after 6 months of study-drug

MAP (mmHg, left pair of bars), systolic blood pressure (SBP, mmHg, second from left pair of bars), diastolic blood pressure (DBP, mmHg, second from right pair of bars), and pulse pressure (PP=SBP – DBP, mmHg, right pair of bars) changes are shown for placebo (Placebo, dark) and spironolactone (Spiro, light) after 6 months of study-drug. *Two-way repeated measures ANOVA were performed to establish significant interactions between Spiro and Placebo after 6 months study-drug for SBP ($P<0.05$) and PP ($P<0.05$). There were no significant interactions between Spiro and Placebo after 6 months study-drug for MAP ($P=0.14$) and DBP ($P=0.52$). Data are means with error bars as standard deviation.

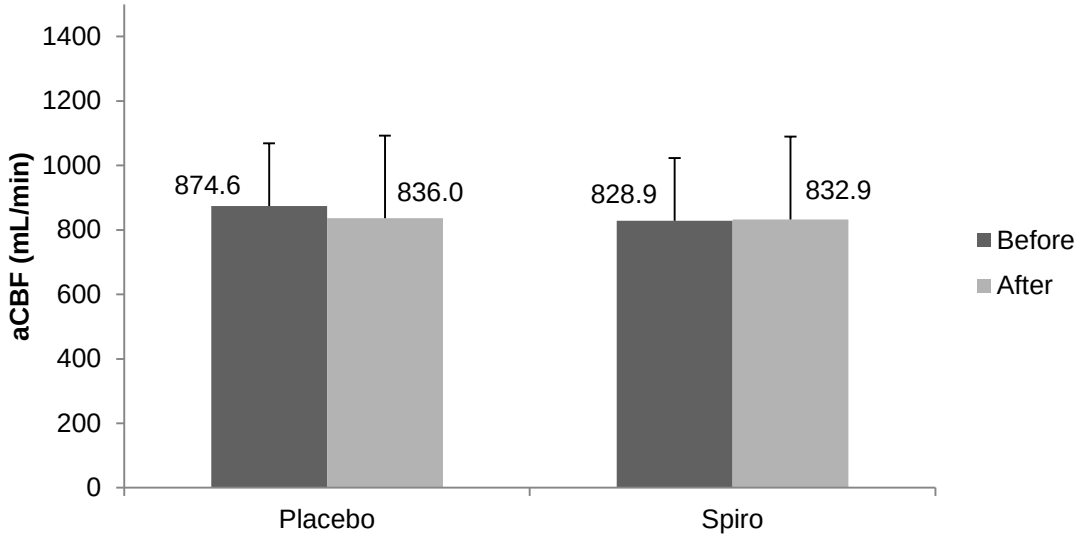


Figure 12. Anterior cerebral blood flow (aCBF) at baseline and after 6 months of study-drug

aCBF (mL/min) at baseline (dark bars) and after 6 months of study-drug (grey bars) are shown for spironolactone (Spiro, right pair of bars) and placebo (Placebo, left pair of bars) groups. Two-way repeated measure ANOVA was performed to establish no significant interaction between or within groups after 6 months of therapy (P=0.52). Data are means with error bars as standard deviation.

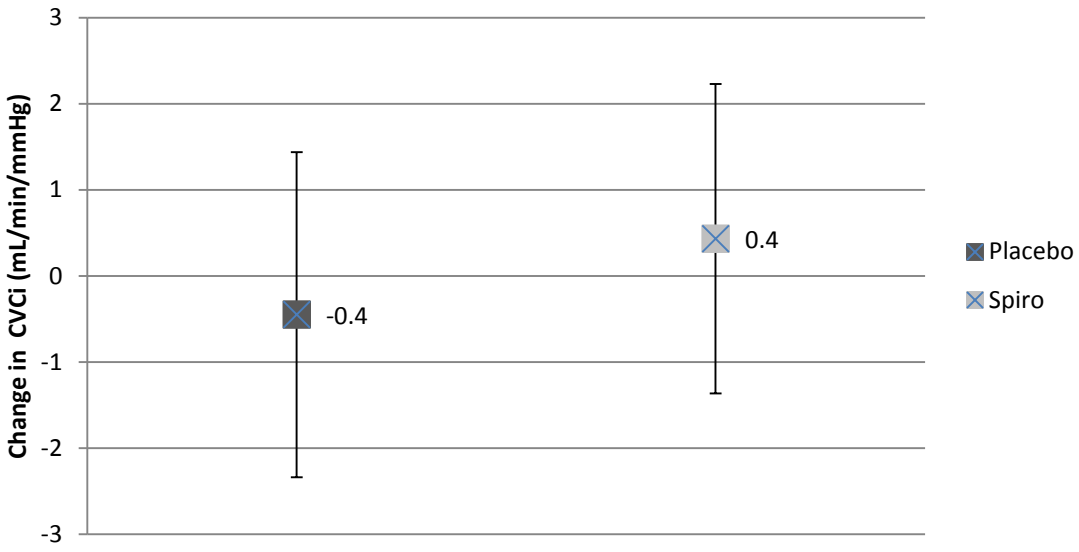


Figure 13. Change in cerebrovascular conductance index (CVC_{index}) after 6 months of study-drug

CVC_i (aCBF/MAP=cerebrovascular conductance index, ((mL/min)/mmHg) changes are shown for placebo (Placebo) and spironolactone (Spiro) after 6 months of study-drug. Two-way repeated measure ANOVA was performed to establish no significant interaction between or within groups after 6 months of therapy (P=0.33). Data are means with error bars as standard deviation.

There were no adverse events that led to discontinuation in the Spiro group. Table 7 shows that three participants randomized to Placebo withdrew due mild diarrhea or hyperkalemia prior to first dose of study-drug. One participant had a single brief episode of symptomatic rapid heart rate. One participant in each group voluntarily withdrew due to concerns of compromising travel health insurance while on vacation during the study. No participants were lost to follow up.

Table 7. Reasons for participant discontinuation from study

Measure	Spiro (n=10)	Placebo (n=13)
Mild diarrhea		1
Hyperkalemia prior to study drug initiation		1
Voluntarily withdrew	1	1
Brief symptom of rapid heart rate		1

Spiro=spironolactone group, Placebo=placebo group, hyperkalemia = serum potassium ≥ 5.0 mmol/L.

4.4 Discussion

The objective of this chapter was to assess the effect of spironolactone on cerebral blood flow and cognitive function in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. It was hypothesized that spironolactone would improve cerebral blood flow and cognition from existing studies that have shown a significant negative correlation between cerebral blood flow (total cerebral blood flow measured using MRI) and cfPWV; and based on evidence of higher cfPWV being independently associated with manifestations of brain small-vessel disease (i.e., greater volume of white matter hyperintensities) in hypertensive patients (Henskens et al., 2008; Tarumi et al., 2014; Xing et al., 2017). In this thesis RCT, aCBF and cognitive function remained unchanged after 6 months of spironolactone. It is possible that aCBF was maintained by the slight non-significant improvement in cerebrovascular conductance to compensate for the reduction in MAP.

This thesis RCT results are consistent with Hajjar et al. (2013) that showed 12 months of RAAS inhibition with an ACE inhibitor (or angiotensin receptor blocker, ARB) preserved cerebral blood flow in older hypertensive adults of similar age and blood pressure. Although, their study used MCA velocity (MCAv) using transcranial Doppler ultrasound as a different marker of cerebral blood flow, and therapy was preceded by a washout period allowing for antihypertensive medication withdrawal. The current thesis RCT showed that absolute or percent change in aCBF (measured as the sum of internal carotid artery blood flow) after 6 months of spironolactone remained unchanged; despite very good adherence to study-drug in both groups (95±2% vs 97±4%, P=0.35). Hajjar et al. (2013) showed that maintenance of cerebral blood flow velocity

was despite significant reductions in blood pressure with ACE inhibitor lisinopril (systolic BP 153 mmHg reduced to 126 mmHg and diastolic BP 85 to 70 mmHg, P<0.001), which is similar to this RCT. In contrast, Lipsitz et al. (2005) showed that RAAS inhibition (6 months of ACE inhibitor or ARB) significantly improved cerebral blood flow estimated by MCA velocity. However, this was only in participants that initially had uncontrolled hypertension (systolic pressure >160 mmHg at study entry) and given RAAS inhibition to achieve the target systolic pressure of <140 mmHg for six months unlike participants in the current study that already had well controlled systolic blood pressure at baseline cerebrovascular and cognitive assessments. The findings in the current RCT are consistent with the controlled-hypertension group (treated hypertensive patients with systolic pressure <140 mmHg at study entry) in Lipsitz et al. (2005) that also showed maintenance of cerebral blood flow after 6 months of RAAS inhibition. Collectively this evidence suggests that cerebral blood flow is preserved despite significant reductions blood pressure. The similar cerebrovascular conductance between the groups in the current RCT is supported by similar cerebral blood flow and MAP changes after 6 months of study-drug. Although one may expect MR antagonism to increase cerebrovascular conductance as predicted from evidence showing a significant negative association between lower cerebral blood flow velocity and high baseline aldosterone (Hajjar et al., 2015). This thesis RCT did not measure serum aldosterone.

The mean MoCA score of 25±2 for each group at baseline validated effective randomization of executive cognitive function between the groups and was above the normative data mean MoCA score of 22±5 for 228 subjects of the age group 65 – 75 years as part of the longitudinal,

population-based, Dallas Heart Study (Rossetti, Lacritz, Cullum, & Weiner, 2011). Unlike Yagi et al. (2011) that found MR antagonism for 6 months added to ongoing hypertension treatment significantly improved cognitive function in 7 matched patients, the current RCT finding was that cognitive function remained similar between the two groups after 6 months of MR antagonism. This finding was unexpected based on the Yagi et al. (2011) results yielded from participants of similar age, MAP, SBP and DBP as this thesis RCT using a less sensitive executive function assessment tool Mini-Mental State Examination (MMSE; unlike the MoCA used in current study) that were only matched by age, sex and MMSE score instead of double-blind randomization as in the current study (Dong et al., 2012). A recent post hoc analysis of the large HOPE 3 study supports this thesis RCT finding: 1626 intermediate cardiovascular risk patients (at least 70 years, no blood pressure medication) over 6 years taking a RAAS inhibitor that lowered blood pressure (systolic mean decline 6 mmHg) did not prevent cognitive decline estimated as Digit Symbol Substitution Test, modified MoCA, and Trails B results being comparable to placebo (Bosch, 2016). Cerebral blood flow was not measured in HOPE-3.

The current RCT finding that maintaining cerebral blood flow preserves cognitive function is consistent with a study in which patients who were unable to maintain cerebral blood flow as a result of a transient ischemic attack (L. Wang, Jia, & Wu, 2013). Wang et al. (2013) showed that temporarily reduced cerebral blood flow was associated with declined executive cognitive function (assessed using the MoCA) and lower MCA peak systolic velocities ($P < 0.001$). This could even be generalized to maintaining Trails B-A times and Digit Span Backward levels as they are simply expanded components of the MoCA assessing various cognitive domains.

Perhaps if participants in this thesis RCT had executive dysfunction at baseline, there could have been a significant improvement in Trails B-A times as demonstrated by Hajjar et al (2015) with RAAS inhibition in patients with executive dysfunction. If the hypothesis of this thesis is still potentially true that the beneficial effects of spironolactone on blood pressure and arterial stiffness will improve cerebral blood flow and cognition, future research is still needed given identifiable limitations of this thesis RCT to address this question (CHAPTER 8, section 8.5).

4.4.1 Limitations

The main limitation is that the small sample size did not provide statistical power to detect significant differences in the primary outcome marker of aCBF. The sample size required to detect a significant difference in mean spironolactone aCBF (832.9 mL/min) and placebo (836.0 mL/min) is estimated from this current study as 330 participants (165 in each group) with a significance level (α) of 0.05 and desired power of 0.8 (Sigmaplot 12.5, Systat Software Inc, San Jose, California, USA). The calculated Cohen's *d* effect size for spironolactone was 0.3 from this thesis RCT data; much smaller than the desired Cohen's *d* effect size of 1.0 that was used to calculate sample size during the trial design. This larger desired effect size was based on observational, non-interventional, pilot data as described in the methods Chapter 2 (2.8). Since it is also possible that the small sample size of 9 participants in each treatment group resulted in a Type II error of a "false negative", due to a low statistical power to detect a true difference in baseline measures between the treatment groups, with SBP and PP in particular, 2-way repeated measures ANOVA tests were conducted. These showed a significant interaction

between treatment group (spironolactone or placebo) and time (pre- or post-treatment) on dependent variable SBP. This was also true for PP. The correlation between the covariate of baseline SBP and post treatment SBP was not similar between the groups, not surprisingly, since spironolactone was expected to reduce blood pressure more effectively than placebo.

Another limitation is that study findings are really only generalizable to older hypertensive patients on stable centrally-active ACE inhibition willing to take spironolactone for 6 months. The third is that the few participants that enrolled and completed the study tended to be motivated male patients. These motivated participants may have introduced bias of an uncommonly health-aware sample of older adults not representative of the general hypertensive population. Although, it should be noted, that these participants were not previously identified as being interested in participating in clinical trials by their Family Physicians which would have been noted in their electronic medical records. The fifth is that internal carotid artery blood flow is only a marker of brain blood flow and does not represent total brain blood flow that includes blood flow from vertebral arteries. Blood viscosity and hematocrit, which are inversely related to brain blood flow velocity, were not measured in the current study (Ameriso et al., 1990; Fiermonte, Aloe Spiriti, Latagliata, Petti, & Giacomini, 1993). Blood flow velocities could increase approximately 20% with a drop in hematocrit from 40% to 30% (Purkayastha & Sorond, 2012). All assessments were done on the background of centrally acting ACE inhibition that may have already optimized marginal benefits of additional RAAS inhibition with an MR antagonist. Since the MoCA is primarily a screening test for MCI, other more sensitive measures of cognitive function could have been applied in this thesis.

Stroop, Eriksen Flanker Task, Wisconsin Card Sorting, or N-back may have provided a greater power to detect changes in cognitive domains.

In conclusion, cerebral blood flow and cognitive function was unchanged from spironolactone administered for 6 months in older hypertensive adults who were receiving stable treatment with centrally acting ACE inhibitors. These data do not support the hypothesis that spironolactone improves cerebral blood flow and cognitive function; likely due to identifiable study limitations.

CHAPTER 5 Effect of Spironolactone on Cerebrovascular Autoregulation in Older Adults with Hypertension

5.1 Objectives

The objective of this chapter was to assess the effect of spironolactone administered for 6 months on cerebrovascular autoregulation in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. The changes in mean arterial pressure (MAP), MCA velocity, and cerebrovascular resistance index (CVRi) responses to posture change from seated to standing upright were estimates of cerebrovascular autoregulation. The secondary objective was to assess the effect of spironolactone on changes in cerebrovascular reactivity to carbon dioxide (CO_2). Since existing evidence that RAAS inhibition in older hypertensive adults significantly reduces blood pressure without compromising orthostatic changes in cerebral blood flow (Lipsitz et al., 2005), it was hypothesized that spironolactone would maintain cerebrovascular autoregulation despite an expected reduction in supine blood pressure.

5.2 Methods

As described in CHAPTER 2 General Methods and Materials.

5.3 Results

There were no significant differences between the spironolactone (Spiro) and placebo (Placebo) groups in hemodynamic responses before, or after 6 months, of study-drug. Specifically, the

posture (from seated to standing) change in MAP, MCA velocity, and cerebrovascular resistance index (CVRi=MAP divided by MCA velocity) was similar during baseline assessments (Table 8). The percent change in CVRi (Spiro 1±9 % vs Placebo -3±9 %, P=0.34) was also similar.

Posture change (absolute and percent) in MCA velocity at baseline was similar between Spiro and Placebo groups (Figure 14). Posture change (absolute and percent) in MAP at baseline was statistically different between Spiro and Placebo groups (Figure 15).

The differences in MAP and MCA velocity responses to posture change were reassessed after 6 months of study-drug. Absolute and percent differences in change of MAP for Spiro were similar to Placebo (Table 9). Absolute and percent differences in change of MCA velocity for Spiro were also similar to Placebo (Table 9). Correspondingly, absolute and percent differences in change of CVRi were similar between the groups (Table 9).

Table 8. Baseline cerebrovascular autoregulation responses to posture change

Measure	Spiro (n=9) mean ±SD	Placebo (n=6) mean ±SD	P-value[a]
Posture change in **MAP** (mmHg)	-9±8	-12±4	0.41
Posture change in **MCA** velocity (cm/s)	-5.0±2	-4.5±3	0.66
Posture change in CVRi (mmHg/(cm/s))	0.03±0.19	-0.04±0.22	0.50

[a]From unpaired t-test, Spiro=spironolactone group, Placebo=placebo group, MAP=mean arterial pressure, MCA = middle cerebral artery, CVRi= cerebrovascular resistance index (MAP/MCA velocity). Posture change was seated to standing.

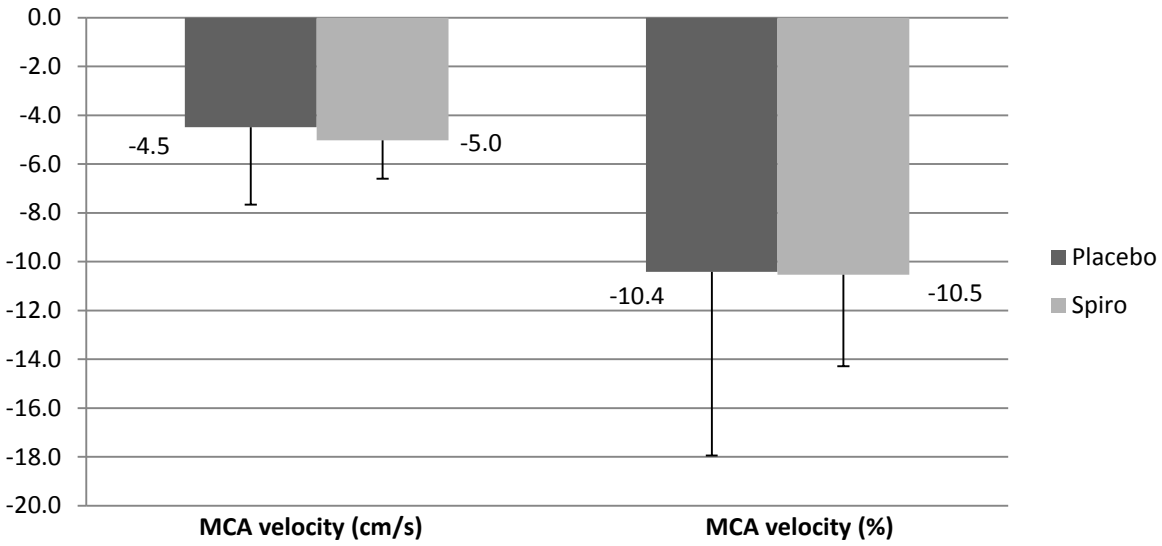


Figure 14. Baseline responses of middle cerebral artery (MCA) velocity to posture change

Baseline posture change in MCA velocity for spironolactone (Spiro, light bars) and placebo (Placebo, dark bars) in absolute (cm/s, left pair of bars) and percent (%, right pair of bars) immediately upon standing upright from resting seated posture. Unpaired t-tests were performed to establish no significant differences between Spiro and Placebo groups for absolute MCA velocity (cm/s, P=0.66) and percent MCA velocity (%, P=0.97) responses. Data are means with error bars as standard deviation.

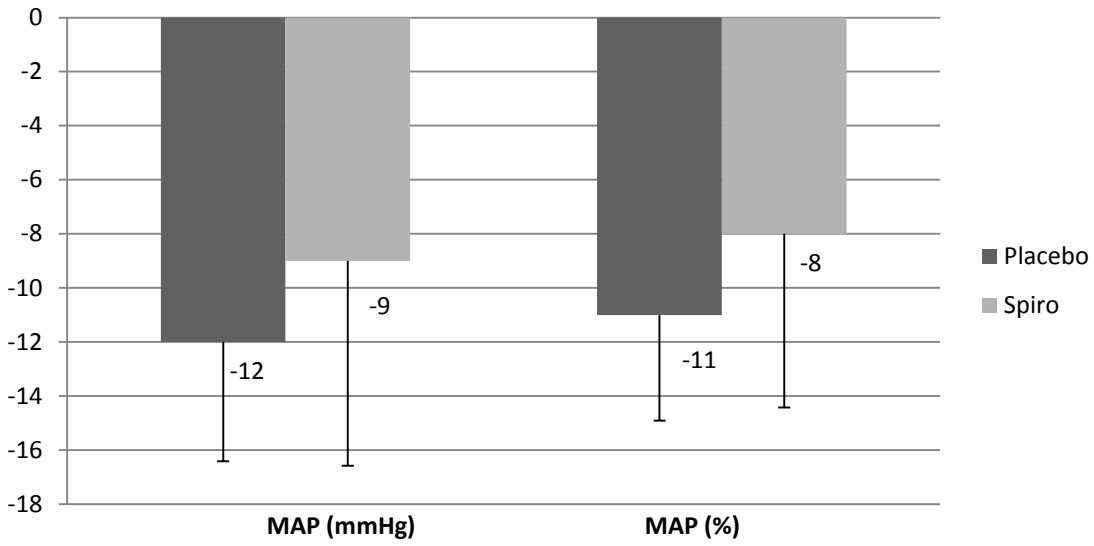


Figure 15. Baseline responses of MAP to sit-to-stand posture change

Baseline posture change in MAP for spironolactone (Spiro, light bars) and placebo (Placebo, dark bars) in absolute (mmHg, left pair of bars) and percent (%, right pair of bars) immediately upon standing upright from resting seated posture. Unpaired t-tests were performed to establish no significant differences between Spiro and Placebo groups for absolute MAP (mmHg, P=0.41) and percent MAP (%, P=0.26) responses. Data are means with error bars as standard deviation.

Table 9. Differences in cerebrovascular autoregulation after 6 months of study-drug

Measure	Spiro Difference (n= 9) mean ±SD	Placebo Difference (n=6) mean ±SD	P-value[a]
Posture change in **MAP** (mmHg)	-2±8	1±11	0.48
Posture change in **MAP** (%)	-3±7	1±12	0.43
Posture change in **MCA** velocity (cm/s)	1±3	1±5	0.88
Posture change in **MCA** velocity (%)	2±7	2±12	0.98
Posture change in CVRi (mmHg/(cm/s))	-0.1±0.2	-1.2±3.2	0.77
Posture change in CVRi (%)	-7.3±10.4	-0.7±24.1	0.68

[a]From unpaired t-test, Spiro=spironolactone group, Placebo=placebo group, MCA=middle cerebral artery, MAP=mean arterial pressure, CVRi=cerebrovascular resistance index (MAP/MCA velocity). Difference=posture change in measure after 6 months of study-drug minus posture change in measure at baseline.

Cerebrovascular reactivity to CO_2

The change in MCA velocity during hypercapnia and hypocapnia for the Spiro group was similar to Placebo during baseline assessments (Table 10). APPENDIX 1.M, section M.5, includes end-tidal CO_2 values measured during assessments. The difference in change in MCA velocity during hypercapnia and hypocapnia for the Spiro group after 6 months of study-drug remained similar to the Placebo group (Table 10).

Table 10. Cerebrovascular reactivity to CO_2 at baseline and after 6 months of study-drug

Measure	Spiro (n=9) mean ±SD	Placebo (n=6) mean ±SD	P-value[a]
Baseline			
Hypercapnia change in MCA velocity (cm/s)	27±8	26±20	0.18
Hypercapnia change in MCA velocity (%)	66±25	56±25	0.48
Hypocapnia change in MCA velocity (cm/s)	-13±5	-13±6	0.87
Hypocapnia change in MCA velocity (%)	-27±9	-27±11	1.00
Difference in change after 6 months of study-drug			
Hypercapnia change in MCA velocity (cm/s)	-7±14	-4±17	0.77
Hypercapnia change in MCA velocity (%)	-18±33	-2±22	0.34
Hypocapnia change in MCA velocity (cm/s)	-1±6	-3±4	0.42
Hypocapnia change in MCA velocity (%)	-5±11	-8±12	0.58

[a]From unpaired t-test, Spiro=spironolactone group, Placebo=placebo group, hypercapnia induced by breath-hold, hypocapnia induced by increased respiratory rate, see APPENDIX 1.M.5 for CO_2 values. Hypercapnia change in MCA velocity=MCA velocity during hypercapnia minus MCA velocity during resting respiratory rate (i.e., normocapnia). Hypocapnia change in MCA velocity=MCA velocity during hypocapnia minus MCA velocity during resting respiratory rate (i.e., normocapnia).

5.4 Discussion

The objective of this chapter was to test the hypothesis that spironolactone would maintain cerebrovascular autoregulation in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. The secondary objective was to assess the effect of spironolactone on cerebrovascular reactivity to CO_2.

The significant reductions in SBP and PP after 6 months of spironolactone, as reviewed in CHAPTER 4, did not appear to compromise dynamic cerebrovascular autoregulation estimated as differences in the immediate change in MCA velocity, MAP, and CVRi to standing upright. After 6 months of study-drug, posture change in MCA velocity as a marker of cerebral blood flow, and posture change in MAP, remained similar between Spiro and Placebo. It is possible that the postural change in MAP for the Spiro group was not as large as the Placebo group due to improved arterial function. Posture change in CVRi for the Spiro group was correspondingly similar to Placebo after 6 months of study-drug. These findings that changes to MCA velocity, MAP and CVRi remain uncompromised despite a significant reduction in blood pressure is consistent with existing evidence that RAAS inhibition (spironolactone is a RAAS inhibitor) in older hypertensive patients significantly reduced blood pressure without compromising orthostatic changes in brain blood flow (Lipsitz et al., 2005). Lipsitz et al. (2005) also conducted a prospective study to observe how significantly lowering systolic blood pressure (delta MAP 17 mmHg) with a RAAS inhibitor in uncontrolled hypertensive older adults did not compromise their cerebrovascular autoregulatory response to posture change; MAP, MCA velocity and CVRi

remained unchanged similar to this thesis RCT. To support these findings, Hajjar et al. (2013) showed that posture changes from (seated to standing) in MCA velocity and CVRi were unaffected after 12 months of an angiotensin receptor blocker in 60 plus year old hypertensive patients. This same study did however show a significant reduction in CVRi after 12 months of an ACE inhibitor in these patients. There does not appear to be published evidence with an MR antagonist (like spironolactone) that supports this thesis RCT finding that cerebral autoregulation remains stable despite a significant blood pressure reduction after 6 months of spironolactone.

Cerebrovascular reactivity to CO_2 was unaffected after 6 months of spironolactone. Observed differences in change in MCA velocity during hypercapnia and hypocapnia for the Spiro group was similar to Placebo after 6 months of study-drug. Cerebrovascular reactivity to CO_2 would have been shown to improve if, for example, there was a greater difference in change in MCA velocity during hypercapnia observed for the Spiro group compared to the Placebo group after 6 months of study-drug.

5.4.1 Limitations

The first limitation is the use of MCA blood flow velocity as a marker of cerebral blood flow; despite the common use of changes in MCA blood flow velocity in response to an active sit-to-stand procedure used to induce hemodynamic changes to assess cerebrovascular autoregulation (Hajjar et al., 2013; Lipsitz et al., 2005; Lipsitz et al., 2000). The second limitation

is variability in the angle of the transmitted ultrasound wave from the transcranial Doppler (TCD) probe. To ensure MCA blood flow velocity during TCD ultrasound accurately represents the velocity of moving red blood cells, the angle Θ of the transmitted ultrasound wave to the direction of moving red blood cells must be zero. If the angle is zero, or the emitted wave is parallel to the direction of flow, the cosine of zero is 1, to achieve the most accurate measure of flow velocity (flow velocity = (Doppler shift frequency x speed of sound in blood)/(2x ultrasound transmission frequency x cosine Θ)). The larger Θ is, the larger cosine Θ is, and the greater the error in the velocity measure. Importantly, Θ less than 30 degrees keeps the error below 15% (Purkayastha & Sorond, 2012). The third limitation is the assumption that MCA diameter, based on a study that used a 1.5T MRI to measure MCA diameter (providing 0.5 mm resolution), remains constant (Serrador et al., 2000). Especially during changes in $ETCO_2$ that could acutely alter the caliber of the MCA and impact resistance to blood flow. This is supported by more recent studies using a higher resolution MRI (3T and 7T with 0.4 mm and 0.2 mm resolution respectively) to measure MCA diameter may change with $ETCO_2$ levels and this change in diameter may be diminish with aging (Coverdale, Badrov, & Shoemaker, 2017; Coverdale et al., 2014; Verbree et al., 2014). The fourth limitation was the strict eligibility criteria of this thesis RCT limits generalizability of findings to generally healthy older adults on stable centrally acting ACE inhibitor therapy; participants with significant comorbidities may have responded differently to 6 months of spironolactone therapy.

The current study findings support the hypothesis that spironolactone would maintain cerebrovascular autoregulation in older hypertensive patients who were receiving stable

treatment with centrally acting ACE inhibitors. Better blood pressure control was not associated with orthostatic declines in cerebral blood flow.

CHAPTER 6 Blood Pressure and Arterial Stiffness in Younger Adults Compared to Older Adults

6.1 Objectives

The primary objective of this chapter was to compare blood pressure and arterial stiffness of younger adults to the older adults in the RCT. Arterial stiffness was estimated by cfPWV, carotid distensibility coefficient and β-stiffness index. Aging is an important risk factor for developing hypertension likely due to changes in artery wall structure, up-regulation of RAAS activity, hypertension, a reduction in compliance and an increase in arterial stiffness (Fukutomi & Kario, 2010; Nilsson, 2008). It is possible that normal arterial aging involves structural and functional changes as an initial mechanism for elevating risk of hypertension. It was hypothesized that older hypertensive adults stably treated by centrally acting ACE inhibitors would have higher blood pressure and greater arterial stiffness, measured by all computed indices, compared to healthy younger adults.

6.2 Methods

As described in CHAPTER 2 General Methods and Materials.

6.3 Results

Blood pressure and arterial stiffness were assessed in 17 older adults (OA, mean age 65±3 years) and 14 younger adults (YA, mean age 26±5 years) as shown in Table 11. The OA group had significantly greater mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic

blood pressure (DBP) and pulse pressure (PP) compared to YA (Table 11). Heart rate however was similar between the groups. Regional stiffness indicator cfPWV in OA showed significantly greater arterial stiffness compared to YA with a percent difference of 24.8% (Table 11). Local stiffness indicators carotid distensibility coefficient (Figure 16) and β-stiffness index (Figures 17) in OA also showed significantly greater arterial stiffness compared to YA. The percent differences were 56.2% and 57.4% respectively.

Transit time estimates $hdTT_i$ (heart-digit transit time from R-peak of QRS complex for pressure pulse wave to reach digit artery divided by SBP) and hcTT (heart-carotid transit time is from R-peak to time for pressure pulse wave to reach carotid artery) were significantly less for OA compared to YA; indicating greater arterial stiffness in OA (Table 11). The percent differences were 15.4% and 23.5% respectively. hdTTi per unit height for YA (9.3 ms/mmHg/m) was significantly greater than OA (7.5 ms/mmHg/m, $P<0.01$), representing less arterial stiffness in YA. The same was true for hcTT per unit height for YA vs OA (516 ms/m vs 385 ms/m, $P<0.01$).

Table 11. Comparison of heart rate, blood pressure, arterial stiffness and transit times of older adults (OA) to younger adults (YA)

Measure	OA (n=17) mean ±SD	YA (n=14) mean ±SD	P-value[a]
Age (years)	65±3	26±5	P<0.05
Heart Rate (beats/min)	68±13	64±7	0.32
Brachial Pressure (mmHg)			
MAP	97±9	86±9	P<0.01
SBP	142±16	124±12	P<0.05
DBP	75±8	67±9	P<0.05
PP	66±13	57±7	P<0.05
Arterial Stiffness			
cfPWV (m/s)	7.22±1.09	5.43±1.13	P<0.001
Transit Times			
$hdTT_i$ (ms/mmHg)	1.3±0.2	1.5±0.2	P<0.05
hcTT (ms)	68±17	84±15	P<0.05

[a]From unpaired t-test, OA = older adults, YA = younger adults, MAP= Mean Arterial Pressure, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, PP=Pulse Pressure, cfPWV=carotid-femoral pulse wave velocity, $hdTT_i$=heart-digit transit time from R-peak of QRS complex for pressure pulse wave to reach digit artery divided by SBP, hcTT= heart-carotid transit time is from R-peak to time for pressure pulse wave to reach carotid. cfPWV was n=13. All measures while participants supine.

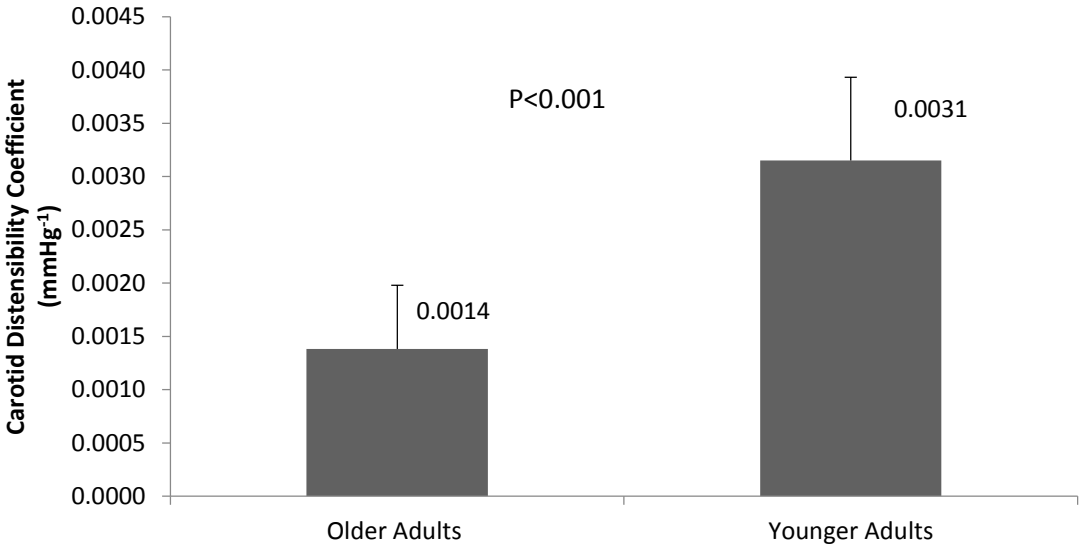


Figure 16. Carotid distensibility coefficient for older adults and younger adults

Carotid distensibility coefficient ($mmHg^{-1}$) for older adults compared to younger adults. Unpaired t-test was performed to establish that carotid distensibility for older adults was significantly less than younger adults (P<0.001). Data are means with error bars as standard deviation.

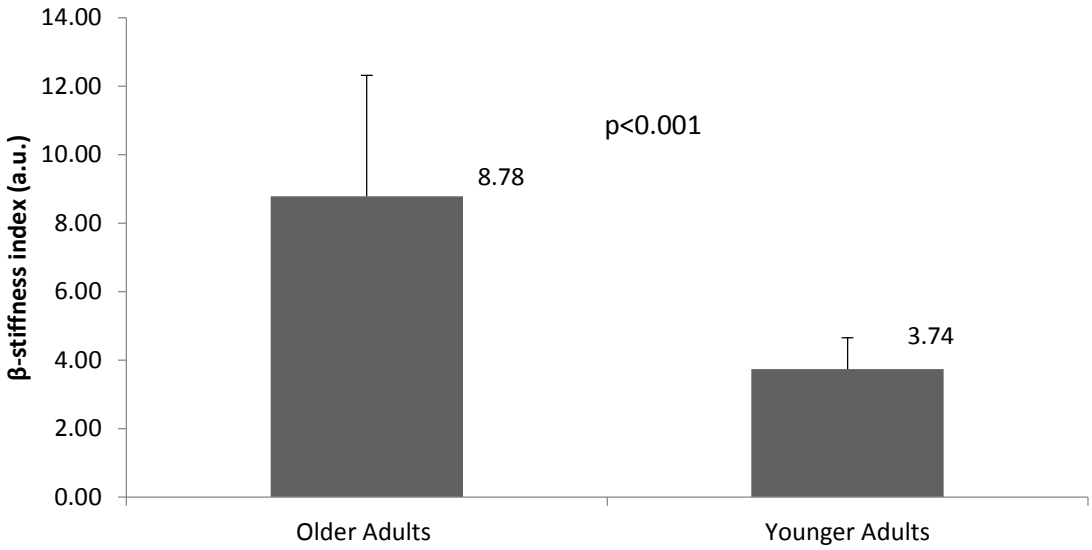


Figure 17. β-stiffness index for older adults and younger adults

β-stiffness index (a.u.) for older adults compared to younger adults. Unpaired t-test was performed to establish that β-stiffness index for older adults was significantly greater than younger adults (P<0.001). Data are means with error bars as standard deviation.

6.4 Discussion

The objective of this chapter was to compare blood pressure and arterial stiffness of younger adults to the older adults in the RCT. Measures of blood pressure and arterial stiffness supported the hypothesis of higher blood pressure and greater arterial stiffness in older adults in the RCT compared to younger adults. The percent differences in the regional stiffness indicator cfPWV, and transit times, were generally smaller than the percent differences for the local stiffness indicators carotid distensibility coefficient and β-stiffness index.

It is accepted that older adults with hypertension have greater arterial stiffness, which results in increasingly faster pulse transmission to the periphery, than younger adults. The older adults in this thesis RCT showed greater arterial stiffness than younger adults represented as faster cfPWV, smaller carotid distensibility coefficient and greater β-stiffness index. These younger adults had comparable blood pressure to other generally healthy younger adults of similar age (Cooke et al,. 2009). These results of increasing PWV with age are supported by existing evidence with similar markers of central arterial stiffness. Choi et al. (2010) showed an increase in central PWV of 1.26 m/s for every 11-year increase in age. As well, Mitchell et al. (2004) showed an estimate cfPWV of 1.04 m/s for every 8.5-year increase in age. Both indicators of central arterial stiffness increase with age to support the current thesis study findings. The very largeobservational study of 11,092 participants by Boutouyrie et al. (2010) was consistent with this thesis data of increasing arterial stiffness with age; amplified by increasing blood pressure. For example, there is a spectrum of increasing cfPWV (9m/s to 13m/s) with increasing blood

pressure (<120/80 to ≥ 160/100) within the same 60-69 year age category (Boutouyrie et al., 2010).

Transit times hdTTi and hcTT, were significantly shorter in older adults of the RCT compared to younger adults, reflecting greater arterial stiffness. This is consistent with the study that demonstrated increasing age as a significant contributor to decreasing hdTT, also measured from R-peak of QRS complex to the finger artery, in 116 adults of mean age 41±13 years of -6 ms per 10-year increase in age (P<0.0001) (J. Allen & A. Murray, 2002). Similarly, a study in 266 healthy adults age 18-78 years showed that hdTT was inversely related to age (β = -0.754, P<0.001) (Zhang, Zheng, Ma, & Sun, 2011).

These younger adults in this thesis experimental study were compared to controlled hypertensive older adults, which may be unlike normotensive older adults. The presence of hypertension may have accelerated normal central arterial aging limiting generalizability of findings to healthy adults with hypertension. The cross-sectional population-based study by Amar et al. (2001) of 993 participants (35-64 years) supports this; it showed that patients treated for hypertension exhibit greater central arterial stiffness than normotensive subjects even after adjusting for age (Amar, Ruidavets, Chamontin, Drouet, & Ferrieres, 2001). Hasegawa et al (1997) also reported acceleration of arterial stiffness indicators hdTT and PWV with the presence of hypertension comparing to age-matched normal controls.

The percent differences in the regional vascular transit indicators were generally smaller than the percent differences for the local carotid artery indicators possibly due to non-uniform stiffness along the arterial tree from differences in properties between elastic and muscular arteries that may be affected by sympathetic vasoconstriction and regional differences in transmural pressures (Tsuchikura et al., 2010). This is supported by the study findings by Paini et al. (2006) that age and blood pressure had a stronger independent influence on local carotid stiffness than regional vascular transit indicator cfPWV; that was amplified by the presence of hypertension. The study by Jurasic et al. (2009) supports the 56% difference in β-stiffness index in this thesis by showing a similar (53%) difference between 25-35 year age group and 65-75 year age group. However, inconsistent with this thesis, findings from large studies show that age had a similar impact on cfPWV as carotid stiffness; although still amplified by hypertension (Boutouyrie et al., 2010; Bruno et al., 2017; Huang, Hu, Huang, Sun, & Zhu, 2008). When considering possible reasons why percent differences in regional transit times, hdTT and hcTT, were generally smaller than the percent differences for the local carotid artery indicators, carotid distensibility coefficient and β-stiffness index, there may be variability introduced by the effect of age and blood pressure on the pre-ejection period.

6.4.1 Limitations

There are several important limitations to consider. The first limitation is that hdTTi has no comparative data, or existing evidence from other studies, to validate the current findings. It is important to note, however, that hdTT is a popular non-invasive pulse transit time estimate

being widely pursued for cuff-less blood pressure monitoring because of the observed association with systolic blood pressure (Gao, Olivier, & Mukkamala, 2016; Mukkamala et al., 2015). The second would be variability introduced from participant's height when calculating $hdTT_i$ since a study by Allen et al. (2002) showed a 0.7 ms increase in transit time per centimeter of height. However, this source of bias would have existed randomly across the age spectrum of adults in the current thesis research as supported by the observation that calculated hdTTi per unit height for YA was still significantly greater than OA, representing less arterial stiffness in YA. The same was true for central transit time hcTT per unit height for YA vs OA. The same study by Allen et al. (2002) showed that central transit times (similar to hcTT) were not associated with subject's height; possibly due to the contribution of a variable pre-ejection period (PEP is the time from the R-peak of the QRS complex to the onset of aortic blood flow) being a substantially larger proportion of hcTT compared to hdTT because of the shorter path length to the carotid site compared to the finger. As will be reviewed in Section 7.3, PEP represents 26 % to 33% of the total hdTT, and 58% to 64% of the total hcTT, which is consistent with existing evidence showing that PEP represents a nontrivial fraction of pulse wave transit time and source of variability (Payne, 2006).

In conclusion, all measures of blood pressure and indicators of arterial stiffness supported the hypothesis of higher blood pressure and greater arterial stiffness in older adults in the RCT compared to younger adults.

CHAPTER 7 Effect of Acute Blood Pressure Changes on Arterial Stiffness Indicators

7.1 Objectives

The primary objective of this chapter was to demonstrate the potential for an acute decrease in systolic and pulse pressure to affect indicators of arterial stiffness that were applied to older adults in the RCT, and to determine if any change in stiffness might simply be a consequence of the reduction in arterial blood pressure anticipated with addition of spironolactone to stable ACE inhibitor therapy. Changes in cardiac dynamics and arterial blood pressure were induced in younger adults by application of mild to moderate levels of lower body negative pressure (mLNBP). The arterial stiffness indicators in this study included cfPWV, a carotid distensibility coefficient, and β-stiffness index. Secondary objectives included the assessment of the impact of acute changes in blood pressure on transit times hdTT (heart-digit transit time from R-peak of QRS complex for pressure pulse wave to reach digit artery) and hcTT (heart-carotid transit time from R-peak for pressure pulse wave to reach carotid artery). The PEP was also assessed (i.e., PEP is the heart-aorta time from the R-peak to the onset of aortic blood flow). Brachial blood pressure measurements included mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP). Stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were also assessed. This chapter also includes the post hoc analysis of the impact that the orthostatic stress on blood pressure changes from quiet standing in older adults (OA) to mLBNP in younger adults (YA). Since acute changes in blood pressure can shift the operational point of the arterial compliance curve versus mechanically stiffer arteries that operate on a different compliance curve (Chirinos, 2012;

McEniery, Wilkinson, & Avolio, 2007; Shibata & Levine, 2011), it was hypothesized that acute changes in blood pressure would affect arterial stiffness indicators.

7.2 Methods

As described in CHAPTER 2 General Methods and Materials.

7.3 Results

There were significant interactions between SBP ($P<0.05$), DBP ($P<0.001$) and PP ($P<0.001$) with level of LBNP as shown in Table 12. Specifically, SBP and PP decreased with progressively increasing LBNP; while DBP and TPR ($P<0.05$) increased. MAP essentially remained stable ($P=0.09$). Heart rate at LBNP -30 mmHg was significantly faster than heart rate at all other LBNP levels ($P<0.01$, Table 12). Importantly, stroke volume (SV, $P<0.001$) and cardiac output (CO, $P<0.001$) were significantly decreased by 31.7% and 22.0% respectively with progressively increasing LBNP to -30 mmHg as shown in Table 12.

Regional arterial stiffness indicator cfPWV (Figure 20) numerically increased by 3.3% to show a non-significant increase in arterial stiffness. Local stiffness indicators carotid distensibility coefficient (Figure 21) and β-stiffness index (Figure 22) numerically decreased by 9.7% and increased 14.7% respectively to also show a non-significant increase in arterial stiffness. Figure 23 shows a strong negative linear correlation between percent change carotid distensibility

coefficient and percent change in β-stiffness index. There was a significant linear correlation between cfPWV and cfPWV index (cfPWV divided by SBP) for each level of LBNP (Figure 24).

There was a significant increase in PEP (heart-aorta time from the R-peak to the onset of aortic blood flow) with progressively increasing LBNP ($P<0.001$, Table 13). Correspondingly, there was a significant increase in regional transit times hdTT ($P<0.001$, Table 13, transit time from R-peak of QRS complex for pressure pulse wave to reach digit artery) and hcTT ($P<0.001$, Table 13, transit time from R-peak of QRS complex for pressure pulse wave to reach carotid artery). Aorta-digit transit time (adTT = hdTT - PEP) significantly decreased by 5.0% ($P<0.05$) and aorta-carotid (acTT = hcTT - PEP) remained unchanged (Table 13). There was a significant increase in PEP/hdTT (%) with progressively increasing LBNP ($P<0.001$, Figure 23). PEP/hdTT (%) at LBNP 0 mmHg was significantly less than PEP/hdTT (%) at -20 mmHg and -30 mmHg. As well, PEP/hdTT (%) at LBNP -10 mmHg was significantly less than at -30 mmHg. There was no interaction of PEP/hcTT (%) with level of LBNP (Figure 24).

Table 12. Hemodynamics of younger adults at four levels of progressively increasing lower body negative pressure (LBNP)

Measure	LBNP 0 mmHg (n=14) mean ±SD	LBNP -10 mmHg (n=14) mean ±SD	LBNP -20 mmHg (n=14) mean ±SD	LBNP -30 mmHg (n=14) mean ±SD	P-value[a]
Heart Rate (beats/min)	64±7	63±6	65±6	70±8[b]	<0.01
Stroke Volume (mL/beat)	82±17	75±20	69±19	56±15[b]	<0.001
Cardiac Output (L/min)	5.0±1.0	4.6±1.0	4.3±0.9	3.9±0.8[c]	<0.001
Brachial Pressure (mmHg)					
MAP	86±9	88±9	87±9	88±10	0.09
SBP	124±12	126±11	123±12	121±13[d]	<0.05
DBP	67±9[b]	69±9	69±9	71±9[d]	<0.001
PP	57±7	57±8	53±9	50±10[b]	<0.001
TPR (mmHg·min· L^{-1})	18±4[b]	21±6	22±5	24±6[d]	<0.001

[a]From 1-way repeated measures ANOVA, [b] Significantly different than all levels using Holm-Sidak Test, [c] Significantly different than LBNP 0 and -10 mmHg, [d] significantly different than LBNP -10 mmHg, using Tukey Test, MAP= Mean Arterial Pressure, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, PP=Pulse Pressure, TPR = total peripheral resistance (MAP/cardiac output). Stroke volume and cardiac output n=13. All measures while participants supine.

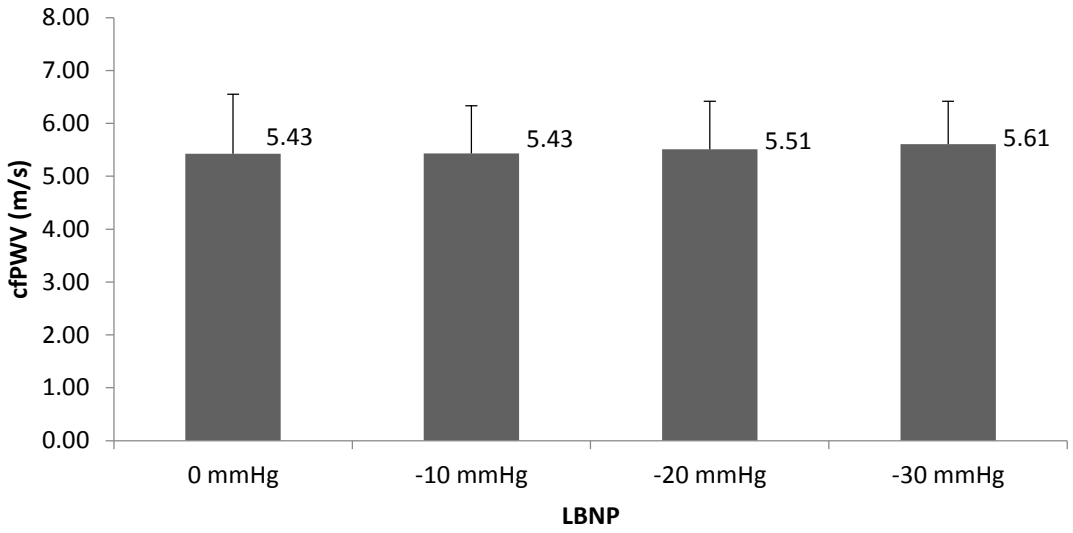


Figure 18. carotid-femoral PWV (cfPWV) at four levels of lower body negative pressure (LBNP)

cfPWV (m/s) at 0 mmHg, -10 mmHg LBNP, -20 mmHg LBNP, and -30 mmHg LBNP. One-way repeated measures ANOVA was used to determine no effect of LBNP on cfPWV (P=0.65). Data are means with error bars as standard deviation.

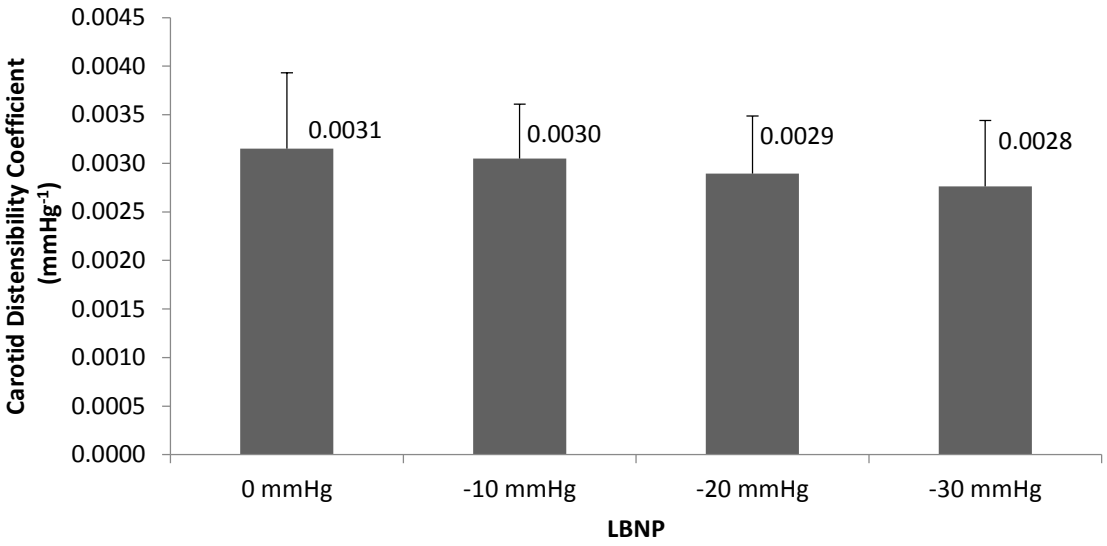


Figure 19. Carotid distensibility coefficient at four levels of lower body negative pressure (LBNP)

Carotid distensibility coefficient ($mmHg^{-1}$) at 0 mmHg LBNP, -10 mmHg LBNP, -20 mmHg LBNP, and -30 mmHg LBNP. One-way repeated measures ANOVA was used to determine no effect of LBNP on carotid distensibility coefficient (P=0.10). Data are means with error bars as standard deviation.

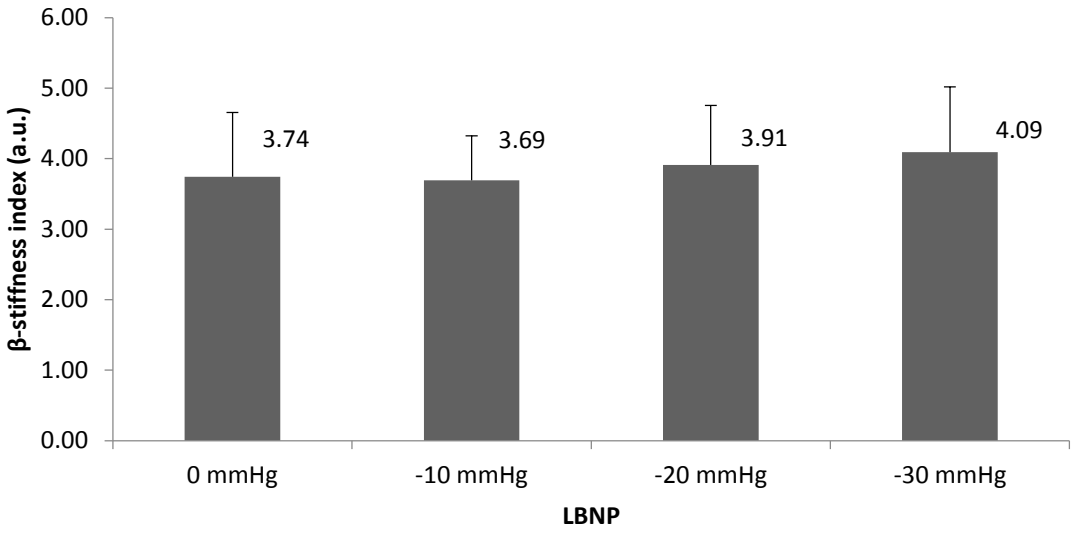


Figure 20. β-stiffness index at four levels of lower body negative pressure (LBNP)

β-stiffness index (arbitrary units=a.u.) at 0 mmHg LBNP, -10 mmHg LBNP, -20 mmHg LBNP, and -30 mmHg LBNP. One-way repeated measures ANOVA was used to determine no effect of LBNP on β-stiffness index (P=0.15). Data are means with error bars as standard deviation.

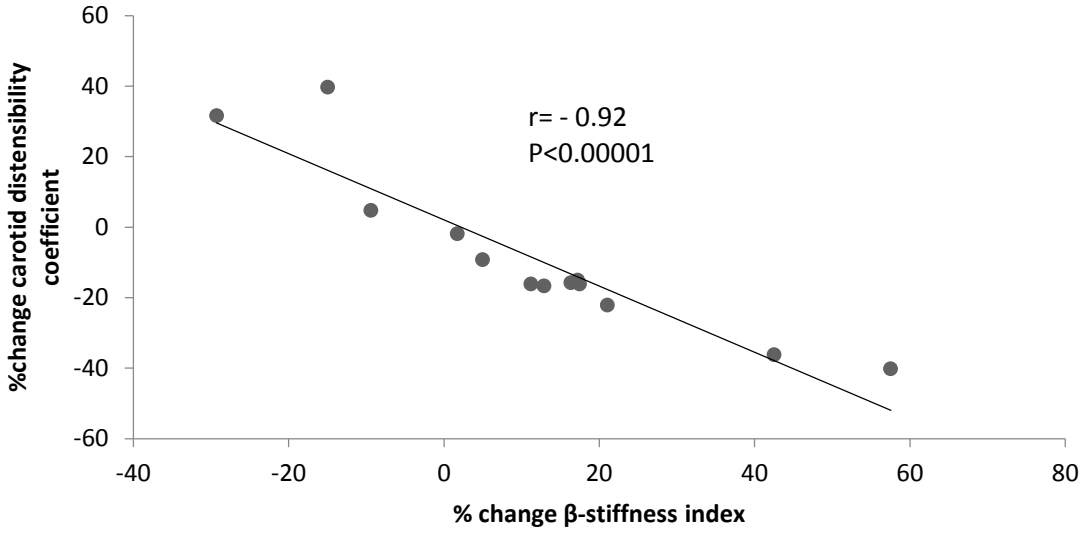


Figure 21 % Change Distensibility Coefficient vs % Change β-stiffness index

A scatterplot of change (from 0 mmHg to -30 mmHg LBNP) in carotid distensibility coefficient (%) vs change in β-stiffness index (%) showing significant negative linear correlation (r= - 0.92, P<0.00001).

Table 13. Pre-ejection period (PEP) and transit times in younger adults at four levels of progressively increasing lower body negative pressure (LBNP)

Measure	LBNP 0 mmHg (n=14) mean ±SD	LBNP -10 mmHg (n=14) mean ±SD	LBNP -20 mmHg (n=14) mean ±SD	LBNP -30 mmHg (n=14) mean ±SD	P-value[a]
PEP (ms)	49±15[b]	56±13[c]	61±12	65±16	<0.001
hdTT (ms)	189±19[b]	196±19	195±20	198±21	<0.001
adTT (ms)	139±18[c]	140±15	134±13	132±13	<0.05
hcTT (ms)	84±15[b]	89±15	94±17	102±19	<0.001
acTT (ms)	35±11	33±9	34±12	36±10	0.22

[a]From 1-way repeated measures ANOVA, [b] Significantly different than all levels, [c] Significantly different than LBNP -30 mmHg, using Holm-Sidak Test, PEP=Pre-Ejection Period (time from the R-peak to the onset of aortic blood flow), hdTT=heart-digit transit time (from R-peak of QRS complex for pressure pulse wave to reach digit artery), adTT=aorta-digit (hdTT – PEP), hcTT= heart-carotid transit time (from R-peak for pressure pulse wave to reach carotid), acTT=aorta-carotid (hcTT – PEP), LBNP=Lower Body Negative Pressure. All measures while participants supine.

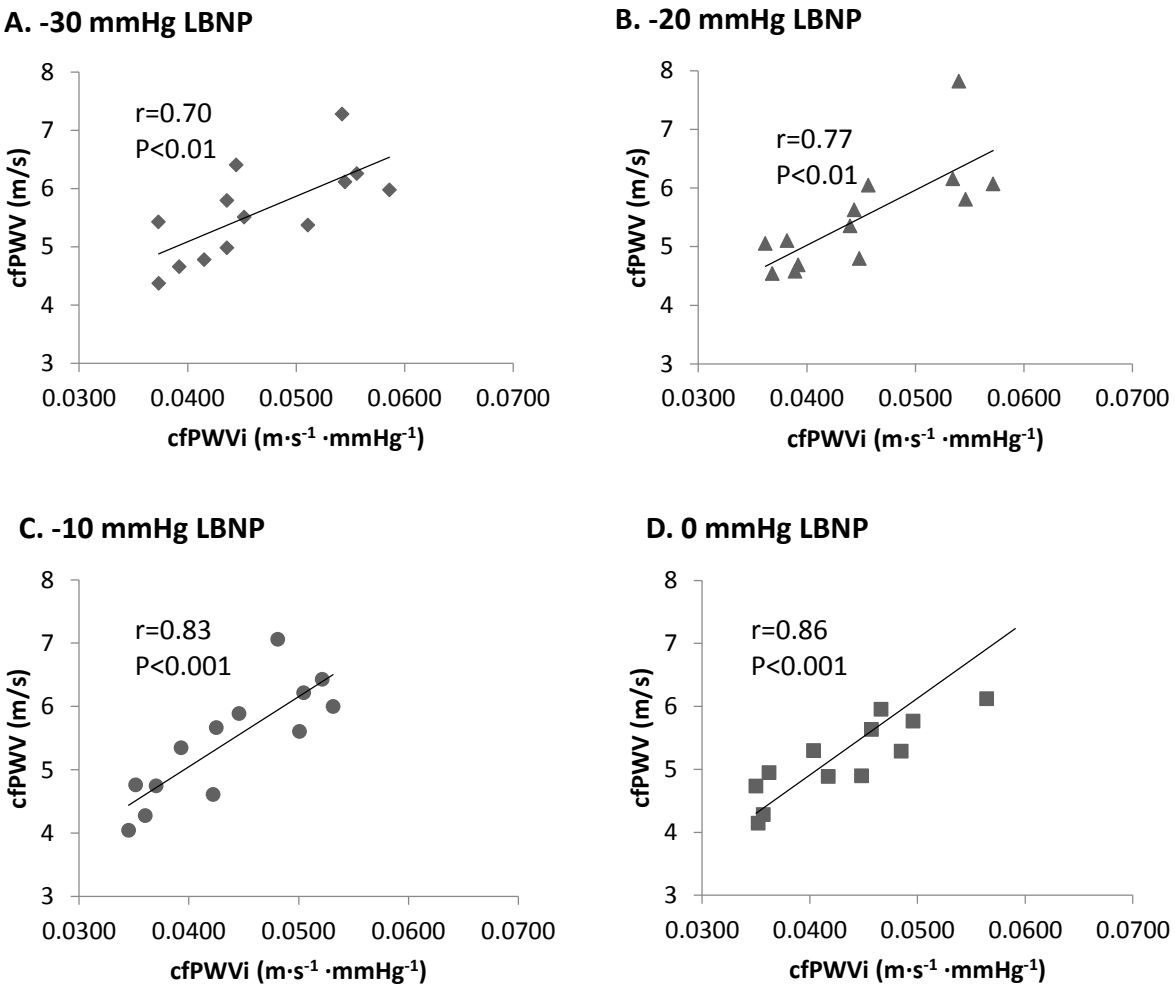


Figure 22. cfPWV vs cfPWVi at four levels of lower body negative pressure (LBNP)

Scatterplots of cfPWV (carotid-femoral PWV) vs cfPWVi (cfPWVi = cfPWV divided by SBP) four levels of LBNP: A. -30 mmHg LBNP, B. -20 mmHg LBNP, C. -10 mmHg LBNP and D. 0 mmHg LBNP all demonstrating significant linear correlations between cfPWV and cfPWVi represented by the trend lines with corresponding P-values.

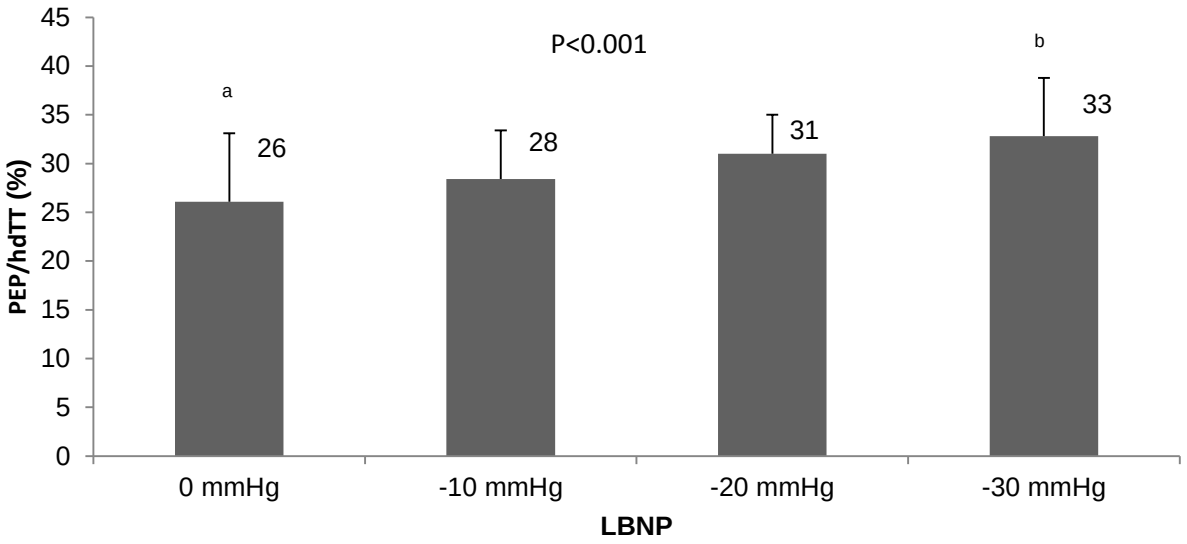


Figure 23. Percent pre-ejection period (PEP) of heart-digit transit time (hdTT) at four levels of graded lower body negative pressure (LBNP)

Percent PEP of hdTT (PEP/hdTT, %) at 0 mmHg LBNP, -10 mmHg LBNP, -20 mmHg LBNP and -30 mmHg LBNP. PEP=Pre-ejection period (heart-aorta time from the R-peak to the onset of aortic blood flow), hdTT=heart-digit transit time from R-peak of QRS complex for pressure pulse wave to reach digit artery. One-way repeated measures ANOVA was used to determine an effect of LBNP on PEP/hdTT (P<0.001). [a] significantly different than LBNP -20 mmHg and -30 mmHg, [b] significantly different than LBNP -10 mmHg. Data are means with error bars as standard deviation.

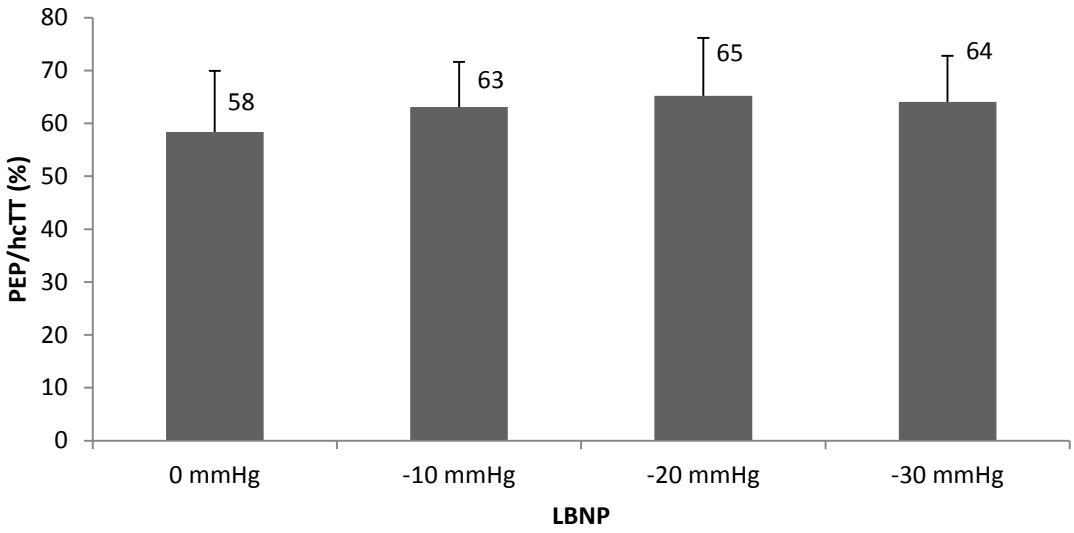


Figure 24. Percent pre-ejection period (PEP) of heart-carotid transit time (hcTT) at four levels of graded lower body negative pressure (LBNP)

Percent PEP of hcTT (PEP/hcTT, %) at 0 mmHg, -10 mmHg LBNP, -20 mmHg LBNP and -30 mmHg LBNP. PEP=Pre-ejection period (heart-aorta time from the R-peak to the onset of aortic blood flow), hcTT=heart-carotid transit time from R-peak of QRS complex for pressure pulse wave to reach carotid artery. One-way repeated measures ANOVA was used to determine no effect of LBNP on PEP/hcTT. Data are means with error bars as standard deviation.

Impact of orthostatic challenge on hemodynamics

In this post hoc analysis, the mean age of the OA was 65±3 years and 26±5 years (P<0.001) for YA. The impact of standing in OA and mLBNP in YA, and comparison, on blood pressure changes are shown in Table 14. Measurements in OA were at 60-90s of standing and at steady-state -30mmHg LBNP for YA. In OA, it was found that MAP, SBP, DBP, PP and heart rate all significantly increased (Table 14). In YA, MAP remained unchanged, both SBP and PP significantly decreased, and DBP significantly increased. If comparing pressure changes in YA to OA, each of MAP, SBP, and DBP were all significantly less affected in YA (P<0.001,Table 14).

Table 14. Change in heart rate and blood pressure for older adults (OA) during standing to younger adults (YA) during moderate LBNP

Measure (change from baseline)	OA (n=14) mean ±SD	P-value [a] OA Change	YA (n=14) mean ±SD	P-value [a] YA Change	P-value [b] OA vs YA % Change
Change in Heart rate (b/m)	9±6	P<0.001	6±10	P<0.05	
Change in Heart rate (%)	14±11		11±19		0.55
Change in MAP (mmHg)	16±7	P<0.001	2±4	0.09	
Change in MAP (%)	17±8		2±5		<0.001
Change in SBP (mmHg)	21±12	P<0.001	-3±7	P<0.05	
Change in SBP (%)	15±8		-2±6		<0.001
Change in DBP (mmHg)	14±6	P<0.001	4±4	P<0.01	
Change in DBP (%)	18±8		7±6		<0.001
Change in PP (mmHg)	8±8	P<0.01	-7±7	P<0.001	
Change in PP (%)	12±13		-13±11		<0.001

[a] From paired t-test; [b] from unpaired t-test, change in OA from standing 60-90 seconds, change in YA from steady-state moderate LBNP at -30 mmHg, b/m=beats per minute, MAP= Mean Arterial Pressure, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, PP=Pulse Pressure.

7.4 Discussion

The objective of this chapter was to demonstrate in young healthy adults the potential for an acute decrease in SBP and PP to affect indicators of arterial stiffness that were applied to older adults in the RCT; spironolactone in the RCT was shown to decrease cfPWV by 9.9%, decrease SBP by 9.7% and decrease PP by 17.1%. Findings from this experimental study were that small acute reductions in SBP by 2.4% and PP by 14.0% were concurrent with a non-significant increase in arterial stiffness (i.e., increase in cfPWV, decrease in carotid distensibility coefficient and increase in β-stiffness index by 3.3%, 9.7% and 14.7% respectively). PEP, and correspondingly regional transit times hdTT and hcTT, increased with these acute reductions in systolic and pulse pressure. These data support the hypothesis that acute changes in blood pressure would affect arterial stiffness indicators as measured on the older adults in the RCT.

This thesis used mLBNP (down to -30 mmHg) that modified cardiac and arterial hemodynamics to show a 31.7% ($P<0.001$) decrease in stroke volume, 22.0% ($P<0.001$) decrease cardiac output, a reduction in both SBP ($P<0.05$) and PP ($P<0.001$), while DBP increased ($P<0.001$) and MAP remained essentially unchanged; which is consistent with existing evidence (William H. Cooke, Caroline A. Rickards, Kathy L. Ryan, Tom A. Kuusela, & Victor A. Convertino, 2009; Fu et al., 2009). The significant increase in heart rate ($P<0.01$) was expected and consistent with other studies using similar LBNP (Bronzwaer et al., 2016; Charalampos Lydakis et al., 2008; Pannier, Bouckaert, & Lefebvre, 1995). It should be noted that there is variability in cardiovascular responses to LBNP as suggested in the study by Phillips et al. (2013) where

systolic blood pressure did not significantly decrease at a similar LBNP applied in this thesis for younger adults.

Regional central arterial stiffness indicator cfPWV numerically increased with acute reductions in SBP and PP, demonstrating slightly faster pressure pulse wave propagation, and correspondingly a non-significant increase in central arterial stiffness. This is consistent with the observed directional changes with local arterial stiffness indicators carotid distensibility coefficient and β-stiffness index; which are strongly correlated. Importantly, previous studies have also shown a numeric increase in central arterial stiffness at a similar LBNP (-30 mmHg) despite an increase in sympathetic nervous system engagement reflex to a decrease in central blood volume and central venous pressure (Lydakis et al., 2008; Phillips, Bredin, Cote, Drury, & Warburton, 2013). Very relevant to this thesis finding that cfPWV numerically increased at -30 mmHg LBNP, Lydakis et al (2008) demonstrated a numeric shortening in transit time of the pulse wave from the heart to the peripheral reflecting sites back to the heart during -30 mmHg down to -50 mmHg LBNP, considered as an increase in PWV, that was independent of the effect of sympathetic activation on the elastic properties and smooth muscle cell tone of the central arteries. Further support to this thesis finding is evidence that cfPWV could significantly increase at maximal levels beyond -50 mmHg LBNP in response to a similar acute decrease in SBP, stroke volume, and cardiac output, and increase in total peripheral resistance (Phillips et al., 2013). Phillips et al. (2013) showed at maximal tolerated LBNP in 8 subjects that cfPWV significantly increased by almost 30%.

Transit time consists of two distinct components: the PEP, which corresponds to the time from the start of ventricular depolarization to the onset of ventricular ejection, and the time it takes the arterial pulse wave to travel from the aortic valve to a point in the peripheral arteries; such as the finger [digit], carotid or femoral artery in this thesis. Essentially, transit time is composed of a delay component during isovolumetric contraction, PEP, and a peripheral component during pressure wave propagation along the artery, aorta to peripheral site. This central component PEP has been shown to be prolonged when either stroke volume, or ventricular filling, is reduced (Newlin & Levenson, 1979; Weissler, Peeler, & Roehll, 1961). PEP got longer with hemodynamic changes associated with progressively increasing LBNP from 49±15 ms to 65±16 ms ($P<0.001$) in the current study. This represents 26 % to 33% of the total hdTT, and 58% to 64% of the total hcTT, which is consistent with existing evidence showing that PEP represents a nontrivial fraction of pulse wave transit time and source of variability (Payne, Symeonides, Webb, & Maxwell, 2006). Notably, diastolic blood pressure significantly increased in the current study, contributing to a corresponding increase in ventricular afterload. The observed lengthening of PEP may be explained by a widening of the pressure gradient leading to a slower rise of left ventricular pressure during isovolumetric contraction secondary to shortened myocardial fiber length from the hypovolemic-reduction in left ventricular end-diastolic volume (Reeves et al., 1960; Wallace, Skinner, & Mitchell, 1963). When PEP contribution to the hcTT is removed, expressed as aorta-carotid TT, central arterial stiffness was estimated to remain unchanged ($P = 0.22$), which is consistent with existing work, and this thesis findings (Lydakis et al., 2008; Pannier, Bouckaert, & Lefebvre, 1995; Phillips et al., 2013).

As an estimate of regional peripheral arterial stiffness in this thesis, aorta-digit TT (heart-digit TT minus PEP) decreased by 5% ($P<0.05$), to demonstrate a slightly shorter time for the pressure pulse wave to arrive at the finger, and possibly increased peripheral arterial stiffness. This increase in stiffness is consistent with an increase in sympathetic nervous system engagement elevating smooth muscle cell tone contributing to the observed significant increase in TPR in this thesis. This increase in peripheral arterial stiffness may have been due to a direct influence of increased baroreceptor-mediated sympathetic vasomotor tone increasing TPR to maintain MAP given the decrease in cardiac output resulting from a decrease in central blood volume. This finding is consistent with the study by Fu et al (2009) also at LBNP of -30 mmHg where they measured a decrease in right atrial pressure and cardiac output with the corresponding increase in TPR; combined with the evidence from Cooke et al (2009) that sympathetic nervous system activity linearly increases with progressively decreasing LBNP to support this point of sympathetic nervous system engagement. The decrease in transit time can likely be explained by the analytical framework by the Moens-Korteweg formula already discussed (Ahlstrom, Johansson, Uhlin, Länne, & Ask, 2005; W. Chen, Kobayashi, Ichikawa, Takeuchi, & Togawa, 2000). PWV depends on the dimension of the vessel and the distensibility of the vessel wall expressed by the Moens-Korteweg formula: $PWV^2 = Eh/2R\rho$. Where PWV reflects aorta-digit TT between two arterial sites, and is directly related to the elastic modulus E, wall thickness h, and inversely related to interior diameter $2R$ and density of blood ρ. An increase in elastic modulus E by increasing smooth muscle cell tone, reflected as increasing

arterial stiffness, would directly increase PWV given less influential changes in the ratio of wall thickness to diameter (Chen et al., 2000; Ahlstrom et al., 2005).

The continuous measurements of heart rate, autonomic nervous system engagement, and blood pressure during quiet standing or LBNP have shown similar changes during head-up tilt (HUT) (Bloomfield et al., 1997; Butler, Yamamoto, Xing, Northey, & Hughson, 1993; Kirbis, Grad, Meglic, & Bajrovic, 2013). Butler et al. (1993) showed that LBNP and HUT in younger adults increased heart rate, MAP, and DBP; and decreased SBP and PP. Their findings are consistent with this thesis finding of increases in heart rate, MAP and DBP, with concurrent decrease in SBP and PP. The recent study by Tymko et al. (2016) also reported LBNP and HUT tilt would increase heart rate and MAP. This thesis compared the impact on heart rate and blood pressure from the orthostatic stress of 60-90s of quiet standing in OA to simulated orthostasis induced from mLBNP in YA. The impact of orthostasis in OA was significantly greater than YA. The current study showed that MAP, SBP, DBP, PP and heart rate all significantly increased during orthostasis in OA; with SBP, DBP, PP and heart rate to a significantly greater extent than YA. This increase in blood pressure in OA is inconsistent with previous studies, using the same technique to monitor beat-by-beat blood pressure while standing, which showed more modest changes in blood pressure and heart rate during orthostasis (Imholz, Dambrink, Karemaker, & Wieling, 1990; van Wijnen et al., 2017). It is possible that mLBNP was insufficient to induce the same hemodynamic effects as true gravity. It may also be possible that less arterial stiffness demonstrated in YA (CHAPTER 6) may be linked to these observed modest changes in blood pressure and heart rate responses to central volume depletion induced by mLBNP.

7.4.1 Limitations

Several limitations exist in this study that require consideration. First, the actual fluid shift leading to a reduction in central blood volume from sub-atmospheric pressure on the lower extremities from progressively increasing LBNP that reduced SV, cardiac output, and blood pressure, was not measured (Bronzwaer et al., 2016; Bronzwaer, Ouweneel, Stok, Westerhof, & van Lieshout, 2015). Consequently, the differences in central hypovolemia between participants could be a confounding factor related to the differential cardiovascular reflex responses between individuals (Bronzwaer et al., 2016). Since orthostatic stress has been shown to shift 300 to 800 mL of blood from the chest to the lower body, the magnitude of central hypovolemia during LBNP may be considered as to affect cardiovascular responses (Sjostrand, 1953). Differences in fluid shift volume between subjects during the same sub-atmospheric box pressure are an intrinsic limitation of LBNP and few studies have addressed how to enable individualization of LBNP-induced central blood volume shifts. In addition to the shift of blood volume to the veins and interstitial spaces of the leg, it is possible that the shift of blood to the small vessels in the leg provide a source of inter-participant variability by influencing the sub-atmospheric pressure feedback loop on baroreceptors (Cirovic, Walsh, Fraser, & Gulino, 2006; Truijen et al., 2012).

Progressively increasing LBNP increases sympathetic nervous system activity (William H. Cooke et al., 2009; Khan, Sinoway, & MacLean, 2002). The second limitation to consider is that sympathetic nervous system engagement was not measured in the current study and may have affected central arterial stiffness (Swierblewska et al., 2010). It is possible that the baroreflex mediated increase in sympathetic nervous system activity is unique to the individual participants as suggested from a recent study investigating inter-participant cardiovascular responses to LBNP compared with consistent and reproducible responses for the individual participant. The study by Bronzwaer et al. (2016) found that distinct and reproducible cardiovascular response patterns of 10 healthy younger adults from LBNP induced sympathetic activity were related to the participant's resting heart rate ($P<0.05$) and gain of the baroreceptors ($P<0.05$) over time. If Bronzwaer et al. (2016) had however measured sympathetic nervous system activity, they may have made the different conclusion that Hinojosa-Laborde et al. (2014) did; that cardiovascular response patterns to central hypovolemia are not actually related to either resting sympathetic nervous system activity (heart rate) or sympathetic baroreflex gain. Measuring sympathetic nervous system activity may have helped clarify how unloading of arterial baroreceptors from reduced central volume affects blood pressure and arterial stiffness indicators.

The third limitation is that LBNP was only to -30 mmHg. Reflex responses to a greater fluid shift from greater sub-atmospheric pressures reductions on the lower extremities may induce cardiovascular responses that are not generalizable to this LBNP level. For example, Philips et

al. (2013) showed a significant increase in markers of central arterial stiffness and sympathetic activity at maximal tolerated LBNP levels.

In conclusion, the acute slight decreases in systolic and pulse blood pressure were associated with a non-significant increase in central stiffness (cfPWV decrease, carotid distensibility coefficient decrease and β-stiffness index increase), slightly elevated peripheral stiffness (aorta-digit TT decrease) and elevated TPR. Despite given limitations, this supports the hypothesis that acute blood pressure changes would affect arterial stiffness indicators representing a confounding variable affecting main study results independent of the expected greater chronic blood pressure changes from spironolactone in the RCT. Therefore this supplementary experimental study showed that it is important to consider how acute variability in blood pressure may affect some indicators of arterial stiffness beyond greater chronic blood pressure changes; caution is necessary when making conclusions on the underlying mechanism of arterial stiffness.

CHAPTER 8 General Discussion

8.1 Overview

Hypertension and central arterial stiffness are demonstrated risk factors for cognitive impairment, likely involving reduced cerebral blood flow during aging (Elias et al., 2009; Hanon et al., 2005; Paran et al., 2003; Ruitenberg et al., 2005; Scuteri et al., 2005; Waldstein et al., 2008). Aldosterone antagonists such as spironolactone in this thesis RCT have been shown to reduce blood pressure and arterial stiffness in older hypertensive adults through arterial structural and functional changes that may explain some evidence of improvements in cognitive function after 6 months of therapy (Edwards et al., 2009; Farquharson & Struthers, 2000; Kithas & Supiano, 2010; Macdonald et al., 2004; Mahmud & Feely, 2005; Savoia et al., 2008). However, there are currently no curative medications available for cognitive decline by increasing cerebral blood flow; and to date, very limited data are available investigating aldosterone antagonism effects on cognitive function (Duron & Hanon, 2010). The main objective of the of this thesis was to conduct a small randomized, double-blind, placebo-controlled trial with Spiro in older hypertensive adults stably treated by centrally acting ACE inhibitors to test the hypothesis that spironolactone, through its influence on blood pressure and arterial stiffness, could improve cerebral blood flow and cognition. Secondary objectives were to examine cerebrovascular autoregulation changes with spironolactone, and to conduct a supplementary experimental study with younger adults. The purpose of the experimental study in younger adults was to test the hypotheses that blood pressure and arterial stiffness are different than older adults in RCT and to demonstrate the potential for acute changes in blood

pressure to impact interpretation of indicators of arterial stiffness that were applied to older adults in the RCT. This final chapter provides a summary of important thesis findings and considers main limitations of the RCT and experimental study. Finally, each of the posed research questions is addressed and a future direction for research is proposed.

8.2 Spironolactone Improves Blood Pressure but Arterial Stiffness, Cerebral Blood Flow and Cognitive Function Remain Unchanged.

The results of chapters 3, 4 and 5 showed that six months of aldosterone antagonism with spironolactone significantly reduced systolic blood pressure and pulse blood pressure while arterial stiffness, cerebral blood flow, cognition, and cerebrovascular autoregulation remained unchanged. This is inconsistent with some evidence that aldosterone antagonism could decrease blood pressure and improve cognition (Yagi et al., 2011; Hajjar et al., 2013), or decrease arterial stiffness while improving cerebral blood flow (Tarumi et al., 2014; Lipsitz et al, 2005). This thesis findings of reduced systolic blood pressure and unchanged cfPWV were consistent with the recent subset of 648 SPRINT trial participants showing that RAAS inhibition (76% ACE inhibitor or angiotensin receptor blocker) leading to a similar SBP reduction as the RCT of this thesis (average delta of 14.8 mmHg) did not significantly reduce cfPWV over the 3 years of follow up (Group et al., 2015; Supiano, 2017). Lower pulse pressure shown in this thesis reflects less arterial stiffness, and although both pulse pressure and cfPWV are independent predictors of cardiovascular outcomes, cfPWV is considered a more direct measure of arterial stiffness (Chirinos, 2012). A potential mechanism to explain how arterial stiffness remained essentially unchanged after aldosterone antagonism (mineralocorticoid receptor specifically, MR) is from recent evidence that acute inhibition of MRs with eplerenone impairs vascular dilation by specifically impairing vascular endothelial function independent of blood pressure change or vascular smooth muscle responsiveness to exogenous vasodilator nitric oxide (Hwang et al., 2016). Hwang et al. (2016) found that vascular endothelial nitric oxide synthase activity, that represents capacity to produce vasodilator nitric oxide in normal healthy aging, was

correspondingly reduced with the MR antagonism compared to placebo. In contrast, aldosterone activation of MR on endothelial cells leads to production of nitric oxide that diffuses to vascular smooth muscle cells and counteracts the MR-dependent vasoconstriction. There is a dynamic balancing of affects from aldosterone MR activation between endothelial-dependent vasodilation and smooth muscle-dependent vasoconstriction (Skott et al., 2006; Galmiche et al., 2014).

The main limitation is that the small sample size did not provide statistical power to detect significant differences in the primary outcome marker of anterior cerebral blood flow (aCBF). The sample size required to detect a significant difference in mean aCBF (832.9 mL/min) after 6 months of spironolactone compared to placebo (836.0 mL/min) is estimated from this current study as 330 participants (165 in each group) with a significance level (α) of 0.05 and desired power of 0.8 (Sigmaplot 12.5, Systat Software Inc, San Jose, California, USA). The calculated Cohen's *d* effect size for spironolactone was 0.3 from this thesis data; much smaller than the desired Cohen's *d* effect size of 1.0 that was used to calculate sample size during the trial design. There is the possibility that stable ACE inhibition in the older adults in this thesis RCT already had improved arterial structure and function enough to minimize further potential changes with spironolactone (Ceconi et al., 2007; Dudenbostel & Glasser, 2012; Hare et al., 2013; Kithas & Supiano, 2010; Mahmud & Feely, 2005). The larger desired effect size used to calculate sample size needed was based on experimental, non-interventional, pilot data as described in the methods CHAPTER 2 (2.8).

Another limitation is the variability in measurement techniques to generate carotid-femoral pulse wave velocity (cfPWV) may make cross-trial comparisons challenging. Notable different techniques include the use of transcutaneous infrared plethysmography or pulse tonometry combined with volumetric changes in a cuff to estimate pulse arrival time (Butlin & Qasem, 2017; A. A. Phillips, S. S. D. Bredin, A. T. Cote, C. T. Drury, & D. E. R. Warburton, 2013). This thesis used Doppler ultrasound to estimate pulse wave transit times based on blood velocity instead of the common mechanotransducer method that estimates pressure pulse wave transit time. However cfPWV generated from Doppler ultrasound method is in strong association with the pressure pulse wave propagation method represented by an intra-class correlation coefficient of 0.91 (Calabia et al., 2011) and, as demonstrated in this thesis, a strong correlation between pressure pulse wave velocity and blood cell pulse wave velocity at the carotid artery site ($r = 0.74$, $P<0.01$, Figure 30, APPENDIX 1.M.2). Even techniques for measuring transit distances between arterial sites can vary across studies making it difficult to compare across studies due to the variability in methodologies (Townsend et al., 2015; Van Bortel et al., 2012). These thesis findings, possibly due to mechanisms and limitations described, do not support the hypothesis that through the effects on blood pressure and arterial stiffness, spironolactone would improve cerebral blood flow and cognition compared to placebo.

8.3 Arterial Stiffness Indicators Affected by Age and Acute Blood Pressure Changes

The main objectives of the supplementary experimental study in younger adults reviewed in chapters 6 and 7 were to test the hypotheses that older hypertensive adults stably treated by centrally acting ACE inhibitors in the RCT would have higher blood pressure and greater arterial stiffness; and acute reductions in blood pressure would affect indicators of arterial stiffness that were applied to these older adults in the RCT. The post hoc analysis was done of hemodynamic changes during central hypovolemia from moderate lower body negative pressure (mLBNP) in younger adults were compared to hemodynamic changes from the orthostatic challenge of quiet standing in older adults.

Aging is an important risk factor for developing hypertension due to changes in artery wall structure, up-regulation of RAAS activity, rise in pulse blood pressure, and correspondingly an increase in vascular stiffness (Fukutomi & Kario, 2010; Nilsson, 2008). The findings from CHAPTER 6 were that measures of blood pressure and indicators of arterial stiffness supported the hypothesis of higher blood pressure and greater arterial stiffness in older adults in the RCT compared to younger adults. This finding was true for each arterial stiffness indicator computed in the study and supports the accepted observation that older adults with hypertension have greater arterial stiffness, which results in increasingly faster pulse transmission to the periphery, than younger adults (J Allen & A Murray, 2002; Amar et al., 2001; Boutouyrie et al., 2010; Choi et al., 2010; Hasegawa et al., 1997; Mitchell et al., 2004; Y.-L. Zhang, Zheng, Ma, &

Sun, 2011). Observing expected, and interesting, pressure and arterial stiffness differences with age was important to validate measures as being consistent with existing evidence.

The findings in CHAPTER 7 during acute manipulations of cardiovascular function in young adults with application of mLBNP were important to support the thesis hypothesis that acute changes in blood pressure affected some indicators of arterial stiffness. The acute reductions in systolic blood pressure (SBP) and pulse pressure (PP) were associated with a non-significant increase in central stiffness (cfPWV increase, carotid distensibility coefficient decrease and β-stiffness index increase), slightly elevated peripheral stiffness (aorta-digit TT decrease) and elevated TPR. Consequently, it is important to consider the potential impact acute changes in BP may have on the interpretation of arterial stiffness indicators applied in the RCT.

There are several important limitations to the supplementary experimental study. The first limitation is that hdTTi (time from R-peak to digit artery divided by SBP) is not a standard method for estimating arterial stiffness in current publications. As well, this thesis showed that PEP represents a nontrivial fraction of heart-digit TT and non-trivial source of variability (Payne, Symeonides, Webb, & Maxwell, 2006). It is however important to note that heart-digit TT is a popular non-invasive pulse transit time estimate being widely pursued for cuff-less blood pressure monitoring because of the observed association with blood pressure (Mukkamala et al., 2015; Gao, Olivier, & Mukkamala, 2016). The actual fluid shift leading to a reduction in central blood volume from progressively increasing LBNP on the lower extremities that reduced stroke volume, cardiac output, and blood pressure, was not measured to account for inter-

participant variability in central hypovolemia that affect cardiovascular responses (Bronzwaer, Ouweneel, Stok, Westerhof, & van Lieshout, 2015; Bronzwaer et al., 2016; William H. Cooke et al., 2009; Khan et al., 2002; Sjostrand, 1953; Swierblewska et al., 2010). The differences in fluid shift volume and sympathetic nervous system engagement between subjects is an intrinsic limitation of LBNP. The shift of blood volume to the small vessels and interstitial spaces of the leg provide a source of inter-participant variability by influencing the LNBP feedback loop on baroreceptors (Truijen et al., 2012; Stewart, 2003; Cirovic, Walsh, Fraser, & Gulino, 2006). Continuous measurement of the sympathetic nervous system activity during assessments may have helped clarify how unloading of arterial baroreceptors from reduced central volume affects blood pressure and arterial stiffness indicators.

8.4 Summary of Findings that Address Thesis Research Questions

The objectives of this thesis were to address posed research questions about the effect spironolactone on blood pressure, arterial stiffness, cerebral blood flow, and cognition when safely added to existing stable ACE inhibitor therapy in older hypertensive adults. Each of the 5 thesis questions are restated here and addressed individually based on research findings:

1. *In older hypertensive adults stably treated by centrally acting ACE inhibitors, will spironolactone decrease blood pressure and arterial stiffness compared to placebo?* No, spironolactone significantly reduced blood pressure but had no effect on arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compared to placebo in this RCT. These data do not support the hypothesis that spironolactone will decrease blood pressure and improve arterial stiffness in older adults on stable ACE inhibitor treatment for hypertension; likely due to mechanisms and limitations described in CHAPTER 3.

2. *In older hypertensive adults stably treated by centrally acting ACE inhibitors, does spironolactone improve cerebral blood flow and cognition compared to placebo?* No, cerebral blood flow and cognitive function were unchanged from spironolactone administered for 6 months in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. These data do not support the

hypothesis that spironolactone improves cerebral blood flow and cognitive function; likely due to identifiable study limitations as described in CHAPTER 4.

3. *In older hypertensive adults stably treated by centrally acting ACE inhibitors, does spironolactone affect cerebrovascular autoregulation compared to placebo?* No, spironolactone maintained cerebrovascular autoregulation in older hypertensive patients who were receiving stable treatment with centrally acting ACE inhibitors. Better blood pressure control was not associated with orthostatic declines in cerebral blood flow as examined in CHAPTER 5.

4. *How does blood pressure and arterial stiffness in older hypertensive adults stably treated by centrally acting ACE inhibitors compare to healthy younger adults?* All measures of blood pressure and indicators of arterial stiffness supported the hypothesis of higher blood pressure and greater arterial stiffness in older adults in the RCT compared to younger adults as examined in CHAPTER 6.

5. *Are indicators of arterial stiffness unaffected by acute changes in blood pressure?* No, acute changes in blood pressure did affect indicators of arterial stiffness. There was an increase in cfPWV, decrease in carotid distensibility coefficient and increase in β-stiffness index to collectively show a non-significant decrease in central arterial stiffness with acute reductions in SBP and PP. There was also a slight increase peripheral arterial stiffness (aorta-digit TT significantly decreased) and increase in

total peripheral resistance (TPR). This supports the hypothesis that acute blood pressure changes would affect arterial stiffness indicators representing a confounding variable affecting main study results independent of chronic blood pressure changes. Therefore it is important to consider how variability in blood pressure, and TPR, may affect indicators of arterial stiffness; and caution is necessary when making conclusions about the underlying mechanism of improved arterial stiffness as described in CHAPTER 7.

The collective findings from this thesis demonstrate that spironolactone, an inexpensive antihypertensive drug, can safely be added to stable centrally acting ACE inhibitor therapy in older hypertensive adults to effectively reduce blood pressure; while arterial stiffness, cerebral blood flow and cognition remain unchanged. These novel RCT data provide necessary input to estimate the sample size necessary to address the research question of the effect of spironolactone, through reduction of blood pressure and arterial stiffness, on CBF and cognition. The thesis findings are limited by the effect of acute blood pressure changes on arterial stiffness indicators, small RCT sample size, and a proposed mechanism how aldosterone antagonism in hypertensive patients may not improve arterial stiffness in contrast to findings from small studies by Kithas et al. (2010) or Yagi et al. (2011) that showed improved arterial stiffness and blood pressure. These limited findings reinforce the need for future studies to better examine research questions 1-3 above.

8.5 Future Research

There is a need for research exploring the effect of an aldosterone antagonist on arterial stiffness, cerebral blood flow and cognitive function could involve a clinical trial with a larger sample size of hypertensive participants than this thesis RCT (with a washout period to RAAS inhibitors) at least 60 years of age and with mild cognitive impairment (MoCA < 22). In a double-blind fashion, participants could be randomly given spironolactone (200mg daily based on patient tolerability) or a calcium channel blocker for 60 months treated to a SBP target of less than 120 mmHg with a non-RAAS inhibitor (Group et al., 2015). It is also possible to use finerenone 20mg once daily (given suitable Health Canada approval); the higher MR selectivity than spironolactone may improve effect size to allow for a smaller sample size. Calcium channel blockers have been shown to reduce blood pressure with less effect on arterial stiffness than RAAS inhibitors (Andreadis et al., 2010; Y. Chen, Shen, Liu, & Yang, 2017). A third observational study arm of generally healthy normotensive participants at least 60 years of age with mild cognitive impairment is also recommended. These participant's outcomes on arterial stiffness and cerebral blood flow would represent normal variability independent of blood pressure changes. A smaller randomized, double-blinded, trial with shorter follow-up could involve participants with compromised cerebral circulation such as vascular type dementia, or prior stroke, that are naïve to RAAS inhibition. Arterial stiffness could be estimated by the gold standard cfPWV and total cerebral blood flow assessed as the sum of bilateral internal carotid and vertebral arteries (mean velocity multiplied by cross-sectional area) using a 3T MRI or duplex ultrasound, then normalized to the participant's grey matter volume. Executive

cognitive function should be assessed by trained clinician using the MoCA, Stroop, Flanker Task and Wisconsin Card Sorting at baseline and end of study.

References

Aaslid, R., Lindegaard, K. F., Sorteberg, W., & Nornes, 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), 769-774. doi:10.3171/jns.1982.57.6.0769

Ahlstrom, C., Johansson, A., Uhlin, F., Länne, T., & Ask, P. (2005). Noninvasive investigation of blood pressure changes using the pulse wave transit time: a novel approach in the monitoring of hemodialysis patients. *Journal of Artificial Organs, 8*(3), 192-197.

Ainslie, P. N., & Subudhi, A. W. (2014). Cerebral blood flow at high altitude. *High Alt Med Biol, 15*(2), 133-140. doi:10.1089/ham.2013.1138

Ainslie, P. N., & Tzeng, Y. C. (2010). On the regulation of the blood supply to the brain: old age concepts and new age ideas. *J Appl Physiol (1985), 108*(6), 1447-1449. doi:10.1152/japplphysiol.00257.2010

Alecu, C., Labat, C., Kearney-Schwartz, A., Fay, R., Salvi, P., Joly, L., . . . Benetos, A. (2008). Reference values of aortic pulse wave velocity in the elderly. *J Hypertens, 26*(11), 2207-2212. doi:10.1097/HJH.0b013e32830e4932

Alessandri, N., Tufano, F., Petrassi, M., Alessandri, C., Lanzi, L., Fusco, L., . . . Tomao, E. (2010). Elasticity/distensibility of the ascending aorta: basal conditions and simulated conditions from space flights. *Eur Rev Med Pharmacol Sci, 14*(5), 421-426.

Allen, J., & Murray, A. (2002). Age-related changes in peripheral pulse timing characteristics at the ears, fingers and toes. *Journal of Human Hypertension, 16*(10), 711-717.

Allen, J., & Murray, A. (2002). Age-related changes in peripheral pulse timing characteristics at the ears, fingers and toes. *J Hum Hypertens, 16*(10), 711-717. doi:10.1038/sj.jhh.1001478

Alosco, M. L., Gunstad, J., Jerskey, B. A., Xu, X., Clark, U. S., Hassenstab, J., . . . Sweet, L. H. (2013). The adverse effects of reduced cerebral perfusion on cognition and brain structure in older adults with cardiovascular disease. *Brain Behav, 3*(6), 626-636. doi:10.1002/brb3.171

Alosco, M. L., Spitznagel, M. B., Raz, N., Cohen, R., Sweet, L. H., Garcia, S., . . . Gunstad, J. (2013). The interactive effects of cerebral perfusion and depression on cognitive function in older adults with heart failure. *Psychosom Med, 75*(7), 632-639. doi:10.1097/PSY.0b013e31829f91da

Amar, J., Ruidavets, J. B., Chamontin, B., Drouet, L., & Ferrieres, J. (2001). Arterial stiffness and cardiovascular risk factors in a population-based study. *J Hypertens, 19*(3), 381-387.

Ameriso, S. F., Paganini-Hill, A., Meiselman, H. J., & Fisher, M. (1990). Correlates of middle cerebral artery blood velocity in the elderly. *Stroke, 21*(11), 1579-1583.

Andreadis, E. A., Sfakianakis, M. E., Tsourous, G. I., Georgiopoulos, D. X., Fragouli, E. G., Katsanou, P. M., . . . Diamantopoulos, E. J. (2010). Differential impact of angiotensin receptor blockers and calcium channel blockers on arterial stiffness. *Int Angiol, 29*(3), 266-272.

Ashendorf, L., Jefferson, A. L., O'Connor, M. K., Chaisson, C., Green, R. C., & Stern, R. A. (2008). Trail Making Test errors in normal aging, mild cognitive impairment, and dementia. *Arch Clin Neuropsychol, 23*(2), 129-137. doi:10.1016/j.acn.2007.11.005

Barrett, K. V., McCurley, A. T., & Jaffe, I. Z. (2013). Direct contribution of vascular mineralocorticoid receptors to blood pressure regulation. *Clin Exp Pharmacol Physiol, 40*(12), 902-909. doi:10.1111/1440-1681.12125

Battisti-Charbonney, A., Fisher, J., & Duffin, J. (2011). The cerebrovascular response to carbon dioxide in humans. *J Physiol, 589*(Pt 12), 3039-3048. doi:10.1113/jphysiol.2011.206052

Bellien, J., Favre, J., Iacob, M., Gao, J., Thuillez, C., Richard, V., & Joannides, R. (2010). Arterial stiffness is regulated by nitric oxide and endothelium-derived hyperpolarizing factor during changes in blood flow in humans. *Hypertension, 55*(3), 674-680. doi:10.1161/HYPERTENSIONAHA.109.142190

Bernini, G., Galetta, F., Franzoni, F., Bardini, M., Taurino, C., Bernardini, M., . . . Salvetti, A. (2008). Arterial stiffness, intima-media thickness and carotid artery fibrosis in patients with primary aldosteronism. *J Hypertens, 26*(12), 2399-2405. doi:10.1097/HJH.0b013e32831286fd

Blacher, J., Amah, G., Girerd, X., Kheder, A., Ben Mais, H., London, G. M., & Safar, M. E. (1997). Association between increased plasma levels of aldosterone and decreased systemic arterial compliance in subjects with essential hypertension. *Am J Hypertens, 10*(12 Pt 1), 1326-1334.

Bloomfield, D. M., Kaufman, E. S., Bigger, J. T., Jr., Fleiss, J., Rolnitzky, L., & Steinman, R. (1997). Passive head-up tilt and actively standing up produce similar overall changes in autonomic balance. *Am Heart J, 134*(2 Pt 1), 316-320.

Bosch, J. (2016). HOPE 3: Effect of BP Lowering and Statin use on Cognitive Function. *AHA Presentation*.

Bosch, J., Yusuf, S., Pogue, J., Sleight, P., Lonn, E., Rangoonwala, B., . . . evaluation, H. I. H. o. p. (2002). Use of ramipril in preventing stroke: double blind randomised trial. *BMJ, 324*(7339), 699-702.

Boutouyrie, P., Achouba, A., Trunet, P., Laurent, S., & Group, E. T. (2010). Amlodipine-valsartan combination decreases central systolic blood pressure more effectively than the amlodipine-atenolol combination: the EXPLOR study. *Hypertension, 55*(6), 1314-1322. doi:10.1161/HYPERTENSIONAHA.109.148999

Breeuwsma, A. C., Hartog, L. C., Kamper, A. M., Groenier, K. H., Bilo, H. J., Kleefstra, N., & Van Hateren, K. J. (2017). Standing orthostatic blood pressure measurements cannot be replaced by sitting measurements. *Hypertens Res, 40*(8), 765-770. doi:10.1038/hr.2017.39

Briet, M., & Schiffrin, E. L. (2013). Vascular actions of aldosterone. *J Vasc Res, 50*(2), 89-99. doi:10.1159/000345243

Bronzwaer, A.-S. G. T., Ouweneel, D. M., Stok, W. J., Westerhof, B. E., & van Lieshout, J. J. (2015). Arterial Pressure Variation as a Biomarker of Preload Dependency in Spontaneously Breathing Subjects – A Proof of Principle. *PLOS ONE, 10*(9), e0137364.

Bronzwaer, A.-S. G. T., Verbree, J., Stok, W. J., van Buchem, M. A., Daemen, M. J. A. P., van Osch, M. J. P., & van Lieshout, J. J. (2016). Cardiovascular Response Patterns to Sympathetic Stimulation by Central Hypovolemia. *Frontiers in Physiology, 7*.

Brown, A. M., & Ransom, B. R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia, 55*(12), 1263-1271. doi:10.1002/glia.20557

Brown, N. J. (2008). Aldosterone and vascular inflammation. *Hypertension, 51*(2), 161-167. doi:10.1161/HYPERTENSIONAHA.107.095489

Bruno, R. M., Cartoni, G., Stea, F., Armenia, S., Bianchini, E., Buralli, S., . . . Ghiadoni, L. (2017). Carotid and aortic stiffness in essential hypertension and their relation with target organ damage: the CATOD study. *J Hypertens, 35*(2), 310-318. doi:10.1097/HJH.0000000000001167

Butler, G. C., Yamamoto, Y., Xing, H. C., Northey, D. R., & Hughson, R. L. (1992). Probing heart rate and blood pressure control mechanisms during graded levels of lower body negative pressure (LBNP). *Microgravity Q, 2*(2), 133-140.

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 (1985), 75*(6), 2602-2612.

Butlin, M., & Qasem, A. (2017). Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel), 4*(4), 180-192. doi:10.1159/000452448

Buus, N. H., Mathiassen, O. N., Fenger-Gron, M., Praestholm, M. N., Sihm, I., Thybo, N. K., . . . Christensen, K. L. (2013). Small artery structure during antihypertensive therapy is an

independent predictor of cardiovascular events in essential hypertension. *J Hypertens, 31*(4), 791-797. doi:10.1097/HJH.0b013e32835e215e

Cahn-Weiner, D. A., Malloy, P. F., Boyle, P. A., Marran, M., & Salloway, S. (2000). Prediction of Functional Status from Neuropsychological Tests in Community-Dwelling Elderly Individuals. *The Clinical Neuropsychologist (Neuropsychology, Development and Cognition: Section D), 14*(2), 187-195.

Cahn-Weiner, D. A., Malloy, P. F., Boyle, P. A., Marran, M., & Salloway, S. (2000). Prediction of functional status from neuropsychological tests in community-dwelling elderly individuals. *Clin Neuropsychol, 14*(2), 187-195. doi:10.1076/1385-4046(200005)14:2;1-Z;FT187

Calabia, J., Torguet, P., Garcia, M., Garcia, I., Martin, N., Guasch, B., . . . Valles, M. (2011). Doppler ultrasound in the measurement of pulse wave velocity: agreement with the Complior method. *Cardiovasc Ultrasound, 9*, 13. doi:10.1186/1476-7120-9-13

Ceconi, C., Fox, K. M., Remme, W. J., Simoons, M. L., Bertrand, M., Parrinello, G., . . . the Statistical, C. (2007). ACE inhibition with perindopril and endothelial function. Results of a substudy of the EUROPA study: PERTINENT. *Cardiovasc Res, 73*(1), 237-246. doi:10.1016/j.cardiores.2006.10.021

Cerasola, G., Mule, G., Cottone, S., Nardi, E., & Cusimano, P. (2008). Hypertension, microalbuminuria and renal dysfunction: the Renal Dysfunction in Hypertension (REDHY) study. *J Nephrol, 21*(3), 368-373.

Chen, W., Kobayashi, T., Ichikawa, S., Takeuchi, Y., & Togawa, T. (2000). Continuous estimation of systolic blood pressure using the pulse arrival time and intermittent calibration. *Med Biol Eng Comput, 38*(5), 569-574.

Chen, Y., Shen, F., Liu, J., & Yang, G. Y. (2017). Arterial stiffness and stroke: de-stiffening strategy, a therapeutic target for stroke. *Stroke Vasc Neurol, 2*(2), 65-72. doi:10.1136/svn-2016-000045

Chirinos, J. (2012). Arterial stiffness: Basic concepts and measurement techniques. *J Cardiovascular Transl Res, 5*(3), 243-255.

Chiu, Y. C., Arand, P. W., Shroff, S. G., Feldman, T., & Carroll, J. D. (1991). Determination of pulse wave velocities with computerized algorithms. *Am Heart J, 121*(5), 1460-1470.

Choi, C. U., Kim, E. J., Kim, S. H., Shin, S. Y., Choi, U. J., Kim, J. W., . . . Oh, D. J. (2010). Differing effects of aging on central and peripheral blood pressures and pulse wave velocity: a direct intraarterial study. *J Hypertens, 28*(6), 1252-1260. doi:10.1097/HJH.0b013e328337dad6

Cipolla, M. (2009). The Cerebral Circulation. *Morgan and Claypool Life Sciences*.

Cirovic, S., Walsh, C., Fraser, W. D., & Gulino, A. (2006). Venous filling and elastance in the calf positioned above and below heart level. *Aviat Space Environ Med, 77*(10), 1009-1014.

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

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. doi:10.1097/01.ta.0000196663.34175.33

Convertino, V. A., Ludwig, D. A., & Cooke, W. H. (2004). Stroke volume and sympathetic responses to lower-body negative pressure reveal new insight into circulatory shock in humans. *Auton Neurosci, 111*(2), 127-134. doi:10.1016/j.autneu.2004.02.007

Cooke, W. H., Rickards, C. A., Ryan, K. L., Kuusela, T. A., & Convertino, V. A. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol, 587*(Pt 20), 4987-4999. doi:10.1113/jphysiol.2009.177352

Cooke, W. H., Rickards, C. A., Ryan, K. L., Kuusela, T. A., & Convertino, V. A. (2009). Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans: Autonomic rhythms and presyncope. *The Journal of Physiology, 587*(20), 4987-4999.

Coverdale, N. S., Badrov, M. B., & Shoemaker, J. K. (2017). Impact of age on cerebrovascular dilation versus reactivity to hypercapnia. *J Cereb Blood Flow Metab, 37*(1), 344-355. doi:10.1177/0271678X15626156

Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A., & Shoemaker, J. K. (2014). Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *Journal of Applied Physiology, 117*(10), 1090-1096.

Coverdale, N. S., Lalande, S., Perrotta, A., & Shoemaker, J. K. (2015). Heterogeneous patterns of vasoreactivity in the middle cerebral and internal carotid arteries. *Am J Physiol Heart Circ Physiol, 308*(9), H1030-1038. doi:10.1152/ajpheart.00761.2014

Crystal, G. J., & Salem, M. R. (2015). Lower Body Negative Pressure: Historical Perspective, Research Findings, and Clinical Applications. *J Anesth Hist, 1*(2), 49-54. doi:10.1016/j.janh.2015.02.005

Daskalopoulou, S. S., Rabi, D. M., Zarnke, K. B., Dasgupta, K., Nerenberg, K., Cloutier, L., . . . Padwal, R. S. (2015). The 2015 Canadian Hypertension Education Program recommendations for blood pressure measurement, diagnosis, assessment of risk, prevention, and treatment of hypertension. *Can J Cardiol, 31*(5), 549-568. doi:10.1016/j.cjca.2015.02.016

Davies, J., Gavin, A., Band, M., Morris, A., & Struthers, A. (2005). Spironolactone reduces brachial pulse wave velocity and PIIINP levels in hypertensive diabetic patients. *British Journal of Clinical Pharmacology, 59*(5), 520-523.

de la Torre, J. C. (2000). Cerebral hypoperfusion, capillary degeneration, and development of Alzheimer disease. *Alzheimer Dis Assoc Disord, 14 Suppl 1*, S72-81.

de Souza, F., Muxfeldt, E., Fiszman, R., & Salles, G. (2010). Efficacy of Spironolactone Therapy in Patients With True Resistant Hypertension. *Hypertension, 55*(1), 147-152.

Dong, Y., Lee, W. Y., Basri, N. A., Collinson, S. L., Merchant, R. A., Venketasubramanian, N., & Chen, C. L. (2012). The Montreal Cognitive Assessment is superior to the Mini-Mental State Examination in

detecting patients at higher risk of dementia. *Int Psychogeriatr, 24*(11), 1749-1755. doi:10.1017/S1041610212001068

Dorrance, A. M. (2008). Stroke therapy: is spironolactone the Holy Grail? *Endocrinology, 149*(8), 3761-3763. doi:10.1210/en.2008-0724

Dudenbostel, T., & Glasser, S. P. (2012). Effects of antihypertensive drugs on arterial stiffness. *Cardiol Rev, 20*(5), 259-263. doi:10.1097/CRD.0b013e31825d0a44

Duprez, D. A. (2007). Aldosterone and the vasculature: mechanisms mediating resistant hypertension. *J Clin Hypertens (Greenwich), 9*(1 Suppl 1), 13-18.

Duron, E., & Hanon, O. (2010). Antihypertensive treatments, cognitive decline, and dementia. *J Alzheimers Dis, 20*(3), 903-914. doi:10.3233/JAD-2010-091552

Edwards, N. C., Steeds, R. P., Stewart, P. M., Ferro, C. J., & Townend, J. N. (2009). Effect of spironolactone on left ventricular mass and aortic stiffness in early-stage chronic kidney disease: a randomized controlled trial. *J Am Coll Cardiol, 54*(6), 505-512. doi:10.1016/j.jacc.2009.03.066

Elias, M. F., & Davey, A. (2009). Predicting conversion to mild cognitive impairment: some error is the price of much truth. *Neurology, 73*(18), 1432-1433. doi:10.1212/WNL.0b013e3181c0668c

Elias, M. F., Robbins, M. A., Budge, M. M., Abhayaratna, W. P., Dore, G. A., & Elias, P. K. (2009). Arterial pulse wave velocity and cognition with advancing age. *Hypertension, 53*(4), 668-673. doi:10.1161/HYPERTENSIONAHA.108.126342

Engbaek, M., Hjerrild, M., Hallas, J., & Jacobsen, I. A. (2010). The effect of low-dose spironolactone on resistant hypertension. *Journal of the American Society of Hypertension, 4*(6), 290-294.

Farquharson, C. A., & Struthers, A. D. (2000). Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation, 101*(6), 594-597.

Farquharson, C. A., & Struthers, A. D. (2002). Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. *Clin Sci (Lond), 103*(4), 425-431. doi:10.1042/

Fiermonte, G., Aloe Spiriti, M. A., Latagliata, R., Petti, M. C., & Giacomini, P. (1993). Polycythaemia vera and cerebral blood flow: a preliminary study with transcranial Doppler. *J Intern Med, 234*(6), 599-602.

Fitzpatrick, A. L., Kuller, L. H., Ives, D. G., Lopez, O. L., Jagust, W., Breitner, J. C. S., . . . Dulberg, C. (2004). Incidence and Prevalence of Dementia in the Cardiovascular Health Study: INCIDENCE OF DEMENTIA IN CHS. *Journal of the American Geriatrics Society, 52*(2), 195-204.

Fogari, R., Mugellini, A., Zoppi, A., Derosa, G., Pasotti, C., Fogari, E., & Preti, P. (2003). Influence of losartan and atenolol on memory function in very elderly hypertensive patients. *J Hum Hypertens, 17*(11), 781-785. doi:10.1038/sj.jhh.1001613

Forette, F., Seux, M.-L., Staessen, J. A., Thijs, L., Birkenhäger, W. H., Babarskiene, M.-R., . . . Fagard, R. (1998). Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. *The Lancet, 352*(9137), 1347-1351.

Franklin, S. S., Gustin, W. t., 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.

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), H480-488. doi:10.1152/ajpheart.00184.2008

Fukutomi, M., & Kario, K. (2010). Aging and hypertension. *Expert Rev Cardiovasc Ther, 8*(11), 1531-1539. doi:10.1586/erc.10.78

Fulesdi, B., Limburg, M., Bereczki, D., Michels, R. P., Neuwirth, G., Legemate, D., . . . Csiba, L. (1997). Impairment of cerebrovascular reactivity in long-term type 1 diabetes. *Diabetes, 46*(11), 1840-1845.

Galmiche, G., Pizard, A., Gueret, A., El Moghrabi, S., Ouvrard-Pascaud, A., Berger, S., . . . Jaisser, F. (2014). Smooth muscle cell mineralocorticoid receptors are mandatory for aldosterone-salt to induce vascular stiffness. *Hypertension, 63*(3), 520-526. doi:10.1161/HYPERTENSIONAHA.113.01967

Ganguli, M., Rodriguez, E., Mulsant, B., Richards, S., Pandav, R., Bilt, J. V., . . . DeKosky, S. T. (2004). Detection and management of cognitive impairment in primary care: The Steel Valley Seniors Survey. *J Am Geriatr Soc, 52*(10), 1668-1675. doi:10.1111/j.1532-5415.2004.52459.x

Gao, M., Olivier, N. B., & Mukkamala, R. (2016). Comparison of noninvasive pulse transit time estimates as markers of blood pressure using invasive pulse transit time measurements as a reference. *Physiol Rep, 4*(10). doi:10.14814/phy2.12768

Gesche, H., Grosskurth, D., Kuchler, G., & Patzak, A. (2012). Continuous blood pressure measurement by using the pulse transit time: comparison to a cuff-based method. *Eur J Appl Physiol, 112*(1), 309-315. doi:10.1007/s00421-011-1983-3

Gillard, J. H., Kirkham, F. J., Levin, S. D., Neville, B. G., & Gosling, R. G. (1986). Anatomical validation of middle cerebral artery position as identified by transcranial pulsed Doppler ultrasound. *J Neurol Neurosurg Psychiatry, 49*(9), 1025-1029.

Goswami, N., Grasser, E., Roessler, A., Schneditz, D., & Hinghofer-Szalkay, H. (2009). The cardiovascular response to lower body negative pressure in humans depends on seal location. *Physiol Res, 58*(3), 311-318.

Grigsby, J., Kaye, K., Shetterly, S. M., Baxter, J., Morgenstern, N. E., & Hamman, R. F. (2002). Prevalence of disorders of executive cognitive functioning among the elderly: findings from the San Luis Valley Health and Aging Study. *Neuroepidemiology, 21*(5), 213-220. doi:65638

Grigsby, J., Kaye, K., Shetterly, S. M., Baxter, J., Morgenstern, N. E., & Hamman, R. F. (2002). Prevalence of Disorders of Executive Cognitive Functioning among the Elderly: Findings from the San Luis Valley Health and Aging Study. *Neuroepidemiology, 21*(5), 213-220.

Group, S. R., Wright, J. T., Jr., Williamson, J. D., Whelton, P. K., Snyder, J. K., Sink, K. M., . . . Ambrosius, W. T. (2015). A Randomized Trial of Intensive versus Standard Blood-Pressure Control. *N Engl J Med, 373*(22), 2103-2116. doi:10.1056/NEJMoa1511939

Guelen, I., Westerhof, B. E., Van Der Sar, G. L., Van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H., & Bos, W. J. (2003). Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Press Monit, 8*(1), 27-30. doi:10.1097/01.mbp.0000057013.67622.97

Hajjar, I., Hart, M., Chen, Y.-L., Mack, W., Novak, V., C. Chui, H., & Lipsitz, L. (2013). Antihypertensive Therapy and Cerebral Hemodynamics in Executive Mild Cognitive Impairment: Results of a Pilot Randomized Clinical Trial. *Journal of the American Geriatrics Society, 61*(2), 194-201.

Hajjar, I., Hart, M., Mack, W., & Lipsitz, L. A. (2015). Aldosterone, cognitive function, and cerebral hemodynamics in hypertension and antihypertensive therapy. *Am J Hypertens, 28*(3), 319-325. doi:10.1093/ajh/hpu161

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. *J Appl Physiol (1985), 100*(3), 1059-1064. doi:10.1152/japplphysiol.00954.2005

Hamner, J. W., Tan, C. O., Lee, K., Cohen, M. A., & Taylor, J. A. (2010). Sympathetic control of the cerebral vasculature in humans. *Stroke, 41*(1), 102-109. doi:10.1161/STROKEAHA.109.557132

Hanon, O., Haulon, S., Lenoir, H., Seux, M.-L., Rigaud, A.-S., Safar, M., . . . Forette, F. (2005). Relationship Between Arterial Stiffness and Cognitive Function in Elderly Subjects With Complaints of Memory Loss. *Stroke, 36*(10), 2193-2197.

Hare, J. L., Sharman, J. E., Leano, R., Jenkins, C., Wright, L., & Marwick, T. H. (2013). Impact of spironolactone on vascular, myocardial, and functional parameters in untreated patients with a hypertensive response to exercise. *Am J Hypertens, 26*(5), 691-699. doi:10.1093/ajh/hpt008

Hasegawa, M., Nagao, K., Kinoshita, Y., Rodbard, D., & Asahina, A. (1997). Increased pulse wave velocity and shortened pulse wave transmission time in hypertension and aging. *Cardiology, 88*(2), 147-151.

Henskens, L. H., Kroon, A. A., van Oostenbrugge, R. J., Gronenschild, E. H., Fuss-Lejeune, M. M., Hofman, P. A., . . . de Leeuw, P. W. (2008). Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients. *Hypertension, 52*(6), 1120-1126. doi:10.1161/HYPERTENSIONAHA.108.119024

Hinojosa-Laborde, C., Ryan, K. L., Rickards, C. A., & Convertino, V. A. (2014). Resting sympathetic baroreflex sensitivity in subjects with low and high tolerance to central hypovolemia induced by lower body negative pressure. *Front Physiol, 5*, 241. doi:10.3389/fphys.2014.00241

Hisdal, J., Toska, K., Flatebo, T., & Walloe, L. (2002). Onset of mild lower body negative pressure induces transient change in mean arterial pressure in humans. *Eur J Appl Physiol, 87*(3), 251-256. doi:10.1007/s00421-002-0630-4

Huang, Y., Hu, B., Huang, P. T., Sun, H. Y., & Zhu, J. A. (2008). Abdominal aortic wall motion of healthy and hypertensive subjects: evaluation of tissue Doppler velocity imaging. *J Clin Ultrasound, 36*(4), 218-225. doi:10.1002/jcu.20452

Hughes, D. J., Babbs, C. F., Geddes, L. A., & Bourland, J. D. (1979). Measurements of Young's modulus of elasticity of the canine aorta with ultrasound. *Ultrason Imaging, 1*(4), 356-367. doi:10.1177/016173467900100406

Hwang, M. H., Yoo, J. K., Luttrell, M., Kim, H. K., Meade, T. H., English, M., . . . Christou, D. D. (2013). Role of mineralocorticoid receptors in arterial stiffness in human aging. *Exp Gerontol, 48*(8), 701-704. doi:10.1016/j.exger.2013.05.058

Hwang, M. H., Yoo, J. K., Luttrell, M., Kim, H. K., Meade, T. H., English, M., . . . Christou, D. D. (2016). Acute effect of mineralocorticoid receptor antagonism on vascular function in healthy older adults. *Exp Gerontol, 73*, 86-94. doi:10.1016/j.exger.2015.11.017

Iadecola, C., & Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. *Nat Neurosci, 10*(11), 1369-1376. doi:10.1038/nn2003

Ihara, M., Okamoto, Y., & Takahashi, R. (2013). Suitability of the Montreal cognitive assessment versus the mini-mental state examination in detecting vascular cognitive impairment. *J Stroke Cerebrovasc Dis, 22*(6), 737-741. doi:10.1016/j.jstrokecerebrovasdis.2012.01.001

Imholz, B. P., Dambrink, J. H., Karemaker, J. M., & Wieling, W. (1990). Orthostatic circulatory control in the elderly evaluated by non-invasive continuous blood pressure measurement. *Clin Sci (Lond), 79*(1), 73-79.

Johnson, B. D., van Helmond, N., Curry, T. B., van Buskirk, C. M., Convertino, V. A., & Joyner, M. J. (2014). Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic responses. *J Appl Physiol (1985), 117*(2), 131-141. doi:10.1152/japplphysiol.00070.2014

Johnston, A. J., Steiner, L. A., Gupta, A. K., & Menon, D. K. (2003). Cerebral oxygen vasoreactivity and cerebral tissue oxygen reactivity. *Br J Anaesth, 90*(6), 774-786.

Jurasic, M. J., Josef-Golubic, S., Sarac, R., Lovrencic-Huzjan, A., & Demarin, V. (2009). Beta stiffness--setting age standards. *Acta Clin Croat, 48*(3), 253-258.

Kay, V. L., & Rickards, C. A. (2015). Reproducibility of a continuous ramp lower body negative pressure protocol for simulating hemorrhage. *Physiol Rep, 3*(11). doi:10.14814/phy2.12640

Kearney-Schwartz, A., Rossignol, P., Bracard, S., Felblinger, J., Fay, R., Boivin, J. M., . . . Zannad, F. (2009). Vascular Structure and Function Is Correlated to Cognitive Performance and White Matter Hyperintensities in Older Hypertensive Patients With Subjective Memory Complaints. *Stroke, 40*(4), 1229-1236.

Khan, M. H., Sinoway, L. I., & MacLean, D. A. (2002). Effects of graded LBNP on MSNA and interstitial norepinephrine. *Am J Physiol Heart Circ Physiol, 283*(5), H2038-2044. doi:10.1152/ajpheart.00412.2001

Kirbis, M., Grad, A., Meglic, B., & Bajrovic, F. F. (2013). Comparison of active standing test, head-up tilt test and 24-h ambulatory heart rate and blood pressure monitoring in diagnosing postural tachycardia. *Funct Neurol, 28*(1), 39-45.

Kithas, P. A., & Supiano, M. A. (2010). Spironolactone and Hydrochlorothiazide Decrease Vascular Stiffness and Blood Pressure in Geriatric Hypertension: SPIRONOLACTONE AND HCTZ EFFECTS IN HYPERTENSION. *Journal of the American Geriatrics Society, 58*(7), 1327-1332.

Knopman, D., Boland, L. L., Mosley, T., Howard, G., Liao, D., Szklo, M., . . . Folsom, A. R. (2001). Cardiovascular risk factors and cognitive decline in middle-aged adults. *Neurology, 56*(1), 42-48.

Koivistoinen, T., Koobi, T., Jula, A., Hutri-Kahonen, N., Raitakari, O. T., Majahalme, S., . . . Kahonen, M. (2007). Pulse wave velocity reference values in healthy adults aged 26-75 years. *Clin Physiol Funct Imaging, 27*(3), 191-196. doi:10.1111/j.1475-097X.2007.00734.x

Kroner, E. S., Lamb, H. J., Siebelink, H. M., Cannegieter, S. C., van den Boogaard, P. J., van der Wall, E. E., . . . Westenberg, J. J. (2014). Pulse wave velocity and flow in the carotid artery versus the aortic arch: effects of aging. *J Magn Reson Imaging, 40*(2), 287-293. doi:10.1002/jmri.24470

Kuo, H. K., Sorond, F., Iloputaife, I., Gagnon, M., Milberg, W., & Lipsitz, L. A. (2004). Effect of blood pressure on cognitive functions in elderly persons. *J Gerontol A Biol Sci Med Sci, 59*(11), 1191-1194.

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., . . . Benetos, A. (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension, 37*(5), 1236-1241.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., . . . European Network for Non-invasive Investigation of Large, A. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J, 27*(21), 2588-2605. doi:10.1093/eurheartj/ehl254

Levick, J. (2010). An Introduction to Cardiovascular Physiology. *Hodder Arnold, 5*.

Lipsitz, L. A., Gagnon, M., Vyas, M., Iloputaife, I., Kiely, D. K., Sorond, F., . . . 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.

Lydakis, C., Momen, A., Blaha, C., Herr, M., Leuenberger, U. A., & Sinoway, L. I. (2008). Changes of elastic properties of central arteries during acute static exercise and lower body negative pressure. *Eur J Appl Physiol, 102*(6), 633-641. doi:10.1007/s00421-007-0637-y

Lydakis, C., Momen, A., Blaha, C., Herr, M., Leuenberger, U. A., & Sinoway, L. I. (2008). Changes of elastic properties of central arteries during acute static exercise and lower body negative pressure. *European Journal of Applied Physiology, 102*(6), 633-641.

Macdonald, J. E., Kennedy, N., & Struthers, A. D. (2004). Effects of spironolactone on endothelial function, vascular angiotensin converting enzyme activity, and other prognostic markers in patients with mild heart failure already taking optimal treatment. *Heart, 90*(7), 765-770. doi:10.1136/hrt.2003.017368

Mackenzie, I. S., McEniery, C. M., Dhakam, Z., Brown, M. J., Cockcroft, J. R., & Wilkinson, I. B. (2009). Comparison of the effects of antihypertensive agents on central blood pressure and arterial stiffness in isolated systolic hypertension. *Hypertension, 54*(2), 409-413. doi:10.1161/HYPERTENSIONAHA.109.133801

Mahmud, A., & Feely, J. (2004). Arterial stiffness and the renin-angiotensin-aldosterone system. *J Renin Angiotensin Aldosterone Syst, 5*(3), 102-108. doi:10.3317/jraas.2004.025

Mahmud, A., & Feely, J. (2005). Aldosterone-to-renin ratio, arterial stiffness, and the response to aldosterone antagonism in essential hypertension. *Am J Hypertens, 18*(1), 50-55. doi:10.1016/j.amjhyper.2004.08.026

Masamoto, K., & Tanishita, K. (2009). Oxygen transport in brain tissue. *J Biomech Eng, 131*(7), 074002. doi:10.1115/1.3184694

Mattace-Raso, F. U., van der Cammen, T. J., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A., . . . Witteman, J. C. (2006). Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation, 113*(5), 657-663. doi:10.1161/CIRCULATIONAHA.105.555235

McCurley, A., & Jaffe, I. Z. (2012). Mineralocorticoid receptors in vascular function and disease. *Mol Cell Endocrinol, 350*(2), 256-265. doi:10.1016/j.mce.2011.06.014

McEniery, C. M., Wilkinson, I. B., & Avolio, A. P. (2007). Age, hypertension and arterial function. *Clin Exp Pharmacol Physiol, 34*(7), 665-671. doi:10.1111/j.1440-1681.2007.04657.x

Meaume, S., Rudnichi, A., Lynch, A., Bussy, C., Sebban, C., Benetos, A., & Safar, M. E. (2001). Aortic pulse wave velocity as a marker of cardiovascular disease in subjects over 70 years old. *J Hypertens, 19*(5), 871-877.

Mellander, S. (1989). Functional aspects of myogenic vascular control. *J Hypertens Suppl, 7*(4), S21-30; discussion S31.

Milan, A. (2010). Age and sex structure: Canada, provinces and territories, 2010. *Statistics Canada, 91*(209X).

Mitchell, G. F. (2008). Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *Journal of Applied Physiology, 105*(5), 1652-1660.

Mitchell, G. F. (2009). Arterial Stiffness and Wave Reflection: Biomarkers of Cardiovascular Risk. *Artery Res, 3*(2), 56-64. doi:10.1016/j.artres.2009.02.002

Mitchell, G. F., Hwang, S. J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., . . . Benjamin, E. J. (2010). Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation, 121*(4), 505-511. doi:10.1161/CIRCULATIONAHA.109.886655

Mitchell, G. F., Parise, H., Benjamin, E. J., Larson, M. G., Keyes, M. J., Vita, J. A., . . . Levy, D. (2004). Changes in Arterial Stiffness and Wave Reflection With Advancing Age in Healthy Men and Women: The Framingham Heart Study. *Hypertension, 43*(6), 1239-1245.

Mitchell, G.F., van Buchem, M.A, Sigurdsson, S., Gotal, J.D., Jonsdottir M.K., …..Kjartansson, O. Garcia (2011). Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility- Reykjavik Study. Brain, 134, 3398-3407.

Mukkamala, R., Hahn, J. O., Inan, O. T., Mestha, L. K., Kim, C. S., Toreyin, H., & Kyal, S. (2015). Toward Ubiquitous Blood Pressure Monitoring via Pulse Transit Time: Theory and Practice. *IEEE Trans Biomed Eng, 62*(8), 1879-1901. doi:10.1109/TBME.2015.2441951

Nasreddine, Z. S., Phillips, N., & Chertkow, H. (2012). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology, 78*(10), 765-766; author reply 766. doi:10.1212/01.wnl.0000413072.54070.a3

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi:10.1111/j.1532-5415.2005.53221.x

Neu, P., Schlattmann, P., Schilling, A., & Hartmann, A. (2004). Cerebrovascular reactivity in major depression: a pilot study. *Psychosom Med, 66*(1), 6-8.

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.

Newlin, D. B., & Levenson, R. W. (1979). Pre-ejection period: measuring beta-adrenergic influences upon the heart. *Psychophysiology, 16*(6), 546-553.

Nichols, W., O'Rourke and Vlachopoulos, C. (2011). McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. *6*.

Nilsson, P. M. (2008). Early vascular aging (EVA): consequences and prevention. *Vasc Health Risk Manag, 4*(3), 547-552.

O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C., Duprez, D., & Plante, G. E. (2002). Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens, 15*(5), 426-444.

Ohrui, T., Tomita, N., Sato-Nakagawa, T., Matsui, T., Maruyama, M., Niwa, K., . . . Sasaki, H. (2004). Effects of brain-penetrating ACE inhibitors on Alzheimer disease progression. *Neurology, 63*(7), 1324-1325.

Osol, G., Brekke, J. F., McElroy-Yaggy, K., & Gokina, N. I. (2002). Myogenic tone, reactivity, and forced dilatation: a three-phase model of in vitro arterial myogenic behavior. *Am J Physiol Heart Circ Physiol, 283*(6), H2260-2267. doi:10.1152/ajpheart.00634.2002

Paini, A., Boutouyrie, P., Calvet, D., Tropeano, A. I., Laloux, B., & Laurent, S. (2006). Carotid and aortic stiffness: determinants of discrepancies. *Hypertension, 47*(3), 371-376. doi:10.1161/01.HYP.0000202052.25238.68

Panerai, R. B. (1998). Assessment of cerebral pressure autoregulation in humans--a review of measurement methods. *Physiol Meas, 19*(3), 305-338.

Pannier, J. L., Bouckaert, J. J., & Lefebvre, R. A. (1995). The antiserotonin agent pizotifen does not increase endurance performance in humans. *European Journal of Applied Physiology and Occupational Physiology, 72*(1-2), 175-178.

Paran, E., Anson, O., & Reuveni, H. (2003). Blood pressure and cognitive functioning among independent elderly. *Am J Hypertens, 16*(10), 818-826.

Payne, R. A. (2006). Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure. *Journal of Applied Physiology, 100*(1), 136-141.

Payne, R. A., Symeonides, C. N., Webb, D. J., & Maxwell, S. R. (2006). Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure. *J Appl Physiol (1985), 100*(1), 136-141. doi:10.1152/japplphysiol.00657.2005

Peters, R., Beckett, N., Forette, F., Tuomilehto, J., Clarke, R., Ritchie, C., . . . investigators, H. (2008). Incident dementia and blood pressure lowering in the Hypertension in the Very Elderly Trial cognitive function assessment (HYVET-COG): a double-blind, placebo controlled trial. *Lancet Neurol, 7*(8), 683-689. doi:10.1016/S1474-4422(08)70143-1

Pfizer, C. (2012). PRODUCT MONOGRAPH ALDACTONE (SPIRONOLACTONE). *Pfizer Canada Inc, 17300 Trans-Canada Highway, Kirkland, QC H9J 2M5*.

Phillips, A. A., Bredin, S. S., Cote, A. T., Drury, C. T., & Warburton, D. E. (2013). Aortic distensibility is reduced during intense lower body negative pressure and is related to low frequency power of systolic blood pressure. *Eur J Appl Physiol, 113*(3), 785-792. doi:10.1007/s00421-012-2489-3

Phillips, A. A., Bredin, S. S. D., Cote, A. T., Drury, C. T., & Warburton, D. E. R. (2013). Aortic distensibility is reduced during intense lower body negative pressure and is related to low frequency power of systolic blood pressure. *European Journal of Applied Physiology, 113*(3), 785-792.

Phillips, A. A., Chan, F. H., Zheng, M. M., Krassioukov, A. V., & Ainslie, P. N. (2016). Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. *J Cereb Blood Flow Metab, 36*(4), 647-664. doi:10.1177/0271678X15617954

Phillips, S. J., & Whisnant, J. P. (1992). Hypertension and the brain. The National High Blood Pressure Education Program. *Arch Intern Med, 152*(5), 938-945.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J. W., Hill, M. N., . . . Public Education Subcommittee, A. H. A. (2005). Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. *J Clin Hypertens (Greenwich), 7*(2), 102-109.

Pitt, B. (2003). Aldosterone blockade in patients with systolic left ventricular dysfunction. *Circulation, 108*(15), 1790-1794. doi:10.1161/01.CIR.0000086776.15268.22

Pitt, B., Remme, W., Zannad, F., Neaton, J., Martinez, F., Roniker, B., . . . Survival Study, I. (2003). Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med, 348*(14), 1309-1321. doi:10.1056/NEJMoa030207

Poels, M. M., Ikram, M. A., Vernooij, M. W., Krestin, G. P., Hofman, A., Niessen, W. J., . . . Breteler, M. M. (2008). Total cerebral blood flow in relation to cognitive function: the Rotterdam Scan Study. *J Cereb Blood Flow Metab, 28*(10), 1652-1655. doi:10.1038/jcbfm.2008.62

Purkayastha, S., & Sorond, F. (2012). Transcranial Doppler ultrasound: technique and application. *Semin Neurol, 32*(4), 411-420. doi:10.1055/s-0032-1331812

Rabbitt, P., Scott, M., Thacker, N., Lowe, C., Jackson, A., Horan, M., & Pendleton, N. (2006). Losses in gross brain volume and cerebral blood flow account for age-related differences in speed but not in fluid intelligence. *Neuropsychology, 20*(5), 549-557. doi:10.1037/0894-4105.20.5.549

Reeves, T. J., Hefner, L. L., Jones, W. B., Coghlan, C., Prieto, G., & Carroll, J. (1960). The hemodynamic determinants of the rate of change in pressure in the left ventricle during isometric contraction. *Am Heart J, 60*, 745-761.

Reul, J. M., Gesing, A., Droste, S., Stec, I. S., Weber, A., Bachmann, C., . . . Linthorst, A. C. (2000). The brain mineralocorticoid receptor: greedy for ligand, mysterious in function. *Eur J Pharmacol, 405*(1-3), 235-249.

Richardson, J. T. (2007). Measures of short-term memory: a historical review. *Cortex, 43*(5), 635-650.

Rigsby, C. S., Ergul, A., Portik Dobos, V., Pollock, D. M., & Dorrance, A. M. (2011). Effects of spironolactone on cerebral vessel structure in rats with sustained hypertension. *Am J Hypertens, 24*(6), 708-715. doi:10.1038/ajh.2011.20

Robertson, A. D., Tessmer, C. F., & Hughson, R. L. (2010). Association between arterial stiffness and cerebrovascular resistance in the elderly. *J Hum Hypertens, 24*(3), 190-196. doi:10.1038/jhh.2009.56

Robitaille, C., Dai, S., Waters, C., Loukine, L., Bancej, C., Quach, S., . . . Quan, H. (2012). Diagnosed hypertension in Canada: incidence, prevalence and associated mortality. *Canadian Medical Association Journal, 184*(1), E49-E56.

Roman, G. C. (2003). Stroke, cognitive decline and vascular dementia: the silent epidemic of the 21st century. *Neuroepidemiology, 22*(3), 161-164.

Rossetti, H. C., Lacritz, L. H., Cullum, C. M., & Weiner, M. F. (2011). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology, 77*(13), 1272-1275. doi:10.1212/WNL.0b013e318230208a

Rowell, L. (1993). Human Cardiovascular Control. *Oxford University Press*.

Royall, D. R., Espino, D. V., Polk, M. J., Palmer, R. F., & Markides, K. S. (2004). Prevalence and patterns of executive impairment in community dwelling Mexican Americans: results from the Hispanic EPESE Study. *Int J Geriatr Psychiatry, 19*(10), 926-934. doi:10.1002/gps.1185

Royall, D. R., Espino, D. V., Polk, M. J., Palmer, R. F., & Markides, K. S. (2004). Prevalence and patterns of executive impairment in community dwelling Mexican Americans: results from the Hispanic EPESE Study. *International Journal of Geriatric Psychiatry, 19*(10), 926-934.

Royall, D. R., Palmer, R., Chiodo, L. K., & Polk, M. J. (2004). Declining executive control in normal aging predicts change in functional status: the Freedom House Study. *J Am Geriatr Soc, 52*(3), 346-352.

Royall, D. R., Palmer, R., Chiodo, L. K., & Polk, M. J. (2004). Declining Executive Control in Normal Aging Predicts Change in Functional Status: The Freedom House Study: DECLINING EXECUTIVE CONTROL PREDICTS CHANGE IN FUNCTIONAL STATUS. *Journal of the American Geriatrics Society, 52*(3), 346-352.

Ruitenberg, A., den Heijer, T., Bakker, S. L., van Swieten, J. C., Koudstaal, P. J., Hofman, A., & Breteler, M. M. (2005). Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. *Ann Neurol, 57*(6), 789-794. doi:10.1002/ana.20493

Ryan, K. L., Rickards, C. A., Hinojosa-Laborde, C., Cooke, W. H., & Convertino, V. A. (2012). Sympathetic responses to central hypovolemia: new insights from microneurographic recordings. *Front Physiol, 3*, 110. doi:10.3389/fphys.2012.00110

Savoia, C., Touyz, R. M., Amiri, F., & Schiffrin, E. L. (2008). Selective mineralocorticoid receptor blocker eplerenone reduces resistance artery stiffness in hypertensive patients. *Hypertension, 51*(2), 432-439. doi:10.1161/HYPERTENSIONAHA.107.103267

Saxby, B. K., Harrington, F., McKeith, I. G., Wesnes, K., & Ford, G. A. (2003). Effects of hypertension on attention, memory, and executive function in older adults. *Health Psychol, 22*(6), 587-591. doi:10.1037/0278-6133.22.6.587

Schiffrin, E. L. (2004). The many targets of aldosterone. *Hypertension, 43*(5), 938-940. doi:10.1161/01.HYP.0000123573.60340.9b

Schiffrin, E. L. (2006). Effects of aldosterone on the vasculature. *Hypertension, 47*(3), 312-318. doi:10.1161/01.HYP.0000201443.63240.a7

Scuteri, A., Brancati, A. M., Gianni, W., Assisi, A., & Volpe, M. (2005). Arterial stiffness is an independent risk factor for cognitive impairment in the elderly: a pilot study. *J Hypertens, 23*(6), 1211-1216.

Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, J. K., & Bondar, R. L. (2000). MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. *Stroke, 31*(7), 1672-1678.

Serrador, J. M., Sorond, F. A., Vyas, M., Gagnon, M., Iloputaife, I. D., & Lipsitz, L. A. (2005). Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains. *J Appl Physiol (1985), 98*(1), 151-159. doi:10.1152/japplphysiol.00471.2004

Settakis, G., Pall, D., Molnar, C., Bereczki, D., Csiba, L., & Fulesdi, B. (2003). Cerebrovascular reactivity in hypertensive and healthy adolescents: TCD with vasodilatory challenge. *J Neuroimaging, 13*(2), 106-112.

Shibata, S., & Levine, B. D. (2011). Biological aortic age derived from the arterial pressure waveform. *J Appl Physiol (1985), 110*(4), 981-987. doi:10.1152/japplphysiol.01261.2010

Silvestrini, M., Troisi, E., Matteis, M., Cupini, L. M., & Bernardi, G. (1996). Effect of smoking on cerebrovascular reactivity. *J Cereb Blood Flow Metab, 16*(4), 746-749. doi:10.1097/00004647-199607000-00027

Sink, K. M., Leng, X., Williamson, J., Kritchevsky, S. B., Yaffe, K., Kuller, L., . . . Goff, D. C., Jr. (2009). Angiotensin-converting enzyme inhibitors and cognitive decline in older adults with hypertension: results from the Cardiovascular Health Study. *Arch Intern Med, 169*(13), 1195-1202. doi:10.1001/archinternmed.2009.175

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

Skoog, I., Lithell, H., Hansson, L., Elmfeldt, D., Hofman, A., Olofsson, B., . . . Group, S. S. (2005). Effect of baseline cognitive function and antihypertensive treatment on cognitive and cardiovascular outcomes: Study on COgnition and Prognosis in the Elderly (SCOPE). *Am J Hypertens, 18*(8), 1052-1059. doi:10.1016/j.amjhyper.2005.02.013

Skott, O., Uhrenholt, T. R., Schjerning, J., Hansen, P. B., Rasmussen, L. E., & Jensen, B. L. (2006). Rapid actions of aldosterone in vascular health and disease--friend or foe? *Pharmacol Ther, 111*(2), 495-507. doi:10.1016/j.pharmthera.2005.10.010

Skow, R. J., MacKay, C. M., Tymko, M. M., Willie, C. K., Smith, K. J., Ainslie, P. N., & Day, T. A. (2013). Differential cerebrovascular CO(2) reactivity in anterior and posterior cerebral circulations. *Respir Physiol Neurobiol, 189*(1), 76-86. doi:10.1016/j.resp.2013.05.036

Sorond, F. A., Serrador, J. M., Jones, R. N., Shaffer, M. L., & Lipsitz, L. A. (2009). The sit-to-stand technique for the measurement of dynamic cerebral autoregulation. *Ultrasound Med Biol, 35*(1), 21-29. doi:10.1016/j.ultrasmedbio.2008.08.001

Staessen, J. A., Birkenhager, W. H., Fagard, R., & Forette, F. (1998). Antihypertensive drugs and cognitive function in older people. *J Am Geriatr Soc, 46*(6), 791; author reply 792.

StatisticsCanada. (2011). The Canadian population in 2011. *Statistics Canada, 98*(311).

Su, J. B. (2015). Vascular endothelial dysfunction and pharmacological treatment. *World J Cardiol, 7*(11), 719-741. doi:10.4330/wjc.v7.i11.719

Supiano, M. A. (2017). ARTERIAL STIFFNESS AS A PREDICTOR OF OUTCOMES IN THE SYSTOLIC BLOOD PRESSURE INTERVENTION TRIAL (SESSION 620: SYSTOLIC BLOOD PRESSURE INTERVENTION TRIAL FINDINGS THAT IMPACT OLDER HYPERTENSIVE PATIENTS). *Innovation in Aging, 1*(1).

Sutton-Tyrrell, K., Najjar, S. S., Boudreau, R. M., Venkitachalam, L., Kupelian, V., Simonsick, E. M., . . . Health, A. B. C. S. (2005). Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation, 111*(25), 3384-3390. doi:10.1161/CIRCULATIONAHA.104.483628

Swierblewska, E., Hering, D., Kara, T., Kunicka, K., Kruszewski, P., Bieniaszewski, L., . . . Narkiewicz, K. (2010). An independent relationship between muscle sympathetic nerve activity and pulse wave velocity in normal humans. *J Hypertens, 28*(5), 979-984.

Tarumi, T., Ayaz Khan, M., Liu, J., Tseng, B. Y., Parker, R., Riley, J., . . . Zhang, R. (2014). Cerebral hemodynamics in normal aging: central artery stiffness, wave reflection, and pressure pulsatility. *J Cereb Blood Flow Metab, 34*(6), 971-978. doi:10.1038/jcbfm.2014.44

Tedesco, M. A., Ratti, G., Mennella, S., Manzo, G., Grieco, M., Rainone, A. C., . . . Iacono, A. (1999). Comparison of losartan and hydrochlorothiazide on cognitive function and quality of life in hypertensive patients. *Am J Hypertens, 12*(11 Pt 1), 1130-1134.

Tombaugh, T. N. (2004). Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol, 19*(2), 203-214. doi:10.1016/S0887-6177(03)00039-8

Torjesen, A., Cooper, L. L., Rong, J., Larson, M. G., Hamburg, N. M., Levy, D., . . . Mitchell, G. F. (2017). Relations of Arterial Stiffness With Postural Change in Mean Arterial Pressure in Middle-Aged

Adults: The Framingham Heart Study. *Hypertension, 69*(4), 685-690. doi:10.1161/HYPERTENSIONAHA.116.08116

Townsend, R. R., Wilkinson, I. B., Schiffrin, E. L., Avolio, A. P., Chirinos, J. A., Cockcroft, J. R., . . . American Heart Association Council on, H. (2015). Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension, 66*(3), 698-722. doi:10.1161/HYP.0000000000000033

Triantafyllidi, H., Arvaniti, C., Lekakis, J., Ikonomidis, I., Siafakas, N., Tzortzis, S., . . . Kremastinos, D. T. (2009). Cognitive impairment is related to increased arterial stiffness and microvascular damage in patients with never-treated essential hypertension. *Am J Hypertens, 22*(5), 525-530. doi:10.1038/ajh.2009.35

Truijen, J., Kim, Y. S., Krediet, C. T. P., Stok, W. J., Kölgen, R. S., Colier, W. N., . . . van Lieshout, J. J. (2012). Orthostatic leg blood volume changes assessed by near-infrared spectroscopy: Orthostatic leg blood volume assessment. *Experimental Physiology, 97*(3), 353-361.

Tsuchikura, S., Shoji, T., Kimoto, E., Shinohara, K., Hatsuda, S., Koyama, H., . . . Nishizawa, Y. (2010). Central versus peripheral arterial stiffness in association with coronary, cerebral and peripheral arterial disease. *Atherosclerosis, 211*(2), 480-485. doi:10.1016/j.atherosclerosis.2010.03.037

Tymko, M. M., Rickards, C. A., Skow, R. J., Ingram-Cotton, N. C., Howatt, M. K., & Day, T. A. (2016). The effects of superimposed tilt and lower body negative pressure on anterior and posterior cerebral circulations. *Physiol Rep, 4*(17). doi:10.14814/phy2.12957

Tzamou, V., Kyvelou, S. M., Karpanou, E., Petras, D., & Vyssoulis, G. (2015). Aldosterone Levels, Aortic Stiffness, and Wave Reflection in Essential Hypertensive Patients. *Am J Hypertens, 28*(7), 852-857. doi:10.1093/ajh/hpu244

Tzourio, C., Anderson, C., Chapman, N., Woodward, M., Neal, B., MacMahon, S., . . . Group, P. C. (2003). Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and

cognitive decline in patients with cerebrovascular disease. *Arch Intern Med, 163*(9), 1069-1075. doi:10.1001/archinte.163.9.1069

Tzourio, C., Dufouil, C., Ducimetiere, P., & Alperovitch, A. (1999). Cognitive decline in individuals with high blood pressure: A longitudinal study in the elderly. *Neurology, 53*(9), 1948-1948.

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. doi:10.1038/jcbfm.2008.13

Van Bortel, L. M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J. K., De Backer, T., . . . European Network for Noninvasive Investigation of Large, A. (2012). Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens, 30*(3), 445-448. doi:10.1097/HJH.0b013e32834fa8b0

Van Spall, H. G. C., Toren, A., Kiss, A., & Fowler, R. A. (2007). Eligibility Criteria of Randomized Controlled Trials Published in High-Impact General Medical Journals: A Systematic Sampling Review. *JAMA, 297*(11), 1233.

van Wijnen, V. K., Finucane, C., Harms, M. P. M., Nolan, H., Freeman, R. L., Westerhof, B. E., . . . Wieling, W. (2017). Noninvasive beat-to-beat finger arterial pressure monitoring during orthostasis: a comprehensive review of normal and abnormal responses at different ages. *J Intern Med*. doi:10.1111/joim.12636

Verbree, J., Bronzwaer, A.-S. G. T., Ghariq, E., Versluis, M. J., Daemen, M. J. A. P., van Buchem, M. A., . . . van Osch, M. J. P. (2014). Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *Journal of Applied Physiology, 117*(10), 1084-1089.

Vlachopoulos, C., Aznaouridis, K., & Stefanadis, C. (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol, 55*(13), 1318-1327. doi:10.1016/j.jacc.2009.10.061

Waldstein, S. R., Rice, S. C., Thayer, J. F., Najjar, S. S., Scuteri, A., & Zonderman, A. B. (2008). Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. *Hypertension, 51*(1), 99-104. doi:10.1161/HYPERTENSIONAHA.107.093674

Waldstein, S. R., Tankard, C. F., Maier, K. J., Pelletier, J. R., Snow, J., Gardner, A. W., . . . Katzel, L. I. (2003). Peripheral arterial disease and cognitive function. *Psychosom Med, 65*(5), 757-763.

Wallace, A. G., Skinner, N. S., Jr., & Mitchell, J. H. (1963). Hemodynamic determinants of the maximal rate of rise of left ventricular pressure. *Am J Physiol, 205*, 30-36.

Wang, H. W., Huang, B. S., Chen, A., Ahmad, M., White, R. A., & Leenen, F. H. (2016). Role of brain aldosterone and mineralocorticoid receptors in aldosterone-salt hypertension in rats. *Neuroscience, 314*, 90-105. doi:10.1016/j.neuroscience.2015.11.055

Wang, L., Jia, J., & Wu, L. (2013). The relationship between cognitive impairment and cerebral blood flow changes after transient ischaemic attack. *Neurol Res, 35*(6), 580-585. doi:10.1179/1743132813Y.0000000160

Wang, M., Kim, S. H., Monticone, R. E., & Lakatta, E. G. (2015). Matrix metalloproteinases promote arterial remodeling in aging, hypertension, and atherosclerosis. *Hypertension, 65*(4), 698-703. doi:10.1161/HYPERTENSIONAHA.114.03618

Weissler, A. M., Peeler, R. G., & Roehll, W. H., Jr. (1961). Relationships between left ventricular ejection time, stroke volume, and heart rate in normal individuals and patients with cardiovascular disease. *Am Heart J, 62*, 367-378.

Wieling, W., Krediet, C. T., van Dijk, N., Linzer, M., & Tschakovsky, M. E. (2007). Initial orthostatic hypotension: review of a forgotten condition. *Clin Sci (Lond), 112*(3), 157-165. doi:10.1042/CS20060091

Willie, C. K., Macleod, D. B., Shaw, A. D., Smith, K. J., Tzeng, Y. C., Eves, N. D., . . . Ainslie, P. N. (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol, 590*(14), 3261-3275. doi:10.1113/jphysiol.2012.228551

Willie, C. K., Tzeng, Y. C., Fisher, J. A., & Ainslie, P. N. (2014). Integrative regulation of human brain blood flow. *J Physiol, 592*(5), 841-859. doi:10.1113/jphysiol.2013.268953

Wray, D. W., & Supiano, M. A. (2010). Impact of Aldosterone Receptor Blockade Compared With Thiazide Therapy on Sympathetic Nervous System Function in Geriatric Hypertension. *Hypertension, 55*(5), 1217-1223.

Xing, C. Y., Tarumi, T., Liu, J., Zhang, Y., Turner, M., Riley, J., . . . Zhang, R. (2017). Distribution of cardiac output to the brain across the adult lifespan. *J Cereb Blood Flow Metab, 37*(8), 2848-2856. doi:10.1177/0271678X16676826

Yagi, S., Akaike, M., Aihara, K.-i., Iwase, T., Yoshida, S., Sumitomo-Ueda, Y., . . . Sata, M. (2011). High plasma aldosterone concentration is a novel risk factor of cognitive impairment in patients with hypertension. *Hypertension Research, 34*(1), 74-78.

Yano, Y., Hoshide, S., Tamaki, N., Nagata, M., Sasaki, K., Kanemaru, Y., . . . Kario, K. (2011). Efficacy of eplerenone added to renin-angiotensin blockade in elderly hypertensive patients: the Jichi-Eplerenone Treatment (JET) study. *J Renin Angiotensin Aldosterone Syst, 12*(3), 340-347. doi:10.1177/1470320310390404

Yano, Y., Hoshide, S., Tamaki, N., Nagata, M., Sasaki, K., Kanemaru, Y., . . . Kario, K. (2011). Efficacy of eplerenone added to renin-angiotensin blockade in elderly hypertensive patients: the Jichi-

Eplerenone Treatment (JET) study. *Journal of the Renin-Angiotensin-Aldosterone System, 12*(3), 340-347.

Yonas, H., Smith, H. A., Durham, S. R., Pentheny, S. L., & Johnson, D. W. (1993). Increased stroke risk predicted by compromised cerebral blood flow reactivity. *J Neurosurg, 79*(4), 483-489. doi:10.3171/jns.1993.79.4.0483

Zannad, F., McMurray, J. J., Krum, H., van Veldhuisen, D. J., Swedberg, K., Shi, H., . . . Group, E.-H. S. (2011). Eplerenone in patients with systolic heart failure and mild symptoms. *N Engl J Med, 364*(1), 11-21. doi:10.1056/NEJMoa1009492

Zhang, Q.-L., & Rothenbacher, D. (2008). Prevalence of chronic kidney disease in population-based studies: Systematic review. *BMC Public Health, 8*(1).

Zhang, Y.-L., Zheng, Y.-Y., Ma, Z.-C., & Sun, Y.-N. (2011). Radial pulse transit time is an index of arterial stiffness. *Hypertension Research, 34*(7), 884-887.

Zhang, Y., Agnoletti, D., Xu, Y., Wang, J. G., Blacher, J., & Safar, M. E. (2014). Carotid-femoral pulse wave velocity in the elderly. *J Hypertens, 32*(8), 1572-1576; discussion 1576. doi:10.1097/HJH.0000000000000187

Zheng, Y. L., Yan, B. P., Zhang, Y. T., & Poon, C. C. (2015). Noninvasive Characterization of Vascular Tone by Model-Based System Identification in Healthy and Heart Failure Patients. *Ann Biomed Eng, 43*(9), 2242-2252. doi:10.1007/s10439-015-1266-y

Zieman, S. J., Melenovsky, V., & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol, 25*(5), 932-943. doi:10.1161/01.ATV.0000160548.78317.29

Zuccala, G., Onder, G., Marzetti, E., Monaco, M. R., Cesari, M., Cocchi, A., . . . Group, G. S. (2005). Use of angiotensin-converting enzyme inhibitors and variations in cognitive performance among patients with heart failure. *Eur Heart J, 26*(3), 226-233. doi:10.1093/eurheartj/ehi058

Appendices

These six appendices cover important methodological considerations that include how and why window sample sizes were selected during waveform analysis (1.M.1). Tailored waveform analysis is important to estimating mean transit times from R-peak of QRS complex to foot of a waveform. Appendices include the comparison of heart to carotid pressure transit time to heart to carotid velocity transit time of waveforms (1.M.2); as well as safety considerations (1.M.3) and generalizability of the outcomes in the RCT (1.M.4). The RCT involved a pharmaceutical. Consequently, careful consideration was made to ensure participant safety, while still maintaining optimal potential for study-drug efficacy. Since most pharmaceutical trials have limited generalizability to the general population due to exclusion criteria, effort was made to clarify how results of this RCT would apply to the general population.

The last section on end-tidal CO_2 (1.M.5) includes important data during hyper- and hypocapnic breathing patterns. This data is included here to supplement Results 5.3 so as to not distract the reader from the main objectives of CHAPTER 5 that did not focus on end tidal CO_2.

Appendices 2.IE, 3.HIF, 4.ECF, 5.T and 6.DS cover inclusion/exclusion criteria, health history forms, and executive cognitive function tests MoCA, Trails and Digit Span respectively. Criteria in APPENDIX 2.IE were used to screen potential subjects in both the RCT and experimental studies; especially important in the RCT. Health History Forms in APPENDIX 3.HIF were an important part of documenting potential drug interactions and baseline criteria during RCT, or

potential health issues that may prevent completion of the thesis studies. Cognitive function tests in the last 3 appendices are validated tests to assess executive function and were used with participants in the RCT before and after six months of study-drug.

APPENDIX 1.M Methodological Considerations

1.M.1 Selection of Sample Window Size for Arterial Waveform Digital Filter Smoothing and 2nd Derivative

A Savitzky-Golay smoothing 3rd order polynomial digital filter was used to increase the signal-to-noise ratio of both arterial velocity and pressure waveforms while preserving the shape of the original signal. Applying this digital filter required selection of a sliding sample window size for each waveform that maintained peak and width of the original waveform. Finger arterial pressure waveform sliding sample windows are shown below in Figure 25 as 11, 21, 51, or 75 points and the 21 point window size was selected for data analysis based on smoothness of the waveform while maintaining peak and width of original waveform.

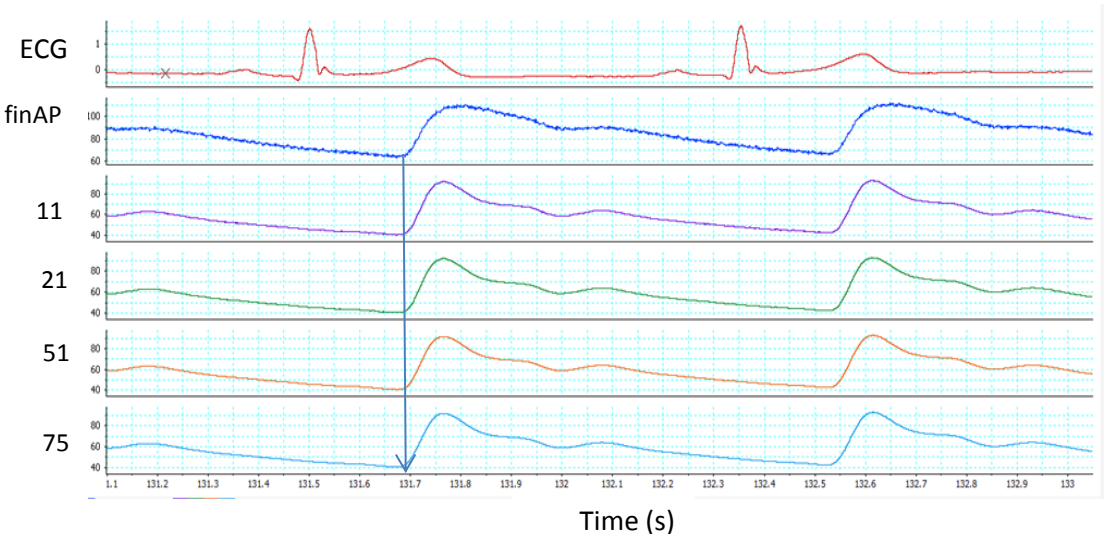


Figure 25. Application of digital filter for smoothing finger arterial pressure (finAP) using a Satvitsky-Golay 3rd order polynomial vs time (seconds)

Channel 1 is ECG, Channel 2 is original finger arterial pressure signal, Channel 3 is finger arterial pressure signal smoothed with a Satvitski-Golay 3rd order polynomial digital filter with sample window 11, Channel 4 is sample window 21, Channel 5 is sample window 51 and Channel 6 is sample window 75.

Figure 26 shows the Savitzky-Golay smoothing 3rd order polynomial smoothed finger arterial waveform (finAP) with a 21 point window size with the 2nd derivative of that waveform using 5 different sample window sizes: 151, 175, 201, 225, and 251. The optimal window size of 151 was selected, after checking larger sizes, for this particular participant based on alignment of foot of pressure waveform (i.e., minimum) with peak of 2nd derivative waveform. Notably, this peak of the 2nd derivative with window sample size 151 occurs *after* the peak of the 2nd derivative with the larger window samples to better identify the foot of the finAP. Once the optimal 2nd derivative sample window size was set, the foot was identified as the time at the peak of the 2nd derivative signal of the smoothed velocity wave form in the same cardiac cycle (Chiu, Arand, Shroff, Feldman, & Carroll, 1991). Selection of each 2nd derivative sample window size was done at the start of each level of lower body negative pressure; and for the RCT at the start of baseline and after study-drug analyses.

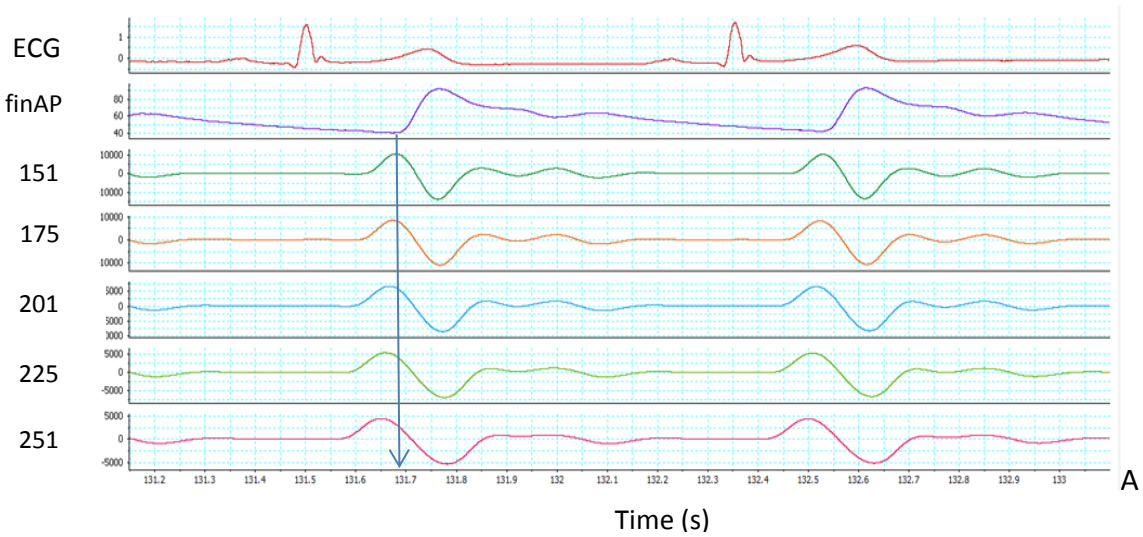


Figure 26. Selection of optimal window sample size for 2nd derivative of smoothed finger arterial pressure (finAP) waveform vs time (seconds)

Channel 1 is ECG, Channel 2 is smoothed finger arterial pressure (finAP) using a Satvitsky-Golay 3rd order polynomial digital filter, Channel 3 is 2nd derivative sample window 151, Channel 4 is sample window 175, Channel 5 is sample window 201 and Channel 6 is sample window 225, and Channel 7 is sample window 251.

Figure 27 shows the Savitzky-Golay smoothing 3rd order polynomial smoothed carotid pressure arterial waveform with a 51 point window size with the 2nd derivative of that waveform using 5 different sample window sizes: 125, 151, 175, 201 and 225. The optimal window size of 125 was selected, after checking larger sizes, for this particular participant based on alignment of foot of pressure waveform (i.e., minimum) with peak of 2nd derivative. The peak of the 2nd derivative with window sample size 125 occurs *after* the peak of the 2nd derivative with window larger sample sizes. Selection of each 2nd derivative sample window size was done at the start of each level of lower body negative pressure; and for the RCT at the start of baseline and after study-drug analyses.

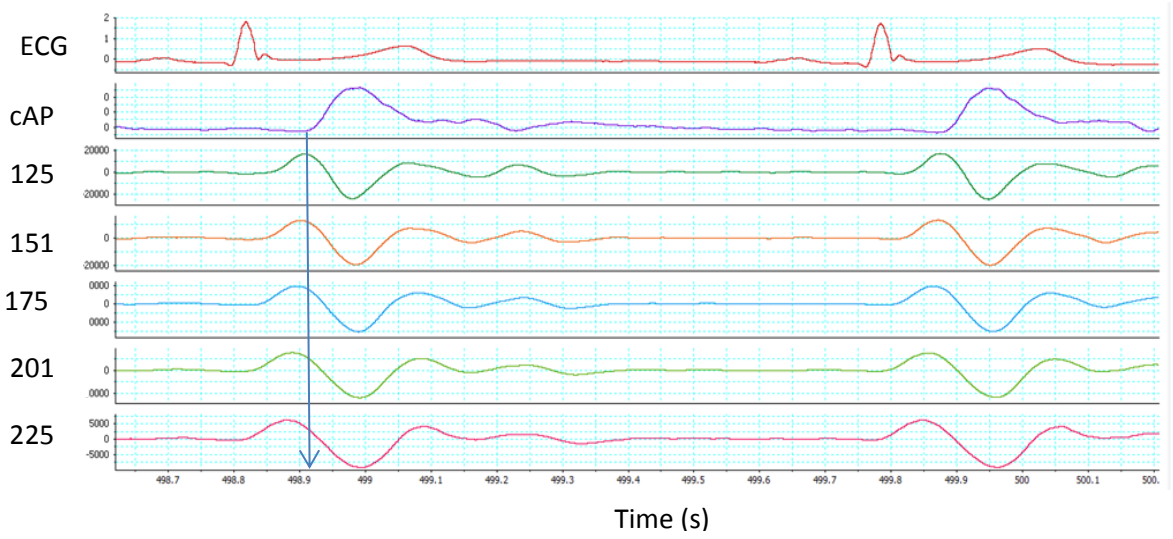


Figure 27. Selection of optimal window sample size for 2nd derivative of smoothed carotid arterial pressure (cAP) waveform vs time (seconds)

Channel 1 is ECG, Channel 2 is smoothed carotid arterial pressure (cAP) using a Satvitsky-Golay 3rd order polynomial window size 51, Channel 3 is 2nd derivative sample window 125, Channel 4 is sample window 151, Channel 5 is sample window 175 and Channel 6 is sample window 201, and Channel 7 is sample window 225.

Similarly, Figure 28 shows the Savitzky-Golay smoothing 3rd order polynomial smoothed aortic velocity waveform with a 75 point window size with the 2nd derivative of that waveform using 3 different sample window sizes: 151, 175, and 201. The optimal window size of 151 was selected for this particular participant, after checking larger sizes, based on alignment of foot of pressure waveform with peak of 2nd derivative. The peak of the 2nd derivative with window sample size 151 occurs *after* the peak of the 2nd derivative with window sample sizes 175 and 201. Selection of each 2nd derivative sample window size was done at the start of each level of lower body negative pressure.

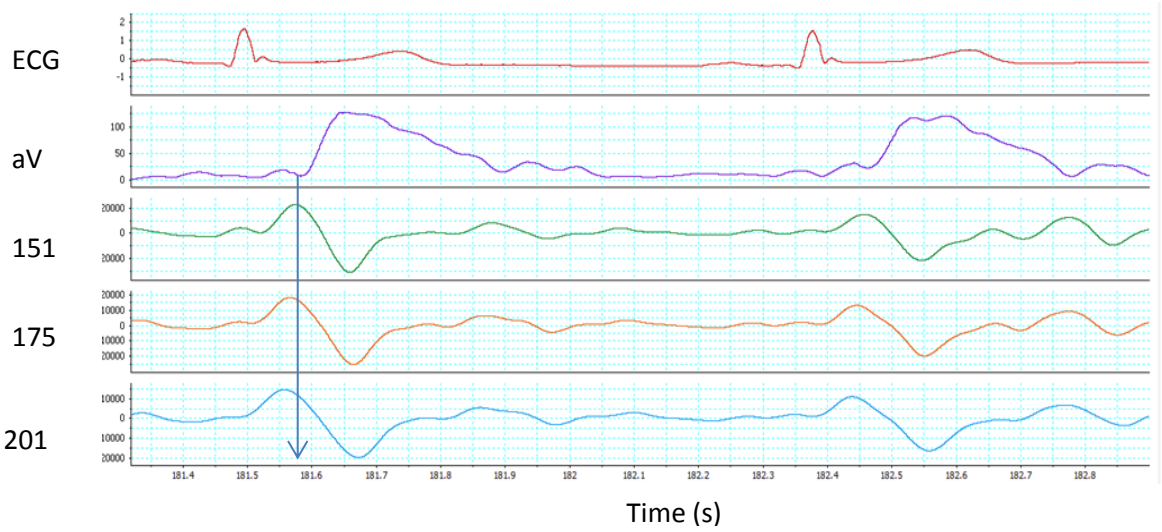


Figure 28. Selection of optimal window sample size for 2nd derivative of smoothed aortic velocity (aV) waveform vs time (seconds)

Channel 1 is ECG, Channel 2 is smoothed aortic velocity waveform (aV) using a Satvitsky-Golay 3rd order polynomial, Channel 3 is 2nd derivative sample window 151, Channel 4 is sample window 175, Channel 5 is sample window 201.

Table 15 provides a summary of sample window sizes for Satvitsky-Golay 3rd order polynomial digital filter and 2nd derivative waveform for all arterial velocity and pressure waveforms in this thesis. Selection of the foot of each waveform as the maximum of the second derivative was done using automatic point selection within LabChart 7 (i.e., macro routine) for all velocity and pressure waveforms except femoral velocity. Selection of the foot of the femoral velocity waveform was done manually using digital markers for transit time measurement set from R-peak of QRS complex to the first peak of second derivative of that waveform during same cardiac cycle---the automatic point selection within LabChart 7 (i.e., macro routine) was unable to consistently identify the first peak unless the amplitude exceeded the second peak of femoral velocity waveform second derivative within the same cardiac cycle.

Table 15. Summary of Satvitsky-Golay 3rd order polynomial digital filter and 2nd derivative window sample sizes for arterial velocity and pressure waveforms

Waveform	Satvitsky-Golay 3rd order polynomial window size applied	2nd Derivative window size options*
Finger pressure	21	151,175,201,225,251
Carotid pressure	21	125,151,175,201,225
Carotid velocity	51	125,151,175,201,225
Femoral velocity	125	175, 201
Aortic velocity	75	151,175,301

*one sample window size was selected from these options based on alignment between foot of smoothed waveform with peak of 2nd derivative waveform at the start of series of cardiac cycles analyzed off-line.

1.M.2 Comparison of Carotid Velocity Pulse Wave Transit Time to Carotid Pressure Pulse Wave Transit Time

The heart to carotid velocity transit time (hcTTv) was compared to heart to carotid pressure transit time (hcTT) to investigate similarity between transit times of these two types (velocity versus pressure) of waveforms. Identical to hcTT, hcTTv was calculated as the time interval between the R-peak of the QRS complex and foot of the smoothed carotid artery velocity waveform within the same cardiac cycle. A Savitzky-Golay smoothing 3rd order polynomial was applied and optimal 2nd derivative samples window size selected as outlined in Table 14 (Section 1.M.1).

Age, heart rate, blood pressure, and transit times of older and younger adults are shown in Table 16. In older adults, hcTT was similar to hcTTv; and significantly shorter than younger adults. There was a strong significant linear correlation between hcTT and hcTTv for both younger adults (YA, Figure 29) and older adults (OA, Figure 30) respectively. Figure 31 shows the Bland-Altman plot of hcTTv and hcTT in OA to show that hcTTv slightly underestimates transit times compared to hcTT. The variability around the mean is fairly constant; except that possibly, hcTTv may overestimate of transit time difference beyond 70 ms. The distance between upper and lower margins is wider than upper and lower margins for hcTTv and hcTT in YA shown in Figure 32. In these YA, hcTTv appears to also slightly underestimate transit time without any observable trend. All but one hcTTv minus hcTT value is within upper and lower limits.

Table 16. Heart rate, blood pressure and carotid arterial transit times of older adults (OA) and younger adults (YA)

Measure	OA (n=17) mean ±SD	YA (n=14) mean ±SD	P-value [a] OA vs YA
Age (years)	65±3	26±5	P<0.05
Heart Rate (beats/min)	68±13	64±7	0.32
Brachial Pressure (mmHg)			
MAP	97±9	86±9	P<0.01
SBP	142±16	124±12	P<0.05
DBP	75±8	67±9	P<0.05
PP	66±13	57±7	P<0.05
Transit times (ms)			
hcTT	68±17	84±15	P<0.05
hcTTv	67±21	81±15	P<0.05

[a]From unpaired t-test, MAP= Mean Arterial Pressure, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, PP=Pulse Pressure, hcTT=heart to carotid pressure transit time is time from R-peak to foot of pressure wave at carotid artery, hcTTv=heart to carotid velocity transit time is time from R-peak to foot of velocity wave at carotid artery. All measures while participants are supine.

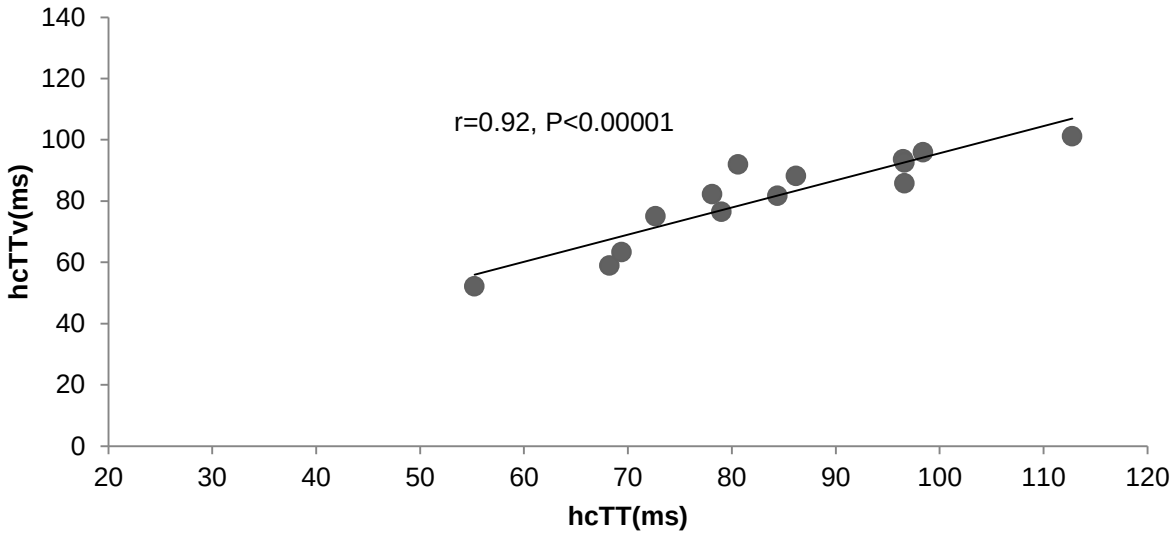


Figure 29. Scatterplot of hcTTv versus hcTT in younger adults (YA)

A scatterplot (black dots) of hcTTv (hcTTv=heart to carotid velocity transit time is time from R-peak to foot of velocity wave at carotid artery, ms) and hcTT (hcTT=heart to carotid pressure transit time is time from R-peak to foot of pressure wave at carotid artery, ms) for younger adults. There was a significant linear correlation between hcTTv and hcTT as represented by the trend line (r=0.92, P<0.00001).

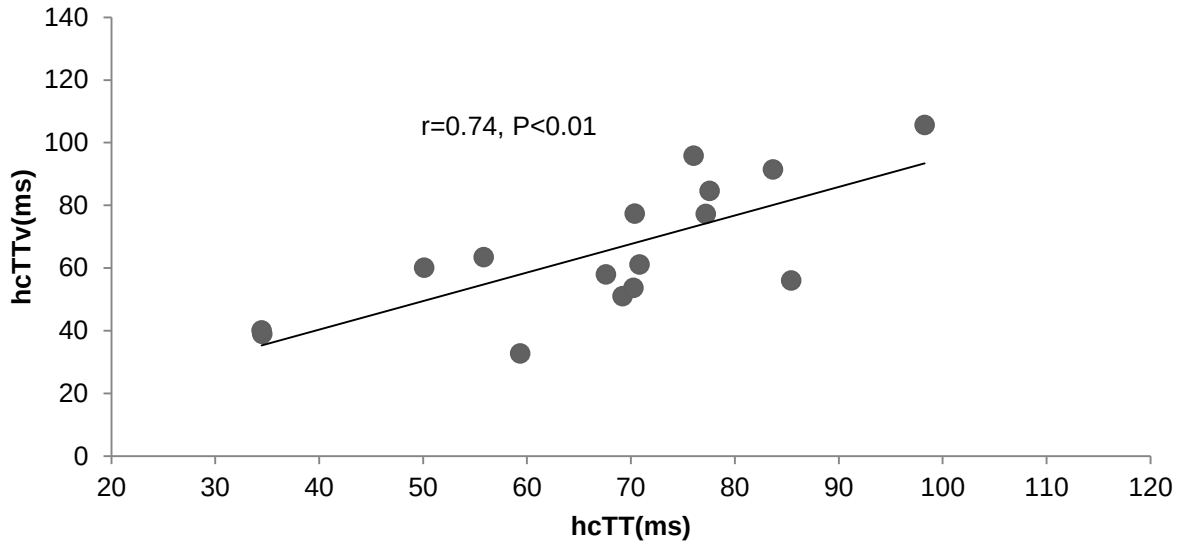


Figure 30. Scatterplot of hcTTv versus hcTT in older adults (OA)

A scatterplot (black dots) of hcTTv (hcTTv=heart to carotid velocity transit time is time from R-peak to foot of velocity wave at carotid artery, ms) and hcTT (hcTT=heart to carotid pressure transit time is time from R-peak to foot of pressure wave at carotid artery, ms) for older adults. There was a significant linear correlation between hcTTv and hcTT as represented by the trend line (r=0.74, P<0.01).

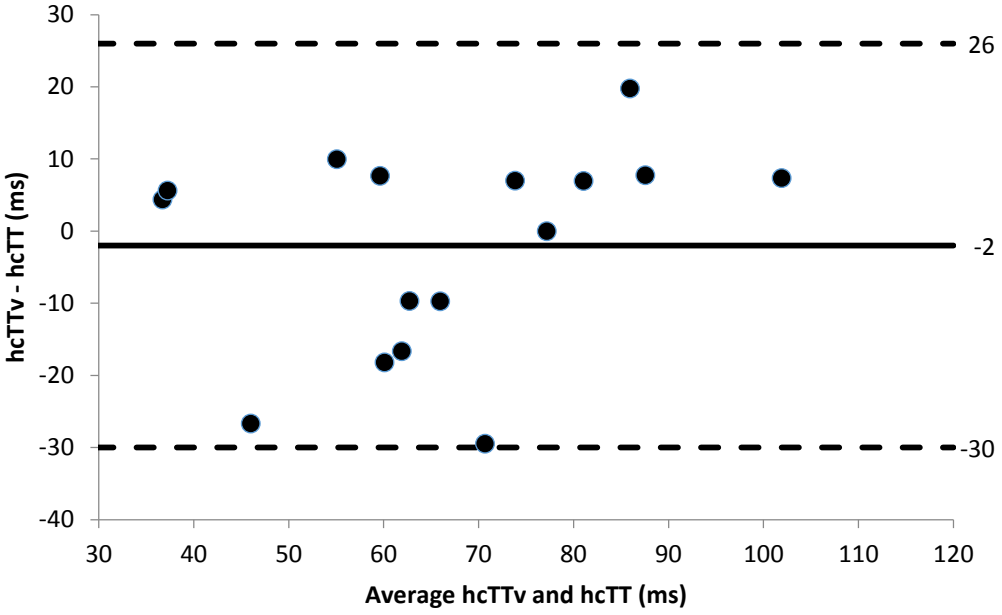


Figure 31. Bland-Altman plots of hcTTv and hcTT for OA

The Bland-Altman plot of heart-carotid TT (hcTTv=heart to carotid velocity transit time is time from R-peak to foot of velocity wave at carotid artery, ms) velocity compared to heart-carotid TT (hcTT=heart to carotid pressure transit time is time from R-peak to foot of pressure wave at carotid artery, ms) pressure for older adults (OA). The mean hcTTv and hcTT difference is -2 ms, upper limit 26 ms and lower limit -30ms.

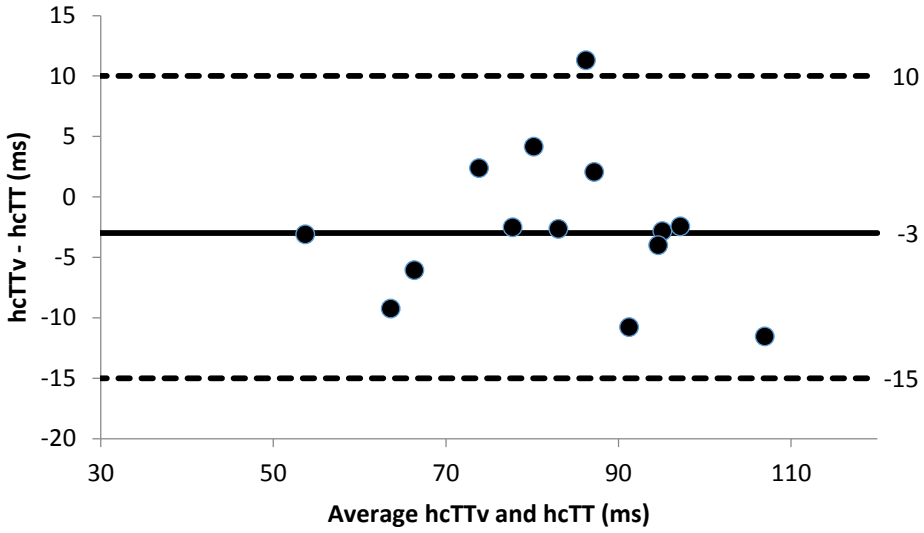


Figure 32. Bland-Altman plots of hcTTv and hcTT for YA

The Bland-Altman plot of heart-carotid TT (hcTTv=heart to carotid velocity transit time is time from R-peak to foot of velocity wave at carotid artery, ms) velocity compared to heart-carotid TT (hcTT=heart to carotid pressure transit time is time from R-peak to foot of pressure wave at carotid artery, ms) pressure for younger adults (YA). The mean hcTTv and hcTT difference is -3 ms, upper limit 10 ms and lower limit -15 ms.

1.M.3 Safety Considerations during Randomized, Double-Blind, Placebo-Controlled Trial

Spironolactone is indicated in Canada for essential hypertension (50-100mg/day); usually in combination with other drugs (Pfizer, 2012). The two most common adverse events are elevation of serum potassium (hyperkalemia) and gynecomastia (breast discomfort), although spironolactone is generally well tolerated in non-diabetic hypertensive patients. Prospective trials have shown 0%-4% risk of hyperkalemia (serum potassium >5.0 mmol per liter) and 0%-5% risk of gynecomastia in older patients with or without diabetes, and hypertension (Davies et al., 2005; Engbaek, Hjerrild, Hallas, & Jacobsen, 2010; Kithas & Supiano, 2010). Hyperkalemia and gynecomastia were not observed in small trials that excluded patients with diabetes or poor renal function, and not observed in this thesis RCT (Kithas & Supiano, 2010; Wray & Supiano, 2010). The open-label trial by Engbaek showed that of the 344 older hypertensive patients (10.5% were diabetic), those with compromised renal function (eGFR 43.3 ml/min/1.73m^2) were at significantly elevated risk of hyperkalemia compared to those with better renal function ($P < 0.05$) (Engbaek et al., 2010). Therefore, blood work for serum potassium levels and renal function were routinely monitored in the current RCT: before initial dose, one week after initial dose or titration, then 12 and 18 weeks from initial dose (Zannad et al., 2011). In the current RCT, serum potassium (K+) was closely monitored before taking the study-drug, after first dose, and after dose adjustments. The protocol involved: Withholding study-drug if K+ was ≥ 5 mmol/L after initial 25 mg once daily dose or ≥ 6 mmol/L at any time; there was a decrease from the 50 mg study-drug to 25 mg if the K+ level was 5.0 to 5.9 mmol/L (Y. Yano et al., 2011; Zannad et al., 2011). Participants were asked to tell their Family Physician if they experience dizziness, diarrhea, vomiting, rapid or irregular heartbeat, lower extremity

swelling, or difficulty breathing. If systolic blood pressure dropped below 100 mmHg or there were symptomatic problems (e.g. dizziness, general fatigue) during the RCT, the protocol was to reduce the dose of study-drug (Yuichiro Yano et al., 2011).

Renal function was monitored during the RCT. If renal function declined to less than eGFR (estimated glomerular filtration rate) of 60 mL/min per 1.73 m^2 participants were withdrawn from the RCT since moderate renal insufficiency (eGFR ≤ 60 mL/min per 1.73 m^2) is not common in generally healthy older adults with hypertension. A systematic review of clinical trials for the prevalence of renal dysfunction in older adults showed that only up to 35% with, or without, hypertension have moderate renal impairment measured as eGFR ≤ 60 mL/min per 1.73 m^2 for three or more months (Q.-L. Zhang & Rothenbacher, 2008). A prospective trial of 1856 older hypertensive patients, free from diabetes like the current study criteria, however showed the prevalence of overt renal insufficiency (eGFR ≤ 60 mL/min per 1.73 m^2) of only 10% (Cerasola, Mule, Cottone, Nardi, & Cusimano, 2008). Concomitant medications were recorded in this thesis RCT to identify potential drug-drug interactions, especially combining study-drug with strong inhibitors of CYP3A4 and potassium supplements (Pfizer, 2012).

Other measurement modalities were of little risk to patient health. Cognitive testing offered very low risk to participant's health. If patients found cognitive testing upsetting, they were free to withdraw themselves from the trial. The equipment used to measure vascular parameters was non-invasive with minimal risk to participants: electrodes for electrocardiogram could have provided risk of skin rash from adhesive, and Doppler ultrasound could have had a risk of skin

burn if transmission power is set excessively high. Minimal power settings were used to obtain optimal signals.

1.M.4 Generalizability of Study Results to Generally Healthy Older Adults

Eligibility criteria in this trial were not intended to be so rigorous as to impair generalizability to the participant population for whom the intervention may be applied. Specifically, generally healthy older adults diagnosed with hypertension, taking stable centrally-active ACE inhibition, in a primary care setting. A systematic review of 283 randomized controlled trials showed that drug intervention trials apply 35% more exclusion criteria versus non-drug intervention trials ($p=0.003$), and more often exclude based on medication and comorbidity that may be poorly justified (Van Spall, Toren, Kiss, & Fowler, 2007).

Inclusion criteria in this thesis RCT capture a sample of older hypertensive patients that could represent 43% of the Canadian population ages 60-85 years (Robitaille et al., 2012). Older adults, from 60 years of age, are at 9% risk of dementia (if <75 years, and 15-20% if 75-79 years) and it is estimated that close to 1 in 3 older adults may suffer with executive dysfunction based on the San Luis Valley Health and Aging Study of 1313 individuals over 60 years old (Fitzpatrick et al., 2004; Jim Grigsby et al., 2002). Executive dysfunction was assessed in study by Fitzpatrick et al. (2004) as voluntary goal-oriented behaviour and self-regulation, and consequently may have significant impairment following medical advice and are more likely to develop disability (Deborah A. Cahn-Weiner, Paul F. Malloy, Patricia A. Boyle, Mary Marran, & Stephen Salloway, 2000; Jim Grigsby et al., 2002; Donald R. Royall, Raymond Palmer, Laura K. Chiodo, & Marsha J. Polk, 2004). Adults over 65 years of age have been shown to experience similar 31% executive function impairment (Donald R. Royall, David V. Espino, Marsha J. Polk, Raymond F. Palmer, & Kyriakos S. Markides, 2004). Two large randomized controlled trials investigating cognitive

impairment and high blood pressure, SYST-EUR and PROGRESS, also preferentially selected adults at least 60 years (Forette et al., 1998; Tzourio et al., 2003). Sixty years of age may also be a hemodynamic transition point, where pulse pressure (systolic blood pressure – diastolic blood pressure) and central pulse wave velocity typically begin to increase, potentially elevating risk of cognitive decline (Hanon et al., 2005; Kearney-Schwartz et al., 2009; Waldstein et al., 2008).

Diagnosed hypertensives with controlled blood pressure (systolic pressure ≤ 140 mmHg and/or diastolic pressure ≤ 90 mmHg) taking standard-care antihypertensive medications is representative of the small matched group prospective study suggesting aldosterone antagonism improved cognition in elderly hypertensives (MMSE 23.7 increased to 25.4, $P < 0.05$) (Yagi et al., 2011). A large longitudinal trial by the ARIC Investigators showed an elevated risk of cognitive decline (attention and memory) over 6 years in 4,800 patients diagnosed with hypertension and over 57 years old (Knopman et al., 2001). Another longitudinal trial of 1373 elderly (59-71 years) over 4 years showed an elevated risk of severe cognitive decline (4 or more points on MMSE) irrespective of antihypertensive medication use in those with high blood pressure (SBP >160 mmHg or DBP >95 mmHg) (Tzourio, Dufouil, Ducimetiere, & Alperovitch, 1999). In summary, diagnosis of hypertension elevates risk of cognitive decline.

Exclusion criteria in this thesis RCT mainly reflect therapeutic contraindications and warnings. Strategies for reducing bias from confounding variables such as age and cardiovascular disease are through study design: randomization, double-blinding, and placebo-controlled.

1.M.5 End-Tidal Carbon Dioxide during Cerebrovascular Assessments in Older Adults

This part of APPENDIX 1.M provides supportive data on end-tidal carbon dioxide ($ETCO_2$) not included in Results section 5.3 so as to not distract the reader from objectives of CHAPTER 5. In these older adults of the RCT, $ETCO_2$ during the assessment of anterior cerebral blood flow (aCBF, Table 17) at baseline and after six months of study-drug were similar between the groups. Baseline $ETCO_2$ during hypocapnia (reduced CO_2 in the blood reflected in expired partial pressure of gas) and during hypercapnia (elevated CO_2 in the blood reflected in expired partial pressure of gas) were similar between the two groups (Table 17) and remained similar after six months of study-drug. At baseline, the hypocapnic stimulus (i.e., controlled mild hyperventilation) lead to a 61±13% decrease in $ETCO_2$ for the Spironolactone group and a 57±13% decrease in $ETCO_2$ for the Placebo group (P=0.67, Figure 33). There was a 30±13% increase in $ETCO_2$ for the Spironolactone group and a 23±14% increase in $ETCO_2$ for Placebo group with the hypercapnic (i.e., maximal voluntary breath-hold) stimulus (P=0.38, Figure 33).

The Spironolactone group normocapnia-R $ETCO_2$ at 41±2 mmHg (Table 17) before hyperventilation (hypocapnic stimulus) was significantly greater than $ETCO_2$ normocapnia-H before breath-hold (hypercapnic stimulus) at 34±7 mmHg ($P<0.05$). The Placebo group $ETCO_2$ at 37±4 mmHg during normocapnia-R before hyperventilation (hypocapnic stimulus) was significantly greater during normocapnia-H before breath-hold (hypercapnic stimulus) at 35±4 ($P<0.05$).

Table 17. End-tidal carbon dioxide ($ETCO_2$) during measurement of cerebral hemodynamics at baseline and changes after 6 months of Study-Drug

Measure	Spironolactone (n=9) mean ±SD[€]	Placebo (n=9) mean ±SD	P-value[a]
$ETCO_2$ Baseline			
During assessment of aCBF supine (mmHg)	42±3	40±4	0.20
Normocapnia-R before hyperventilation (mmHg)	41±2	37±4	<0.05
Hypocapnia (mmHg)	16±5	16±5	0.93
Change from normocapnia-R (%)	-61±13	-57±13	0.67
Normocapnia-H before breath-hold (mmHg)	34±7*	35±4*	0.84
Hypercapnia (mmHg)	44±8	43±5	0.76
Change from normocapnia-H (%)	30±13	23±14	0.38
$ETCO_2$ change from Baseline			
During assessment of aCBF supine (mmHg)	1±1	3±1	0.30
Normocapnia-R before hyperventilation (mmHg)	1±3	5±2	<0.05
Hypocapnia (mmHg)	-2±3	-1±5	0.44
Change from normocapnia-R (%)	-5±7	-6±6	0.85
Normocapnia-H before breath-hold (mmHg)	3±0	5±2	0.53
Hypercapnia (mmHg)	3±1	6±1	0.28
Change from normocapnia-H (%)	-4±6	3±3	0.44

[a]From unpaired t-test, *P<0.05 Normocapnia-H before breath-hold vs Normocapnia-R before hyperventilation supine, Spiro=spironolactone group, Placebo=placebo group, aCBF=anterior Cerebral Blood Flow, $ETCO_2$=end-tidal carbon dioxide, [€] n=8 for hypocapnia in spironolactone group.

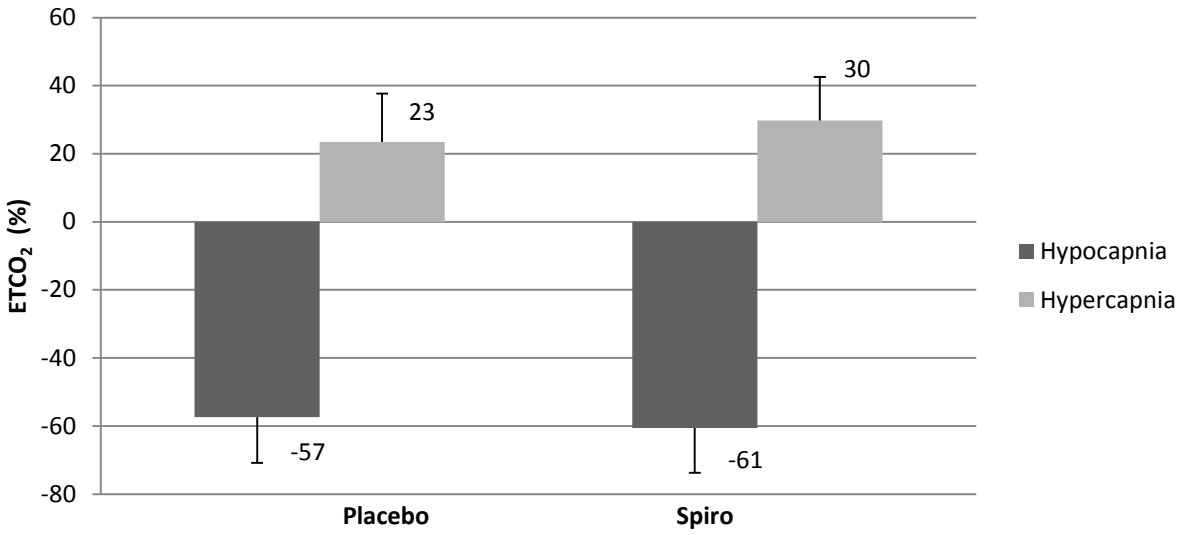


Figure 33. Baseline changes in end-tidal carbon dioxide ($ETCO_2$) during hypocapnia and hypercapnia for placebo (Placebo) and spironolactone (Spiro) groups in the RCT

End-tidal carbon dioxide ($ETCO_2$) during hypocapnia (%, dark bars) and hypercapnia (%, light bars) are shown for placebo (Placebo, left pair of bars) and spironolactone (Spiro, right pair of bars) at baseline. Unpaired t-tests were performed to establish no significant differences in % change $ETCO_2$ between placebo and spironolactone during either hypocapnia (P=0.67) or hypercapnia (P=0.38). Data are means with error bars as standard deviation.

APPENDIX 2.IE Inclusion and Exclusion Criteria for Studies

Table 18. Inclusion and exclusion criteria for younger adults in experimental study

Inclusion
18-35 years of age
Provide a signed Consent Form
Exclusion
History of fainting
Respiratory illness
Resting heart rate >110 beats/minute
Confirmed essential hypertension diagnosis according to Hypertension Canada guidelines (≥ 140mmHg/≥ 90mmHg)
Diabetes
Peripheral Vascular Disease
Reynaud's Syndrome
Pregnancy

Table. 19 Inclusion criteria for older adults in RCT

≥ 60 years of age (maximum 75 years)
Confirmed essential hypertension diagnosis according to Hypertension Canada guidelines (≥ 140mmHg/≥ 90mmHg)
On stable maintenance therapy of a centrally acting ACE inhibitor for at least 6 months (including perindopril, ramipril, captopril, fosinopril, lisinopril, and trandolapril)
Provide a signed Consent Form

Table 20. Exclusion criteria for older adults

Serum potassium ≥ 5.0 mmol/L
Taking potassium or salt supplements
History of hyperkalemia
Hepatic impairment (contraindicated in Child-Pugh Class C)
Diagnosis of congestive heart failure
Renal dysfunction (contraindicated in moderate to severe renal impairment): eGFR < 60 mL/min/1.73m^2 using MDRD equation (eGFR = 186 x (serum Creatinine, mg/dL)$^{-1.154}$ x (age)$^{-0.203}$ [x 0.742 female] x [1.21 Black])
Use of aldosterone antagonist in last 6 months
Diagnosis of diabetes with microalbuminuria
Taking insulin and/or oral hypoglycemic agents, angiotensin receptor blockers, direct renin inhibitors
Taking strong CYP3A4 inhibitors (ketoconazole, itraconazole, nefazodone, telithromycin, clarithromycin, ritonavir, and nelfinavir), lithium, or St John's Wort
Systolic blood pressure ≤ 100 mmHg and diastolic blood pressure ≤ 60 mmHg
Montreal Cognitive Assessment score < 20
Pregnancy

APPENDIX 3.HIF Health History Forms

HEALTH HISTORY FORM (RCT)

The Effect of Spironolactone on Vascular Dynamics and Cognitive Function

Researchers and Contact Information:

Richard Hughson, PhD	phone: 519-888-4567 ext 32516	email: hughson@uwaterloo.ca
Jason Xeni, MSc	phone: 519-591-1113	email: jxeni@uwaterloo.ca
Katelyn Fraser, MSc	phone: 519-571-4981	email: k4fraser@uwaterloo.ca
Danielle Greaves, MSc	phone: 519-888-4567 ext 32127	email: dgreaves@uwaterloo.ca

Name :_____ [Code:]
Phone Number:_____
Email:_____
Family Doctor:_____

1. **Date of Birth** (day/ month/year):_____
2. **Sex** (male or female):____ _____
3. Reason(s) why the participant **can't follow instructions** and complete this study:
   (Yes or No) _____ . If yes, explain why.
4. Years of formal **education** (from grade 1 to now):
   _____ years of formal education.
5. **Diplomas, certificates or degrees**:
   (check all that apply, twice if two of the same level)

□ None
□ High School
□ Trade certificate/diploma
□ Community College diploma
□ University undergraduate degree
□ University Graduate degree

6. Check all the boxes apply (mark every box participant **diagnosed** with):

□ Chest Pain (Heart Attack or Angina) □ Hypertension

□ Atrial Fibrillation □ Stroke or TIA

□ Mild Cognitive Impairment □ Depression

7. **Medications** (including supplements) taken within the last 6 months:

Medication Name	**Dose (mg or g)**	**Route (mouth, on skin, inhaled, or injected)**	**Frequency (1,2, or 3 times daily)**

a. Frequency participant **forgets** to take these above medications:

Never Rarely Sometimes Often Always

b. Reason(s) why the participant may **not** take a medication:

□ No □ Yes If yes, explain.

8. Current **smoking status** (cigarettes, a pipe, or cigars):

□ Yes □ No

UNIVERSITY OF WATERLOO

Health History Form Experimental Study

Changes in Arterial Stiffness Indicators

Researchers and Contact Information: Date:
Richard Hughson, PhD phone: 519-888-4567 ext 32516 email: hughson@uwaterloo.ca
Jason Xeni, MSc phone: 519-591-1113 email: jxeni@uwaterloo.ca
Katelyn Fraser, MSc phone: 519-571-4981 email: k4fraser@uwaterloo.ca
Danielle Greaves, MSc phone: 519-888-4567 ext 32127 email: dgreaves@uwaterloo.ca
Kinesiology, Applied Health Sciences, University of Waterloo, Waterloo ON N2L 3G1

SELF-REPORT CHECK LIST Participant ID: _____

Exclusion Criteria: If any of the following apply, you should not participate in this study

- Diabetes
- Resting heart rate > 110bpm
- High blood pressure (≥ 140/90)
- Peripheral vascular disease
- -Respiratory illness
- -Raynaud's syndrome
- -History of fainting
- -Pregnancy

One or more of these applies: **Yes** or **No**

Do you have any allergies or sensitivities to water based gels or adhesives? Yes or No

Current Health (within the past 3 months)

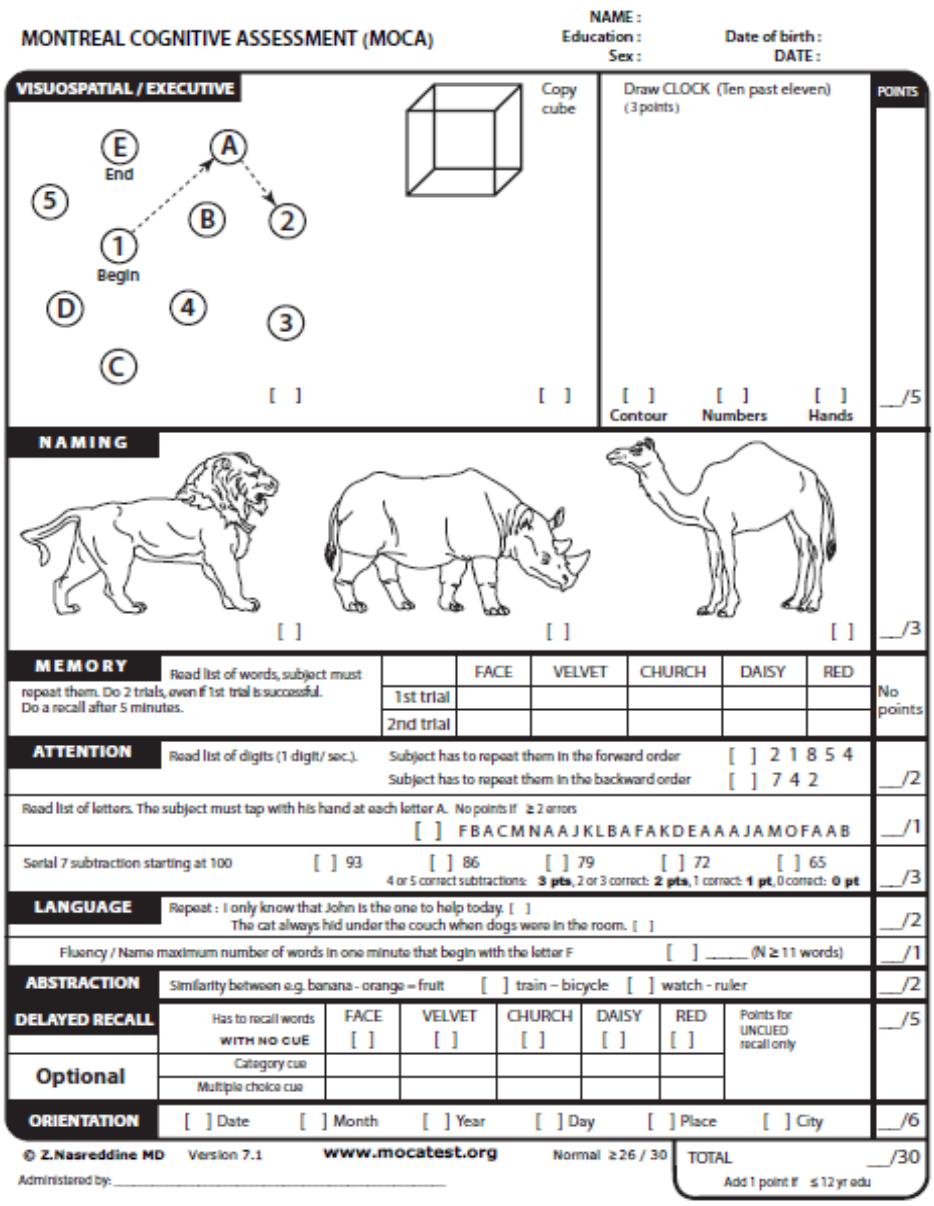
List current health issues:

1. 5.
2. 6.
3. 7.
4. 8.

List current medications (including supplements and vitamins):

1. 5.
2. 6.
3. 7.
4. 8.

APPENDIX 4.ECF Montreal Cognitive Assessment (MoCA) Form



MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS

Copy cube

Draw CLOCK (Ten past eleven) (3 points)

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ]

[ ] Contour [ ] Numbers [ ] Hands

__/5

NAMING

[ ] [ ] [ ]

__/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

__/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

__/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

__/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

__/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]
Optional	Category cue					
	Multiple choice cue					

Points for UNCUED recall only

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

© Z.Nasreddine MD Version 7.1 www.mocatest.org Normal ≥ 26 / 30

TOTAL __/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

APPENDIX 5.T Trail Making Test Parts A & B

Trail Making Test (TMT) Parts A & B

Instructions:

Both parts of the Trail Making Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the patient should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Time the patient as he or she connects the "trail." If the patient makes an error, point it out immediately and allow the patient to correct it. Errors affect the patient's score only in that the correction of errors is included in the completion time for the task. It is unnecessary to continue the test if the patient has not completed both parts after five minutes have elapsed.

Step 1: Give the patient a copy of the Trail Making Test Part A worksheet and a pen or pencil.
Step 2: Demonstrate the test to the patient using the sample sheet (Trail Making Part A – *SAMPLE*).
Step 3: Time the patient as he or she follows the "trail" made by the numbers on the test.
Step 4: Record the time.
Step 5: Repeat the procedure for Trail Making Test Part B.

Scoring:

Results for both TMT A and B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment.

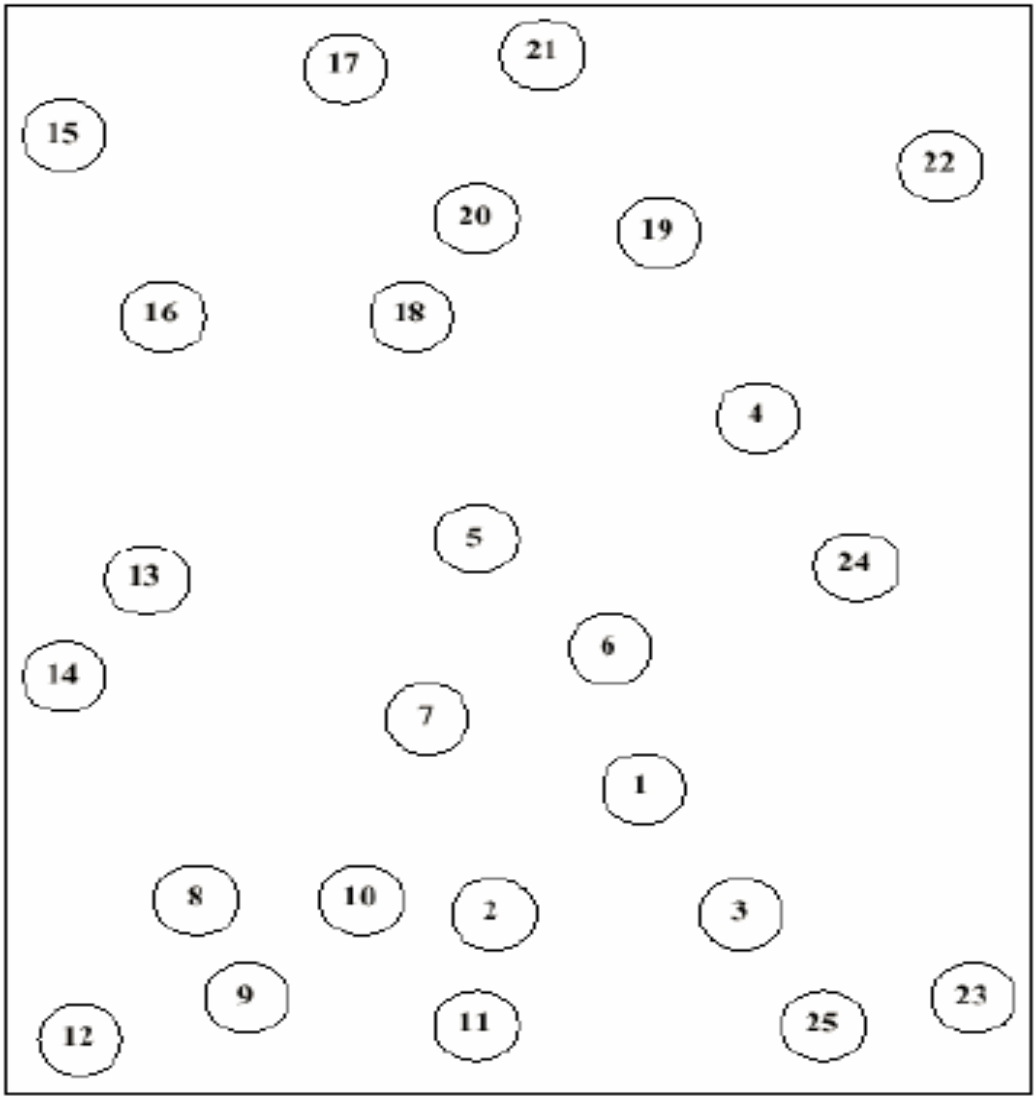
	Average	Deficient	Rule of Thumb
Trail A	29 seconds	> 78 seconds	Most in 90 seconds
Trail B	75 seconds	> 273 seconds	Most in 3 minutes

Sources:

- Corrigan JD, Hinkeldey MS. Relationships between parts A and B of the Trail Making Test. *J Clin Psychol*. 1987;43(4):402–409.
- Gaudino EA, Geisler MW, Squires NK. Construct validity in the Trail Making Test: what makes Part B harder? *J Clin Exp Neuropsychol*. 1995;17(4):529-535.
- Lezak MD, Howieson DB, Loring DW. *Neuropsychological Assessment*. 4th ed. New York: Oxford University Press; 2004.
- Reitan RM. Validity of the Trail Making test as an indicator of organic brain damage. *Percept Mot Skills*. 1958;8:271-276.

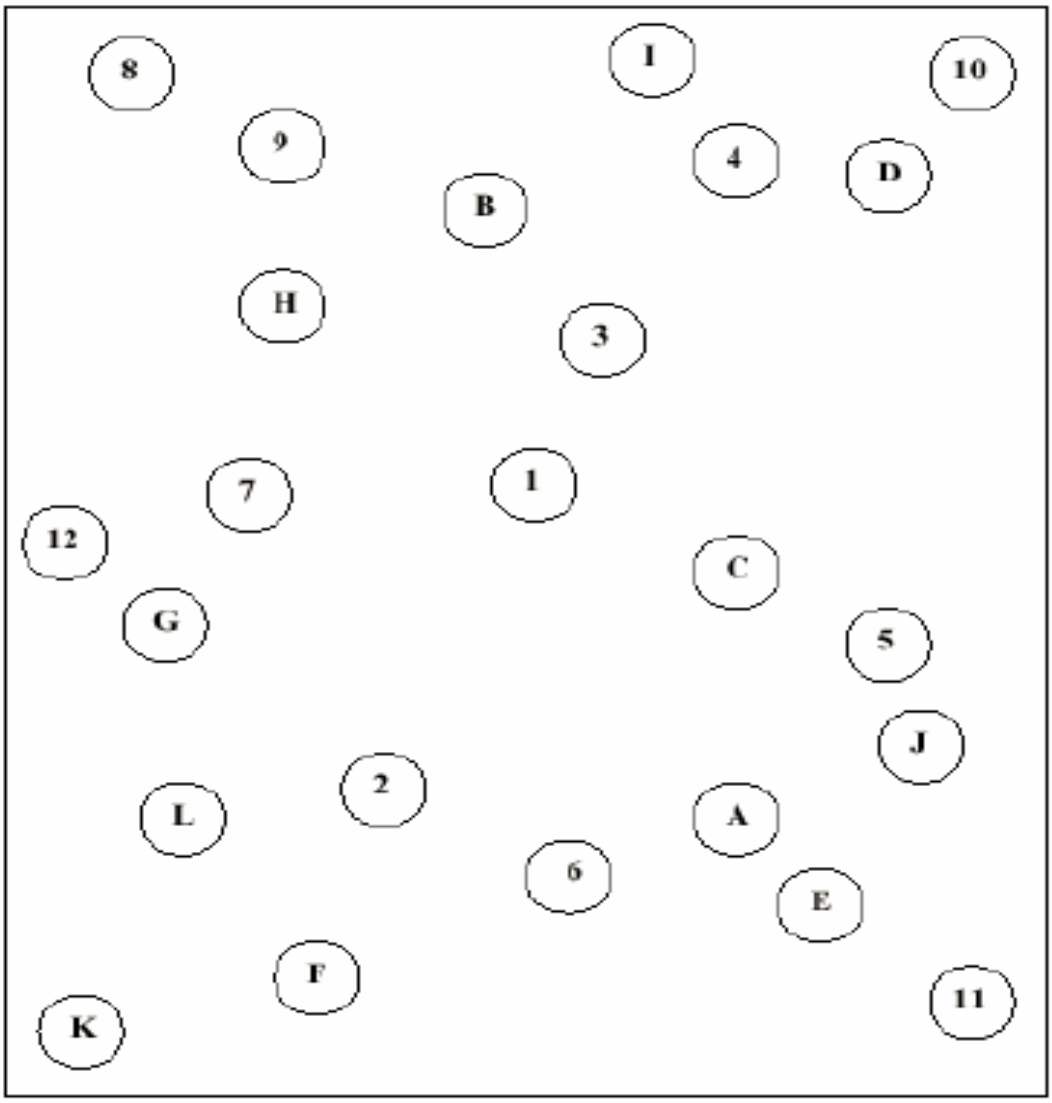
Trail Making Test Part A

Patient's Name: _____ Date: _____



Trail Making Test Part B

Patient's Name: _____ Date: _____



APPENDIX 6.DS Digit Span Tests Forwards and Backwards

Digit Span

Following Numbers

I am going to say some numbers. Listen carefully, and when I'm finished you repeat them back to me in exactly the same order as I say them.

Item	Trial 1	Trial 2
1	5 - 8 - 2	6 - 9 - 4
2	6 - 4 - 3 - 9	7 - 2 - 8 - 6
3	4 - 2 - 7 - 3 - 1	7 - 5 - 8 - 3 - 6
4	6 - 1 - 9 - 4 - 7 - 3	6 - 9 - 5 - 4 - 8 - 7
5	5 - 9 - 1 - 7 - 4 - 2 - 8	4 - 1 - 7 - 9 - 3 - 8 - 6
6	5 - 8 - 1 - 9 - 2 - 6 - 4 - 7	3 - 8 - 2 - 9 - 5 - 1 - 7 - 4
7	2 - 7 - 5 - 8 - 6 - 2 - 5 - 8 - 4	7 - 1 - 3 - 9 - 4 - 2 - 5 - 6 - 8

Numbers Backwards

Now, I am going to say other numbers, but this time when I stop, you give them to me backwards. For example, if I say 7-1-9 you say (pause) 9-1-7. (If the patient doesn't understand, give another example).

Item	Trial 1	Trial 2
1	2 - 4	5 - 8
2	6 - 2 - 9	4 - 1 - 5
3	3 - 2 - 7 - 9	4 - 9 - 6 - 8
4	1 - 5 - 2 - 8 - 6	6 - 1 - 8 - 4 - 3
5	5 - 3 - 9 - 4 - 1 - 8	7 - 2 - 4 - 8 - 5 - 6
6	8 - 1 - 2 - 9 - 3 - 6 - 5	4 - 7 - 3 - 9 - 1 - 2 - 8
7	9 - 4 - 3 - 7 - 6 - 2 - 5 - 8	7 - 2 - 8 - 1 - 9 - 6 - 5 - 3

*Give both trials of a series. Discontinue after failing two trials of the same series.