Cerebrovascular hemodynamics in older adults: Associations with lifestyle, peripheral vascular health and functional decline

by

Andrew Donald Robertson

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AUTHOR'S DECLARATION

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

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

ABSTRACT

In today's aging population, cerebrovascular health plays a pivotal role in maintaining independence. The identification of early markers of change might help to plan more appropriate preventative and/or therapeutic measures. Recent focus has been placed on the relationship between peripheral vascular characteristics and cerebral hemodynamics. Given the compliant nature of the cerebral circulation, examination of passive properties, including critical closing pressure (CrCP) and resistance area product (RAP), might provide sensitive information about early functional changes. The purpose of this thesis was to provide a comprehensive view of peripheral vascular and cerebrovascular regulation in community-living older adults. In doing so, the thesis covered a spectrum, ranging from an examination of lifestyle factors, including habitual physical activity and sleep quality, to the impact of cerebrovascular health on functional status, characterized by gait speed. Key findings included the observation that while participants showed the ability to regulate cerebral blood flow (CBF) appropriately in most circumstances, the underlying mechanisms used to achieve this regulation was dependent on baseline vascular tone. During sit-to-stand transitions, individuals with lower baseline resistance relied primarily on fluctuations in RAP, which have been suggested to more closely reflect myogenic pathways. In contrast, individuals with elevated resistance had lower baseline CBF and relied relatively more on fluctuations in CrCP during the dynamic transition. The greater reliance on CrCP might indicate that these individuals were required to tap further into reserve pools to avoid hypoperfusion during the transition. Notably, those who exhibited a smaller dynamic RAP response during the posture change also had slower gait speed and higher occurrence of falls over the past year. These results provide evidence that passive cerebrovascular dynamics are sensitive markers linking peripheral and cerebrovascular properties with functional consequences for brain health in the elderly.

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ABP	Arterial blood pressure
AEE	Active energy expenditure
BP_{MCA}	Mean arterial blood pressure at the level of the middle cerebral artery
CA	Cerebral autoregulation (prefix: d – dynamic, s – static)
CBF	Cerebral blood flow
CCA	Common carotid artery
CO	Cardiac output
CO_2	Carbon dioxide
cPP	Pulse pressure (common carotid artery)
CR_{CO2}	Cerebrovascular reactivity to carbon dioxide
CrCP	Critical closing pressure
CRP	C-reactive protein
CVR	Cerebrovascular resistance (MAP/Flow)
CVRi	Cerebrovascular resistance index (MAP/Flow velocity)
DBP	Diastolic blood pressure
ECA	External carotid artery
GDS	Geriatric Depression Scale
ICA	Internal carotid artery
IMT	Intima-media thickness
MAP	Mean arterial blood pressure
MBF	Mean blood flow
MCA	Middle cerebral artery
MFV	Mean flow velocity
MoCA	Montreal Cognitive Assessment
PAEE	Physical activity-related energy expenditure
PCO_2	Partial pressure of carbon dioxide (subscripts: ET – end-tidal, a – arterial)
PI	Pulsatility index
PWV	Pulse wave velocity (prefix: cf – carotid-femoral, ba – brachial ankle)
RAP	Resistance area product
SBP	Systolic blood pressure
TCD	Transcranial Doppler ultrasound
VA	Vertebral artery

CHAPTER 1. CEREBROVASCULAR HEALTH IN AN AGING POPULATION

Western society is in the midst of an aging boom. Over the last 50 years, Canada's population of adults over 65 years of age has increased from 8 % to 14 % of the general population. Even under moderate growth predictions, this *silver tsunami* is expected to push towards 24 % by 2036, led by a 2.6-fold increase in the population over 80 years of age to 3.3 million (Statistics Canada, 2010). Cerebrovascular impairment is a leading cause of disability in older adults. Seven percent of Canadians over 74 years of age live with the effects of an overt stroke (Public Health Agency of Canada, 2009), and many more live with covert impairment. Population-based studies suggest that silent ischemia, identified by growing magnetic resonance imaging accessibility, has five times the prevalence of stroke (Bryan *et al.*, 1997; Vermeer *et al.*, 2003; Das *et al.*, 2008).

These aging projections forecast that the population with cerebrovascular dysfunction will continue to rise, and the impact of their impairment will be felt throughout the circles in which they keep. Stroke survivors lead more dependent lives than the general aging population with 84% reporting restrictions from their normal activities (Public Health Agency of Canada, 2009) and, while 'silent' covert episodes do not have the same devastating effects, they do contribute to a steady deterioration of executive function and quality of life (Swartz *et al.*, 2008). Physiologically, low cerebral blood flow (CBF) has been shown to alter critical protein synthesis pathways involved in the cortical plasticity necessary for learning and memory (Mies *et al.*, 1991). Given the impact of ischemia and the accumulative effect of even subtle hypoperfusion, more clarity surrounding the underlying factors associated with low CBF and impaired cerebrovascular regulation, as well as a greater understanding of the early functional consequences of such impairment, is needed.

Consistently, CBF has been shown to decrease with increasing age (Scheel *et al.*, 2000b; Bakker *et al.*, 2004; Albayrak *et al.*, 2007; Ainslie *et al.*, 2008a; Chen *et al.*, 2011). However, a wide range of CBF is commonly reported in "healthy" older adults, from ~ 350 to 850 mL/min, suggesting particular individuals might be at a greater risk for periodic hypoperfusion (Scheel *et al.*, 2000a; Dorfler *et al.*, 2000; Albayrak *et al.*, 2007; Henriksen *et al.*, 2012). The lower end of this range of flows is similar to those noted in clinical cohorts of dementia, cerebral atrophy and stroke (Scheel *et al.*, 1999; Albayrak *et al.*, 2006; Han *et al.*, 2007). A greater understanding of how CBF is regulated in older adults might provide insight to the influence of vascular risk in individuals with cognitive impairment, but no dementia (Di Carlo *et al.*, 2000).

Further, a recent focus on the role of peripheral vascular health has found relationships between changes in arterial function, cerebrovascular resistance, and brain health. Faster pulse wave velocity (PWV), a marker of increased arterial stiffness, is related to increased pulsatility (Webb *et al.*, 2012) and resistance (Robertson *et al.*, 2010) in the cerebral vasculature. In addition, increases in stiffness and atherosclerotic progression are associated with increased white matter lesions and infarctions (Henskens *et al.*, 2008; Kearney-Schwartz *et al.*, 2009; Kim *et al.*, 2011a), as well as deficits in various cognitive traits, concentrating in the frontal lobes (Gorelick *et al.*, 2011; Mitchell *et al.*, 2011; Watson *et al.*, 2011). The cerebral circulation is a highly compliant, high-flow system that might be particularly susceptible to changes in hemodynamics associated with peripheral vascular aging. This thesis has provided a comprehensive examination of CBF regulation in a cohort of community-living older adults. The chapters, herein, have examined how lifestyle characteristics, including habitual physical activity and sleep quality, and peripheral vascular function, including arterial stiffness and

intima-media thickness, are associated with CBF and cerebrovascular regulation. Specifically, cerebrovascular reactivity to carbon dioxide (CR_{CO2}) and cerebral autoregulation (CA) during posture change have been addressed. In addition, the impact of these changes on functional status, with particular attention to gait speed and falls, was considered. The following literature review examined aspects of CBF regulation, with insight into the influences of aging and vascular risk. This review helped to provide the rationale for the specific objectives of this thesis, which were outlined at the end of this chapter.

Literature Review

The brain is arguably the most metabolically active organ in the human body. Relative to other organs, however, the brain has little energy storage (Peters *et al.*, 2004) and thus relies on a tight coupling of CBF to cerebral metabolic rate of oxygen consumption, which ensures an adequate supply of oxygen and glucose (Hoge *et al.*, 1999). The importance of constant perfusion is evident from the observation that, although the brain weighs only 2-3 % of the body's total mass, CBF accounts for approximately 20 % of resting cardiac output (CO) and oxygen uptake (Mchedlishvili, 1986). Insufficient blood supply leads to energy imbalance and depletion of adenosine triphosphate, followed closely by the inactivation of ion pumps, depolarization of neuronal membranes, accumulation of intracellular calcium, and cellular dysfunction (for review, see Allen & Bayraktutan, 2009). Consequently, the brain relies on a complex network of vessels and regulatory mechanisms to maintain appropriate perfusion and avoid the deleterious effects of even subtle levels of ischemia.

Anatomy of Cerebral Arteries

The macrovascular supply of CBF begins in the four main arteries transporting blood toward the cranial vault. The bilateral internal carotid arteries (ICA) are responsible for ~76 % of total

CBF (Schoning *et al.*, 1994). After passing through the carotid canal, the ICA branches into the middle and anterior cerebral arteries (MCA and ACA, respectively) to form the rostral loop of the Circle of Willis at the base of the brain. The bilateral vertebral arteries (VA), carrying the remaining 24 %, converge to form the basilar artery, before splitting into bilateral posterior cerebral arteries (PCA), comprising the caudal loop of the Circle of Willis. Communicating branches between these basal arteries, as well as cerebral vessels at more distal sites, provide the brain with a robust collateral blood supply (Edvinsson & Mackenzie, 2002). Key cerebral regions relying on these arteries include the frontal cortex, globus pallidus and amygdala (ACA); the temporal and parietal cortices, and subcortical nuclei (MCA); and, the occipital cortex, cerebellum and diencephalon, including the hypothalamus where important cardiovascular regulatory nuclei are located (PCA) (Edvinsson & Mackenzie, 2002).

Moving distally from the Circle of Willis, cerebral arteries cover the pial surface of the cerebral cortex before penetrating the parenchymal tissue and branching extensively into the microcirculation (Edvinsson & Mackenzie, 2002). This thesis focused on the characteristics of the cerebral macrovasculature, with particular attention to the ICA, VA and MCA. Although tissue perfusion is highly-dependent on the function of smaller arterioles and capillary networks, the characteristics of the hemodynamics through larger basal arteries can provide important insight into CBF regulation.

Local Regulation of Cerebral Blood Flow

In addition to the collateral anatomy mentioned above, tight regulation of CBF is achieved through a complex interaction of functional mechanisms. According to the hydraulic analogy of Ohm's law, pressure gradient and vascular resistance are two inversely-related physiological factors affecting cerebral perfusion, (Equation 1-1).

CBF = (arterial blood pressure – intracranial pressure) / resistance

Equation 1-1. Ohm's law analogy of cerebral blood flow regulation.

An inherent caveat when describing flow using this linear relationship is that flow is assumed to be both constant and laminar. Although flow is pulsatile and, at times, turbulent in the macrovasculature, this linear relationship is regularly interpreted as a generalized model to describe the circulatory system (Rowell, 1993). This assumption was held throughout this thesis. The physiological factors identified in Equation 1-1 can be appropriated into local [intracranial pressure (ICP) and resistance (CVR)] and systemic [arterial blood pressure (ABP)] factors.

In the absence of overt cerebrovascular disease or injury, ICP is assumed to be relatively constant and close to zero (Aaslid *et al.*, 1989). Although posture change might induce differential hydrostatic responses in ICP and ABP (Rosner & Coley, 1986), the magnitude of the drop in ABP is considered dominant to any fluctuation in ICP (Hughson *et al.*, 2001). Given these considerations, physiological studies in healthy subjects often assume limited involvement of ICP (Aaslid *et al.*, 1989; Edwards *et al.*, 2004; Zhang *et al.*, 2004). Accordingly, most models submit local control of perfusion to changes in vascular resistance. Poiseuille's law (Equation 1-2) states that resistance is a function of vessel length, blood viscosity, and vessel radius.

Vascular resistance = $8\eta \cdot L/\pi r^4$; where η = viscosity, L = vessel length and r = vessel radius

Equation 1-2. Contributors to pure resistance of flow.

The exponential influence of vessel radius indicates that change in arteriolar diameter is the primary local mechanism regulating flow. Control over vascular diameter results from the sum

effect of a complex set of pathways, of myogenic, metabolic, and neurological origin (Paulson *et al.*, 1990). This section of the review provides a brief introduction to these underlying mechanisms regulating CBF.

Myogenic Regulation

Pure myogenic regulation speaks to the apparent reflexive response of arteries to changes in transmural pressure (Bayliss, 1902). This regulation is primarily attributed to intrinsic properties of the vascular smooth muscle that adjusts the concentration of intracellular calcium through the opening or closing of voltage-gated calcium channels (Harder *et al.*, 2011). Evidence of this mechanism comes from isolated human pial resistance arteries, where the systematic increase in vascular tone mediated by increases in transmural pressure was dependent on calcium being freely available in the physiological bath (Wallis *et al.*, 1996). Confirmed removal of the endothelium did not alter the response suggesting that this observation was specific to the smooth muscle cells (Wallis *et al.*, 1996), and not the endothelial lining.

The endothelium-independent nature of control is consistent with *in vivo* findings from studies in humans (Lavi *et al.*, 2003; Zhang *et al.*, 2004; Lavi *et al.*, 2006). Nitric oxide (˙NO) is a major contributor to endothelial-mediated vascular regulation (Ignarro *et al.*, 1987; Palmer *et al.*, 1987), so the influence of the endothelium can be tested by altering its bioavailability. Neither sodium nitroprusside, which supplements ˙NO, nor N(G)-monomethyl-L-arginine (L-NMMA), which blocks ˙NO production, altered the mean CBF velocity (MFV) response to a hypertensive-stimulus, suggesting that endothelial factors are not involved in pressure-flow autoregulation (Lavi *et al.*, 2003; Zhang *et al.*, 2004). Further, in patients with hypertension or diabetes mellitus, two conditions associated with endothelial dysfunction, the cerebrovascular

response to changes in pressure induced by phenylephrine infusion were similar to healthy controls (Lavi *et al.*, 2006). In contrast, White *et al.* (2000) reported that L-NMMA negatively influenced the MFV response to a hypotensive stimulus supporting at least a partial role for endothelial-mediated processes in autoregulation.

Vasoconstriction in response to increased intraluminal pressure has been shown in isolated human cerebral arteries taken from the pial surface of the brain (Wallis *et al.*, 1996); however, *in vivo* monitoring during hyper- and hypotensive challenges found little or no change in MCA diameter (Giller *et al.*, 1993; Serrador *et al.*, 2000). This suggests the myogenic mechanism is functional primarily in smaller resistance arteries and/or arterioles distal to the MCA. This is an important observation in consideration of the validity of transcranial Doppler ultrasound (TCD) monitoring of CBF (see Chapter 2).

Metabolic Regulation

The metabolic regulatory mechanism refers to the local coupling between neuronal activity and cerebral perfusion (reviewed by Girouard & Iadecola, 2006). Briefly, action potentials associated with neuronal activity alter extracellular potassium (K^+) concentration which can directly or indirectly influence vascular tone through alteration of endothelial and vascular smooth muscle membrane potentials or enzyme-mediated pathways (Filosa *et al.*, 2006). In parallel, neurotransmitters (*e.g.*, glutamate) act through neighbouring interneurons and astrocytes to modulate the release of vasoactive epoxyeicosatrienoic acids (EETs) (Lecrux *et al.*, 2012). Glutamate-stimulated $^{\bullet}$NO and prostaglandin release are believed to play supportive roles in the process (Girouard & Iadecola, 2006). In addition to these byproducts of synaptic activity, metabolites from energetic processes, including lactate and adenosine, can directly influence vascular smooth muscle tone (Liu *et al.*, 2012). Although transient reductions of

oxygen (O_2) and elevations of carbon dioxide (CO_2) occur locally with increased neuronal activity (Li *et al.*, 2011), these changes are of small amplitude and are quickly reversed. Thus, they are not likely to play a significant role in metabolic coupling under normal conditions (Iadecola, 2004). The strong influence of O_2 and CO_2 on cerebral circulation is much more evident under modulation of systemic arterial blood gases (CO_2 reactivity is discussed below).

Neurological Regulation

In contrast to both myogenic and metabolic regulation, neurological influences on CBF are likely restricted to the larger cerebral arteries. While terminal microvascular arterioles and capillaries lack direct innervation, larger arteries on the brain's surface are innervated by sympathetic, parasympathetic and sensory neurons (for review, see Faraci & Heistad, 1990; Farkas & Luiten, 2001). Sympathetic nerves originate in the superior cervical ganglia and reach towards the basal and pial arteries. These nerves directly innervate the vascular smooth muscle and act through the release of norepinephrine and neuropeptide Y (Farkas & Luiten, 2001). Recordings of sympathetic nerve activity from the superior ganglia of sleeping lambs showed increased activity with increases in ABP, suggesting they are involved in protecting the cerebral vasculature against high pressure (Loos *et al.*, 2005; Cassaglia *et al.*, 2009). Despite the apparent influence of sympathetic innervation, cerebral vessels appear to be predominantly unresponsive to circulating alpha-agonists (Kimmerly *et al.*, 2003), possibly due to the tightly-knit blood brain barrier.

The parasympathetic nervous system influences the cerebral vasculature, via the pterygopalatine ganglia (Agassandian *et al.*, 2003), through the release of acetylcholine, vasoactive intestinal polypeptide, substance-P, and calcitonin gene-related peptide (reviewed by Farkas & Luiten, 2001). In rodent models, stimulation of these nerves induced increases in

CBF (Agassandian *et al.*, 2003), and ganglion removal lowered CBF, independent of ABP (Boysen *et al.*, 2009), suggesting that the parasympathetic system is involved in regulating CBF through dilation.

The precise role for the autonomic nervous system is still disputed, which might in part be due to species differences (this topic was recently debated in the Journal of Applied Physiology's Point-Counterpoint Series: Strandgaard & Sigurdsson, 2008; Van Lieshout & Secher, 2008). In humans, a ganglionic blockade inhibiting both sympathetic and parasympathetic responses was associated with increased gain and reduced phase differences in the relationship between ABP and MFV from beat to beat (Zhang *et al.*, 2002). As well, spectral analysis has revealed impaired autoregulation, in particular at higher frequencies, following separate blockade of either the cholinergic (Hamner *et al.*, 2012) or sympathetic (Hamner *et al.*, 2010) pathways. The observation of a specific influence on higher frequency fluctuations (> 0.05 Hz or within 20 s) suggest that autonomic influences play a bigger role in dynamic, short term aspects of regulation (Hamner *et al.*, 2010). A hypothesis bringing together all sides could be that neurohumoral influences modulate the parameters of autoregulation, but fine tuning of CBF is ultimately controlled by downstream myogenic and metabolic influences (Zhang *et al.*, 2002; Mitsis *et al.*, 2009; Hamner *et al.*, 2010).

Measurement of Regulatory Mechanisms

The development of TCD has provided the ability to monitor CBF velocity through the basal arteries (ACA, MCA and PCA) with excellent temporal resolution (Aaslid *et al.*, 1982). Although regulatory systems are controlled by vessels which lie further downstream, indirect evidence of the contribution of these cerebrovascular mechanisms can be inferred by monitoring dynamic changes in the macrovasculature. Two commonly used methods to assess

cerebrovascular regulation include the systematic perturbation of ABP (Aaslid *et al.*, 1989; Giller *et al.*, 1993; Levine *et al.*, 1994; Lipsitz *et al.*, 2000; Hughson *et al.*, 2001; Panerai *et al.*, 2001) and arterial pressure of CO_2 (P_aCO_2; Kety & Schmidt, 1948; Lavi *et al.*, 2006; Carrera *et al.*, 2011; Mardimae *et al.*, 2012).

Cerebral Autoregulation

First introduced in a review over 50 years ago (Lassen, 1959), cerebral autoregulation (CA) describes mechanisms that limit the increase or decrease in CBF following directionally similar changes in ABP, and is functional between perfusion pressures of 60 and 150 mmHg (Paulson *et al.*, 1990; van Beek *et al.*, 2008). In doing so, these mechanisms help to protect the brain against mismatches in perfusion and metabolism in the face of oscillating pressure (Lassen, 1959). Outside of this range of pressures, CBF is more passive to the change in blood pressure. Although the myogenic mechanism is often referred to as the dominant mechanism of CA (Paulson *et al.*, 1990), *in vitro* (Harder *et al.*, 2011) and *in vivo* (White *et al.*, 2000) evidence exists supporting at least a supplementary role of surrounding tissues including the endothelium, neuronal glia and/or autonomic nervous system.

Autoregulation can be classified as static or dynamic, which is differentiated based on the labile characteristics of ABP. CBF responses to longer term changes in ABP, after the CBF and pressure changes have stabilized, is referred to as static autoregulation (sCA), whereas dynamic autoregulation (dCA) characterizes the rate of change in CBF during step or transient changes in ABP. The utility of TCD and continuous blood pressure monitoring (*e.g.*, radial tonometry, finger-cuff plethysmography) provides excellent temporal resolution with which to monitor these dynamic changes that are either spontaneous in nature (Zhang *et al.*, 2000) or induced. A variety of experimental methods have been demonstrated to alter ABP, including

posture change (Lipsitz *et al.*, 2000), head-up tilt (Hughson *et al.*, 2001), lower body negative pressure (Levine *et al.*, 1994), thigh cuff release (Aaslid *et al.*, 1989), and pharmacological intervention (Giller *et al.*, 1993), or a combination thereof.

Cerebrovascular Reactivity to Carbon Dioxide

The cerebral vasculature is unique relative to other vascular beds in its response to P_aCO_2. Lennox and Gibbs (1932) published a landmark observation demonstrating differences in circulatory control between the brain and the leg. Specifically, increasing P_aCO_2 resulted in a marked increase in cerebral perfusion, while little or no change was observed in leg blood flow, thus demonstrating cerebrovascular reactivity to CO_2 (CR_{CO2}) in humans. At the time, it was proposed that the hypercapnic-induced changes were related to an acid-base phenomenon, however, it wasn't until 45 years later that Kontos *et al.* (1977) empirically demonstrated that the effects were indeed related to changes in $[H^+]$, and not the result of any molecular interaction of CO_2 or bicarbonate ion.

Despite this clarification, the exact pathway through which P_aCO_2 (or pH) induces vasodilation remains controversial. The resultant effect appears to be achieved through the interaction of ˙NO- and prostaglandin-mediated pathways from both the endothelium and neighbouring interneurons/astrocytes, resulting in K^+-channel activation and modification of intracellular Ca^{2+} within the vascular smooth muscle cell (Brian, Jr., 1998). In the early 1990's, multiple groups independently demonstrated a relationship between P_aCO_2 and/or pH with ˙NO in animal models (Iadecola, 1992; Wang *et al.*, 1992; Niwa *et al.*, 1993; Pelligrino *et al.*, 1993; Bonvento *et al.*, 1994). Results from ˙NO synthase blockade studies involving adult humans have been less consistent (Schmetterer *et al.*, 1997; White *et al.*, 1998; Ide *et al.*, 2007). Only Schmetterer *et al.* (1997) reported attenuation of the hypercapnic response, as was

reported from animal models. These inconsistent results might be a consequence of redundant pathways. In a rodent model, blocking *both* ˙NO and prostaglandin pathways, with co-administration of L-N^G-nitroarginine methyl ester and indomethacin, resulted in complete attenuation of CR_{CO2} (Wang *et al.*, 1994).

The apparent co-involvement of prostaglandin and ˙NO in hypercapnic hyperemia has led to the supposition that CR_{CO2} is a marker of endothelial dysfunction specific to the cerebral circulation (Lavi *et al.*, 2006). Indeed, the hypercapnic response is lower in diabetic and essential hypertensive patients who display impaired flow-mediated dilation, an indicator of endothelial dysfunction typically measured in the brachial artery. The difference in the hypercapnic response between the patient group and a healthy control group was eliminated by infusion of sodium nitroprusside, a ˙NO donor, implying endothelium-independent mechanisms were intact (Lavi *et al.*, 2006). However, evidence has also suggested that CR_{CO2} is not solely an endothelium-mediated event. The relaxing property of P_aCO_2 on isolated helical strips of dog and monkey cerebral arteries was maintained when the endothelium was removed (Toda *et al.*, 1989; Toda *et al.*, 1993). Confusion surrounding the exact mechanism is likely a function of difficulties in separating vascular from neural tissues, differences between arterial and arteriolar segments, and species differences. *In vivo*, the bulk of evidence suggests the hypercapnic response is due to a combination of dilating effectors produced by endothelial cells, with possible contribution from neuronal and glial cells (Brian, Jr., 1998). Further, the confounding influences of blood pressure and sympathetic neural activity that parallel changes in PCO_2 complicate the overall interpretation. Elevated PCO_2 acts as both a hypertensive stimulus, which initiates autoregulatory mechanisms that might attenuate the hyperemic response (Garnham *et al.*, 1999; Hetzel *et al.*, 1999), and a modulator of the autoregulatory

response, through changes in baseline vascular tone (Edwards *et al.*, 2004). In addition, sympathetic inhibition (Jordan *et al.*, 2000) and excitation (Zhang *et al.*, 2011) have been shown to alter CR_{CO2}, confounding the ability to delineate endothelial mechanisms within and the observed hemodynamic response.

Regardless of the exact mechanism, CR_{CO2} has been identified as a valid marker of cerebral vasomotor reserve (Herold *et al.*, 1988). Further, impaired CR_{CO2} has been associated with cerebrovascular pathology, including subcortical and lacunar infarctions (Molina *et al.*, 1999; Cupini *et al.*, 2001), white matter lesions (Bakker *et al.*, 1999; Fu *et al.*, 2006; Liem *et al.*, 2009), incident and hereditary cerebral angiopathy (Terborg *et al.*, 2000; Pfefferkorn *et al.*, 2001); as well as functional cognitive deficits, including depression (Lemke *et al.*, 2010), and vascular and Alzheimer's dementia (Ruitenberg *et al.*, 2005; Silvestrini *et al.*, 2006; Vicenzini *et al.*, 2007). Thus, CR_{CO2} appears to be a reproducible (Mayberg *et al.*, 1996) and valid indicator of cerebrovascular health and reserve capacity (Herold *et al.*, 1988), partially describing the state of endothelial function within this vascular bed (Lavi *et al.*, 2006).

Modeling Regulatory Pathways

Just as important as the methods used to perturb the physiological pathways can be the model chosen to describe the cerebrovascular response. The simultaneous measurement of CBF velocity and ABP allows cerebrovascular regulation to be assessed with respect to indices of vascular resistance (Hughson *et al.*, 2001). This paradigm, which assessed resistance based on mean ABP (MAP) and MFV, relied on assumptions regarding the diameter of the MCA (*i.e.*, the relationship between CBF and MFV) and ICP. Importantly, the MCA diameter has been shown to be relatively stable under conditions of changing ABP (Giller *et al.*, 1993; Serrador *et al.*, 2000) and P_aCO_2 (Giller *et al.*, 1993; Valdueza *et al.*, 1997; Serrador *et al.*, 2000). In

addition, in healthy patients, where intracranial hypertension is unlikely and invasive ICP measurement is not practical, an assumption that ICP is both insignificant relative to MAP and relatively stable is considered (Newell *et al.*, 1992) resulting in Equation 1-3 as an index of CVR.

$$CVRi = MAP/ MFV$$

Equation 1-3. Cerebrovascular resistance index.

Using this model, MFV is affected by two components – MAP and CVRi.

An alternative model takes advantage of the within-beat temporal resolution of continuous TCD and ABP monitoring to estimate downstream pressure, and provides a better approximation of cerebral perfusion pressure. Resistance characteristics based on the passive intra-beat relationship between pressure and velocity allow the estimation of critical closing pressure (CrCP; Aaslid *et al.*, 2003; Panerai, 2003; Panerai *et al.*, 2011). CrCP refers to the ABP at which flow approaches zero (Burton, 1951). Extrapolation of the passive resistance relationship between pressure and velocity identifies an approximate CrCP (Figure 1-1). CrCP is believed to be influenced by both ICP and vasomotor tension (Panerai, 2003), a combination which might better reflect effective downstream pressure compared to ICP alone, in the absence of intracranial hypertension (Weyland *et al.*, 2000). Substituting CrCP into Equation 1-1 might then provide a more robust estimation of factors contributing to CBF (Equation 1-4). In this expanded three-component model of the cerebral circulation, the resistance area product (RAP) represents the passive resistance between flow velocity and pressure (Figure 1-1).

$$MFV = (MAP – CrCP)/RAP$$

Equation 1-4. Model of MFV considering critical closing pressure.

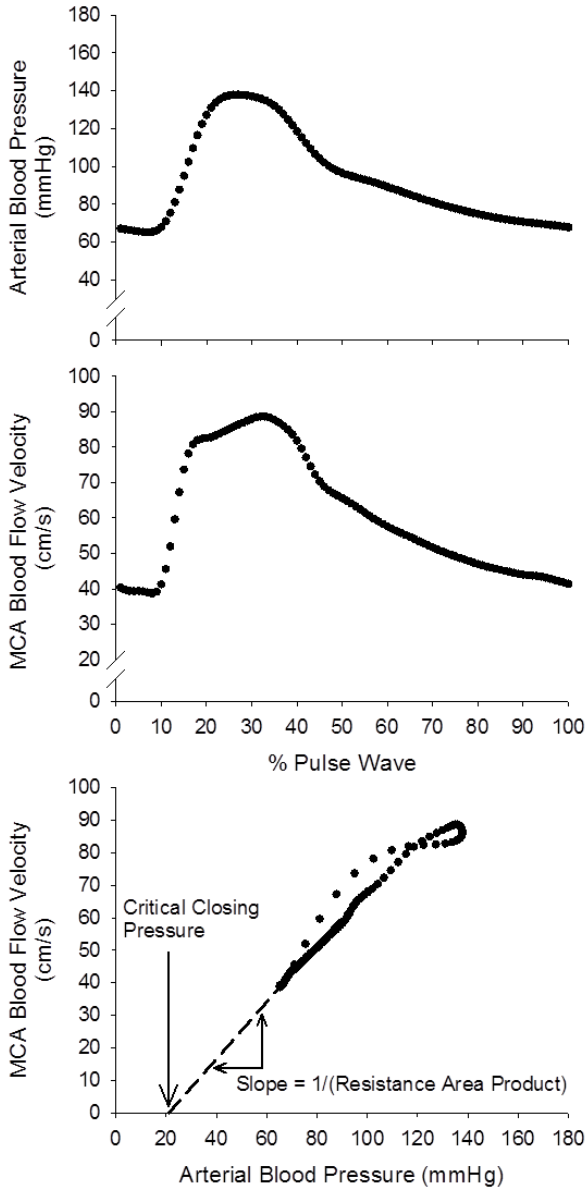

Figure 1-1. Estimation of critical closing pressure.
The within-beat dynamics of arterial blood pressure (top) and middle cerebral artery (MCA) blood flow velocity (middle), as well as the relationship between them (bottom), are shown (dotted lines). Data points were collected at 100 Hz and interpolated to 100 % of the cardiac cycle for direct comparison between pressure and velocity. Critical closing pressure was estimated from linear regression of the relationship between the mean and diastolic values of these measures. The slope derived from the linear regression was extrapolated (dashed line) to a flow velocity of zero (down arrow). See Chapter 2 for full consideration of methods.

In a comparison of multiple extrapolation methods, estimated CrCP appears to be most reliable when using the first harmonic of the ABP and CBF velocity waves, or the relationship between the mean and diastolic values of each measure (Panerai *et al.*, 2011). Of note, preliminary evidence suggests partial independence between CrCP and RAP, where CrCP reflects changes in MFV due to metabolic influences and RAP reflects changes in MFV due to myogenic influences, while both CrCP and RAP appear to be influenced by $P_{ET}CO_2$ (Carey *et al.*, 2001; Panerai *et al.*, 2005; Panerai *et al.*, 2012; Zuj *et al.*, 2013). Therefore, this three-component model might provide a method to distinguish relative involvement of different regulatory mechanisms in the cerebral circulation.

These two- and three-component models can be used to assess regulatory responses in the time domain as well as the frequency domain. Implicitly, the time domain examines changes in the characteristics of interest (*e.g.*, MFV, CVRi, CrCP, RAP) at discrete points in time, usually following a perturbation. Whereas, frequency analysis identifies distinct repetitive patterns with a given cycle period that are found within the complex continuous signal (Giller, 1990). In frequency analysis, the gain of the transfer function between the input (*e.g.*, MAP) and the output (*e.g.*, MFV) provides information about how autoregulatory factors buffer the effect of blood pressure on flow. Higher frequency fluctuations in ABP are associated with higher gain, suggesting that intact dCA acts like a high pass filter, providing efficient attenuation of slower blood pressure oscillations (Zhang *et al.*, 1998). Phase shift and coherence are two additional factors considered in transfer function analysis that relate to the time delay between changes in MAP and MFV, as well as the linear relationship between these two variables (van Beek *et al.*, 2008). This thesis focused only on cerebrovascular responses reported in the time domain.

Aging and Cerebral Blood Flow

A decrease in CBF with aging has been consistently reported (Reich & Rusinek, 1989; Ackerstaff *et al.*, 1990; Krejza *et al.*, 1999; Dorfler *et al.*, 2000; Scheel *et al.*, 2000a; Kamper *et al.*, 2004; Demirkaya *et al.*, 2008). Whether this reduction is secondary to decreased metabolic demand as a result of cerebral atrophy (Appelman *et al.*, 2008) or is a result of changes in arterial structure and function (Beason-Held *et al.*, 2007; Robertson *et al.*, 2010) is unclear, but probably is subject to both etiologies. Although CBF is tightly regulated to the metabolic rate of the underlying cerebral tissue (Hoge *et al.*, 1999), reductions of regional CBF observed in older adults are not tightly correlated to underlying atrophy (Chen *et al.*, 2011). Further, although 'normal' CBF for adults is reported to be in the range of 50 mL/100g/min (Lassen, 1985) (or ~700 mL/min assuming an average brain weight of 1.4 kg); this ignores observations of individual differences in resting CBF both when expressed as global flow (Schoning *et al.*, 1994) and tissue perfusion (Henriksen *et al.*, 2012). Evaluation of the association of CBF with cardiovascular risk factors and underlying pathology might provide insight into the range of reported CBF. Kamper *et al.* (2004) noted that L-NMMA reduced basal CBF in elderly but not young participants, suggesting that redundant mechanisms, including a role for $^{\bullet}$NO, prostaglandins and endothelial-derived hyperpolarizing factors, might be responsible for differences observed between studies, and that these redundant mechanisms might be impaired to different degrees in the elderly. Lower resting CBF in certain individuals might suggest that they are at a greater risk for ischemia, but might also reflect a lower cerebrovascular reserve necessary for CR_{CO2} and CA mechanisms to draw upon as they respond to transient CBF challenges.

In addition to a reduction in resting CBF, the ability to regulate changes in CBF appears to be attenuated with aging. Evidence from both animal (Mayhan *et al.*, 1990; Park *et al.*, 2007; Mayhan *et al.*, 2008; Mitschelen *et al.*, 2009) and human (Yamaguchi *et al.*, 1979; Yamamoto *et al.*, 1980; Reich & Rusinek, 1989; Tsuda & Hartmann, 1989; Kastrup *et al.*, 1998; Matteis *et al.*, 1998; Lipsitz *et al.*, 2000; Ito *et al.*, 2002) models suggest that both neuronal coupling and endothelial regulation of CBF is impaired. In healthy adults, a 1 mmHg increase in P_aCO_2 is associated with 4-5 % increase in CBF velocity (Ide *et al.*, 2003). In cohorts of older adults, mean CR_{CO2} has been shown to be as high as 3.5 %/mmHg (Kastrup *et al.*, 1998) and as low as -2 %/mmHg (Glodzik *et al.*, 2011), where negative responses might reflect a vascular steal effect due to relative differences in reactivity of collateral vascular beds. The extent of age-related alterations to CR_{CO2}, in particular, appears to vary depending on sex (Kastrup *et al.*, 1998), hormonal status (Matteis *et al.*, 1998), the region under examination (*i.e.*, grey vs. white matter; Tsuda & Hartmann, 1989), and the state of underlying atherosclerotic progression (Yamamoto *et al.*, 1980). The link to variation in underlying atherosclerosis is consistent with the involvement of endothelial processes in effective CR_{CO2}.

Basic and clinical research findings have presented complementary evidence supporting the hypothesis that impairment in the ability to regulate CBF with increasing age is secondary to endothelial dysfunction. In 22-24 month old Wistar rats, the dilatory response to acetylcholine was reduced to 3 %, from 11 % in their 6-8 month old counterparts (Mayhan *et al.*, 1990). Similar dilatory responses between the groups to nitroglycerin suggested that the attenuation is secondary to endothelial factors rather than changes to the integrity of the vascular smooth muscle. Interestingly, the reduction was associated with an up-regulation of superoxide-producing nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and

could be ameliorated by scavengers of reactive oxygen species (ROS) (Mayhan *et al.*, 2008). ROS alters the activity of cyclooxygenase enzymes, such that arachidonic acid metabolism is shifted from the production of a vasodilator, prostacyclin, to a vasoconstrictor, thromboxane A_2 (Feletou *et al.*, 2010). Similar to the animal research, attenuated CR_{CO2} in humans appears to be related to endothelial function and ROS. Barnes *et al.* (2012) recently demonstrated that inhibition of cyclooxygenase, with indomethacin, eliminated age-related differences in CR_{CO2} supporting the hypothesis that impairment is endothelial rather than myogenic in nature.

In contrast to CR_{CO2}, CA, which is primarily myogenic in origin, has proven to be a robust physiological mechanism that is well-maintained in older adults (Carey *et al.*, 2000; Lipsitz *et al.*, 2000; Carey *et al.*, 2003; Serrador *et al.*, 2005; Sorond *et al.*, 2005; Franke *et al.*, 2006; Dineen *et al.*, 2011). At least two reports, however, found impaired CA in older compared to younger adults, as indicated by a greater relative dip in MCA MFV during the transition from supine lying to standing postures (Lucas *et al.*, 2008), and greater reduction in cortical oxygenation between steady-state supine lying and standing (Mehagnoul-Schipper *et al.*, 2000). Collectively, these studies used a range of methodologies, including postural transitions, thigh-cuff release, lower body negative pressure and spontaneous fluctuations in ABP; and analysis techniques in both the time and frequency domain. The consistent observations of retained efficiency of CA in aging suggest it is a robust mechanism of regulation.

Two caveats should be considered with respect to these aforementioned studies. First, the authors have characterized their populations as older adults, though few have included participants over 75 years of age. Increasing longevity within the older population suggests new research is needed to cover this more extreme age category. Importantly, Deegan *et al.*

(2011) reported on a group of older adults with a mean age of ~ 78 years and found that elderly women had better dCA than men; however, both groups were within the normal range. Secondly, in most cases these studies examined the relationship between MFV and MAP, considering CVRi as their measure of resistance. Vascular related changes with aging involve an increase in the pulsatility of cerebrovascular hemodynamics (Webb *et al.*, 2012) that might not be reflected in mean values. In addition, the CBF velocity response to a hypotensive stimulus involves an increase in the pulsatile characteristics of the velocity wave profile despite a reduction in the pulsatility of the pressure wave profile (Lipsitz *et al.*, 2000; Rosengarten & Kaps, 2002). These pulsatile characteristics of the cerebral circulation that are not considered by mean beat-by-beat measurements (*e.g.*, MAP, MFV, and CVRi). Heckmann *et al.* (2003) noted that autoregulatory capacity in older adults was similar to younger participants in response to the pressor effect of exercise, but that the rate of the autoregulatory response – characterized by a repeated measures analysis on the increase in pulsatility index (PI) – was delayed. PI is a function of the systolic to diastolic fluctuations in the CBF velocity pulse wave across a cardiac cycle (Equation 2-3); however, it does not always reflect changes in downstream resistance (Czosnyka *et al.*, 1996). An examination into the relationship between CVRi, PI, CrCP and RAP during hypercapnia revealed that PI was more closely related to CrCP than CVRi, and that CVRi and RAP were highly correlated (Hsu *et al.*, 2004). Given these relationships, the observation by Heckmann *et al.* (2003) suggests that a regulatory model involving CrCP and RAP (Equation 1-4) might be more sensitive to subtle age-related changes in CA.

The relationship between CrCP and RAP in the three-component model of local cerebrovascular regulation (Equation 1-4) is relatively novel and has not been well

characterized in older adults. In a group of 12 older adults, Ogoh *et al.* (2011) reported lower resting CrCP and an exaggerated increase in CrCP with cycling exercise, compared to younger adults. Of note, their method of calculating CrCP was from linear extrapolation of systolic and diastolic points (rather than the first harmonic or mean and diastolic points, as described earlier). Age-related and exercise-induced changes in arterial stiffness can significantly alter the systolic portion of the waveform (O'Rourke & Hashimoto, 2007), thereby influencing their CrCP estimation and contributing to a systematic bias in their age-based comparison. The mean-diastolic linear extrapolation is less likely to be biased by age-related changes in arterial stiffness, as well as important waveform differences between the finger and the brain (O'Rourke *et al.*, 2001). However, the novel analytical technique and findings of Ogoh *et al.* (2011) do provide reason for questioning the influences of CrCP and RAP in cerebrovascular regulation in an aging population.

The final sections of this literature review discuss the particular interests of the thesis with respect to CBF and CBF regulation. First, lifestyle characteristics of older adults, including sleep quality and habitual physical activity, will be considered. Metabolic and inflammatory mechanisms are proposed as a link between behavior and cerebrovascular health. Second, intima-media thickness (IMT) and arterial stiffness will be explored as structural links mediating the relationship between cardiovascular risk factors and cerebrovascular function. Finally, the link between cerebrovascular health and functional independence will be addressed with a particular focus on gait speed.

Influence of Habitual Sleep and Physical Activity

A growing number of studies have linked lifestyle characteristics to vascular health (Aizawa *et al.*, 2009; Dod *et al.*, 2010). Two characteristics that are pertinent to the concerns of the older

population include sleep duration and habitual physical activity. The prevalence of sleep disturbances among community-living and assisted-living older populations is estimated at 50 % and 69 %, respectively (Foley *et al.*, 1995; Rao *et al.*, 2005). Regarding habitual exercise, beneficial effects are noted in the protection against morbidity and mortality in the elderly (Chipperfield, 2008; Stessman *et al.*, 2009), including stroke (Gillum *et al.*, 1996), yet half of older Canadians are physically inactive (Azagba & Sharaf, 2012). The association of both sleep and active lifestyle characteristics with cerebral impairment (*e.g.*, white matter lesions, atrophy, or cognitive impairment) has received more attention than associations with cerebrovascular health, which might be an important mediating factor.

Sleep Duration

Older adults commonly experience longer sleep latency, as well as more fragmented sleep (*i.e.*, more frequent awakenings), which contribute to shorter sleep duration. Disturbed sleep is a central concern for the aging population both because of its pervasiveness (Foley *et al.*, 1995; Rao *et al.*, 2005) and its consequences for immediate and long-term health. A distinct U-shape association links sleep duration to mortality and morbidity. Both short duration ($\leq$ 6 hours) and long duration ($\geq$ 9 hours) sleep are associated with greater risk for myocardial infarction, heart disease, and stroke (Sabanayagam & Shankar, 2010; Magee *et al.*, 2012).

The focus of the literature examining CBF and sleep disturbances typically involves chronic disease, including obstructive sleep apnea (Kiratli *et al.*, 2010) or dementia (Ismail *et al.*, 2009), with little evidence examining the relationship between sleep characteristics and CBF in healthy aging. However, some insight can be gained by examining conditions associated with poor sleep. Compared to age-matched controls, patients with idiopathic rapid eye movement (REM) sleep behavior disorder exhibit reduced regional perfusion through the

frontal cortex and medial parietal areas, yet elevated perfusion in subcortical regions of the pons, putamen and hippocampus (Vendette *et al.*, 2011). A similar divergence between cortical hypoperfusion and subcortical hyperperfusion was noted in a subset of Parkinson's disease patients exhibiting excessive daytime sleepiness (Matsui *et al.*, 2006). Due to these region-specific responses, the hypotheses surrounding altered CBF with sleep disturbances center around arousal state (hypoperfusion) and brain regions involved in sleep onset (hyperactivity); however, the potential for a role of vascular health remains less explored.

Recently, studies of habitually short-duration sleepers, as well as sleep deprivation, found shorter sleep to be associated with endothelial dysfunction (Weil *et al.*, 2010; Sauvet *et al.*, 2010). Insight into the pathway through which sleep deprivation affects vascular health might be gained from examination of traditional risk factors. In a recent review, Knutson (2010) posited the link between sleep duration and obesity, diabetes mellitus and hypertension, to be mediated by altered glucose utilization, hormonal balance and/or sympathetic drive.

Habitual Physical Activity

Higher levels of habitual active energy expenditure (AEE) are associated with improved functional performance, prolonged independence (Brach *et al.*, 2004; Sattler *et al.*, 2011), and reduced incidence of cognitive impairment (Laurin *et al.*, 2001; Hillman *et al.*, 2008; Middleton *et al.*, 2011; Vercambre *et al.*, 2011; Vidoni *et al.*, 2012). This functional relationship is in agreement with structural benefits of physical activity. Improved white matter integrity (Johnson *et al.*, 2012) and reduced cerebral atrophy (Szabo *et al.*, 2011; Weinstein *et al.*, 2012) have both been associated with increased aerobic fitness. However, only recently has focus been placed on the role of vascular regulation in mediating the relationship between exercise and brain health.

Physical fitness is generally associated with increased CBF or an attenuation of the age-related decline in CBF (Rogers *et al.*, 1990). MFV was reported to be 17 % higher in endurance-trained men and women, aged 18 to 79 years, compared to their sedentary counterparts. The authors equated this difference to a 10-year savings in age-predicted CBF (Ainslie *et al.*, 2008b). This reduction is consistent with an earlier observation of reduced MFV in unfit versus fit elderly participants (Franke *et al.*, 2006). While these studies have demonstrated a link between fitness and CBF, there is less literature supporting the benefits of habitual physical activity for cerebrovascular health. Animal research supporting the hypothesis reported that voluntary wheel running by mice recovering from an ischemic insult showed increased angiogenesis and CBF in the striatum (Gertz *et al.*, 2006).

The benefits of exercise from a vascular viewpoint are purported to be mediated through modulation of oxidative stress and endothelial function (Rush *et al.*, 2005). To confirm this hypothesis, Pialoux *et al.* (2009) examined oxidative stress in a cohort of post-menopausal women. Physical activity was negatively associated with lipid peroxidation and DNA oxidation, and cerebrovascular conductance was negatively correlated with lipid peroxidation, nitrotyrosine formation and positively correlated with end-products of nitric oxide. Consequently, CR_{CO2}, which is believed to be sensitive to endothelial health, might provide the most sensitive marker for the determination of the benefit of exercise (Brown *et al.*, 2010; Eskes *et al.*, 2010; Davenport *et al.*, 2012). In animal models, CR_{CO2} appears to be modulated by fitness. An elevated hyperemic response to hypercapnia was noted in adult rodents after 30 days of habitual wheel running (Swain *et al.*, 2003). This increased reactivity was observed despite no change in resting CBF, suggesting an increased cerebrovascular reserve capacity, believed to be a result of exercise-induced angiogenesis (Swain *et al.*, 2003). Cerebrovascular

adaptations were isolated to the area of neural activation (*i.e.*, motor cortex) (Swain *et al.*, 2003), so whether activity provides general cerebrovascular benefit is not clear from this study. Although evidence in humans is sparse, cerebrovascular conductance was noted to be higher at baseline and during hypercapnia in fit volunteers compared to a less fit group of post-menopausal women (Brown *et al.*, 2010). In addition, exercise training has been shown to reverse obesity-related differences in retinal arteriolar dilation (Hanssen *et al.*, 2011). Retinal vessel characteristics predict small vessel disease in the cerebral vasculature (Haan *et al.*, 2012), suggesting a possible interaction between regular physical activity and obesity in CBF regulation.

Influence of Central Arterial Structure

Patent and asymptomatic cerebrovascular disease share vascular risk factors. These include increasing age and blood pressure, dyslipidemia, affirmed smoking status, male sex, diabetes mellitus, pre-existing cardiovascular disease and hypertensive therapy (D'Agostino *et al.*, 1994; Longstreth, Jr. *et al.*, 1998). By definition, risk factors identify the threat of an event, but only account for a small portion of the subclinical variance in older adults, tending to underestimate the prevalence of asymptomatic pathology (Michos *et al.*, 2006). Carotid IMT and carotid-femoral pulse wave velocity (cf-PWV) are quantifiable measures of vascular structure and function with direct application to cerebrovascular health. Both population-based and clinical studies have demonstrated relationships between subclinical brain pathology and increasing IMT (Bots *et al.*, 1993; Pico *et al.*, 2002; Inoue *et al.*, 2007; Matsumoto *et al.*, 2007; Nomura *et al.*, 2010), as well as elevated arterial stiffness (Ohmine *et al.*, 2008; Kearney-Schwartz *et al.*, 2009; Hatanaka *et al.*, 2011; Kim *et al.*, 2011a; Mitchell *et al.*, 2011). These cerebral impairments might be the expression of elevated ischemic burden secondary to vascular

dysfunction imparted by atherosclerosis or increasing arterial stiffness; however, the relationship between central arterial factors and CBF has not been extensively studied.

The cerebral vessels, which are low resistance/low impedance vessels, might be at particular risk to changes in the cushioning properties of central arteries. Observations of a direct relationship between arterial stiffness and the pulsatility of MCA blood flow velocity (Kim *et al.*, 2010; Webb *et al.*, 2012) demonstrate that pulsatile characteristics of the central circulation are propagated into downstream vessels. In addition, stiffness has been associated with reduced CBF (Kielstein *et al.*, 2006; Tarumi *et al.*, 2011) and increased cerebrovascular resistance (Robertson *et al.*, 2010), which might be a consequence of damage imparted by the pulsatile flow or a functional adaptation to protect the microcirculation. Interestingly, increased IMT was associated with reduced CBF in the lingual, inferior occipital and superior temporal gyri, but with increased CBF in medial frontal gyri, putamen and hippocampal regions (Sojkova *et al.*, 2010). This suggests that specific regions of the brain might be more susceptible to pro-atherosclerotic environments. While these recent findings demonstrate that central vascular structure and function do appear to impact the cerebral circulation – as evidenced by increased resistance and pulsatile characteristics – little is known about their impact on CBF regulation including CR_{CO2} and CA (Park *et al.*, 2007).

Inflammation as a Mediator of Vascular Health

Although the underlying physiological pathways relating lifestyle risk factors, such as physical activity and sleep, to central arterial structure and function and cerebrovascular health remain poorly understood, a background of chronic inflammation is widely believed to play an important role (Ross, 1999; Rost *et al.*, 2001; van Dijk *et al.*, 2005). Atherosclerosis is an inflammatory disease (Ross, 1999) and chronic inflammation has been associated with cognitive impairment (Yaffe *et al.*, 2004; Roberts *et*

al., 2009) and increased risk for cerebrovascular disease (Rost *et al.*, 2001; van Dijk *et al.*, 2005). High-sensitive C-reactive protein (CRP) is an acute-phase marker of inflammation that has received particular attention related to vascular health (Kampus *et al.*, 2004; Yasmin *et al.*, 2004; Jenny *et al.*, 2012). Cellular research has demonstrated the involvement of CRP in multiple pathways associated with vascular impairment. These include decreasing production of nitric oxide (Verma *et al.*, 2002b), facilitating the migration of leukocytes into the vascular wall (Pasceri *et al.*, 2000), and increasing the production of the vasoconstrictor endothelin-1 (Verma *et al.*, 2002a), among others. In a cross-sectional study of community-dwelling older adults, fatigue was independently related to CRP level and self-reported physical activity (Valentine *et al.*, 2009), suggesting that inflammation may mediate the relationship between disturbed sleep, low physical activity and cerebrovascular health.

Association between Cerebrovascular Health and Gait Speed

Slow gait is associated with disability and functional dependence in community-living older adults (Guralnik *et al.*, 2000; Cesari *et al.*, 2005). The prevalence of cardiovascular risk factors has been purported as a contributor to gait difficulties, yet the physiological mechanism through which this relationship is realized remains unclear. Several studies have associated obstructive peripheral arterial disease as an important mediator of the link between cardiovascular risk factors, declining gait speed, and functional dependence (Brach *et al.*, 2008; Kuo & Yu, 2008). As a marker of central arterial disease, IMT has also been associated with slower gait speed (Elbaz *et al.*, 2005). Impaired peripheral vascular health might compromise muscle blood flow and contribute to faster onset of fatigue, as well as reduced muscle strength, mediating a decline in gait speed (Kuo & Yu, 2008).

As an alternative to the peripheral impairment hypothesis, a cognitive hypothesis has gained traction recently. The triumvirate of slow gait, depression and executive dysfunction was proposed as a classic phenotype of aging that might be related to vascular dysfunction,

particularly in the frontal lobes (Hajjar *et al.*, 2009). Rosano *et al.* (2012) further supported this hypothesis, noting a direct relationship between prefrontal area volume and gait speed which was attenuated after adjusting for processing speed. Further, arterial stiffness was found to be an independent mediator of cognitive deficits in community-living older adults (Elias *et al.*, 2009; Watson *et al.*, 2011), perhaps confounding the relationship between peripheral vascular health and gait speed (Brach *et al.*, 2008; Kuo & Yu, 2008).

Given this apparent relationship between cognition and gait speed, impaired cerebrovascular regulation might be an important contributor to functional outcomes. Sorond *et al.* have reported slower gait in community-living older adults who exhibit impaired neurovascular coupling (Sorond *et al.*, 2011) and CR_{CO2} (Sorond *et al.*, 2010). Although CA is relatively stable in healthy aging (Lipsitz *et al.*, 2000; Dineen *et al.*, 2011), typical posture-related drops in CBF (Sorond *et al.*, 2005; Alperin *et al.*, 2005a; Deegan *et al.*, 2011; Sato *et al.*, 2012) superimposed upon an age-related lower CBF (Scheel *et al.*, 2000b) might place an individual at greater risk for symptoms of subtle hypoperfusion, including light-headedness and dizziness, which might contribute to unsteadiness, slow gait or falls.

Summary

In summary, the cerebral circulation involves a complex network of collateral vessels and complimentary regulatory mechanisms to ensure CBF matches the demand for energy and oxygen. CBF is reduced in aging, but the individual differences in CBF, as well as regulatory mechanisms, places some individuals at greater ischemic risk. Individual risk profiles might be the result of structural and functional changes to central arteries, associated with poor metabolic and inflammatory profiles. A three-component model of cerebral regulation, including ABP, CrCP and RAP might be more sensitive to address changes in the passive

within-beat pressure-flow relationships that characterize changes in the pulsatile nature of CBF. A more thorough understanding of how aging impacts CBF and cerebrovascular regulation is necessary due to the potential impact on brain health, function, independence and quality of life in our aging population.

Thesis Objectives

General Objective

The general objectives of this thesis were to examine CBF and cerebrovascular function with respect to habitual physical activity and sleep characteristics, as well as underlying central arterial aging. Further, the association between cerebrovascular properties and functional manifestations of brain health in older adults was investigated.

Specific Questions

The objectives noted above were subsequently addressed by answering four main questions:

A. Are habitual physical activity levels and sleep duration associated with cerebrovascular hemodynamics and CBF regulation in community-living older adults? (Chapter 3).

B. Are peripheral vascular structure and function characteristics associated with cerebrovascular hemodynamics and CBF regulation in community-living older adults? (Chapter 4).

C. Do peripheral vascular structure and function alter the local pressure-velocity relationship, and affect CBF regulation during posture change and steady-state upright posture? (Chapter 5).

D. Is lower upright CBF or effectiveness of dynamic cerebral autoregulation related to gait speed and falls in older adults? (Chapter 6).

Hypotheses

A. It was hypothesized that low levels of physical activity, and both short and long sleep duration, would be associated with lower CBF and impaired CR_{CO2}. This relationship is expected to be mediated by the presence of metabolic syndrome and markers of chronic inflammation.

B. It was hypothesized that the inverse relationship between aging and CBF will be modulated by arterial stiffening and elevated IMT. Specifically, these markers of vascular structure and function will contribute to lower CBF, greater cerebrovascular resistance, and lower CR_{CO2}.

C. It was hypothesized that older adults with increased arterial stiffness and IMT, characterizing elevated vascular risk, would exhibit a greater drop in steady-state CBF between supine and upright postures and impaired dCA. A linear model including RAP and CrCP, which considers the passive pressure-flow relationships subject to arterial stiffening, will be more sensitive to differences than a model including CVRi.

D. It was hypothesized that lower upright CBF and impaired dCA in older adults would be associated with frailty characteristics. This would be expressed through slow gait speed and history of falls. This relationship is expected to be primarily mediated through executive dysfunction.

Organization of Thesis

This thesis was written to guide the reader through as though it was a single piece of literature covering pertinent issues in cerebrovascular health of older adults; however, divisions were maintained in parts with an eye toward expected independent publications. Chapter 2 provides an in-depth description of the methods used to characterize cerebrovascular health, referred to throughout the thesis; however, each chapter includes supplementary methodology that is specific to the issue at hand. Chapters 3 to 6 cover a spectrum of issues ranging from an examination of habitual physical activity and sleep quality, to the impact of cerebrovascular health on functional status. The majority of the thesis draws on the main cohort described in Chapter 2; however, Chapter 5 also draws on evidence from a small study of 29 young participants examining the CBF response to upright posture. Chapter 7 serves to provide a general discussion of the issues raised throughout the thesis, as well as areas for future exploration.

CHAPTER 2. GENERAL METHODS AND MATERIALS

In this chapter, the main participant cohort is introduced and a description of the main cardiovascular and cerebrovascular assessment methods referred to throughout the thesis document is described. Additional methodologies and analytical techniques specific to chapters of interest are highlighted in their respective chapters.

Participant Description

In the main study, a convenient sample of high-functioning older adults (≥ 65 years) was recruited from the community (N = 81). The primary recruitment tool was the University of Waterloo Research in Aging Participant Pool (WRAP), based in the University's Department of Psychology. The WRAP database is a listing of adults who have identified themselves as being interested in participating in research involved in aging. The WRAP program was advertised by flyers in the community, in local newspapers, on local television and radio stations and through word of mouth. Listings from this database were requested using an age restriction of at least 65 years. From WRAP, 146 candidates for enrolment were contacted by phone, from which 87 (59.6 %) expressed interest in the study (Figure 2-1). A further 23 were excluded after reporting at least one exclusion criterion (Table 2-1), resulting in 64 (43.8 %) candidates being enrolled into the study through the WRAP database. Additional participants were recruited through 'snowballing', as well as the placement of posters, specific to this study, in local older adult community centers. The final enrolment included 31 men and 50 women between the ages of 65.3 and 89.7 years. Following enrolment, participants arrived at the University of Waterloo in the morning and completed a comprehensive health screen, a battery of neuropsychological tests, and a familiarization session in the vascular imaging suite

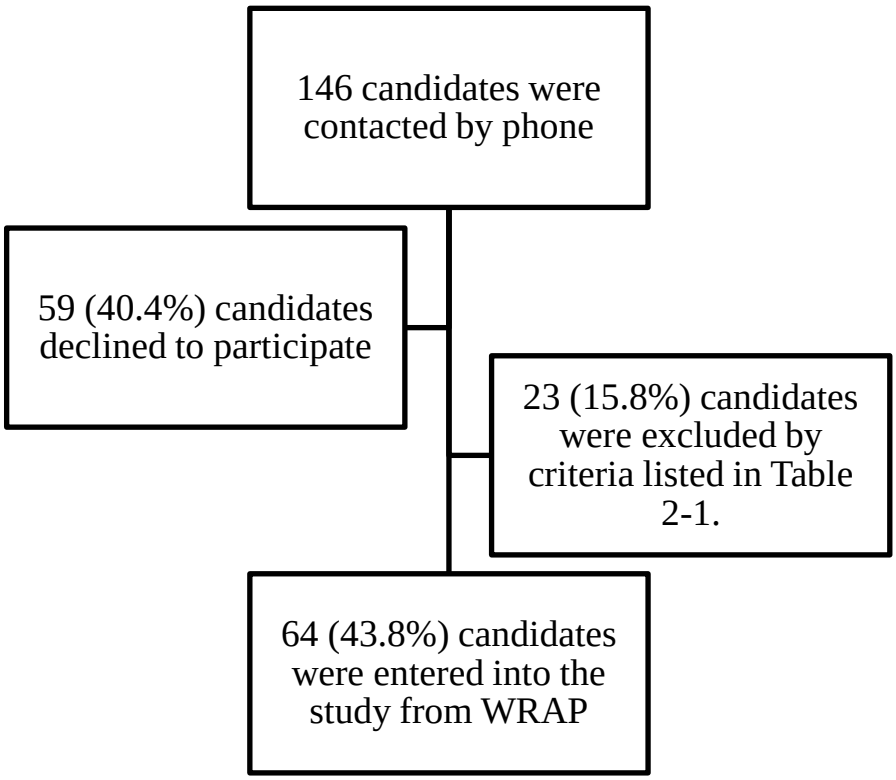

Figure 2-1. Participant recruitment through Waterloo Research in Aging Participant Pool (WRAP).

Table 2-1. Exclusion Criteria

Stage II hypertension (≥ 160/90 mmHg)
Stroke
Transient ischemic attack
Carotid endarterectomy or stent
Atrial fibrillation
Open heart surgery
Congestive heart failure
Systemic inflammatory disease
Metastatic or current cancer
Chronic kidney disease
Neurological impairment
Unstable health (overnight hospitalization within the past 12 months)
Dementia

Seated blood pressure was the average of the second and third measurements taken in the morning, while fasting and before medication. Evidence of stroke or transient ischemic attack was determined by a National Institute of Health Stroke Scale score > 0. Presence of dementia was determined by the Montreal Cognitive Assessment (MoCA Version 7.0; http://www.mocatest.org/; Nasreddine *et al.*, 2005) score < 24. All other conditions were self-reported.

with a Registered Nurse. They also provided a fasting blood sample at this time. On a separate day (approximately one week later), participants returned for a vascular health assessment in the afternoon, at least two hours following a light meal. Participants were requested to avoid exercise, as well as caffeine, nicotine and alcohol on the day of the vascular assessment. 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. Each participant was made aware of his/her right to withdraw from the study at any time.

Health Questionnaire and Classification of Cardiovascular Risk Factors

A comprehensive questionnaire was provided and reviewed with a Registered Nurse (see Appendix A). Information on demographic background, health history, current medications, and family history of cardiovascular disease was obtained. Prior cardiovascular disease was scored as positive if the participant reported a prior myocardial infarction or peripheral vascular disease. Familial history was scored as positive if any first-degree relative was known to have had a myocardial infarction, peripheral vascular disease, a stroke, or a transient ischemic attack. Participants were asked to bring their medication with them to their first visit to authenticate the type and quantity. Waist circumference was measured half way between the top of the iliac crest and the lower rib at the end of a normal expiration. Hip circumference was measured at the widest part of the buttocks.

Smoking, alcohol, sleeping and physical activity habits were assessed by self-report. Participants were scored as current smokers if they reported smoking cigarettes, cigars, pipes or using smokeless tobacco during the past 30 days. Participants were scored as former smokers if they reported smoking at least 100 cigarettes in their lifetime (Trosclair & Dube, 2010). Alcohol consumption was assessed using a frequency/quantity approach, without

distinguishing between wine (5 oz.), beer (12 oz.) or liquor (1.5 oz.). Alcohol risk was stratified by sex. A high risk intake was scored if consumption averaged more than 1 drink per day for women and more than 2 drinks per day for men (Chobanian *et al.*, 2003), or if either sex reported 5 or more drinks per session at least twice per month. Scoring of sleep quality and habitual physical activity are discussed in detail in Chapter 3.

Seated blood pressure was assessed using the auscultation method, taking the average of the second and third measurements in the morning while fasting and before medication. Hypertension was indicated if measured systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or the participant was currently receiving antihypertensive therapy. A fasting blood sample was taken for assessment of metabolic disorders (see more details on blood draw and preparation below). Hyperlipidemia was indicated from low density lipoprotein (LDL) > 3.5 mmol/L, total cholesterol:high-density lipoprotein ratio > 5 mmol/L (consistent with Canadian Cardiovascular Society guidelines to initiate therapy for moderate risk individuals; Genest *et al.*, 2009), or lipid lowering therapy. Diabetes mellitus was indicated from glucose ≥ 7.0 mmol/L, glycolated hemoglobin (HbA1c) ≥ 6.5%, or insulin or hypoglycemic therapy (Nathan *et al.*, 2009). Metabolic syndrome was indicated from having 3 of the following 5 characteristics – (1) waist circumference: in standing ≥ 102 cm (men) or ≥ 88 cm (women); (2) fasting triglyceride (TG) ≥ 1.7 mmol/L or medication; (3) fasting high density lipoprotein (HDL) < 1.03 mmol/L (men), < 1.30 mmol/L (women) or medication; (4) blood pressure: SBP ≥ 130 mmHg, DBP ≥ 85 mmHg, or medication; (5) fasting glucose ≥ 5.55 mmol/L or medication (Grundy *et al.*, 2005).

Cerebrovascular Assessment

Cardiovascular Hemodynamics

Participants reported to an environmentally-controlled laboratory and rested supine for at least 15 minutes prior to data collection. Heart rate was monitored using a 3-lead electrocardiogram (ECG; Pilot 9200, Colin Medical Instruments, San Antonio TX USA). Continuous arterial blood pressure (ABP) was monitored by finger-cuff photoplethysmography, calibrated to brachial artery SBP using a return-to-flow technique, and reconstructed to brachial arterial pressure waveforms using a proprietary transfer function (Finometer, Finapres Medical Systems, Amsterdam NL). The reconstructed brachial waveforms were corrected to heart level and have been shown to estimate intra-arterial pressures within international standards (Guelen *et al.*, 2003). Blood flow velocity through the middle cerebral artery (MCA) was monitored by transcranial Doppler ultrasound (TCD; see below). Exhaled gas was captured by nasal cannula and the concentration of carbon dioxide (CO_2) was measured by infrared spectroscopy (Pilot 9200). These four measures were collected continuously at 1000 Hz by a data acquisition system (PowerLab; AD Instruments, Colorado Springs CO USA) using the associated software (Chart v5.5.1). Subsequent analysis was performed in Excel 2010 (Microsoft Corp., Redmond WA USA) and MatLab v7.9 (The MathWorks Inc., Natick MA USA).

Off-line analysis involved beat-to-beat averaging triggered to the ECG R-wave. The continuous blood pressure signal was monitored throughout the study and compared to periodic auscultation measurements. When drift was observed (defined as a mismatch between Finometer brachial artery pressure and auscultation methods of > 5 mmHg), the finger-cuff value was recalibrated with the traditional method. A warming pad was placed around the hand to improve signal quality from the finger. Further, mean arterial pressure (MAP) was corrected

to brain level (BP_{MCA}) to account for the orthostatic pressure gradient between the brain and the heart when in an upright posture (distance·0.78 mmHg/cm). Modelflow analysis of the arterial pressure wave (Finometer) was used to estimate cardiac output (CO; stroke volume x heart rate). The 3-element model flow algorithm for estimating stroke volume from the non-invasive finger pressure has been shown to reliably track changes in stroke volume during orthostatic stress (Harms *et al.*, 1999). Breath-by-breath values for end-tidal CO_2 concentration were obtained and time-matched to the beat-by-beat data for analysis. The partial pressure of CO_2 ($P_{ET}CO_2$) was calculated from the product of CO_2 concentration and barometric pressure.

Cerebral Blood Flow

The right MCA was insonated using a 2-MHz pulsed TCD system (Neurovision Transcranial Doppler Ultrasound Model 500V, Multigon Industries, Yonkers NY USA, or Doppler Box, Compumedics DWL, Singen DE) using standard search techniques (Aaslid *et al.*, 1982). Briefly, the transducer was placed against the temporal window, with a slightly forward orientation. The insonation depth was placed between 50 and 60 mm, consistent with the proximal M1 segment of the artery (Gillard *et al.*, 1986). Traceability, velocity profile, signal strength, auditory pitch, and probe angle were used to confirm insonation of the MCA (Zwiebel & Pellerito, 2005).

Mean flow velocity (MFV) through the MCA has been shown to be a reliable marker of changes in CBF when compared to more invasive techniques (*e.g.*, SPECT, Xe-enhanced CT) (Bishop *et al.*, 1986; Dahl *et al.*, 1992b; Dahl *et al.*, 1992a; Kofke *et al.*, 1995; Ulrich *et al.*, 1995). Despite these assurances, using velocity to track changes in flow is strictly reliant on a constant diameter within the MCA (Equation 2-1). A small change in the diameter could augment or diminish the association between flow and velocity.

$$\text{Blood flow} = \text{velocity} \cdot \pi \cdot \text{diameter}^2/4$$

Equation 2-1. Mean blood flow.

A number of studies have provided some evidence that the MCA is relatively stable under conditions of changing orthostatic stress and hypercapnia (Giller *et al.*, 1993; Valdueza *et al.*, 1997; Serrador *et al.*, 2000). However, results from prolonged hypoxia (Wilson *et al.*, 2011) and maximal hypercapnia (Clark *et al.*, 1996; Valdueza *et al.*, 1999; Willie *et al.*, 2012) have suggested that the MCA diameter does change in extreme conditions. Therefore, conclusions about CBF from TCD measures need to be interpreted carefully. Not understating these concerns in the within-subject comparison, perhaps of greater import is the need to exercise caution for comparison between subjects, as differences will most certainly be affected by individual variation of arterial diameters. This is especially relevant in the current population of interest who are more likely to have structural changes in the intracranial vessels due to age or atherosclerosis (Vorstrup *et al.*, 1992; Brauer *et al.*, 1998; Ozdogmus *et al.*, 2008). Despite these concerns, TCD remains a valuable tool for the non-invasive characterization of CBF and cerebrovascular properties, including resistance, pulsatility, reactivity and autoregulation – discussed in detail below.

To supplement the TCD-derived CBF velocity in this study, extracranial CBF was assessed through the bilateral internal carotid (ICA) and vertebral arteries (VA). This method assessed both velocity and diameter of the arteries to provide a quantitative value of CBF (Figure 2-2). In addition, flow through the external carotid artery (ECA) was included as a late revision to the protocol. Duplex ultrasonography (HFL38 6-13 MHz linear array transducer with Micromaxx system, Sonosite, Bothwell WA USA, or L14-6s 8-12 MHz linear array

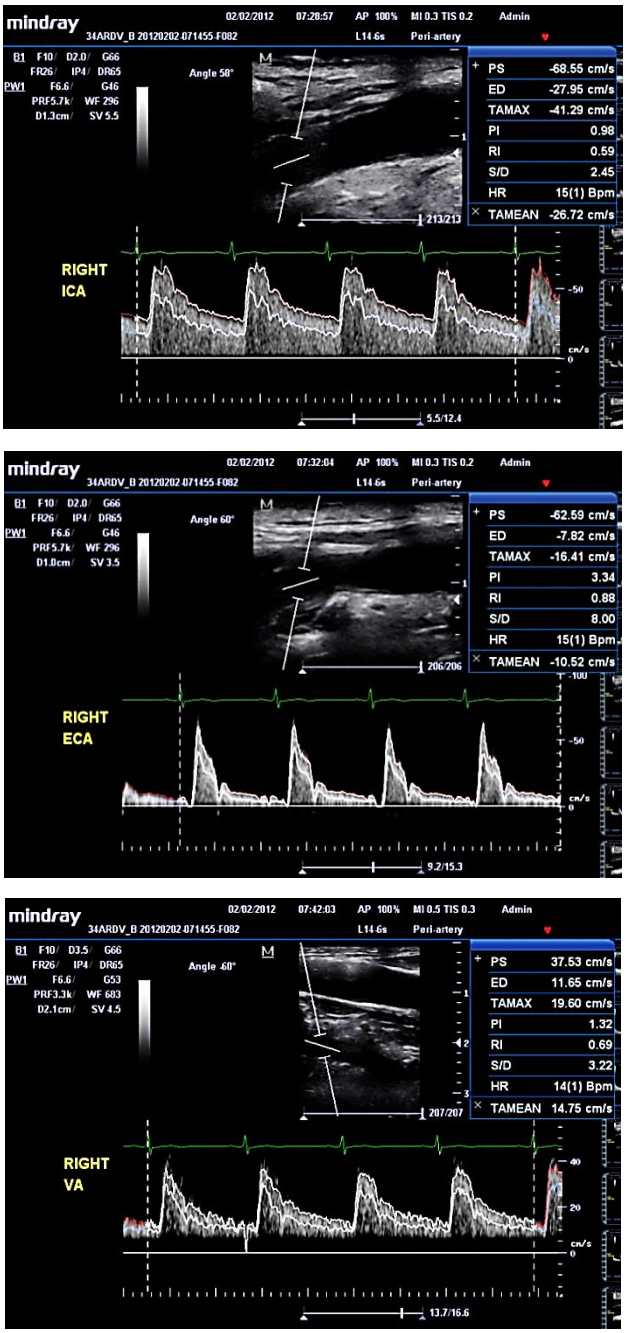

Figure 2-2. Measurement of blood flow velocity in the extracranial vessels.
Representative images of B-mode (insets) and Doppler traces for sequential measurements of the internal carotid artery (ICA, top), external carotid artery (ECA, middle) and vertebral artery (VA, bottom).

transducer with M5 system, Mindray Bio-Medical Electronics Co., Shenzhen CN) captured arterial diameter (gated to the R-spike on three consecutive beats) and blood flow velocity (time- averaged over 10-15 cardiac cycles). Diameter was measured during the diastolic phase by electronic calipers in triplicate for each of the three cardiac cycles. Mean blood flow (MBF) through each vessel was computed from the diastolic diameter and time-averaged MFV (Equation 2-1). Vessels were insonated between 50° and 65°. As needed, the participants' head was tilted back and away from the sonographer to facilitate imaging of the ICA. ICA flow was measured at least 1 cm distal to the carotid bifurcation to minimize the influence of turbulent flow in the carotid bulb. VA flow was measured just proximal to entering the vertebral column or between the transverse processes of the third and fifth cervical vertebrae, depending on the optimal window for insonation. Anterior and posterior measures of CBF were computed for analysis. This technique has shown low inter- and intra-observer variability in the literature (Schoning & Scheel, 1996). In the current study, all imaging was completed by the author. A repeatability study of ICA imaging on 13 young participants showed good reliability for arterial diameter (coefficient of variation (CV): 0.6-6.5 %; intraclass correlation coefficient (ICC): 0.91). Mean flow velocity was more variable (CV: 2.3-20.1 %; ICC: 0.67). This is consistent with literature which reported that structural characteristics were more reproducible than velocity (Sutton-Tyrrell *et al.*, 1992). Although the velocity ICC observed here is greater than documented by Sutton-Tyrrell *et al.* (0.81-0.84), the physiological moment-to-moment variability in CBF velocity secondary to fluctuations in PCO_2 and ABP has been documented, with individual fluctuations as high as 30 % (Diehl *et al.*, 1991; Panerai *et al.*, 2003).

Cerebrovascular Function

Cerebrovascular Resistance

Resistance indices were used to examine CBF with respect to ABP. Four indices were calculated, including cerebrovascular resistance (CVR; rearranged from Equation 1-1), a cerebrovascular resistance index (CVRi; Equation 1-3), critical closing pressure (CrCP), and resistance area product (RAP). CrCP and RAP were computed by the two-point method, using mean and diastolic values based on a linear model, where the x-intercept represented the theoretical pressure at zero velocity (see Figure 1-1; Eq. 2-2; Panerai *et al.*, 2011). CrCP and RAP were assessed using the peak blood flow velocity (*i.e.,* outer envelope) in the artery of interest.

A: velocity = $a \cdot$ pressure + b

B: CrCP = $- b / a$

C: RAP = $1 / a$

Equation 2-2. Linear regression model for estimating critical closing pressure and resistance area product.

Cerebrovascular Pulsatility

As mentioned above, the temporal resolution is one of the major benefits of TCD, allowing for the assessment of pulsatile CBF within the cardiac cycle. These pulsations were quantified through the calculation of Gosling's pulsatility index (PI; Equation 2-3).

PI = (systolic velocity – diastolic velocity) / MFV

Equation 2-3. Gosling's pulsatility index.

Cerebrovascular Reactivity

Reactivity was assessed by monitoring the change in MFV through the MCA while breathing a hypercapnic gas mixture (5% CO_2, 21% O_2, balance N_2) or while hyperventilating to produce a hypocapnic stimulus. While sitting, participants breathed through a disposable facemask, which formed a tight seal surrounding the nose and mouth to ensure no ambient contamination. Following 3 minutes of rest, participants hyperventilated at 20 breaths per minute for 2 minutes, returned to spontaneous breathing for 3 minutes, and breathed from the hypercapnic gas mixture for 3 minutes. A manually-controlled valve (3-way T-shape Stopcock Type, Hans Rudolph, Shawnee KS USA) determined whether participants received room air or a hypercapnic gas mixture (5% CO_2, 21% O_2, balance N_2) from a non-diffusing gas bag (Series 6000, Hans Rudolph). During hyperventilation, participants were coached to achieve at least a 5 mmHg decrease in $P_{ET}CO_2$ by increasing their tidal volume, if necessary. Measurements for analysis were averaged from the final 30 s in each condition (Figure 2-3). Total CR_{CO2} (hypercapnia – hypocapnia) was computed by dividing the percentage change in MFV by the absolute change in $P_{ET}CO_2$ (Equation 2-4). An *a priori* threshold of a minimum 5 mmHg change in $P_{ET}CO_2$ was considered necessary for a valid CR_{CO2} test to achieve sufficient signal-to-noise ratio. A threshold CR_{CO2} of 1.3 %/mmHg was used to indicate high cerebrovascular risk (Kleiser & Widder, 1992).

$$CR_{CO2} = [\ (MFV_{(t2)} - MFV_{(t1)}) / MFV_{(t1)} \cdot 100\%] / (P_{ET}CO_{2(t2)} - P_{ET}CO_{2(t1)})$$

Equation 2-4. Cerebrovascular reactivity to carbon dioxide.

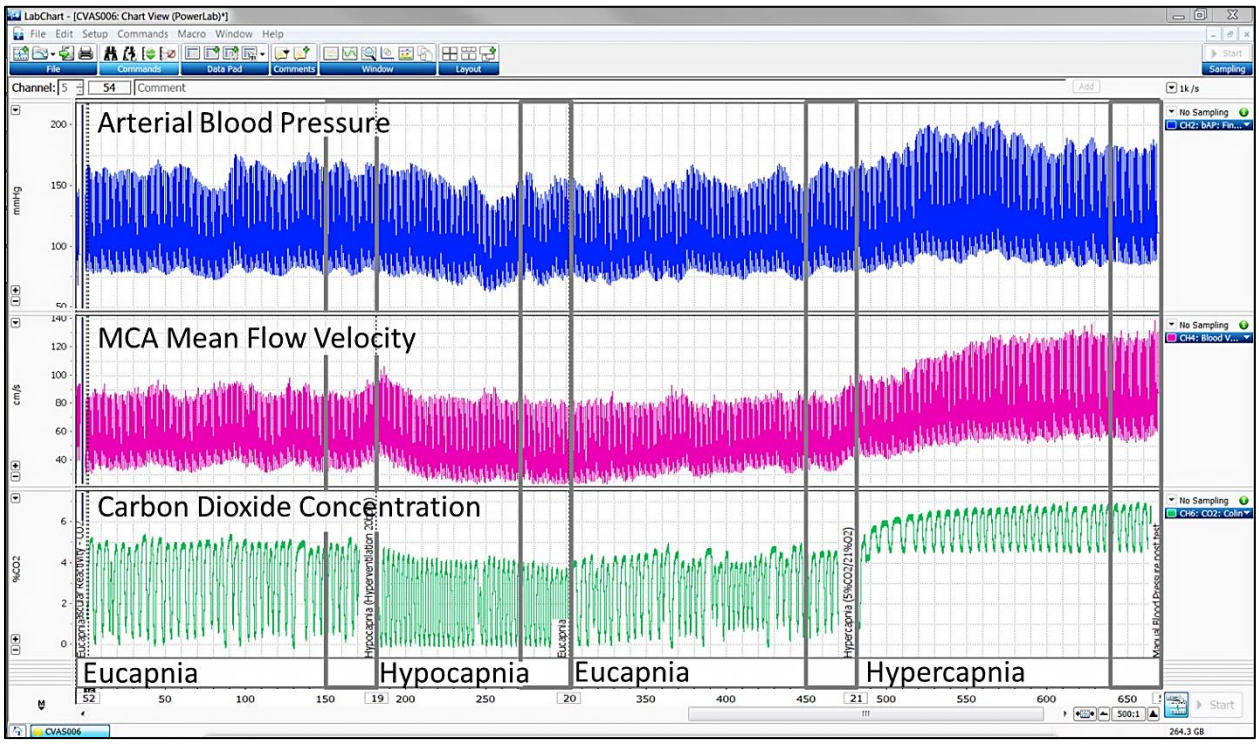

Figure 2-3. Representative data strip for cerebrovascular response to CO_2.
A sample data strip showing the influence of eucapnia, hypocapnia and hypercapnia on arterial blood pressure and cerebral blood flow velocity. The three rows of data (top to bottom) reflect arterial blood pressure, blood flow velocity through the middle cerebral artery (MCA), and carbon dioxide concentration at the level of the mouth. The four rectangles represent the last 30 s of each condition, from which data were averaged for analysis.

Cerebral Autoregulation

Static and dynamic cerebral autoregulation (sCA and dCA, respectively) were assessed through changes in posture. During this task, MBF through the ICA, ECA and VA; MFV through the MCA; BP_{MCA}; and $P_{ET}CO_2$ were measured. After resting supine for 3 minutes, participants fluidly transitioned to the sitting posture, with researcher assistance (~2-4 s), for 2 minutes, and then stood for a further 2 minutes. Measurements were averaged over the final 30 s in each posture for analysis of sCA (Figure 2-4). sCA is the classical assessment of autoregulatory function, looking at the steady state change in CBF after BP_{MCA} has reached a new stable plateau (Panerai, 1998). sCA was examined by linear regression analysis of % MBF (or MFV) on BP_{MCA}, with consideration for changes in $P_{ET}CO_2$, in lying and sitting positions for ICA, VA and ECA; and in lying, sitting and standing positions for the MCA. A smaller slope fitting the regression reflects better autoregulation (*i.e.*, smaller change in CBF).

dCA was determined from the relative change in MFV through the MCA in relation to the relative dynamic change in BP_{MCA} observed immediately following postural transitions from lying to sitting and from sitting to standing (Equation 2-5; Sorond *et al.*, 2009). Lipsitz *et al.* (2000) developed a simple, non-invasive sit-to-stand procedure that examines temporal dynamics of MFV in response to rapid changes in BP_{MCA}. This technique has since been validated against other analytical techniques (Serrador *et al.*, 2005) and blood pressure manipulations (Sorond *et al.*, 2009). Beat-by-beat data were resampled at 1 Hz (MatLab) and time-matched for comparison between participants. The point of interest for comparison was taken at the lowest point (nadir) of the BP_{MCA} response. An average value from the 3 s surrounding the nadir was used for analysis (Figure 2-4, arrows). Similar to sCA, a smaller dCA represents better autoregulation.

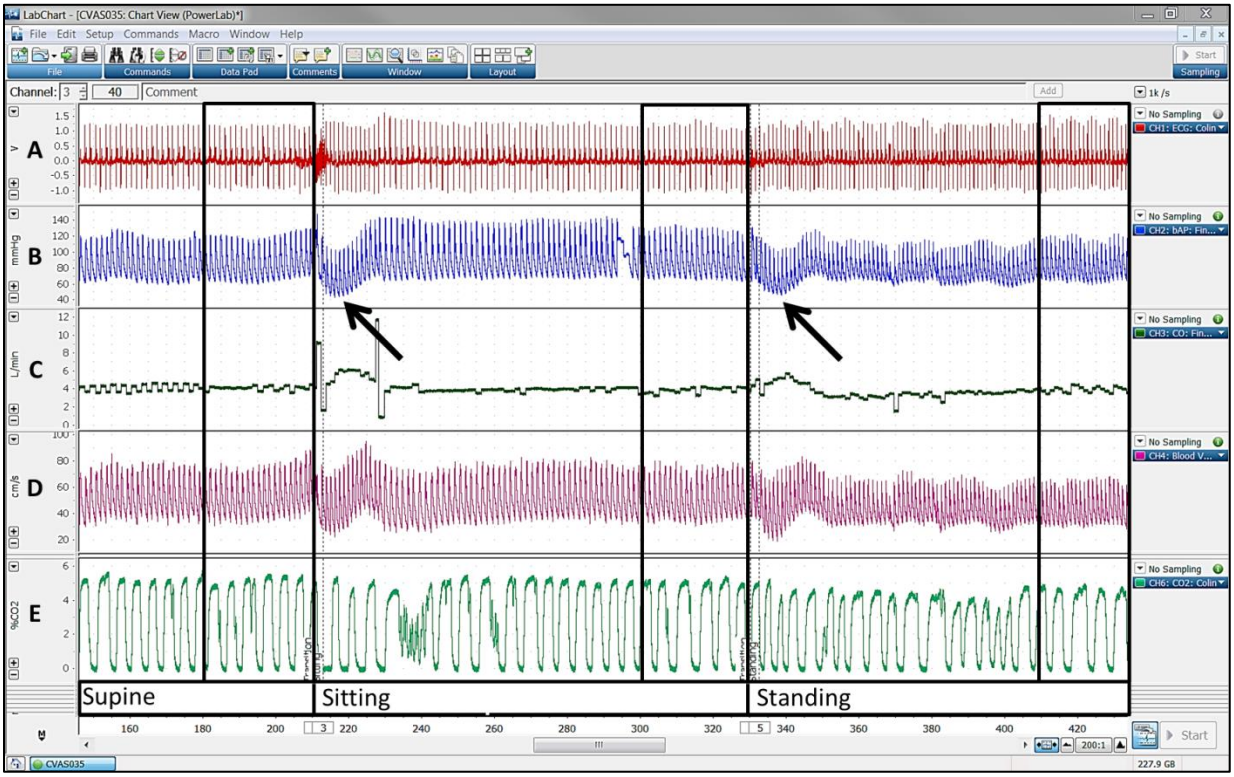

Figure 2-4. Representative data strip for postural transition.
Sample data strip showing the cardiovascular and cerebrovascular responses to postural change. Posture transitions from supine to sitting and from sitting to standing occurred in sequential fashion following a period of steady conditions [3 min (supine), 2 min (sitting) and 2 min (standing)]. The five rows of data reflect heart rate (A), arterial blood pressure at heart level (B), cardiac output (C), blood flow velocity through the middle cerebral artery (D), and carbon dioxide concentration at the mouth (E). The three vertical rectangles represent the final 30 s in each position, used to assess static cerebral autoregulation. The two arrows indicate the nadir of mean arterial pressure following the supine-to-sitting (left) and sitting-to-standing (right) postural transitions, used to assess dynamic cerebral autoregulation.

$$dCA = [(MFV_{(t2)} - MFV_{(t1)}) / MFV_{(t1)}] / [(MAP_{(t2)} - MAP_{(t1)}) / MAP_{(t1)}]$$

Equation 2-5. Dynamic cerebral autoregulation.

Arterial Structure and Function

Carotid Intima-Media Thickness (IMT)

High resolution B-mode ultrasonography (Sonosite or Mindray) was performed on vessel walls in the common carotid artery (CCA), bilaterally, within 1.5 cm of the carotid bifurcation. On frozen longitudinal images, captured at the ECG's R-wave, electronic calipers were placed on the lumen-intima and media-adventitia interfaces of the far wall. Eight distinct sets of calipers were equally distributed over a vessel segment approximately 1 cm in length (Figure 2-5). In a subset of 33 participants (those imaged with the Sonosite ultrasound system), the images were captured on videotape (Sony Handycam DCR-HC42, Sony Corp., Tokyo JP). The video was digitized using Adobe Premiere 6.5 (Adobe Systems Inc., San Jose CA USA) before the IMT was measured using an in-house edge detection program (GDM © Hanif M. Ladak 1998-1999; MatLab v5.3, The MathWorks Inc.). Mean and peak measurements were averaged across three consecutive cardiac cycles. The right and left sides were averaged for analysis. A threshold for mean IMT of 0.8 mm for women and 0.9 mm for men (associated with 75th percentile for the Caucasian population; Howard *et al.*, 1993) was used to indicate high cerebrovascular risk.

Central Pulse Pressure

The pulse pressure in the CCA (cPP) was measured by applanation tonometry. As the arterial pulse moves distally along the arterial tree, the waveform is augmented by the relative increase in stiffness in peripheral arteries compared to central arteries (O'Rourke & Hashimoto, 2007). Examining the pulse at the CCA rather than the brachial or finger arteries might therefore

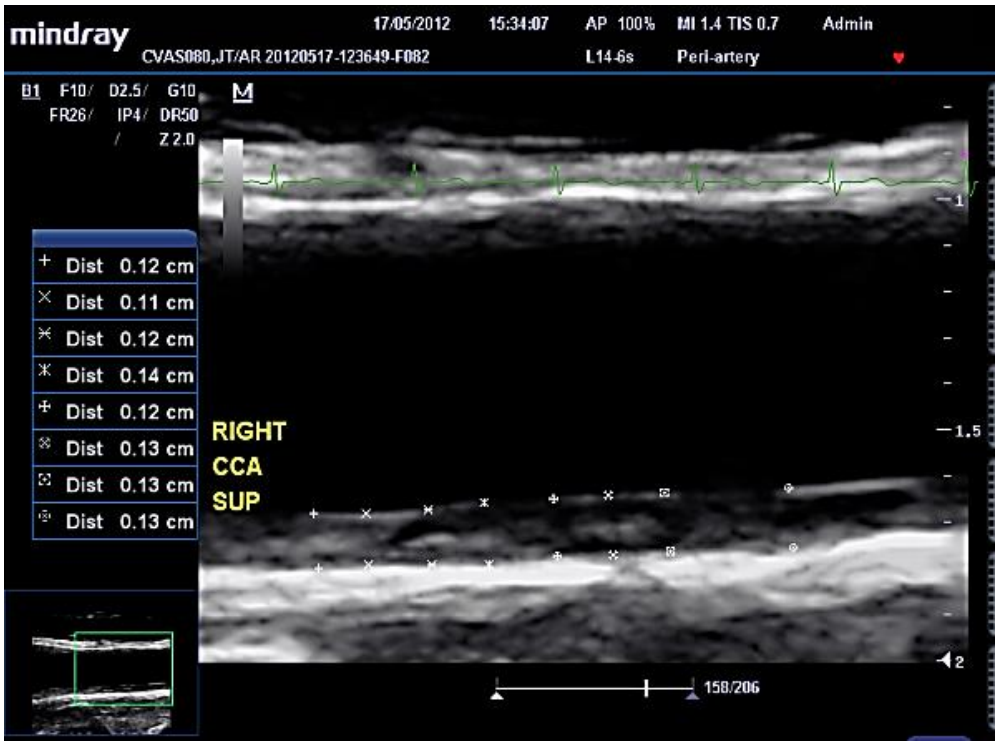

Figure 2-5. Measurement of intima-media thickness.
Representative image for measurement of intima-media thickness of the far wall of the right common carotid artery (CCA) while lying supine (SUP). An average of 8 caliper measurements was taken over approximately 1 cm, at least 0.5 cm proximal to the carotid bifurcation.

provide a better evaluation of arterial pressure leading to the brain. In addition, the carotid pulse wave has been found to be a valid estimate of aortic pulse pressure (Kelly *et al.*, 2006). Briefly, a high-fidelity tonometer (SPT-301, Millar Instruments, Houston TX USA) was held against the right CCA over 20-30 consecutive cardiac cycles. To correct for hold down pressure, the mean and diastolic pressure of the CCA pulse was calibrated against that of the brachial artery as recorded by the Finometer. Mean and diastolic pressures do not change considerably between the carotid and the radial arteries (< 3mmHg), thereby allowing these measures to be used for a two-point calibration of the carotid pulse wave amplitude (O'Rourke *et al.*, 2001).

Arterial Stiffness

Pulse wave velocity was measured within the carotid-femoral (cf-PWV) and brachial-ankle (ba-PWV) circulatory regions (Laurent *et al.*, 2006; Yu *et al.*, 2008). Blood flow velocity pulse waves were recorded for 20-30 cardiac cycles, simultaneously at the common carotid and common femoral arteries, or the brachial and posterior tibial arteries. Pulse waves were recorded by Doppler ultrasound (DWL). The foot of each pulse wave was identified by a combined low-pass filter (15Hz) and 2^{nd} derivative (maximum) algorithm (Figure 2-6; Chiu *et al.*, 1991). The direct distance between the suprasternal notch and each location was measured with an inelastic tape. PWV was computed by dividing the difference in distance between each site by the difference in arrival time of the foot of the wave at each site (Equation 2-6). A threshold cf-PWV of 10 m/s was used to indicate high cerebrovascular risk (Van Bortel *et al.*, 2012).

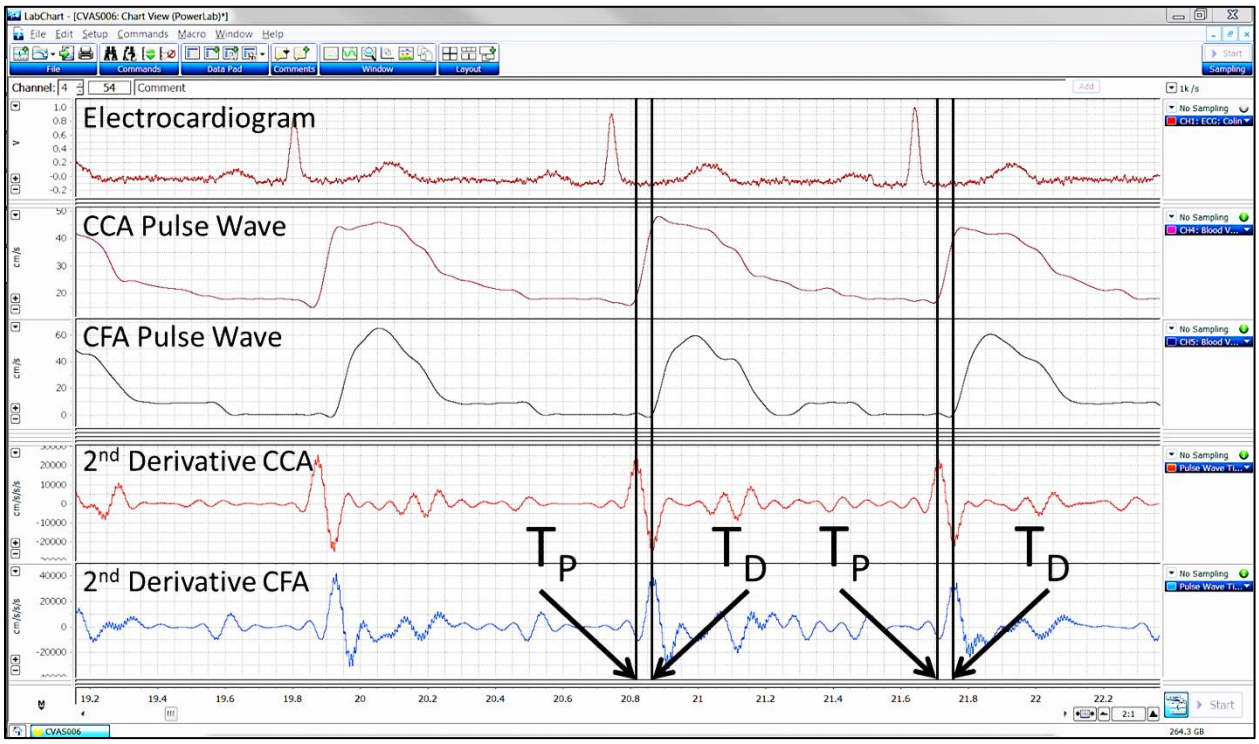

Figure 2-6. Identification of the foot of the pulse wave.
A sample data strip showing the identification of the foot of pulse waves in the common carotid artery (CCA) and common femoral artery (CFA) by calculating the maximum of the 2nd derivative of the waveform (vertical lines). T_P and T_D refer to the time of the proximal and distal waveforms, respectively. The time difference ($T_D - T_P$) is the duration used to calculate carotid-femoral pulse wave velocity (PWV).

$$PWV = (L_D - L_P) / (T_D - T_P)$$

where, L_D and L_P represent the lengths of the distal and proximal arterial sites, and T_D and T_P represent the arrival time of the pulse wave at the distal and proximal sites.

Equation 2-6. Pulse wave velocity

Blood Analysis

A venous blood sample was collected for the determination of a fasting metabolic profile, as well as biomarkers of subclinical inflammation and target organ damage. A fasting 35-mL blood sample was collected by venipuncture (Becton Dickinson, Franklin Lakes NJ, USA) from an antecubital vein (predominantly from the right arm). Following the blood draw, samples were gently inverted and immediately placed on ice. The blood sample was assessed for glucose and HbA1c from whole blood droplets using commercially available kits (Glucose: Accu-Chek Aviva, Roche, Mannheim DE; HbA1c: A1cNow$^+$, Bayer Health Care, Sunnyvale CA USA).

A lipid profile, including total cholesterol, HDL, and TG was completed by a local commercial laboratory (LifeLabs Medical Laboratory Services, Waterloo ON CA). Following the blood draw, samples were allowed to clot, centrifuged at 2500 rpm and stored in a refrigerator (within 45 minutes) until ready for analysis (within 48 hours). Specifically, all three lipid markers were analyzed by enzymatic assays using appropriate reagents with Siemens' ADVIA Chemistry System (Siemens Healthcare Diagnostic Inc., Tarrytown NY USA). LDL was calculated using the Friedewald equation (Friedewald *et al.*, 1972).

To obtain an indication of subclinical inflammation, as well as target organ damage, serum samples were analyzed for C-reactive protein (CRP) and creatinine, respectively. Following the blood draw, samples were allowed to clot before centrifugation at 2500 rpm. Serum was separated by a manual pipetting technique and stored in 1.5 mL Eppendorf tubes

and placed in a -80°C freezer for up to 2 years prior to analysis. CRP was analyzed by a colorimetric enzyme-linked immunosorbent assay (CRP sensitiv [Cat. No. K9710s], ALPCO Immunoassays, Salem NH USA). This test has a reported intra-assay coefficient of variation of 6.0 % and a detection limit of 0.92 ng/mL, which was equivalent to ~ 1% of the lowest value measured in the study. Creatinine was analyzed by modified Jaffé reaction assay (Cat. No. 700460, Cayman Chemical Co., Ann Arbor MI USA). This test has a reported intra-assay coefficient of variation of 6.4 % and a detection limit of 0.1 mg/dL, which was equivalent to ~ 18% of the lowest value measured in the study.

Cognitive Assessment

The Montreal Cognitive Assessment (MoCA) was used to assess general cognitive function. The MoCA incorporates shorter versions of a battery of common neuropsychological tests to assess competency across multiple cognitive domains (Nasreddine *et al.*, 2005). The MoCA has recently been demonstrated to be particularly sensitive to cognitive impairments associated with vascular dysfunction when compared to the more prevalent Mini-Mental State Exam (Ihara *et al.*, 2012; Sikaroodi *et al.*, 2013). In addition, the Trail Making Test (TMT) was used to assess more specific executive-oriented cognitive domains. A close relationship between TMT performance and executive function (*i.e.*, attention, speed, and mental flexibility), as well as frontal lobe integrity, has been demonstrated (Strauss *et al.*, 2006). Depression was assessed by the 15-item Geriatric Depression Scale (Sheikh & Yesavage, 1986). A score > 3 was taken as showing depressive symptoms (de Craen *et al.*, 2003).

Statistical Considerations

All statistical analyses were completed using Statistical Analysis Software v9.2 (SAS Institute, Cary NC, USA). Significance was inferred at $P < 0.05$, and trends were noted if $P < 0.10$.

Normal probability and quantile-quantile plots were assessed to determine if the data were normally distributed. If the data fit a normal distribution, they were expressed as mean ± standard deviation. Non-normal data were expressed as median (interquartile range). Categorical variables were expressed as proportion (count). Accordingly, parametric and non-parametric tests were used in the analysis. Variables of interest were sorted into clinically-relevant risk categories or sex-specific tertiles, where appropriate for analysis. Specific statistical models were discussed within each chapter as they related to the analysis in question.

Methodological Considerations

Participants

The older participants involved in the main component of this thesis were recruited from a database in which they self-enrolled due to an interest in participating in research studies. This self-selection presents the distinct possibility that the sample is biased towards an unusually health-conscious group within the larger population of community-dwelling older adults. As such, extrapolating findings to the general population should be done with caution.

Doppler Ultrasound

Doppler ultrasound has been used as a method to measure blood flow for over 50 years (Franklin *et al.*, 1961). Both peripheral and TCD ultrasound techniques offer distinct advantages of being non-invasive, easy to use, and provide high temporal resolution for blood flow within a cardiac cycle (10-100 Hz). As an incident sound wave hits a moving object, the frequency of that sound wave is shifted in proportion to the speed of the moving object (Equation 2-7).

$$\text{Velocity} = \frac{c}{(2 \cdot F_o \cdot \cos\theta)} F_d,$$

where, c represents the speed of sound through biological tissue; F_o is the transmitted frequency; θ represents the angle on insonation; and F_d represents the Doppler shift (*i.e.*, frequency shift from striking the moving red blood cells), which is directly proportional to velocity.

Equation 2-7. Doppler shift equation for velocity estimation.

Blood flow can then be calculated when considering the diameter or cross-sectional area of the vessel (Equation 2-1). Despite its widespread use, there are a number of sources of error inherent in ultrasonic investigation of blood flow that should be considered. The five major considerations include diameter measurement, angle correction, off-axis sampling, arterial pulsatility, and turbulent or non-laminar flow – each of which is discussed briefly.

Equation 2-1 calculates the area of the vessel from the diameter, thus assuming a circular vessel which is not always held, especially within vessels of older adults, which exhibit age-related structural changes (Ozdogmus *et al.*, 2008). In addition, area is calculated by $\frac{\pi d^2}{4}$ so any error in diameter, d, is amplified in exponential fashion (Hoskins, 1990). To optimize the accuracy of the vessel diameter, attempts can be made to image the artery with a 90° incidence between the ultrasound beam and the arterial wall. This produces short duration, high intensity echoes that provide accurate visualization of the vessel wall (Gill, 1985).

To obtain an accurate velocity, the angle of insonation is of the utmost importance. Optimal Doppler shifts are obtained when the angle of insonation is zero; however, this is not possible in almost all situations (an exception being TCD where the angle is not known, but a zero angle of insonation is assumed for the MCA when the greatest amplitude signal is achieved). As the angle of insonation increases from zero, the impact of measurement error rises. For example, when assuming an insonation angle of 0°, a 5° error would result in a 0.4 %

difference in velocity; however, when assuming an insonation angle of 45°, a 5° error would result in a 9.1 % difference in velocity. To minimize variation in error between participants, the angle of insonation was held in most cases within a relatively small range (53 to 63°) across all participants for the ICA, ECA and VA. Angle of insonation was confirmed by duplex ultrasound allowing the image and Doppler signal to be viewed, simultaneously. The angle was obtained by tilting the ultrasound transducer; however, in doing so, the angle of the vessel has rotated off of 90°, thereby increasing the error in vessel diameter, as discussed above (Gill, 1985).

In addition to the angle of insonation, accurate determination of blood flow velocity depends on proper placement of the sampling window. If the sample volume is too small to encompass the entire vessel or if the sampling is off the axis of the vessel, slower velocities near the vessel wall will be missed, leading to an overestimation of the mean signal. Further, sampling can be impacted by arterial pulsations and respiratory movement which might shift the vessel off of the sampling site (Hoskins, 1990). Care was taken to ensure that the edges of the sampling window were placed on the edges of the vessel and measurements were taken only when a stable pattern of blood flow was observed, increasing confidence that the entire lumen of the vessel was captured.

Arterial pulsations also impact the precise measurement of flow through the changing diameter. For technical reasons, it was not practical to adjust flow measures by tracking pulsatile change in diameter across the cardiac cycle. Rather, an *a priori* decision was made to measure arterial diameter gated to the R-spike in the ECG, representative of diastolic diameter. It is acknowledged that ‘trapping’ the diameter in the diastolic phase leads to a slight underestimation of actual flow. Future work involving the use of novel echo-tracking edge-

detection software during pulsatile ultrasound measurements is needed to establish the magnitude of any bias introduced by using this diastolic-only method.

Finally, turbulence gives rise to blood moving in multiple directions in relation to the ultrasound beam, making a determination of flow error prone (Hoskins, 1990). Turbulent flow is often found at points of branching. To help alleviate any influence of turbulence, a point at least 1 cm distal to the carotid bifurcation was chosen for imaging of the ICA and ECA.

In addition to the precautions mentioned above, replicate measures were taken in each position to help strengthen the validity of the flow. Vessel diameter was measured using 3 sets of calipers and averaged over 3 cardiac cycles. Blood flow velocity was averaged over 10-15 cardiac cycles to help maximize signal-to-noise and minimize low frequency fluctuations in flow, as well as incidental deviations of the sampling window from the vessel axis. Periodic arrhythmias that were observed to dramatically alter MFV were not included in the analysis.

The extracranial method of quantifying CBF used in the current study has a number of benefits including its ease of use and suitability for bed side or clinical exams. In addition, it is more advantageous than TCD in that it can measure quantitative flow. However, there is a subset of the population that is not suited to this technique. Individuals with high carotid bifurcations (under the mandible) make it difficult or impossible to image the internal and external branches of the CCA. As well, increasing tortuosity with age and a high degree of stenosis creates turbulent flow and off-axis sampling, increasing the potential for measurement inaccuracies.

In the main component of this thesis, 81 individuals were recruited and underwent the vascular assessment. Measures in the bilateral ICA were obtained in 73 individuals and bilateral vertebral arteries were obtained in 63 individuals. Reasons for exclusion included

carotid bifurcation located too far under the mandible or unclear Doppler tracing. Bilateral ICA and VA blood flow was obtained in 76 % of participants while supine. Imaging of the ECA was added to the protocol at a later date, thus only 22 measures of bilateral ECA flow were obtained. Further, a change in ultrasound equipment from Sonosite to Mindray after 33 participants allowed for off-line measurement of the peak velocity trace. This peak trace was needed to calculate PI, CrCP and RAP in the extracranial vessels, thus fewer participants were included in the analysis of these data.

TCD monitoring was successful in 68 of 81 participants (note that there were individuals with whom a TCD signal, but not a reliable ICA measure, was obtained and vice versa). TCD was only measured on the right side. Assumptions were made about bilateral symmetry (Schmidt *et al.*, 2003), which is supported by comparing the right and left extracranial measurements (Table 2-2). Traditionally, TCD is limited in about 25 % of older adults because of structural interference of the temporal bone on the ultrasound signal (Itoh *et al.*, 1993). Characteristics of the participants with and without TCD are compared in Table 2-3. Participants without TCD were generally older and had lower anterior CBF. Although extracranial CBF and transcranial MFV can be used together to provide valuable measures of cerebral hemodynamics, these techniques cannot provide information on cerebral metabolism (Sugimori *et al.*, 1995) or tissue perfusion, and thus findings need to be viewed through a lens of the macrovasculature.

Cerebrovascular Reactivity

The presence of a sigmoidal relationship between CBF and PCO_2 when examined over a wide range of PCO_2 suggests that hypocapnic and hypercapnic responses should be examined separately (Ide *et al.*, 2003; Claassen *et al.*, 2007). In the current study, CR_{CO2} was determined

from a combination of hypercapnic gas and hypocapnic hyperventilation, assuming a linear relationship across the range of PCO_2 that was achieved. A linear relationship was assumed *post-hoc* based on the observation that hypercapnic and hypocapnic responses were similar (hypercapnic CR_{CO2}: 3.5 ± 1.3 vs. hypocapnic 3.5 ± 1.6 %/mmHg CO_2) and the range of PCO_2 achieved using the current interventions was narrow (~ 15 mmHg).

Blood Samples

Of note, the creatinine assay recommends performing the test within 2 months of collection. Due to the need to build the sample size prior to performing the analysis, earlier samples were stored for up to 2 years at -80° C. To assess whether this delay had a systematic bias against more recent samples, the measurements were examined by participant order. No effect was observed (Spearman correlation coefficient: -0.18, $P > 0.10$).

Table 2-2. Comparison of extracranial hemodynamics by hemisphere

Artery	Variable	n	Hemisphere		*P* value
			RIGHT	LEFT	
External Carotid	Diameter, cm	23	0.40 ± 0.07	0.40 ± 0.08	0.657
	Mean Velocity, cm/s	22	15.1 ± 4.5	16.4 ± 4.1	0.284
	Pulsatility Index	20	2.48 ± 0.60	2.40 ± 0.66	0.186
	CrCP, mmHg	17	46.2 ± 12.2	45.9 ± 11.8	0.439
	RAP, mmHg/cm/s	17	1.96 ± 0.62	1.96 ± 0.51	0.664
Internal Carotid	Diameter, cm	73	0.51 ± 0.10	0.51 ± 0.09	0.730
	Mean Velocity, cm/s	73	22.8 ± 6.6	24.7 ± 7.4	0.051
	Pulsatility Index	37	1.18 ± 0.25	1.12 ± 0.14	0.084
	CrCP, mmHg	32	20.8 ± 14.5	23.9 ± 14.6	0.247
	RAP, mmHg/cm/s	32	2.31 ± 0.83	2.24 ± 0.73	0.648
Vertebral	Diameter, cm	63	0.36 ± 0.06	0.39 ± 0.06	**0.022**
	Mean Velocity, cm/s	63	12.1 ± 4.7	12.4 ± 3.7	0.451
	Pulsatility Index	30	1.53 ± 0.42	1.57 ± 0.46	0.981
	CrCP, mmHg	26	34.8 ± 16.4	38.1 ± 16.2	0.779
	RAP, mmHg/cm/s	26	3.14 ± 1.24	3.21 ± 1.46	0.738

Abbreviations: CrCP – critical closing pressure; RAP – resistance area product.

Data were presented as mean ± SD.

P values represent side-to-side differences determined by a paired *t*-test.

Table 2-3. Comparison of participants with and without successful TCD window

Characteristic	With TCD (n= 68)	Without TCD (n = 13)	*P* value
Age, years	73.3 ± 5.6	78.1 ± 7.0	**0.008**
Sex, % women (n)	60.3 (41)	71.4 (10)	0.183
Education, years	14.9 ± 3.8	15.1 ± 4.6	0.853
MoCA, score of 30	28.3 ± 1.3	27.4 ± 2.0	0.139
GDS, score of 15	0.8 ± 1.2	0.7 ± 0.8	0.893
Body Mass Index, kg/m^2	26.7 ± 3.8	28.8 ± 4.2	0.068
Waist:Hip, ratio	0.89 ± 0.09	0.92 ± 0.10	0.256
TMT A, s	29.0 ± 7.6	34.2 ± 12.8	0.164
TMT B, s	70.4 ± 32.3	92.2 ± 47.8	0.122
TMT B-A, s	41.3 ± 30.0	58.0 ± 39.8	0.071
Dom. Grip Pressure, kPa	51 ± 23	44 ± 22	0.255
Dom. Finger Tapping, count (57/12)	42 ± 8	39 ± 10	0.188
Usual Walking Speed, m/s	1.18 ± 0.18	1.13 ± 0.26	0.405
Steps, count/day	7300 ± 2890	6220 ± 3160	0.219
Physical Activity, kcal/day	930 ± 690	770 ± 680	0.428
Falls in past year, % yes (n)	25.0 (17)	21.4 (3)	0.264
Arterial Structure/Function			
cPP, mmHg	52.6 ± 14.5	55.8 ± 14.6	0.479
cf-PWV, cm/s	9.0 ± 1.7	9.5 ± 1.8	0.356
ba-PWV, cm/s	11.0 ± 1.6	11.6 ± 1.6	0.216
IMT, mm	0.78 ± 0.21	0.78 ± 0.11	0.911
CRP, mg/L	2.2 ± 2.0	2.6 ± 2.3	0.518
Creatinine, μmol/L	92.9 ± 22.0	89.9 ± 29.5	0.675
Supine CBF Characteristics			
Total, mL/min (49/10)	740 ± 150	630 ± 80	**0.002**
Anterior, mL/min (60/13)	560 ± 130	490 ± 90	**0.041**
Posterior, mL/min (53/10)	170 ± 60	150 ± 40	0.288

Abbreviations: MoCA – Montreal Cognitive Assessment; GDS – Geriatric Depression Scale; TMT – Trail Making Test; cPP – carotid pulse pressure; PWV – pulse wave velocity (cf – carotid-femoral, ba – brachial-ankle); IMT – intima-media thickness; CRP – C-reactive protein; CBF – cerebral blood flow.

Data were presented as mean ± SD or proportion (count). Sample size variation for Finger Tapping and CBF Characteristics (with/without TCD) is indicated in parentheses under 'Characteristic'.

P values represent side-to-side differences determined by an unpaired *t*-test.

CHAPTER 3. ASSOCIATIONS OF PHYSICAL ACTIVITY AND SLEEP HABITS WITH CEREBROVASCULAR REGULATION IN OLDER ADULTS

Introduction and Rationale

Lifestyle factors play an important role in the outlook of long term health and independence. Habitual physical activity has been shown to protect against morbidity and mortality in older adults (Stessman *et al.*, 2009). Further, higher levels of active energy expenditure are associated with improved functional performance, prolonged independence (Brach *et al.*, 2004; Sattler *et al.*, 2011), and reduced incidence of cognitive impairment (Middleton *et al.*, 2011; Vercambre *et al.*, 2011; Vidoni *et al.*, 2012). In addition to physical activity, sleep quality is an important predictor of health with a distinct U-shape distribution linking sleep duration to mortality and morbidity. Both short ($\leq$ 6 hours) and long ($\geq$ 9 hours) sleep duration are associated with greater risk for myocardial infarction, heart disease, and stroke (Sabanayagam & Shankar, 2010; Magee *et al.*, 2012). Recently, the interaction of habitual physical activity and sleep has been explored with exercise training leading to improved sleep patterns and reduced fatigue in older adults (Reid *et al.*, 2010; Lira *et al.*, 2011; Valentine *et al.*, 2011).

Despite significant evidence linking physical activity to brain function, little is known about how these lifestyle characteristics impact cerebrovascular health, and cerebral blood flow (CBF) in particular as a mediating factor. Increased physical activity levels were shown, in a 1990 study, to attenuate a 4-year decline in CBF in older adults (Rogers *et al.*, 1990). However, only recently has considerable focus been given to the role of cerebrovascular health within the constraints of the habitual physical activity-cognition relationship (Brown *et al.*, 2010; Eskes *et al.*, 2010; Davenport *et al.*, 2012). Particular focus has been placed on the role of exercise training or fitness on CBF. Elevated middle cerebral artery (MCA) mean blood flow velocity

(MFV) has been reported in endurance-trained compared to sedentary older adults (Franke *et al.*, 2006; Ainslie *et al.*, 2008b). In addition to these indices of greater flow, cerebrovascular reactivity appears to benefit from fitness. A 6-month aerobic training program was found to increase cerebrovascular reactivity to carbon dioxide (CR_{CO2}) in stroke survivors (Ivey *et al.*, 2011). As well, cerebrovascular conductance during hypercapnia was directly related to fitness in a group of post-menopausal women (Brown *et al.*, 2010). Although the evidence is supportive of the notion that exercise training can raise CBF and can improve cerebrovascular health, the impact of a habitually active lifestyle remains uncertain.

Sleep quality is a second lifestyle characteristic that has, until recently, received little attention from a cerebrovascular perspective in older adults. Sleep is a major issue in the elderly population as the prevalence of sleep disturbances is over 50 % in community living older adults (Foley *et al.*, 1995). Qureshi *et al.* (1999) observed that sleep fragmentation in middle-aged adults was associated with morning reductions in MFV and CR_{CO2}. Studies involving habitually short-duration sleepers, as well as sleep deprivation techniques, reported that shorter sleep was associated with endothelial dysfunction in peripheral vascular beds (Weil *et al.*, 2010; Sauvet *et al.*, 2010). These latter studies examined middle-aged and young adult cohorts, respectively. Most research examining the link between sleep and cerebrovascular health involves sleep apnea, which has been associated with increased oxidative stress and endothelial dysfunction (Furtner *et al.*, 2009; Kiratli *et al.*, 2010). However, there is a lack of literature regarding sleep quality and cerebrovascular characteristics in healthy older adults.

Insight into the biological mechanism relating habitual physical activity and sleep duration to cerebrovascular health might be gained from examination of metabolic and inflammatory profiles (Valentine *et al.*, 2009; Giannopoulos *et al.*, 2010). In a recent review, Knutson (2010) posited the link between sleep duration and obesity, diabetes mellitus and

hypertension, to be mediated by altered glucose utilization and/or hormonal balance. The interaction of these two lifestyle characteristics might significantly influence health outcomes and remains an important area of interest for our aging population.

The purpose of this chapter was to characterize cerebrovascular health in older adults from the perspective of habitual physical activity and sleep quality, using both objective and subjective methods. It was hypothesized that individuals who engaged in a more active lifestyle would exhibit greater CBF and CR_{CO2}. Further, a curvilinear relationship between sleep duration and these same cerebrovascular characteristics was expected, such that both very short and very long sleep duration would be associated with poorer vascular health. Metabolic profile and C-reactive protein (CRP), as a marker of inflammation, were surveyed as potential mediators of a relationship between behaviour and vascular health.

Methods

This chapter discusses the cohort and cerebrovascular assessment described in Chapter 2.

Habitual Physical Activity

Active Energy Expenditure

Physical activity characteristics were measured using the Sensewear™ Body Monitoring System™ (Body Media Inc., Pittsburgh PA USA). This system continuously monitors energy expenditure by an armband that is worn around the lateral head of the triceps on the right arm. The armband samples data at 1-minute epochs from a 2-axis (longitudinal x transverse) accelerometer (Range: ± 2.00 g; Resolution: 0.01 g), heat flux monitor (0-300 W/m^2; 1 W/m^2), skin temperature thermometer (20 – 40 °C; 0.05 °C), galvanic skin response gauge (0 – 17 μSiemens; 0.0083 μSiemens), and near body temperature thermometer. These data were used in a proprietary algorithm (Sensewear Professional V6.1), along with participant sex, age,

height, weight, smoking status and handedness, to estimate energy expenditure (EE). The Sensewear system differentiates between activity intensity by examining EE over 2 consecutive minutes. A standard threshold of 3 METS was used to classify moderate intensity levels (Pate *et al.*, 1995). The Sensewear system has been shown to be a reliable estimator of resting EE in community-living adults 60-87 years of age, even though it tended to overestimate compared to indirect calorimetry (Heiermann *et al.*, 2011). A separate validation study in older adults found agreement between the doubly-labeled water method and Sensewear-predicted measures of total EE ($r = 0.89$, intraclass correlation coefficient = 0.90). Agreement was lower, yet still acceptable, for active EE ($r = 0.76$, ICC = 0.64) with the armband underestimating true EE (Mackey *et al.*, 2011).

Participants were asked to wear the Sensewear for 3 consecutive days (removing only for personal care or water activities) and instructed to behave in their usual manner. Three days of accelerometer-based physical activity tracking has previously been shown to provide sufficient accuracy for physical activity in older adults (Hart *et al.*, 2011). In addition, Chipperfield (2008) noted no difference in everyday physical activity between weekdays and weekends for older adults using an accelerometer, so no specific preference of wearing schedule was controlled. The 79 g armband was not expected to cause any discomfort, however a number of participants removed it for brief periods due to reported discomfort. The final analysis included participants who wore the armband for at least 90% of the time over at least 48 hours, and was expressed as daily averages. Lifestyle activity was characterized as active EE (AEE), which controlled for differences in resting metabolic rate (RMR). Sleeping metabolic rate, used as a proxy for RMR, was taken as the average EE over the last 30 minutes prior to waking up after a night's sleep. AEE was then calculated as (Total EE – RMR) x 0.9, where 10 % is assumed for the thermal effect of meals (Middleton *et al.*, 2011). Physical

activity EE (PAEE) is the energy expended by activities at greater than 3 METS. AEE of 2719 kcal/week, representing the 25th percentile of physical activity in the large Health ABC population study cohort, was used to identify inactive participants (Brach *et al.*, 2004).

Self-Reported Physical Activity

To supplement the Sensewear data, participants were asked to describe their habitual physical activity in the recent past. Participants were oriented to physical activity as "*any form of body movement that requires effort, but does not include routine activities of daily living such as self-care and cooking. Physical activity can be required for work or transportation or pleasure*". Examples of *Light, Moderate* and *Hard* intensities were given in relation to a numerical scale (where 0 is *sitting quietly*, and 10 is *all-out effort*), as well as a descriptive scale (relative sweating and breathing rates). Participants were asked to list all specific physical activities that they performed regularly over the past 4 months, specifying the frequency, duration and intensity of each activity. Activities listed as being performed at least 3 days/week and 20 min/day, with at least 50% of the time spent at *Moderate* or *Hard* intensities were considered as an indication of regular exercise. Participants were also asked to gauge the number of days/week on average they engaged in *Light*, *Moderate* or *Hard* activities (minimum 20-minute bouts) over the past year, and were subsequently grouped into Sedentary, Active and Highly Active categories (see Appendix A (section 6) for PA questionnaire; see Appendix B for questionnaire scoring).

Sleep Analysis

Actigraph-derived Sleep Quality

Sleep quality was assessed using the Sensewear armband over 3 consecutive days. The armband predicts sleep in binary (0 = wakefulness, 1 = any stage sleep), as well as determines when the wearer is lying down vs. upright. Determination of sleep or wakefulness is based on a

proprietary algorithm (incorporating data from the bi-axial accelerometer, heat flux sensor, and skin temperature) and parceled into 1-minute epochs. Sleep efficiency is calculated as time spent sleeping / time spent lying down. Sensewear has provided an acceptable sensitivity (0.83-0.88), but low specificity (0.50-0.73), for sleep/wake determination when compared to sleep diaries and polysomnographs (van Wouwe *et al.*, 2011; O'Driscoll *et al.*, 2013). Consequently, the device provided reliable measures of sleep quantity, but not sleep efficiency (O'Driscoll *et al.*, 2013).

Self-reported Sleep Quality

Participants were asked the following sleep hygiene questions: *"How many hours do you sleep in 24 hours? (6 h or less, 7 h, 8 h or 9 h or more); Have you had any of the following symptoms within the last 30 days? Sleeping disorders or insomnia (Y/N), Fatigue or tiredness (Y/N)"*. In addition, participants completed the Epworth Sleepiness Scale as an indicator of daytime sleepiness (http://www.sleepfoundation.org/sleep-scale; Johns, 1991).

Statistical Analysis

Continuous data were presented as mean ± SD and categorical data were presented as proportions (counts). Non-normal data were presented as median (interquartile range). Student's *t*-test and chi-square analyses were used for comparison of continuous and categorical variables, respectively. Between groups, non-normal data were analyzed using the Mann-Whitney U test. Where category sample size was ≤ 5, Fisher's exact test (FET) was used. Non-normal data were transformed where appropriate prior to correlation and regression analyses. Specifically, age, physical activity duration and CRP underwent log transformation. Bivariate Pearson product correlations following adjustment for age and sex, and multivariate linear regression analyses were used to assess relationships between habitual physical activity levels and sleep duration with cerebrovascular characteristics. A progressive series of models

were used involving the following covariates: age, sex, metabolic syndrome, and CRP. The relationships between cerebrovascular regulation (anterior CBF and CR_{CO2}) and both self-reported sleep duration and physical activity level were examined by a one-way analysis of covariance (ANCOVA), with adjustment for age and sex. Where F-tests were significant, post-hoc comparison between least square means was performed with the Tukey-Kramer adjustment. The interaction between habitual and physical activity was assessed by correlation. Significance was inferred at $P < 0.05$, and trends were noted at $P < 0.10$. All statistical analyses were completed using Statistical Analysis Software v9.2 (SAS Institute, Cary NC USA).

Results

For 17 participants, technical difficulties prevented 3 consecutive days of Sensewear monitoring. Data from 2 consecutive days were used to estimate activity in 12 of these participants. Bland-Altman analyses compared 2-day average to 3-day average AEE and PAEE from the 64 participants for whom 3-day observations were available (Bland & Altman, 1999). The limits of agreement between 2-day and 3-day data were close to zero (estimated bias ± 1.96SD; AEE: -9 ± 247 kcal/day; log(PAEE/[kcal/day]): -0.01 ± 0.30), and this difference was consistent across the range of absolute activity levels (Pearson correlation between difference and average; AEE: $r_{(n=64)} = -0.16$, $P = 0.203$; log(PAEE/[kcal/day]): $r_{(n=64)} = -0.11$, $P = 0.384$). Therefore, the 2-day averages were incorporated into the sample with confidence. In addition, no differences in activity levels were noted for participants recruited during the winter months (November-April; $P > 0.6$) or for participants whose activity period included weekends ($P > 0.4$).

The final cohort for analysis in this chapter included 29 men and 47 women. Participant characteristics as a group are presented in Table 3-1. There were no sex-based differences for age, race, education, and scores on the Montreal Cognitive Assessment (MoCA) or Geriatric Depression Scale (GDS). A few metabolically relevant differences were noted between men and women. Men had a larger waist-to-hip ratio than women (men: 0.98 ± 0.06 vs. women: 0.84 ± 0.07; $t_{72} = 8.89$, $P < 0.001$). In contrast, men had lower cholesterol levels (total cholesterol: 4.49 ± 0.82 vs. 5.46 ± 0.84 mmol/L; $t_{74} = -4.95$, $P < 0.001$; low-density lipoprotein: 2.66 ± 0.69 vs. 3.30 ± 0.77 mmol/L; $t_{74} = -3.70$, $P < 0.001$; and high-density lipoprotein: 1.22 ± 0.33 vs. 1.60 ± 0.36 mmol/L; $t_{74} = -4.57$, $P < 0.001$) and a greater proportion of men were taking statin medication [44.8 % (13 of 29) vs. 17.0 % (8 of 47); $\chi^2_{(1,n=76)} = 6.9$, $P = 0.008$]. The presence of metabolic syndrome was assessed as a comprehensive metabolic score. No difference in the proportion of men and women with metabolic syndrome was observed [men: 48.3 % (14 of 29) vs. women: 34.0 % (16 of 47); $\chi^2_{(1,n=76)} = 1.5$, $P = 0.218$].

Subjective and objective physical activity and sleep characteristics, separated by sex, are presented in Table 3-2. Objectively-measured physical activity parameters showed that men had greater AEE and PAEE than women. Although men did not spend significantly more time engaging in physical activity, the intensity of their activity was greater, leading to the higher daily energy output. As a whole, the older cohort examined here was considered active. Only 6.9 % (2 of 29) of men and 21.3 % (10 of 47) of women averaged activity levels below 2719 kcal/week (a threshold of AEE based on published guidelines; Brach *et al.*, 2004) ($FET_{(n=76)}$, $P = 0.116$). In addition, 75.8 % of men and 70.2 % of women averaged at least 30 minutes of moderate-intensity physical activity per day over the monitoring period ($\chi^2_{(1,n=76)} = 0.3$, $P = 0.593$).

No differences were noted between men and women for subjectively reported physical activity levels, although in contrast to the objectively measured data, 59.1 % considered themselves to lead a sedentary lifestyle. Ten participants did not complete the self-report questionnaire.

Overall, both objective and subjective sleep characteristics were similar between men and women (Table 3-2). Two exceptions included a trend for more women to report feeling fatigued, and for men to score higher on the Epworth sleepiness scale as an indication of their daytime drowsiness.

Cerebrovascular characteristics are presented in Table 3-3. Quantitative anterior CBF tended to be lower in women than men; however, women had a higher MFV and lower cerebrovascular resistance index (CVRi) in the MCA. A significant relationship between anterior CBF and self-reported physical activity level was noted, after adjusting for age and sex (Figure 3-1, top). Post-hoc analysis revealed a trend for greater anterior CBF in exercising individuals compared to the sedentary group ($P = 0.060$). In contrast, no effect of self-reported physical activity was noted for CR_{CO2} (Figure 3-1, bottom). The relationship between self-reported sleep duration and cerebrovascular characteristics showed that longer sleep had a negative association with anterior CBF (Figure 3-2, top), including a trend for lower CBF with sleep longer than 7 hours ($P = 0.071$) in post hoc analysis. Also, sleep duration of 6 hours or less was associated with lower CR_{CO2} than 8 hours of sleep (Figure 3-2, bottom; $P = 0.027$).

A partial correlation analysis using objectively-measured continuous variables of physical activity and sleep duration, adjusting for age and sex, revealed similar relationships to the subjective measures (Table 3-4). Anterior CBF showed a trend for a direct relationship with PAEE (n=70, $P = 0.085$) and duration of physical activity (n = 70, $P = 0.070$). This apparent

connection was only present in the anterior circulation, and not the posterior CBF. In contrast, other characteristics of cerebrovascular resistance were not related to habitual physical activity.

As was noted with the subjective measures, CR_{CO2} was directly related to sleep duration (n = 58, $P = 0.034$), but not physical activity levels. Sleep duration was unrelated to any other cerebrovascular characteristic, although there was a trend for reduced posterior CBF with longer sleep (n = 59, $P = 0.055$). The self-reported Epworth Sleepiness Scale was not related to any cerebrovascular measure. Eight of the 76 participants (10.5 %) reported a diagnosis of sleep apnea. Participants who reported being diagnosed with sleep apnea had similar anterior CBF ($P = 0.544$), posterior CBF ($P = 0.510$) and CR_{CO2} ($P = 0.399$), compared to the rest of the sample.

Metabolic factors and inflammation were considered as possible underlying factors mediating the relationship between low physical activity level, short sleep duration and cerebrovascular health. However, when considering sex-adjusted tertiles, the rates of metabolic syndrome and high-risk concentration of CRP in the lowest tertile of PAEE and the lowest tertile of sleep duration were similar to those in the whole collection of older adults (Table 3-5). Multiple linear regression analyses (Table 3-6) found that physical activity duration was directly related to greater anterior CBF. This relationship tended to hold independent of age and sex; however, the further inclusion of the presence of metabolic syndrome and CRP levels did not improve the amount of variance explained by the model. Models predicting CR_{CO2} with sleep duration became significant only after age and sex were included as covariates, and not when metabolic syndrome and CRP were added. Of note, all models that were examined had explained variances below 15 %, suggesting that alternative unaccounted for factors are primary contributors to differences in CBF and CR_{CO2} within the current elderly cohort.

A valid model for anterior CBF or CR_{CO2} that included both physical activity duration and sleep duration was not found. Unexpectedly, inverse relationships between AEE and sleep duration ($r_{(n=76)}$ = -0.30, P = 0.009), as well as between steps per day and sleep duration ($r_{(n=76)}$ = -0.25, P = 0.031) were observed.

Table 3-1. Participant characteristics

Characteristic	All (N = 76)
Age, years §	73.7 (8.2)
Sex (women), % (n)	62 (47)
Race/Ethnicity, % (n)	
Non-Hispanic, White	96 (73)
Chinese	1.3 (1)
Euroasian	1.3 (1)
American Indian	1.3 (1)
Education, % (n)	
< 12 years	23.7 (18)
≥ 12 years	76.3 (58)
Montreal Cognitive Assessment, score out of 30 §	28.2 (2.0)
Geriatric Depression Scale, score out of 15 §	0 (1)
Vascular Risk Factors	
Systolic Blood Pressure, mmHg	127.8 ± 16.2
Diastolic Blood Pressure, mmHg	69.4 ± 8.9
Body Mass Index, kg/cm^2	27.0 ± 4.0
Waist:Hip, ratio	0.89 ± 0.09
Low Density Lipoprotein, mmol/L	3.05 ± 0.80
High Density Lipoprotein, mmol/L	1.45 ± 0.39
Total Cholesterol, mmol/L	5.09 ± 0.95
Triglycerides, mmol/L §	1.15 (0.80)
Glucose, mmol/L §	5.40 (0.70)
HbA1c, % §	5.30 (0.50)
Smoking (ever), % (n)	47.4 (36)
Smoking (current), % (n)	6.6 (5)
Frequent Alcohol Intake, % (n)	2.6 (2)
Prior CVD†, % (n)	2.6 (2)
Family History – CVD, % (n)	34.2 (26)
Family History – stroke, % (n)	15.8 (12)
Medications	
Polypharmacy, % (n)‡	9.2 (7)
Anti-hypertensive, % (n)	
ACEi	14.5 (11)
ARB	11.8 (9)
CCB	2.6 (2)
β-Blocker	7.9 (6)
Diuretic	22.4 (17)
ASA	21.0 (16)
Anti-hyperlipidemic, % (n)	
Statin	27.6 (21)
Anti-diabetics/Hypoglycemic, % (n)	
Insulin	2.6 (2)
Hypoglycemic	4.0 (3)

Abbreviations: HbA1c – glycolated hemoglobin; CVD – cardiovascular disease; ACEi – angiotensin converting enzyme inhibitor; ARB – angiotensin II receptor blocker; CCB – calcium channel blocker; ASA – acetylsalicylic acid.

Data were presented as mean ± SD for continuous variables and proportion (count) for categorical variables.

§ Age, scores on the Montreal Cognitive Assessment and Geriatric Depression Scale, triglycerides, glucose and HbA1c were not normally distributed and are presented as median (interquartile range).

† CVD is inclusive of myocardial infarction and peripheral arterial disease.

‡ Polypharmacy represents taking 4 or more medications, including all that are listed, as well as sleep or sedative medication (see Table 3-2).

Table 3-2. Prevalence of physical activity and sleep characteristics

Characteristic	All (N = 76)	Men (n = 29)	Women (n = 47)	*P* value
Physical Activity				
Self-report (questionnaire)				
Activity Level , % (n)				0.143
Sedentary	59.1 (39)	44.4 (12)	69.2 (27)	
Active	21.2 (14)	29.6 (8)	15.4 (6)	
Highly Active	19.7 (13)	25.9 (7)	15.4 (6)	
Perform at least one regular activity, % (n) †	66.2 (43)	76.9 (20)	59.0 (23)	0.183
Belongs to fitness club or group, % (n)	42.7 (32)	32.1 (9)	48.9 (23)	0.155
Objective (Sensewear Armband)				
Active Energy Expenditure, kcal/week	4530 ± 1808	5330 ± 1820	4040 ± 1630	**0.002**
PA Energy Expenditure, kcal/week §	1550 (2020)	2650 (2630)	1430 (1490)	**0.018**
Duration of PA, min/day §	59 (70)	72 (77)	53 (65)	0.165
PA Intensity, kcal/min	3.7 ± 0.8	4.2 ± 0.9	3.4 ± 0.6	**0.001**
Steps, count/day	7130 ± 2960	7320 ± 3180	7010 ± 2840	0.667
Sleep				
Self-report (questionnaire)				
Sleep Duration, % (n)				0.271
≤ 6 hours	13.2 (10)	10.3 (3)	14.9 (7)	
7 hours	47.4 (36)	44.8 (13)	48.9 (23)	
8 hours	34.2 (26)	44.8 (13)	27.7 (13)	
≥ 9 hours	5.3 (4)	0 (0)	8.5 (4)	
Insomnia/Sleeping Difficulty, % (n)	26.3 (20)	17.2 (5)	31.9 (15)	0.188
Fatigue/Tiredness, % (n)	50 (38)	37.9 (11)	57.4 (27)	0.098
Sleep Apnea, % (n)	10.5 (8)	17.2 (5)	6.4 (3)	0.247
Sedative Medications, % (n) ‡	14.5 (11)	6.9 (2)	19.2 (9)	0.189
Epworth Sleepiness Score, score out of 21	7.2 ± 3.4	8.2 ± 3.1	6.6 ± 3.4	**0.045**
Objective (Sensewear Armband)				
Sleep Duration, min/day	400 ± 90	410 ± 100	390 ± 80	0.462
Sleep Efficiency, % lying down §	84 (11)	84 (9)	84 (13)	0.831

Data were presented as mean ± SD for continuous variables and proportion (count) for categorical variables, except where noted.

§ Physical activity (PA) energy expenditure, duration of physical activity, and sleep efficiency were not normally distributed and are presented as median (interquartile range).

† A specific activity which participants reported performing at least 3 times per week, at least 20 minutes per session, at a moderate or higher effort for at least 50% of the time. Only 65 participants (26 men, 39 women) completed this question.

‡ Medications considered to assist sleep included benzodiazepines (n = 5), non-benzodiazepines (1), anti-depressants (5), and dimenhydrinates (1).

Table 3-3. Cerebrovascular hemodynamics of older adults separated by sex

Characteristic	n (men/women)	All	Men	Women	*P* value
Total CBF, mL/min	21/36	720 ± 150	730 ± 120	710 ± 160	0.505
Anterior CBF, mL/min	27/43	550 ± 130	580 ± 100	530 ± 140	0.063
Posterior CBF, mL/min	21/38	170 ± 60	160 ± 50	170 ± 60	0.369
CVR, mmHg/L/min §	27/43	170 (70)	170 (40)	190 (80)	0.130
MFV, cm/s §	28/37	53.4 (14.4)	48.4 (9.8)	58.1 (18.1)	**<0.001**
CVRi, mmHg/cm/s §	28/37	1.78 (0.66)	2.11 (0.61)	1.58 (0.53)	**<0.001**
PI, ratio	28/37	0.90 ± 0.16	0.86 ± 0.14	0.93 ± 0.16	0.123
CrCP, mmHg	28/37	31.9 ± 12.0	34.9 ± 12.8	29.7 ± 11.1	0.086
RAP, mmHg/cm/s §	28/37	1.18 (0.39)	1.23 (0.66)	1.08 (0.41)	**0.042**
CR_{CO2}, %/mmHg CO_2	26/32	4.15 ± 1.09	3.91 ± 1.24	4.34 ± 0.93	0.133

Abbreviations: CBF – cerebral blood flow; CVR – cerebrovascular resistance; CVRi – cerebrovascular resistance index; MCA MFV – middle cerebral artery mean flow velocity; PI – pulsatility index; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide.

Data were presented as mean ± SD, except where noted.

§ CVR, MFV, CVRi, and RAP were not normally distributed and are presented as median (interquartile range).

Complete cerebrovascular measures were not obtained. Sample sizes are indicated under ‘n’.

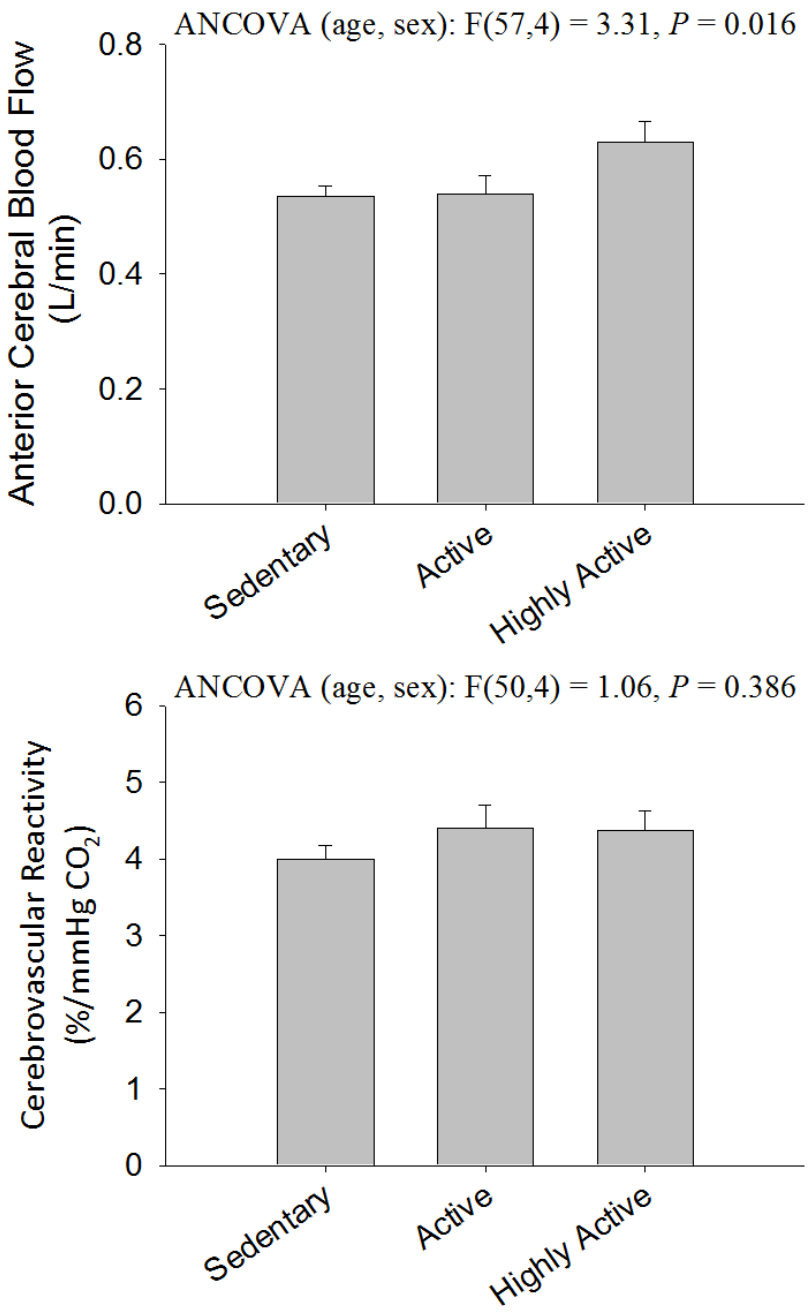

Figure 3-1. Cerebral blood flow and cerebrovascular reactivity by self-reported physical activity level.

Anterior cerebral blood flow (top) and cerebrovascular reactivity to carbon dioxide (bottom) as related to physical activity level from a self-report questionnaire. Data were presented as least square means ± standard error; output from one-way ANCOVA, with age and sex as covariates. Sample sizes for each category are listed in Table 3-2.

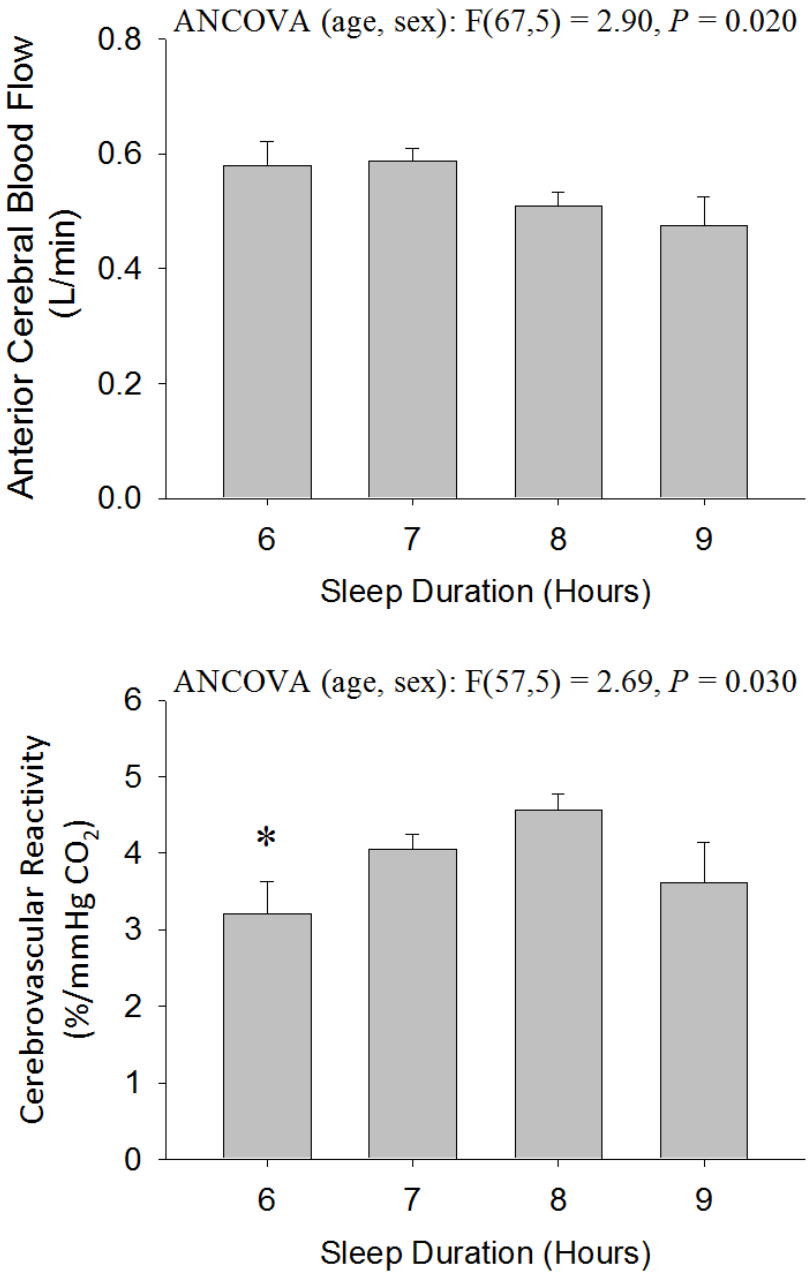

Figure 3-2. Cerebral blood flow and cerebrovascular reactivity by self-reported sleep duration.

Anterior cerebral blood flow (top) and cerebrovascular reactivity to carbon dioxide (bottom) as related to sleep duration on self-report questionnaire. Data were presented as least square means ± standard error; output from one-way ANCOVA, with age and sex as covariates.

* different from 8-hour sleep duration, $P < 0.05$ in post-hoc analysis

Table 3-4. Partial correlations between cerebrovascular hemodynamics and objective characteristics of physical activity and sleep in older adults, adjusted for age and sex

	AEE	PAEE §	PA Duration §	PA Intensity	Steps	Sleep Duration	Epworth Score
Total CBF	0.19	0.18	0.18	-0.03	0.18	-0.14	0.13
Anterior CBF	0.18	**0.21***	**0.22***	-0.08	0.18	-0.12	0.05
Posterior CBF	0.10	0.02	0.01	0.03	0.02	**-0.26***	0.22
CVR §	-0.14	-0.16	-0.18	0.05	-0.16	0.01	-0.03
MFV §	-0.06	-0.06	-0.06	-0.05	-0.03	0.00	-0.07
CVRi §	0.03	0.04	0.02	0.03	0.02	-0.10	0.02
PI	0.02	0.14	0.14	-0.13	0.06	0.18	-0.10
CrCP	0.08	0.12	0.08	0.08	0.05	-0.03	0.08
RAP §	-0.04	-0.04	-0.04	-0.03	-0.03	-0.10	-0.07
CR_{CO2}	-0.22	-0.14	-0.16	0.07	-0.10	**0.28****	0.16

Abbreviations: AEE – active energy expenditure; PAEE – physical activity energy expenditure; CBF – cerebral blood flow; CVR – cerebrovascular resistance in anterior circulation; CVRi – cerebrovascular resistance index; PI – pulsatility index; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide.

§ CVR, MFV, CVRi, and RAP were natural log transformed. PAEE and PA Duration were square root transformed.

Data were presented as Pearson correlation coefficients. All correlations are adjusted for age (log transformed) and sex.

* $P < 0.10$

** $P < 0.05$

Table 3-5. Metabolic and inflammatory profiles of participants in lowest tertile of physical activity and sleep duration.

	All (n = 76)	Low Activity (n = 24)	Low Sleep (n = 24)
Metabolic Syndrome			
No	60.5 (46)	58.3 (14)	70.8 (17)
Yes	39.5 (30)	41.7 (10)	29.2 (7)
C-Reactive Protein			
< 3 mg/L	75.0 (57)	66.7 (16)	83.3 (20)
≥ 3 mg/L	25.0 (19)	33.3 (8)	16.7 (4)

Data were presented as proportion (count). 'Low Activity' is group in the lowest tertile of physical activity energy expenditure (PAEE). 'Low Sleep' is group in the lowest tertile of sleep duration. No differences were noted between proportions in subgroups and proportions in overall sample when assessed by the exact binomial test.

Table 3-6. Standardized parameter estimates for multiple linear regressions of cerebrovascular characteristics on physical activity and sleep duration

	PA duration§			Sleep Duration		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Anterior CBF	**0.27****	**0.22***	**0.22***	-0.11	-0.12	-0.14
Model R^2	0.07	0.11	0.11	0.01	0.08	0.08
CVR§	**-0.24****	-0.18	-0.18	0.02	0.01	0.06
Model R^2	0.06	0.10	0.10	0.00	0.08	0.07
CR_{CO2}	-0.16	-0.16	-0.13	0.21	**0.29****	0.17
Model R^2	0.03	0.09	0.13	0.04	0.14	0.14

Abbreviations: Anterior CBF – anterior cerebral blood flow; CVR – cerebrovascular resistance in anterior circulation; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; PA – physical activity.

Models are presented as standardized parameter coefficients for PA duration or Sleep Duration, and explained variance.

§ CVR was natural log transformed and PA Duration was square root transformed prior to analysis.

Model 1: Unadjusted.
Model 2: Adjusted for age(log transformed) and sex.
Model 3: Adjusted for age(log transformed), sex, metabolic syndrome, and C-reactive protein(log transformed).

Standardized parameter estimate significance level is defined by the following symbols:
* $P < 0.10$
** $P < 0.05$

Discussion

The main contributions of this work were the observations that greater habitual physical activity tended to be associated with increased anterior CBF, whereas sleep deficiency was linked to the low CR_{CO2}. Contrary to the hypothesis, no relationship between physical activity levels and CR_{CO2} was observed. Underlying metabolic and inflammatory conditions have been proposed as a potential mechanisms through which the relationships between sleep quality, physical activity level and vascular health exist (Knutson, 2010; Lira *et al.*, 2011). However, no evidence in support of this proposal was observed in the current study, which included 29 men and 47 women, over 65 years of age, living independently in the community. The group of participants in the lowest tertile of PAEE, or the lowest tertile of sleep duration, did not exhibit differences in the prevalence of metabolic syndrome or high-risk CRP levels from the cohort as a whole. Surprisingly, the presence of an inverse relationship between physical activity characteristics and sleep duration was noted, which might have confounded expected relationships with metabolic dysfunction and inflammation.

Habitual Physical Activity

Although physical inactivity is a well-documented modifiable risk factor for cardiovascular and cerebrovascular disease in older adults (Heckman & McKelvie, 2008), the relationship between habitual physical activity and cerebrovascular hemodynamics in healthy aging has received little attention. Rogers *et al.* (1990) observed that maintaining a physically active lifestyle after reaching retirement age was associated with maintained cerebral perfusion and cognitive function. Here, both objectively-measured PAEE and subjectively-reported activity suggested that the most active individuals had greater CBF than the least active individuals. Despite few reports on the association between physical activity levels and CBF, similar relationships to those reported here can be inferred from evidence linking increased fitness to

greater MFV (Franke *et al.*, 2006; Ainslie *et al.*, 2008b) and cerebral conductance (Brown *et al.*, 2010) through the MCA. The reliance on CBF velocity as an indicator of flow needs to be considered with caution, however, as differences in diameter could contribute to velocity-independent changes in flow. This concern is especially prudent when examining older cohorts and comparing between fit and unfit individuals, who might exhibit greater group variance in the degree of atherosclerosis (Ozdogmus *et al.*, 2008), thereby confounding the results. Importantly, the current study found an association with CBF, where vessel diameter was taken into consideration.

Interestingly, the physical activity-CBF effect was only present for flow through the internal carotid arteries and not the vertebral arteries. This is consistent with a line of evidence that suggests the frontal regions of the brain are preferentially benefited by exercise. Recently, an 8-year prospective study found that objectively-measured habitual physical activity was associated with attenuated progression of frontal lobe atrophy, while changes in the temporal lobe were unrelated (Yuki *et al.*, 2012). In addition, an association between physical activity and executive dysfunction was related to volume of the prefrontal gyrus (Weinstein *et al.*, 2012). The carotids deliver 75-80% of the total CBF and, as such, any effect may be more readily detected in the anterior circulation. Indeed, a link between physical activity and grey matter volume has also been demonstrated to be more widespread, comprising of effects in the frontal, occipital, hippocampal and entorhinal brain regions (Erickson *et al.*, 2010).

The exact mechanism through which habitual physical activity contributes to elevated CBF can only be speculated from the current findings. There is strong evidence that habitual physical activity attenuates age-related changes in vascular structure and endothelial function in the periphery (Tanaka *et al.*, 1998; Desouza *et al.*, 2000; Pierce *et al.*, 2011). The endothelial lining of cerebral arteries and arterioles plays an important role in the regulation of resting

cerebrovascular tone, as well as the response to physical and metabolic stimuli (Andresen *et al.*, 2006). CR_{CO2} is at least partially mediated through endothelial processes (Lavi *et al.*, 2006; Ainslie *et al.*, 2007) and increased fitness has been associated with improved CR_{CO2} (Brown *et al.*, 2010). In an animal model, a direct link between habitual-wheel running and increased CR_{CO2}, but not resting CBF, has been demonstrated (Swain *et al.*, 2003). In addition, enhanced neovascularization in mice recovering from an ischemic event has been related to voluntary wheel running (Gertz *et al.*, 2006). In humans, CR_{CO2} was increased with aerobic training in sedentary older adults (Vicente-Campos *et al.*, 2012) and stroke survivors (Ivey *et al.*, 2011); however, there was no relationship between reactivity and activity level in the current study. The discrepancy between the current findings and these training studies might be a consequence of the relatively active standard of living of the adults examined here. Estimations from 3 days of monitoring suggested that the sample was highly active, with less than 20 % being considered inactive (*i.e.*, AEE less than 2719 kcal/week; Brach *et al.*, 2004) and over 70 % engaging in at least 30 minutes of regular moderate daily activity (Chodzko-Zajko *et al.*, 2009). Consequently, even the less active individuals in the group may have been more active and in better general health than the sedentary and stroke cohorts of the training studies discussed above.

Sleep Habits

Older adults commonly experience altered sleep patterns (*e.g.*, more frequent awakenings, longer sleep onset latency) which contribute to shorter sleep duration. The prevalence of sleep disturbances among the elderly population living in the community is estimated at 50% (Foley *et al.*, 1995). In the current sample, only 26% reported insomnia or sleep disturbances, including 11 taking sedative medications. However, 50% reported frequently feeling tired, so

although they might underrepresent the general population, a definite range of sleep quality was present to examine the relationships of interest.

The observation of reduced CR_{CO2} in individuals with subjectively- and objectively-measured shorter sleep duration is consistent with previous research in peripheral vascular beds linking endothelial dysfunction to short sleep duration (Sauvet *et al.*, 2010; Weil *et al.*, 2010). In addition, Qureshi *et al.* (1999) observed that sleep fragmentation was associated with morning reductions in MFV and CR_{CO2} through the middle cerebral artery. Despite the prevalence of a wide variety of sleep disorders with deleterious consequences in older adults (Gangwisch *et al.*, 2008; Crowley, 2011), the majority of sleep-related research as it relates to cerebrovascular health is concentrated within the background of sleep apnea (Furtner *et al.*, 2009; Kiratli *et al.*, 2010). In the present sample, 8 of 76 reported a diagnosis of sleep apnea, however there was no difference between these individuals and the rest of the cohort with respect to anterior or posterior CBF or CR_{CO2}. In sleep apnea patients, periodic hypoxia leading to elevated reactive oxygen species has been proposed as a mediating factor linking endothelial dysfunction and cardiovascular and cerebrovascular events (Jelic & Le Jemtel, 2008; Kohler & Stradling, 2010). Continuous, positive airway pressure therapy, which was used by 6 of 8 participants in the current sample, might have limited the occurrence of hypoxic insults, helping to maintain cerebrovascular health. In addition, severity of the apneic condition, as well as duration of exposure, would have likely contributed to the relationship between apnea and impaired CR_{CO2}; however, this was not determined as part of the current study.

Metabolic Profile and C-Reactive Protein

Metabolic and inflammatory disturbances were hypothesized as potential mechanisms through which physical inactivity and shorter sleep duration contribute to cerebrovascular impairment. Regression modeling found that AEE was associated with increased anterior CBF independent

of age, sex, metabolic syndrome and CRP. In addition, the bivariate relationship between AEE and anterior CVR was attenuated when age and sex were entered into the model; however, further inclusion of metabolic syndrome and CRP did not increase the amount of variance explained by the model. Further, the relationship between CR_{CO2} and sleep duration was strongest when age and sex, but not metabolic syndrome and CRP, were also considered.

Based on the literature, these variables were hypothesized to mediate the relationship between physical activity, sleep and cerebrovascular health. In animal models, habitual exercise has been shown to reduce age- and hypertension-related increases in pro-inflammatory cytokines, as well as levels of oxidative stress (Agarwal *et al.*, 2011; Lesniewski *et al.*, 2011). In humans, impaired CR_{CO2} is associated with conditions such as metabolic syndrome and diabetes – both of which are consistent with endothelial dysfunction (Lavi *et al.*, 2006; Giannopoulos *et al.*, 2010). Along the same vein, Knutson (2010) reported that short sleep duration was associated with metabolic risk. In the current sample, ~40 % exhibited metabolic syndrome and 25 % had elevated serum CRP levels. Therefore, the exposure level was expected to be sufficient to observe an effect. The population investigated by Giannopoulos *et al.* (2010) included patients with contralateral carotid artery stenosis (50 %) and ipsilateral stroke (32 %). These patients were at a highly advanced stage of cerebrovascular impairment relative to participants in the current study, which may account for inconsistencies in the relationship between metabolic syndrome and CR_{CO2}.

The link between physical activity, sleep duration, metabolic syndrome and inflammation was hypothesized to be a direct relationship. More active participants were expected to exhibit better sleep patterns and be at a lower risk for metabolic disorders (Lira *et al.*, 2011). However, the prevalence of high risk levels of CRP, as well as metabolic syndrome, was unrelated to either low physical activity or short sleep duration. Unexpectedly, there was

an inverse correlation between physical activity and sleep duration. Consequently, any relationship between short sleep duration and cerebrovascular health would have been mitigated by increased physical activity levels, and vice versa.

Objective vs. Subjective Tracking

This study incorporated both objectively measured and subjectively reported measures of physical activity and sleep. Even with objective data, the consideration of self-report data in the current study helps to relate to the literature, which still relies heavily on questionnaires to determine habitual physical activity (Brach *et al.*, 2004; Vercambre *et al.*, 2011). As well, it provides insight into longer term physical activity routines, rather than relying on only 3 days of monitoring. The main relationships noted here, that physical inactivity is associated with low anterior CBF and short sleep duration is associated with low cerebrovascular reactivity, were consistent between both measurement modalities.

Despite known benefits, the Canadian Community Health Survey estimated that approximately 50 % of older Canadians do not engage in sufficient physical activity on a regular basis (Azagba & Sharaf, 2012). In the current study, objective measures of AEE and PAEE suggested that over 70 % of this group of older adults were engaging in recommended levels of physical activity (Brach *et al.*, 2004; Chodzko-Zajko *et al.*, 2009). Still, 59 % of participants subjectively considered themselves as sedentary; perhaps indicating that the Sensewear monitors overestimated physical activity levels. Previously, the Sensewear monitor has been reported to overestimate resting EE compared to indirect calorimetry (Heiermann *et al.*, 2011) and underestimate active EE compared to the doubly-labeled water method (Mackey *et al.*, 2011). Alternatively, the discrepancy between the objective and subjective data might indicate that older adults had difficulty in subjectively rating their own activity levels. Because of uncertainties in subjective reports, objective measurements of physical activity are

considered superior to subjective questionnaires and valid for ranking activity levels within the context of the current research (Colbert *et al.*, 2011). No relationship between the intensity of physical activity and cerebrovascular health was observed. Although any such finding might have been confounded by the ability to quantify absolute EE, an alternate possibility includes the likelihood that the current sample was too active and too homogenous to observe a relationship. Even low levels of activity, including walking, have been associated with a reduced risk of cognitive impairment and slowing of cognitive decline in older men and women (Abbott *et al.*, 2004; Weuve *et al.*, 2004); however, relatively little is known about the role exercise intensity plays in cerebrovascular health.

Conclusions

The current study provided a comprehensive evaluation of cerebrovascular health in community-living older adults, taking into consideration physical activity and sleeping habits. Higher PAEE and longer duration of daily physical activity were related to greater CBF and a lower cerebrovascular resistance profile. The relationship between anterior CBF and duration of daily physical activity persisted, even after adjusting for age, sex, metabolic syndrome and CRP. Short sleep duration was related to lower CR_{CO2}. Contrary to the hypothesis, these relationships did not appear to be mediated through the presence of metabolic syndrome or elevated CRP. These results have potentially important policy implications regarding elder care as poor sleep quality and low physical activity levels are each highly modifiable characteristics. As part of a general discussion, these implications are discussed in Chapter 7.

CHAPTER 4. THE ASSOCIATION OF ARTERIAL STRUCTURE AND FUNCTION WITH CEREBRAL BLOOD FLOW CHARACTERISTICS

Introduction and Rationale

Across the lifespan, there is a progressive reduction in CBF (Scheel *et al.*, 2000b; Pagani *et al.*, 2002; Ainslie *et al.*, 2008a; Bertsch *et al.*, 2009); however, the extent to which individuals experience a drop is heterogeneous within the population. Flows ranging between ~ 350 and 850 mL/min are commonly observed in older adults without overt cardiovascular, cerebrovascular or neurodegenerative disorders (Scheel *et al.*, 2000a; Dorfler *et al.*, 2000; Schreiber *et al.*, 2005; Albayrak *et al.*, 2007). The lower end of this spectrum is consistent with flow in patients with dementia and cerebral atrophy (Scheel *et al.*, 1999; Schreiber *et al.*, 2005; Albayrak *et al.*, 2006; Bateman *et al.*, 2006; Han *et al.*, 2007), and low CBF might be predictive of future cognitive impairment (Maalikjy *et al.*, 2005). A greater understanding of the factors contributing to low CBF is needed to better understand the development of disease, as well as plan appropriate preventative care.

The change in CBF with aging may be incompletely accounted for by underlying changes in neuronal atrophy/diminished metabolism (Chen *et al.*, 2011; Henriksen *et al.*, 2012). An alternative basis for differences in flow between individuals might lie within cerebrovascular integrity and/or vasoconstrictor tone (Ito *et al.*, 2008). Subtle changes in arterial structure and function that are inadequate to trigger clinical events, but sufficient to alter cerebral hemodynamics, have only recently received attention in the literature (Gorelick *et al.*, 2011). Carotid intima-media thickness (IMT) and carotid-femoral pulse wave velocity (cf-PWV) are quantifiable measures denoting atherosclerotic risk and arterial stiffness, respectively (Allan *et al.*, 1997; Van Bortel *et al.*, 2012). Epidemiological and clinical studies

have demonstrated associations of IMT and PWV with subclinical brain pathology (Bots *et al.*, 1993; Pico *et al.*, 2002; Inoue *et al.*, 2007; Matsumoto *et al.*, 2007; Ohmine *et al.*, 2008; Kearney-Schwartz *et al.*, 2009; Mitchell *et al.*, 2011). However, less focus has been given to the understanding of their weight on cerebrovascular hemodynamics (Robertson *et al.*, 2010; Sojkova *et al.*, 2010; Webb *et al.*, 2012; Xu *et al.*, 2012).

In addition to these structural changes, blood-borne biomarkers might provide information related to early changes in underlying vascular pathology. C-reactive protein (CRP), a widely recognized marker of inflammation (Pearson *et al.*, 2003), is an independent risk factor for stroke and transient ischemic attack (Pikula *et al.*, 2012), as well as for vascular changes related to cognitive dysfunction (Gorelick *et al.*, 2011). Elevated serum creatinine, a marker of kidney dysfunction (Leoncini *et al.*, 2004; Rule *et al.*, 2006), has been associated with cardiovascular endpoints (Fried *et al.*, 2003), and might reflect the presence of vascular dysfunction with common deleterious effects in the kidney and brain (O'Rourke & Safar, 2005). Together, these markers of inflammation and end-organ damage might provide insight into the association of arterial structure and function with CBF regulation and early end organ disease.

The objective of this study was to assess CBF and cerebrovascular reactivity from the viewpoint of heterogeneity in underlying vascular health in community-living older adults, free of patent cerebrovascular disease. Specific focus was placed on physical and serum biomarkers of underlying central artery structure and function. It was hypothesized that the presence of greater IMT and elevated cf-PWV would be associated with lower CBF, increased cerebrovascular resistance (CVR), and impaired cerebrovascular reactivity to carbon dioxide (CR_{CO2}). Inflammation, as indicated by CRP, was expected to be a mediator of this association,

and creatinine was expected to reflect the severity of vascular dysfunction based on end-organ involvement. Also, it was hypothesized that the presence of both IMT and cf-PWV above established risk thresholds would be associated with cumulative risk and result in lower CBF, lower CR_{CO2}, and higher levels of both CRP and creatinine than individuals with only one, or none of these risk markers.

Methods

This chapter discusses the cohort and cerebrovascular assessments described in Chapter 2.

Statistical Analysis

Continuous data were presented as mean ± SD and categorical data were presented as proportions (counts). Non-normal data were presented as median (interquartile range). Sample characteristics and cerebrovascular hemodynamics were separated by sex due to possible dimorphic cerebrovascular changes with aging (Deegan *et al.*, 2011). Student's *t*-test and chi-square analyses were used for comparison of continuous and categorical variables, respectively. Where non-normal distributions were noted or where large sample size differences existed between groups, the Mann-Whitney U-test and Fisher's exact test (FET) replaced their corresponding parametric tests. Non-normal data were transformed where appropriate prior to correlation and regression analyses. Specifically, age; CVR in the anterior circulation; mean IMT; the middle cerebral artery's (MCA) mean flow velocity (MFV), cerebrovascular resistance index (CVRi), and resistance area product (RAP); as well as CRP; and creatinine underwent natural logarithm transformation. Pearson product correlation was used to assess relationships between subclinical vascular health and cerebrovascular hemodynamic variables, after adjustment for age and sex. Linear regression modeling was used to assess the amount of variance in anterior CBF, anterior CVR and CR_{CO2}, stratified by sex,

explained by the arterial stiffness and IMT alone, and after adjustment for risk factors of ischemic events (D'Agostino *et al.*, 1994). The sum effect of risk associated with blood pressure, plasma lipids and glucose level was considered by the presence of metabolic syndrome. The list of covariates used for multiple linear regression included age, metabolic syndrome and current smoking status. Considering all variables, a model of best fit was determined by Akaike's Information Criterion (AIC; Beal, 2005). Secondary to the pleiotropic effects of statins (Athyros *et al.*, 2009), regression modeling was repeated considering only those individuals not receiving statin therapy and including sex as a covariate. Finally, a combined risk effect was assessed by examining individuals who exhibited elevated risk threshold values for IMT and cf-PWV. Thresholds for IMT were set at the 75^{th} percentile for the Caucasian population adjusted for sex (men: 0.9 mm; women: 0.8 mm; based on Howard *et al.*, 1993). A single cf-PWV threshold was used for both men and women (10 m/s; based on Van Bortel *et al.*, 2012). High risk thresholds were also set for CRP (3.0 mg/L; based on Pearson *et al.*, 2003) and creatinine (men: 115 umol/L; women: 96 umol/L; based on Rule *et al.*, 2006). Chi-square analyses were performed to examine the relationship between the risk thresholds of these 4 variables. Finally, a Kruskal-Wallis non-parametric analysis of the cumulative vascular risk examined characteristic differences between individuals within the high risk range of both IMT and cf-PWV, of only one variable, or of neither variable. Significance was inferred at $P < 0.05$. Trends are highlighted at $P < 0.10$. All statistical analyses were completed using Statistical Analysis Software v9.2 (SAS Institute, Cary NC, USA).

Results

Valid IMT and cf-PWV were not obtained in two men from the overall study cohort. Thus, 50 women and 29 men (65 – 89 years of age), living independently in the community, were considered for this analysis. General participant characteristics, separated by sex, are listed in Table 4-1. Age, race and education were similar between sexes. Both men and women were highly educated, showed normal cognitive function on the Montreal Cognitive Assessment, and self-reported infrequent indications of depressive symptoms. There were a few notable differences in cardiovascular risk factors between men and women. Waist-to-hip ratio was smaller for women (t_{75} = -8.84, $P < 0.001$), but mean body mass index (BMI) indicated both men and women were, on average, overweight. Systolic blood pressure was similar between the sexes (women: 142.7 ± 20.2 vs. men: 137.6 ± 14.0 mmHg; $t_{73.9}$ = 1.31, P = 0.195), but women had lower diastolic pressure (women: 69.4 ± 10.4 vs. men: 74.6 ± 7.9; t_{76} = -2.30, P = 0.024). Both men and women were characterized by a high prevalence of hypertension and hyperlipidemia (55-60 %). Thirty-nine percent (n = 29) were noted to express the metabolic syndrome phenotype, with no differences between men and women. Although the overall prevalence of current smoking was small, a trend was noted that men were more likely to smoke ($FET_{(n = 79)}$, P = 0.058). Men were also more likely to be receiving statin therapy ($\chi^2_{(1,n = 79)}$ = 5.14, P = 0.023), but other mediations had similar use profiles between men and women. The self-reported prevalence of myocardial infarction and diabetes mellitus was low, and there were no reported cases of peripheral vascular disease indicating that symptomatic disease was rare within the sample.

Although total CBF was similar between men and women (Table 4-1), women exhibited lower CBF through the bilateral internal carotid arteries (ICA) (anterior CBF;

$t_{68.2}$ = -2.05, P = 0.044). In contrast, women had higher MCA MFV ($t_{63.6}$ = 4.20, P < 0.001). In the extracranial vasculature, women had a smaller ICA diameter (0.48 ± 0.09 vs. 0.55 ± 0.10 cm; t_{75} = -3.16, P = 0.002), but a similar ICA velocity (P = 0.194). In the vertebral circulation, women had a similar diameter compared to men (P = 0.776), but a trend for a greater velocity was noted (12.8 ± 5.0 vs. 10.5 ± 3.9; t_{66} = 1.92, P = 0.059). Due to the sex-related differences in anterior CBF, further analysis was adjusted for sex. The range of CBF was greater for women in both anterior and posterior circulations (Figure 4-1, top). No significant relationship between anterior CBF and age was observed in either sex; however, a trend for an inverse relationship between posterior CBF and age was noted in men ($F_{1,19}$ = 4.18, P = 0.055). CR_{CO2} was similar between men and women, on average, but exhibited a large coefficient of variation of 26 %. CR_{CO2} was not related to age in men, but a trend was noted for an inverse relationship in women ($F_{1,33}$ = 3.13, P = 0.086; Figure 4-1, bottom).

Linear relationships between cerebrovascular characteristics and central artery structure and function are shown in Figure 4-2 to 4-4. In women, but not men, a trend for an inverse relationship between anterior CBF and mean IMT was observed ($F_{1,43}$ = 3.86, P = 0.056; Figure 4-2, top). In contrast, anterior CBF was not related to cf-PWV in either men or women (Figure 4-2, bottom). The effect of mean IMT on the anterior circulation in women was strengthened when mean arterial pressure (BP_{MCA}) was considered and the anterior hemodynamic characteristics were expressed as CVR. Mean IMT was directly related to increased CVR in women ($F_{1,43}$ = 7.67, P = 0.008; Figure 4-3, top); however, still no effect of cf-PWV was observed in either sex (Figure 4-3, bottom). In contrast to CBF and CVR, CR_{CO2} was not related to either IMT or cf-PWV (Figure 4-4).

Pearson product correlations exploring the relationship between cerebrovascular characteristics and biomarkers of subclinical vascular risk, adjusted for age and sex, are shown in Table 4-2. Carotid pulse pressure (cPP) and brachial-ankle pulse wave velocity (ba-PWV) were considered as indicators of arterial stiffness in addition to cf-PWV. Peak IMT was considered in addition to mean IMT as an indicator of atherosclerotic progression. Elevated arterial stiffness was concomitant to increased pulsatility index in the MCA (cPP: n = 68, P = 0.002; ba-PWV: n = 68, P = 0.031). Higher cPP was also associated with elevated total CBF (n = 56, P = 0.047).Similar to the unadjusted relationship shown in Figure 4-3, an inverse relationship was identified between IMT and CVR (mIMT: n = 69, P = 0.012; pIMT n = 69, P = 0.013) after adjustment for age and sex. IMT was also associated with an elevated pulsatility index (mIMT: n = 68, P = 0.001; pIMT: n = 68, P = 0.002) and a lower dynamic cerebral autoregulation (dCA) index (mIMT: n = 48, P = 0.045; pIMT: n = 48, P = 0.044) – an unexpected result given that the index of dCA is such that a lower value reflects improved autoregulation. In addition to these significant observations, trends were noted between markers of arterial stiffness, resistance area product and critical closing pressure; as well as for IMT with MFV and anterior CBF (all $P < 0.10$). Serum CRP level was directly related to RAP (n = 66, P = 0.024), but no other cerebrovascular characteristics.

Multiple regression modeling confirmed that mean IMT was an independent contributing factor to anterior CVR in older women, but not men (Table 4-3). Mean IMT accounted for 15% of the variance in CVR in an unadjusted model, and remained a significant factor in all models of CVR for women, even after adjustment for age, metabolic profile and smoking status. While the model of best fit for CVR in men, according to AIC, included mean IMT, the model itself was not significant. Of the covariates examined, age explained the most

variance in CR_{CO2} in older women and the presence of metabolic syndrome did the same for older men. Cf-PWV was not a significant contributor to CVR or CR_{CO2} in any of the models that were examined (Table 4-4). However, the AIC best fit model for estimating CVR in older men indicated that cf-PWV was perhaps better than any other covariate (excluding IMT). Overall, the adjusted models were only able to explain 25% of the total variance in anterior CVR and 20% of the total variance in CR_{CO2}.

On average, participants taking either anti-hypertensive or anti-hyperlipidemic medications were the same age (P = 0.830), had similar anterior CBF (P = 0.384), posterior CBF (P = 0.245) and CR_{CO2} (P = 0.122) as participants not on therapy. In multivariate modeling excluding individuals on statin therapy, mean IMT, age, sex and smoking status contributed significantly to the estimation of anterior CVR; while sex alone was the optimal predictor of CR_{CO2}.

A clinically-relevant threshold risk assessment of vascular health revealed 22% of participants had advanced IMT (13 women/4 men), 29% had elevated cf-PWV (15/8), 26% had elevated CRP (15/6), and 20% had elevated serum creatinine (8/8). Cf-PWV risk was associated with CRP risk ($\chi^2_{(1, N = 79)} = 4.75$, $P = 0.040$); however, no other significant associations between the markers of subclinical vascular health were noted.

Seven individuals exhibited both cf-PWV and IMT above clinical risk threshold values. Unfortunately, a failure to obtain successful CBF or TCD signals in all participants reduced the effective sample size of the highest risk group to between 2 and 4, depending on the characteristic of interest (Table 4-5). A non-parametric Kruskal-Wallis comparison was performed between three groups: participants with both IMT and cf-PWV, with only one of the vascular characteristics, or with neither characteristic exceeding risk thresholds. The results

showed elevated risk from subclinical changes in arterial structure and function was associated with increased CVR in the anterior circulation and an elevated RAP in the MCA.

Table 4-1. Participant Characteristics

Characteristic	Men (n = 29)	Women (n = 50)	*P* value
Physical Status			
Age, years §	73.0 (10.1)	73.2 (7.5)	0.498
Race – white, % (n)	97 (28)	96 (48)	1.000
Height, cm	175.2 ± 7.8	161.3 ± 6.6	**<0.001**
Weight, kg	83.6 ± 15.3	69.8 ± 11.8	**<0.001**
BMI, kg/cm^2	27.1 ± 3.9	26.8 ± 4.0	0.731
Waist:Hip, ratio	0.98 ± 0.06	0.84 ± 0.07	**<0.001**
Cognitive Status			
Education, years	15.2 ± 3.9	14.7 ± 3.9	0.636
MoCA, total score	28.3 ± 1.2	28.0 ± 1.6	0.254
GDS, total score	1.0 ± 1.5	0.6 ± 1.0	0.295
Risk Factors and Medications			
Hypertension, n (%)	55 (16)	62 (31)	0.551
ACEi, n (%)	10 (3)	14 (7)	0.738
ARB, n (%)	10 (3)	14 (7)	0.738
CCB, n (%)	0 (0)	8 (4)	0.291
β-Blocker, n (%)	7 (2)	8 (4)	1.000
Diuretic, n (%)	21 (6)	24 (12)	0.735
ASA, n (%)	28 (8)	18 (9)	0.318
Hyperlipidemia, n (%)	55 (16)	60 (30)	0.675
Statin, n (%)	41 (12)	18 (9)	**0.023**
Diabetes Mellitus, n (%)	7 (2)	6 (3)	1.000
Insulin, n (%)	3 (1)	2 (1)	1.000
Hypoglycemic, n (%)	7 (2)	2 (1)	0.551
Metabolic Syndrome, n (%)	45 (13)	36 (18)	0.439
Prior CVD, n (%)	0 (0)	2 (1)	1.000
Smoking (ever), n (%)	52 (15)	44 (22)	0.507
Smoking (current), n (%)	14 (4)	2 (1)	0.058
Frequent Alcohol Intake, n (%)	0 (0)	6 (3)	0.294
Physical Activity, kcal/day	330 ± 210	200 ± 140	**0.005**
Family History – CVD, n (%)	31 (9)	40 (20)	0.426
Family History – stroke, n (%)	24 (7)	14 (7)	0.255
Cerebrovascular Hemodynamics			
Total CBF, mL/min (20/38)	700 ± 120	710 ± 160	0.424
Anterior CBF, L/min (26/45)	590 ± 90	530 ± 140	**0.044**
Posterior CBF, L/min (20/41)	160 ± 50	170 ± 50	0.352
CVR, mmHg (26/45) §	170 (40)	190 (80)	0.093
MFV, cm/s (29/40) §	48.9 (9.8)	58.4 (17.6)	**<0.001**
CR_{CO2}, %/mmHg (28/35)	4.0 ± 1.2	4.3 ± 0.9	0.269

Abbreviations: BMI – body mass index; MoCA – Montreal Cognitive Assessment; GDS – Geriatric Depression Scale; CBF – cerebral blood flow; CVR – cerebrovascular resistance in the anterior circulation; MFV – middle cerebral artery mean flow velocity; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; ACEi – angiotensin converting enzyme inhibitor; ARB – angiotensin II receptor blocker; CCB – calcium channel blocker; ASA – acetylsalicylic acid; CVD – cardiovascular disease (myocardial infarction or peripheral vascular disease); Family History – the presence of CVD or stroke in immediate family member.

Data were presented as mean ± SD for continuous variables and proportion (count) for categorical variables. Sample size for CBF and MFV measures are identified in parentheses (men/women).

§ Age, CVR, and MFV were not normally distributed and are presented as median (interquartile range).

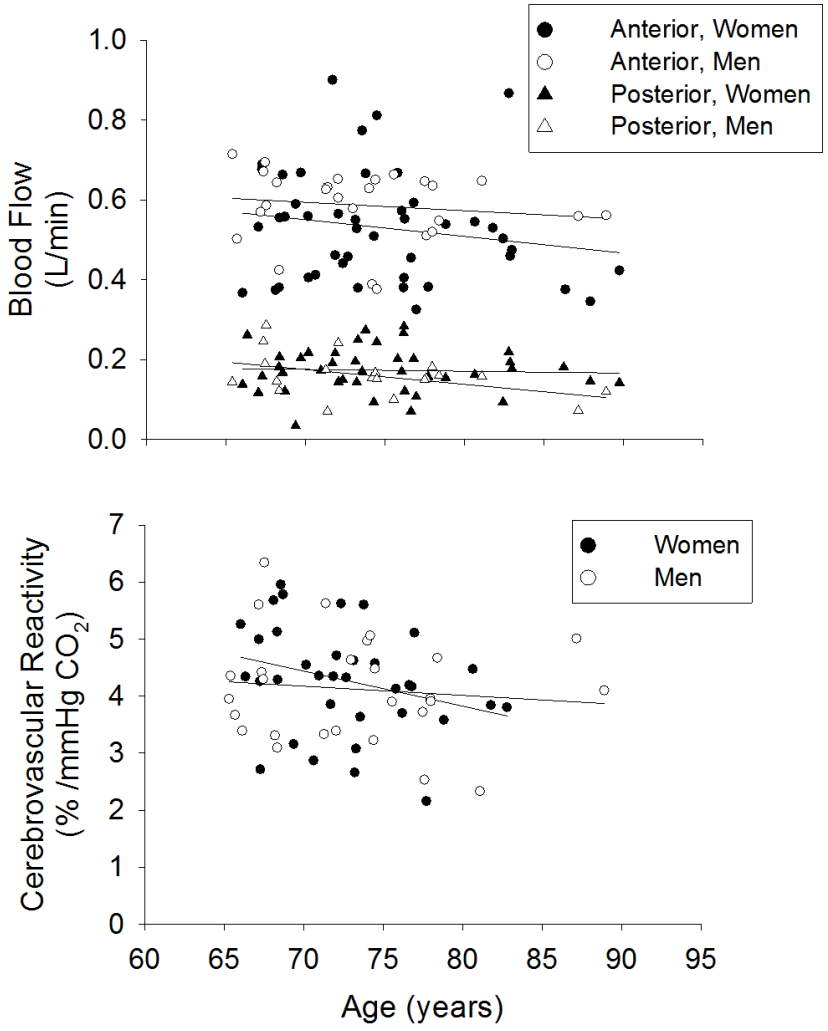

Figure 4-1. Relationship of cerebral blood flow and cerebrovascular reactivity to age stratified by sex.
Top: Anterior (●) and posterior (▲) cerebral blood flow (CBF) plotted against age for men (open symbols) and women (closed symbols), separately. Raw values are shown for clarity. Linear regression analysis was performed with logarithmic transformation of age. Bivariate analysis stratified by sex found no relationship between age and anterior CBF in either men or women (women: $P = 0.265$, men: $P = 0.344$), or between age and posterior CBF in women ($P = 0.787$). A trend was observed in men (standardized $\beta = -0.42$, $t_{19} = -2.04$, $P = 0.055$).
Bottom: Cerebrovascular reactivity to carbon dioxide plotted against age for men and women, separately. No relationship with age was noted for men ($P = 0.733$). A trend was observed in women (standardized $\beta = -0.29$, $t_{33} = -1.77$, $P = 0.086$). Differences in sample sizes between plots were noted in Table 4-1.

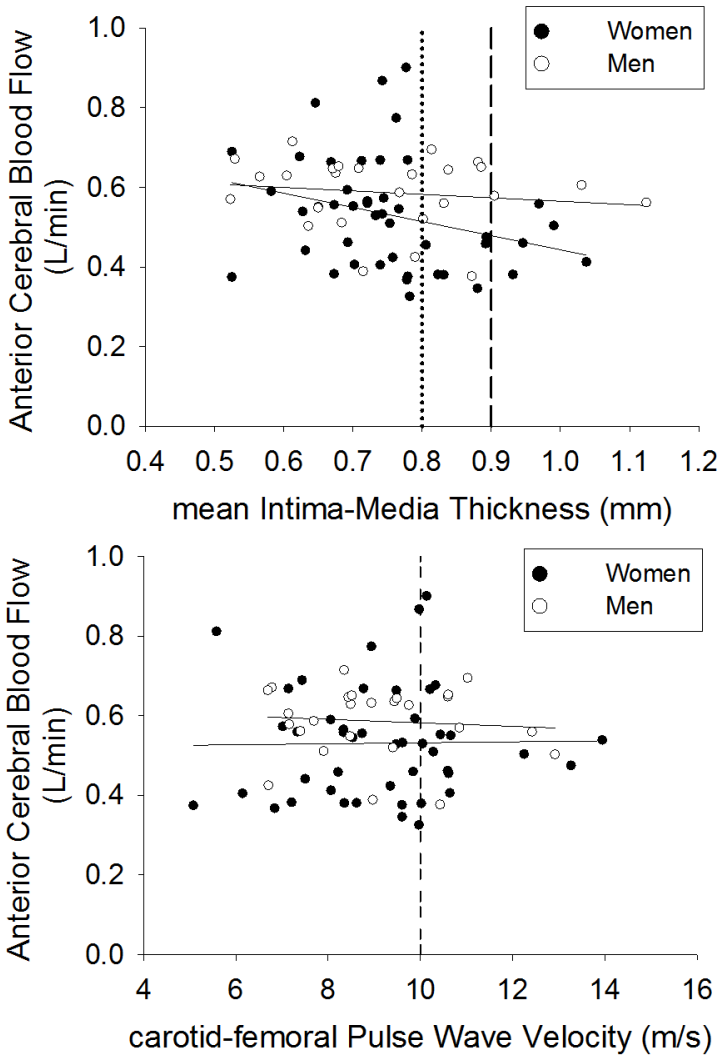

Figure 4-2. Relationship of anterior cerebral blood flow to intima-media thickness and carotid-femoral pulse wave velocity.
Top: Anterior cerebral blood flow (CBF) plotted against mean intima media thickness (IMT) in men (open circles) and women (closed circles). Vertical lines represent threshold value for elevated risk in women (0.8 mm, dotted line) and men (0.9 mm, dashed line). Raw values are shown for clarity. Linear regression analysis was performed with logarithmic transformation of mean IMT. Bivariate analysis showed a trend between anterior CBF and IMT in older women (standardized $\beta = -0.29$, $t_{43} = -1.97$, $P = 0.056$). No relationship was observed in men ($P = 0.471$). **Bottom:** Anterior CBF plotted against carotid-femoral pulse wave velocity. Vertical, dashed line represents threshold value for elevated risk in women and men (10 m/s). No relationships were noted (women: $P = 0.917$, men: $P = 0.535$). Differences in sample sizes between plots were noted in Table 4-1.

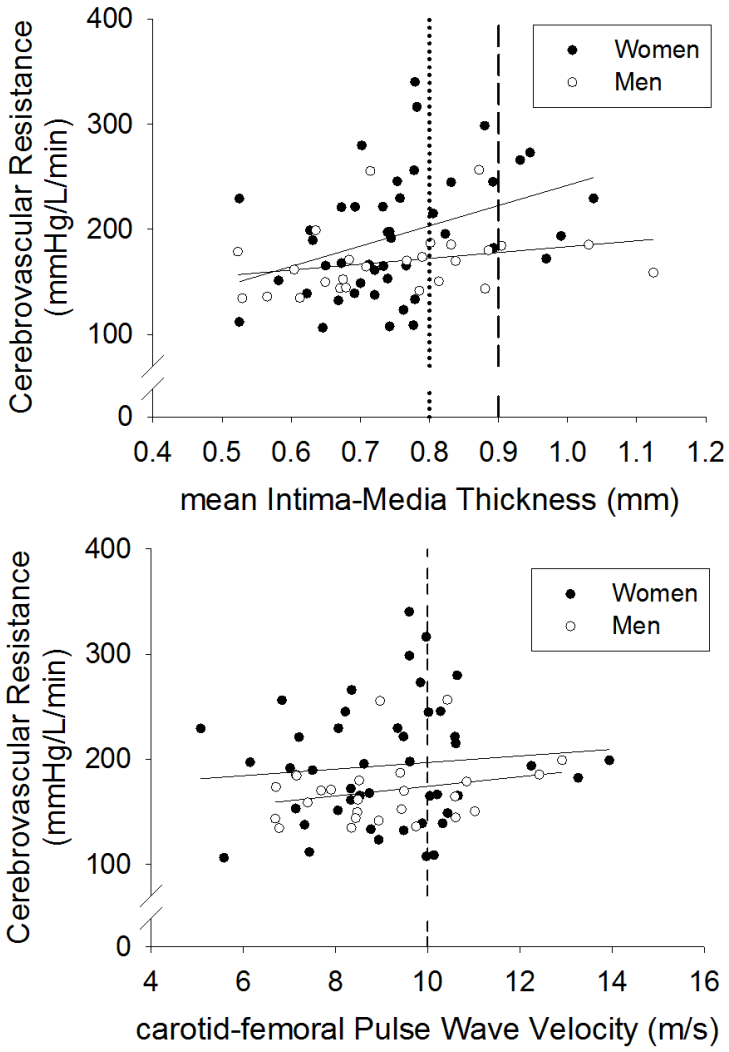

Figure 4-3. Relationship of cerebrovascular resistance to intima-media thickness and carotid-femoral pulse wave velocity.

Top: Anterior cerebrovascular resistance (CVR) plotted against mean intima media thickness (IMT) in men (open circles) and women (closed circles). Vertical lines represent threshold value for elevated risk in women (0.8 mm, dotted line) and men (0.9 mm, dashed line). Raw values are shown for clarity. Linear regression analysis was performed with logarithmic transformation of mean IMT and CVR. Bivariate analysis showed a direct relationship between CVR and mean IMT in older women (standardized $\beta = 0.39$, $t_{43} = 2.77$, $P = 0.008$). No relationship was noted in men ($P = 0.137$). **Bottom:** Anterior CVR plotted against carotid-femoral pulse wave velocity. Vertical, dashed line represents threshold value for elevated risk in women and men (10 m/s). No relationships were noted (women: $P = 0.484$, men: $P = 0.211$). Differences in sample sizes between plots were noted in Table 4-1.

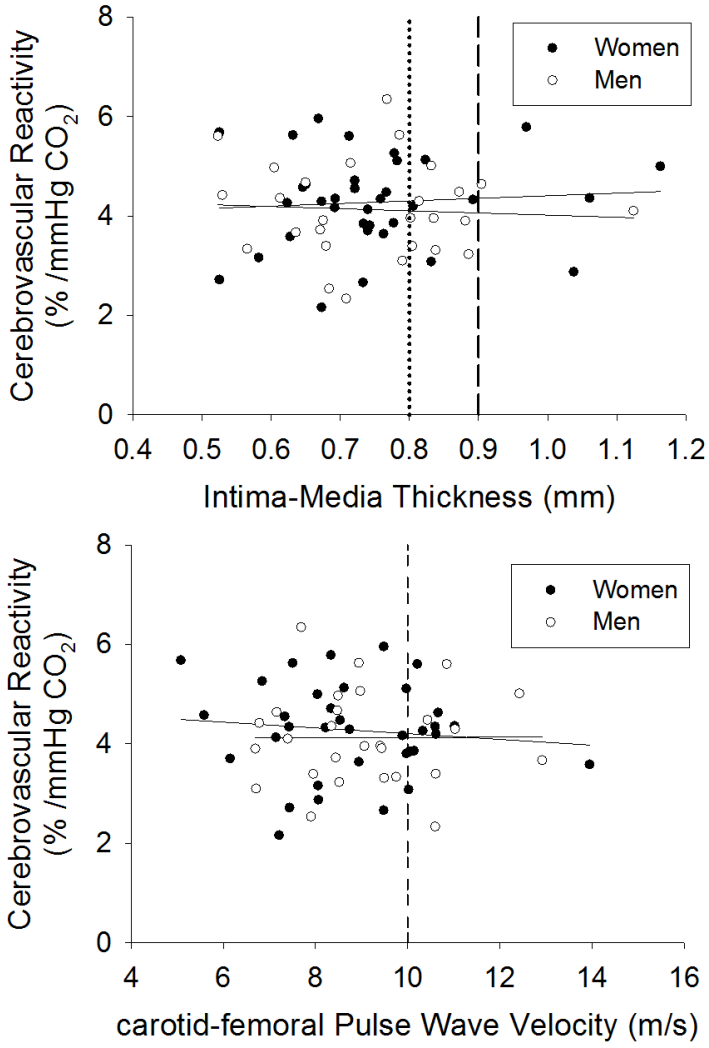

Figure 4-4. Relationship of cerebrovascular reactivity to intima-media thickness and carotid-femoral pulse wave velocity.
Top: Cerebrovascular reactivity to carbon dioxide (CR_{CO2}) plotted against mean intima media thickness (IMT) in men (open circles) and women (closed circles). Vertical lines represent threshold value for elevated risk in women (0.8 mm, dotted line) and men (0.9 mm, dashed line). Raw values are shown for clarity. Linear regression analysis was performed with logarithmic transformation of mean IMT. Bivariate analysis found no relationships between CR_{CO2} and mean IMT in either sex (women: $P = 0.662$, men: $P = 0.136$). **Bottom:** CR_{CO2} plotted against carotid-femoral pulse wave velocity. Dashed line represents cut-off value for both men and women (10 m/s, dashed). No relationships were noted (women: $P = 0.534$, men: $P = 0.447$). Differences in sample sizes between plots were noted in Table 4-1.

Table 4-2. Partial correlation of vascular structure and cerebral hemodynamics

Cerebrovascular Index	Subclinical Vascular Risk						
	cf-PWV	ba-PWV	cPP	mIMT §	pIMT	CRP §	Creatinine §
Total CBF	-0.06	0.03	**0.27***	-0.17	-0.19	-0.07	-0.10
Anterior CBF	0.05	0.09	0.23	-0.20	-0.22	-0.04	-0.06
Posterior CBF	-0.21	-0.20	0.03	0.04	0.03	-0.19	-0.10
CVR §	0.06	-0.10	-0.13	**0.30***	**0.30***	0.05	-0.10
MFV §	0.01	-0.02	0.17	0.21	0.20	-0.08	-0.01
CVRi §	0.21	0.02	-0.01	-0.08	-0.08	0.19	-0.15
MCA PI	0.14	**0.27***	**0.38****	**0.41 †**	**0.38 †**	-0.19	-0.07
CrCP	0.02	0.09	-0.24	-0.04	0.02	-0.11	0.04
RAP §	0.24	-0.03	0.23	0.01	-0.02	**0.28***	-0.21
CR_{CO2}	0.05	0.00	-0.11	-0.13	-0.18	0.19	0.18
dCA	-0.04	0.09	0.19	**-0.30***	**-0.30***	-0.09	-0.01

Abbreviations: CBF – cerebral blood flow; CVR – cerebrovascular resistance; MFV – middle cerebral artery mean flow velocity; CVRi –cerebrovascular resistance index; PI –pulsatility index; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; dCA – dynamic cerebral autoregulation from sitting to standing; cf-PWV – carotid-femoral pulse wave velocity; ba-PWV – brachial-ankle pulse wave velocity; cPP – carotid pulse pressure; mIMT – mean intima media thickness, pIMT – peak intima media thickness.

§ CVR, MFV, CVRi, RAP, mIMT, CRP and creatinine were natural log transformed prior to analysis.

Data were presented as Pearson correlation coefficients, adjusted for age (log transformed) and sex.

* $P < 0.05$
** $P < 0.01$
† $P < 0.001$

Table 4-3. Linear regression of intima-medial thickness with cerebrovascular resistance and cerebrovascular reactivity to carbon dioxide

Dependent Variable	Sex	Model	β	*P value*	Model R^2
Full Sample					
CVR §	Women (n=45)	mIMT §	**0.39**	**0.008**	0.15
		Adjusted	**0.34**	**0.024**	0.25
		Best Fit (mIMT §, current smoker)	**0.40**	**0.005**	0.21
	Men (n=26)	mIMT §	0.30	0.137	0.09
		Adjusted	0.33	0.156	0.13
		Best Fit (mIMT §)	0.30	0.137	0.09
CR_{CO2}	Women (n=35)	mIMT	0.08	0.662	0.00
		Adjusted	0.06	0.750	0.09
		Best Fit (age §)	--	--	0.09
	Men (n=28)	mIMT §	-0.29	0.136	0.08
		Adjusted	-0.25	0.219	0.20
		Best Fit (metabolic syndrome)	--	--	0.12
No Statin Use (n = 58)					
CVR §	Both	mIMT §	0.17	0.474	0.03
		Adjusted	0.25	0.083	0.25
		Best Fit (mIMT §, age §, sex and current smoker)	0.25	0.078	0.25
CR_{CO2}	Both	mIMT §	-0.15	0.309	0.02
		Adjusted	-0.11	0.467	0.11
		Best Fit (sex)	--	--	0.08

Abbreviations: CVR– anterior cerebrovascular resistance; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; mIMT – mean carotid intima-media thickness.

§ CVR, age and mIMT were natural log transformed prior to analysis.

Data were presented as standardized parameter estimate (β) for mIMT, significance of the parameter estimate, and explained variance (R^2) of the model. Adjusted models included age, metabolic syndrome, and current smoker as covariates. For 'no statin use' models, sex was also included as a covariate. 'Best Fit' model was nested within the adjusted model and determined by lowest Akaike's Information Criterion.

Table 4-4. Linear regression of arterial stiffness with cerebrovascular resistance and cerebrovascular reactivity to carbon dioxide

Dependent Variable	Sex	Model	β	*P value*	Model R^2
Full Sample					
CVR §	Women (n=45)	cf-PWV	0.11	0.484	0.01
		Adjusted	-0.04	0.784	0.14
		Best Fit (age §, current smoker)	--	--	0.14
	Men (n=26)	cf-PWV	0.24	0.211	0.06
		Adjusted	0.22	0.284	0.13
		Best Fit (cf-PWV)	0.24	0.211	0.06
CR_{CO2}	Women (n=35)	cf-PWV	-0.11	0.534	0.01
		Adjusted	-0.01	0.941	0.09
		Best Fit (age §)	--	--	0.09
	Men (n=28)	cf-PWV	0.15	0.447	0.02
		Adjusted	0.11	0.585	0.16
		Best Fit (metabolic syndrome)	--	--	0.12
No Statin Use (n = 58)					
CVR §	Both	cf-PWV	0.16	0.264	0.02
		Adjusted	0.02	0.886	0.20
		Best Fit (age §, sex and current smoker)	--	--	0.20
CR_{CO2}	Both	cf-PWV	0.16	0.285	0.02
		Adjusted	0.23	0.137	0.15
		Best Fit (sex)	--	--	0.08

Abbreviations: CVR– anterior cerebrovascular resistance; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; cf-PWV – carotid femoral pulse wave velocity.

§ CVR, age and mIMT were natural log transformed prior to analysis.

Data were presented as standardized parameter estimate (β) for cf-PWV, significance of the parameter estimate, and explained variance (R^2) of the model. Adjusted models included age, metabolic syndrome, and current smoker as covariates. For 'no statin use' models, sex was also included as a covariate. 'Best Fit' model is nested within the adjusted model and determined by lowest Akaike's Information Criterion.

Table 4-5. Cumulative impact of vascular risk based on thresholds for IMT and cf-PWV

Characteristic	No Vascular Risk Markers (n = 41)	1 Vascular Risk Marker (n = 25)	2 Vascular Risk Markers (n =4)	*P* value
Age, years	73.2 (8.6)	72.7 (7.5)	75.0 (8.0)	0.596
Sex – women, % (n)	57.4 (27)	64.3 (18)	83.3 (5)	0.732
Metabolic Syndrome, % (n)	42.6 (20)	28.6 (8)	66.7 (4)	0.108
Hemodynamics				
MAP, mmHg	91.1 (11.3)	101.3 (12.4)	93.0 (3.5)	**0.006**
$P_{ET}CO_2$, mmHg	37.5 (5.5)	36.1 (3.5)	39.2 (2.5)	0.128
Total CBF, mL/min (33/16/2)	760 (180)	680 (190)	580 (100)	0.183
Anterior CBF, L/min (37/24/2)	590 (150)	550 (170)	420 (80)	0.175
Posterior CBF, L/min (35/16/3)	160 (60)	190 (70)	170 (180)	0.900
CVR, mmHg/L/min (37/24/2)	160 (50)	180 (60)	230 (30)	**0.027**
MCA MFV, cm/s	53.4 (17.1)	53.4 (13.2)	52.5 (17.2)	0.997
MCA CVRi, cm/s	1.67 (0.64)	1.82 (0.44)	1.88 (0.46)	0.497
MCA PI, ratio	0.88 (0.17)	0.91 (0.21)	1.11 (0.28)	0.103
CrCP, mmHg	36.3 (19.7)	29.9 (12.8)	25.0 (7.7)	0.159
RAP, mmHg/cm/s	1.06 (0.36)	1.30 (0.24)	1.27 (0.45)	**0.013**
CR_{CO2}, %/mmHg (38/22/3)	4.15 (1.32)	4.31 (1.33)	4.20 (1.28)	0.801
dCA, ratio (31/15/3)	0.34 (0.35)	0.16 (0.44)	0.00 (0.06)	0.136
Blood Markers				
CRP, mg/L (38/25/4)	1.14 (2.31)	2.12 (2.64)	2.08 (2.70)	0.568
Creatinine, μmol/L (38/24/4)	92.2 (27.0)	91.5 (22.4)	92.2 (62.2)	0.529
Cognitive Function				
MoCA, total score	28.0 (2.0)	29.0 (1.0)	28.5 (1.0)	0.939
GDS, total score	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.632

Abbreviations: MAP – mean arterial pressure; $P_{ET}CO_2$ – end-tidal pressure of carbon dioxide; CBF – cerebral blood flow; CVR – anterior cerebrovascular resistance; MCA MFV – middle cerebral artery mean flow velocity; MCA CVRi – cerebrovascular resistance index; MCA PI – pulsatility index; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; dCA – dynamic cerebral autoregulation during sit-to-stand transition; MoCA – Montreal Cognitive Assessment; GDS – Geriatric Depression Scale(short scale).

Data were presented as median (interquartile range) for continuous variables and proportion (count) for categorical variables. Sample sizes for variables with missing data are identified in parentheses. Risk markers included carotid-femoral pulse wave velocity (≥ 10.0 m/s) and mean intima media thickness (men ≥ 0.90 mm; women ≥ 0.80 mm).

Significance determined by Kruskal-Wallis Test for continuous variables and Fisher's Exact Test for categorical variables.

Discussion

This chapter examined the impact of underlying arterial structure and function on cerebrovascular characteristics in a community-based sample of older adults, free of cerebrovascular disease. IMT, but not cf-PWV, proved to be a robust predictor of anterior CBF and CVR in older women. The association with CVR remained after adjusting for age, the presence of metabolic syndrome and smoking status. Although the relationship between arterial structure and cerebrovascular health was hypothesized to be mediated by inflammatory processes, CRP was unrelated to IMT. The influence of arterial stiffness on cerebrovascular health was not as persuasive as that of IMT. While cPP and ba-PWV were both related to the pulsatile cerebral hemodynamics, a consistent relationship with cf-PWV – the consensus measure of arterial stiffness (Laurent *et al.*, 2006) – was not observed. However, when the influence of cf-PWV was combined with that of IMT, the combined effects were associated with an increased cerebrovascular resistance profile and a non-linear blood pressure profile that appeared to suggest cumulative risk. In contrast to the hypothesis, CR_{CO2} was not significantly related to any underlying vascular characteristic.

Total CBF in the current study was 722 ± 147 mL/min, which is in line with values reported in the literature. Schreiber *et al.* (2005) reported a mean CBF of 733 ± 54 mL/min in a group of older adults (n = 12, mean age 65 ± 8 years). Others, however, have reported lower mean values ranging from 567 to 603 mL/min, with individual variation ranging from 350 to 850 mL/min (Dorfler *et al.*, 2000; Scheel *et al.*, 2000a; Albayrak *et al.*, 2007). The individual range of total CBF in the current sample was 430 to 1090 mL/min, which was within the limits of previous reports from middle-aged and older adults (Scheel *et al.*, 2000b). In addition, antihypertensive and statin therapy have been shown to reduce cerebrovascular resistance and

increase indices of CBF (Lipsitz *et al.*, 2005; Carlsson *et al.*, 2012), and might have contributed to a mildly elevated CBF in the current sample. Sixty percent of the sample was taking hypertensive or hyperlipidemic therapy, whereas the study by Scheel *et al.* (2000a) reported only one individual on mild antihypertensive medication. In the current study, CBF characteristics in participants taking anti-hypertensives or anti-hyperlipidemics were not different compared to participants not taking medications. The true influence of medication use on CBF in the current sample would have required a gradual tapering of medication use prior to the cerebrovascular exam and was not the focus of this research.

The observation that 76 % of total CBF was directed towards the anterior cerebral circulation is consistent with the literature (77 %; Scheel *et al.*, 2000a). No sex-specific differences in total CBF were observed; however, men had ~ 11 % greater anterior CBF than women. A trend was noted for an inverse relationship between posterior CBF and age in men; however, no relationship was observed in the anterior circulation for either men or women. Although Scheel *et al.* (2000b) reported a gradual decrease in CBF across the lifespan (*i.e.*, age 20-85 years); they did not identify significant relationships within each 20-year grouping, which is consistent with the shorter age range in the current study. In multiple regression models, age was a significant predictor of elevated CVR in women suggesting that resistance characteristics might be a more robust indicator of vascular change in healthy aging than flow.

After stratification by sex, the relationship between IMT and the anterior circulation was significant only in women. Interestingly, MCA MFV was greater in women despite a similar total CBF, and even a lower anterior CBF, suggesting a smaller intracranial vessel diameter. The MFV in older men was ~ 20 % less than in older women. Even assuming equal CBF, this would relate to a ~ 10 % greater diameter in men than women, which is consistent

with angiography evidence showing that the MCA diameters of men were 9.3 % larger than women (Muller *et al.*, 1991). Smaller vessels would be at greater risk for luminal encroachment that occurs with intima-medial thickening, perhaps contributing to the stronger relationship between IMT and CBF observed in women than men. In addition, more women than men exhibited IMT levels that exceeded recommended sex-specific thresholds for cardiovascular risk (n_{women} = 11 vs. n_{men} = 3; Figure 4-2 to 4-4; Howard *et al.*, 1993). The Baltimore Longitudinal Study of Aging noted that elevated IMT was associated with lower perfusion through the occipital-temporal region but higher perfusion through the frontal-temporal region in older adults without patent cerebrovascular disease (Sojkova *et al.*, 2010). These differences in regional susceptibility to atherosclerotic development might partially account for discrepancies in the association between CBF and cerebral atrophy in aging (Chen *et al.*, 2011). The current study was interested in global relationships and is unable to distinguish regional differences, other than to say the anterior circulation appeared to be more variable than the posterior circulation.

From studies in both the extracranial and intracranial vasculature, a consistent relationship between increasing stiffness, increasing cerebrovascular resistance and lowered perfusion has emerged (Hirata *et al.*, 2006; Kielstein *et al.*, 2006; Kwater *et al.*, 2009; Robertson *et al.*, 2010; Mitchell *et al.*, 2011; Tarumi *et al.*, 2011; Xu *et al.*, 2012). A direct correlation between the pulsatility of both ICA and MCA flow velocity and indices of arterial stiffness was noted, which provides further support that changes in central stiffness are propagated into the cerebral circulation (Kwater *et al.*, 2009; Mitchell *et al.*, 2011; Webb *et al.*, 2012). However, no relationship was observed between arterial stiffness and mean CBF or resistance. Evidence from animal models has shown that controlled exposure to increased pulse

pressure induces hypertrophic remodelling in cerebral arterioles (Baumbach, 1996). In agreement with this hypothesis, we have previously observed a relationship between brachial-ankle PWV and cerebrovascular resistance e. The mean age of the current sample was ~ 3 years younger than this prior report, and despite similar ba-PWV, the carotid pulse pressure in the current sample is lower (53 vs. 64 mmHg). Differences in the magnitude and duration of the exposure of the cerebral circulation to the stresses associated with arterial stiffening might have contributed to the discrepancies noted. Both samples were cross-sectional in nature and a prospective study examining the relationship between arterial stiffness and cerebrovascular health over time is warranted. Further exploration into this association is pertinent due to the apparent relationship between stiffness, cerebral lesions and cognitive function (Waldstein *et al.*, 2008; Mitchell *et al.*, 2011; Scuteri *et al.*, 2011).

CR_{CO2} has been linked to endothelial dysfunction (Lavi *et al.*, 2006) and might be an early marker of changes in cerebrovascular health. Hypercapnic cerebrovascular reactivity in the current study was similar to that reported by Kastrup *et al.* (1998) in the 7th decade for men and women (~3.5 %/mmHg CO_2). However, CR_{CO2} in healthy older adults has been reported as low as 1 %/mmHg CO_2 (Glodzik *et al.*, 2011). CR_{CO2} was not associated with aging in men although there was a trend for an inverse relationship between CR_{CO2} and age in older women. Kastrup *et al.* (1998) reported that aging was associated with a reduction in CR_{CO2} in women between the 4th and 5th decade, such that sex-based differences in reactivity in young adults are lost in older age. Further, no relationship with CR_{CO2} was found for any marker of vascular structure and function. In multiple linear regression analyses, the models of best fit for women and men were age and the presence of metabolic syndrome, respectively. Glodzik *et al.* (2011) reported an inverse relationship between cortical CR_{CO2} and the Framingham risk score, which

considers age, as well as some of the same characteristics as the definition of metabolic syndrome. The current sample of older adults had prevalent hypertension (50%) but other risk factors were generally well controlled, with a low prevalence of pre-existing CVD and diabetes mellitus. Therefore, the convenient sampling technique used to recruit participants for the current study might have been biased towards a healthy group within the larger community. Further, the length of time that the participants have been exposed to risk factors, or the duration of medical therapy, was unknown. Longer exposure to hypertension has been linked with reduced CR_{CO2} (Birns *et al.*, 2009), and might have been a factor in the current study. Considering the current results, perhaps the vascular impact of IMT and arterial stiffness had not had a sufficient incubation time to impact CR_{CO2} in the current sample.

Despite the absence of overt cerebrovascular disease, 20-30% of participants had arterial stiffness, arterial wall thickening, and inflammatory characteristics above established risk thresholds. The systemic properties of vascular aging, suggest that while a single marker might provide some insight into subclinical cerebrovascular risk, an index of multiple risk measurements can provide added benefit by considering the influence of complementary factors (Greenland *et al.*, 2010). Arterial stiffness and IMT have previously been shown to provide complementary information regarding vascular risk (Kobayashi *et al.*, 2004; Tu *et al.*, 2010), suggesting both measures should be included into a comprehensive subclinical risk score. We examined a group of individuals characterized by both elevated IMT and cf-PWV. This small group of 'accelerated vascular aging' was characterized by increased cerebrovascular resistance in the anterior circulation and elevated RAP in the MCA. Anterior CBF was progressively lower in each level of risk, but did not reach significance. Notably, although not significant ($P = 0.128$), $P_{ET}CO_2$ was marginally higher in the group with elevated

risk for both IMT and cf-PWV, which might have attenuated the differences in CBF through a vasodilator effect. While elevated $P_{ET}CO_2$ may have masked what would have otherwise been a greater discrepancy in CBF between the groups, it may be reasonable to ponder whether these secondary observations are protective responses in vascular regulation to mitigate the potential for hypoperfusion. Also, the relationship between the number of risk factors and MAP is worth noting. MAP was elevated in individuals with either risk factor, but decreased when both were present. Lower MAP in the highest risk group might be secondary to increased diastolic decay associated with arterial stiffness (O'Rourke & Hashimoto, 2007). Consequently, the relationship between arterial structure/function and cerebrovascular hemodynamics is not a linear one across the aging spectrum. The point where MAP begins to drop might represent a fulcrum point that imparts new challenges on the ability to maintain sufficient perfusion pressure. A larger sample size is necessary to confirm how multiple measures of arterial structure/function contribute to cerebrovascular risk.

Limitations

This study involved a cross-sectional design, and, as such, conclusions cannot infer any causal relationships, but rather demonstrate the associations to be explored by future prospective studies. The study sample was predominantly of Caucasian race and the findings might not be generalizable to other ethnic groups. Further still, no indication of the length of exposure to many of the cerebrovascular risk factors or the duration on medication was recorded. A hypothesized latent period between the appearance of vascular change and changes in the brain might have confounded the current findings (Iadecola, 2004). Taking into consideration the scoring on the Montreal Cognitive Assessment and Geriatric Depression Scale, as well as the overall elevated CBF, the current cohort appeared to be one with good cognitive health.

A few technical limitations merit consideration when discussing these findings. The extracranial technique for CBF measurement requires the sequential measurement of blood flow through the ICA and VA on the right and left sides. Hence, rather than measuring CBF at one point in time, CBF is the sum of four measurements taken over ~20-30 minutes. This process is subject to two main concerns: (1) the summation or interference of error from each of the individual measurement has the potential to diminish or exaggerate CBF, and (2) non-stationarities secondary to spontaneous fluctuations in blood pressure and PCO_2 may contribute to real differences in flow at the time of measurement in one or more of the extracranial arteries (Panerai *et al.*, 2000; Mitsis *et al.*, 2004). In the current study, simultaneous measurement of TCD, $P_{ET}CO_2$ and ABP were only recorded for the ipsilateral ICA measurement. In the future, maintaining recording of these variables throughout measurement of the 4 extracranial vessels will allow for the determination of CBF stability across the measurement period. Finally, extracranial measurement of CBF provides a global measure of cerebral perfusion and the comprehensive collateralization of the cerebral circulation precludes any conclusions on regional CBF. Still, the extracranial technique has been shown to be sufficiently sensitive to identify clinically important differences in CBF (Maalikjy *et al.*, 2005; Bai *et al.*, 2007).

Conclusion

This study examined the relationships between vascular health characteristics and the range of CBF observed in a cohort of older, community-living adults, free of diagnosed cerebrovascular disease. In women, increased IMT was related to lower CBF and increased CVR, independent of age, metabolic syndrome and smoking status. IMT, age and sex were the factors which contributed most to increased CVR. However, a large amount of variance in CBF, CVR and

CR_{CO2} remained unexplained even after the consideration of subclinical markers of vascular function and classic cardiovascular risk factors. Prospective examination of this relationship, involving methods that are more spatially sensitive, might help contribute to the better understanding of the relationship of CBF and aging vasculature.

CHAPTER 5. INFLUENCE OF ELEVATED RESISTANCE ON POSTURAL CEREBRAL AUTOREGULATION IN OLDER ADULTS

Introduction and Rationale

Orthostatic intolerance is a major contributing factor to hospitalizations of older adults, with syncope being a prevalent cause of admittance to emergency departments (Shibao *et al.*, 2007). Intolerance is a consequence of a reduction in arterial blood pressure (ABP) in the upright posture and subsequent cerebral hypoperfusion. To maintain cerebral blood flow (CBF) despite a drop in ABP, the body relies on cerebral autoregulation – a process regulating near constant flow across a range of perfusion pressures (typically defined as 60 to 150 mmHg) (Paulson *et al.*, 1990). Static cerebral autoregulation (sCA) refers to fluctuations in CBF in response to stable changes in ABP. sCA has been shown to be altered in aged animals (with an upward shift of the lower pressure limit) (Hoffman *et al.*, 1982); however, autoregulatory efficiency in humans appears to be tolerant of aging and hypertension (Eames *et al.*, 2003). This robust effectiveness of sCA was demonstrated across a battery of pressor tests, including lower body negative pressure, isometric handgrip exercise and bilateral thigh-cuff inflation (Eames *et al.*, 2003). Yet, despite apparently intact sCA, steady state CBF of both young and older adults is lower in upright postures (Savin *et al.*, 1995; Ouchi *et al.*, 2001; Alperin *et al.*, 2005a; Sato *et al.*, 2012) and cerebral oxygen delivery is strained (Mehagnoul-Schipper *et al.*, 2000).

Though believed to be primarily controlled by myogenic mechanisms (Wallis *et al.*, 1996; Harder *et al.*, 2011), assessment of CA by posture change is confounded by local metabolic and sympathetic influences (Shoemaker *et al.*, 2001), as well systemic changes in vasoactive $PaCO_2$ (Serrador *et al.*, 2006; Immink *et al.*, 2009; Edgell *et al.*, 2012) and central hemodynamics (Wilson *et al.*, 2002; Harms *et al.*, 2010). The parallel anatomies of the external and internal carotid arteries (ECA; ICA) provide a within-subject comparison of intracranial

vs. extracranial circulations which are subject to the same central hemodynamics, but have differing local regulatory mechanisms. This comparison might be useful in elucidating cerebrovascular autoregulatory characteristics (Savin *et al.*, 1997; Arbeille *et al.*, 2012). After just 3 minutes of upright posture, a large differential in the decrease of mean flow velocity (MFV) through the middle cerebral artery (MCA) and superficial temporal artery was noted: 14 % and 53 %, respectively (Savin *et al.*, 1997). Further, during head-up tilt, a significant increase in resistance in the temporal artery vascular bed was proposed to be an important marker of cardiac output (CO) redistribution towards the cerebral circulation (Arbeille et al., 2012).

In addition to sCA, the use of transcranial Doppler ultrasound (TCD) and finger-cuff plethysmography have contributed to the field of dynamic CA (dCA) introduced by Aaslid *et al.* (1989). The high temporal resolution of these methods has allowed examination of beat-to-beat, and even within-beat, relationships between arterial pressure and CBF velocity (for review, see Panerai, 2009). Evidence in healthy older adults has consistently shown that dCA is preserved (Lipsitz *et al.*, 2000; Carey *et al.*, 2000; Carey *et al.*, 2003; Deegan *et al.*, 2011; Kim *et al.*, 2011b). In contrast, the gradual development of vascular pathology might be related to impairment of dCA, as has been reported in diabetes mellitus (Kim *et al.*, 2008) and stroke populations (Immink *et al.*, 2005; Aoi *et al.*, 2012).

Most reports of dCA involve time or frequency domain characterization of changes in MFV and mean arterial pressure (MAP). Using this approach, Edwards *et al.* (2002) demonstrated that cerebrovascular resistance index (CVRi; Equation 1-3) is a sensitive measure of the cerebrovascular response to changes in MAP. Use of this two-component model (*i.e.*, MAP → CVRi) has led to a better understanding of the dynamic regulation of

CBF. However, age-related increases in arterial stiffness are associated with changes to the pulsatile nature of the cerebral circulation (Kim *et al.*, 2010; Webb *et al.*, 2012). Examination of mean signals across the cardiac cycle do not reflect these pulsatile changes and might not be the most appropriate method for examining autoregulation in association with aging-related changes in vascular health. Critical closing pressure (CrCP) and resistance area product (RAP) are two derived variables to characterize the passive pressure-velocity relationship in the cerebral circulation according to within-beat pulsatile changes. Carey *et al.* (2001) noted that the onset of syncope following head-up tilt was associated with a hypocapnic-mediated increase in CrCP and reduction in MFV, despite a compensatory reduction in CVRi suggesting that this latter resistance index was not driving the observed change in flow. Interestingly, Ogoh *et al.* (2011) found CrCP to be lower in older adults compared to young adults at rest, but that it increased further in older adults during aerobic exercise, suggesting that CrCP, which can change independent of blood pressure (Panerai, 2003), plays a critical role in autoregulatory function. RAP has been used to estimate sCA in hypertensive challenges through noradrenaline and phenylephrine infusion (Daley *et al.*, 2002; McCulloch & Turner, 2009); however, the roles of RAP and CrCP in the dynamic response to hypotensive stimuli have yet to be explored.

Recently, Panerai *et al.* (2005) introduced a three-component model of cerebrovascular regulation. Their model proposed to differentiate the independent contributions of MAP, CrCP and RAP, to the MFV response during a cognitive task. In doing so, they were able to consider both between- and within-beat variations in the pressure-velocity relationship in response to a given stimulus. Given the potential role of CrCP and RAP in mediating autoregulatory responses, it is relevant to examine orthostatic stress from the viewpoint of this new model.

The purpose of this analysis was to explore sCA and dCA in response to upright posture using quantitative extracranial imaging ultrasound and TCD methods. To study the impact of gradual changes in vascular health associated with aging, older adults were divided into sex-specific tertiles of anterior cerebrovascular resistance (CVR). In Chapter 4, CVR was shown to be associated to underlying changes in central vascular structure and function. Secondary to these underlying changes, it was hypothesized that both sCA and dCA would be impaired in the group with elevated CVR. With respect to sCA, differences in ICA_{BF} and vertebral artery blood flow (VA_{BF}) between supine lying and upright sitting were expected to be exaggerated in the tertile with the highest CVR. Posture-related changes in ECA_{BF}, CO, $P_{ET}CO_2$ and BP_{MCA} were examined as potential modifiers of sCA. With respect to dCA, it was hypothesized that advanced changes in vascular structure and function, as embodied by higher CVR, would be associated with greater impairment. A model of dCA which evaluates CrCP and RAP, because of these variables' relationship to the pulsatile changes in the vascular characteristics of aging, was expected to be more sensitive to the differences in vascular health than the traditional model incorporating mean characteristics (MAP and CVRi).

Methods

In this chapter, data from older adults refers to the cohort and cerebrovascular assessments described in Chapter 2. Where indicated, older adults are grouped according to sex-adjusted tertiles of CVR (LOW, MID, and HIGH). For this chapter, a group of young participants was examined, in addition to the main elderly cohort of the thesis, to further assess the influence of central hemodynamics in both intracranial and extracranial vascular beds. This younger cohort is described here, along with slight differences in methodology. Twenty-nine young, healthy participants (57% women; age 24.8 ± 3.1 years (mean ± SD); height: 171 ± 10 cm; weight: 74

± 18 kg) completed a self-reported health questionnaire to rule out pre-existing cardiovascular, neurological and psychomotor pathology, and medication use. Participants were asked to refrain from exhaustive exercise and alcohol for 24 hours and from caffeinated beverages for 12 hours prior to testing. Participants were encouraged to eat a light snack at least 2 hours before testing. All procedures were reviewed and approved by the Office of Research Ethics at the University of Waterloo and all participants provided written, informed consent.

The procedures performed with the young cohort are similar to those outlined in the 'Cardiovascular Hemodynamics', 'Cerebral Blood Flow' and 'Cerebrovascular Function (Cerebral Autoregulation)' sections found in Chapter 2, with a few exceptions. Notably, in the younger cohort, blood flow was measured in the common carotid artery (CCA) in addition to the ICA and ECA; however not in the vertebral artery (VA). In addition, blood flow was measured unilaterally on the right side in this group, relying on the assumption of symmetry between the right and left sides (Schmidt *et al.*, 2003).[1] Finally, steady state extracranial hemodynamics were assessed in standing (STA), in addition to the supine lying (SUP) and sitting (SIT) postures that had been completed in the older cohort (Figure 5-1). ECA measurements were added to the protocol in older adults as an amendment to the original data collection. Consequently, only 24 older adults have data on ECA characteristics and 19 of them have valid ECA flow in both supine and sitting postures.

In this chapter dCA was assessed using a three component model of CBF regulation, in addition to the two-component model described in Chapter 2. The three-component model considers the influence of ABP, CrCP and RAP on MFV, as shown in Equation 1-4, where

[1] Refer to Table 2-2 for evidence of hemispheric congruence of carotid artery structure and function in the older cohort.

MFV represents blood flow. During the transition from sitting to standing, each component (ABP, CrCP and RAP) can change (Δp, Δcp and Δr, respectively), independently contributing to the overall ΔMFV. For small changes in RAP, Panerai *et al.* (2005) derived the following equations to estimate the contribution to the ΔMFV from each component:

$$\text{A: } \Delta V\_ABP = \Delta p / RAP_0$$
$$\text{B: } \Delta V\_CrCP = -\Delta cp / RAP_0$$
$$\text{C: } \Delta V\_RAP = -\Delta r \cdot MFV_0 / RAP_0$$
$$\text{D: } \Delta MFV = \Delta V\_ABP + \Delta V\_CrCP + \Delta V\_RAP,$$

where ΔV is the velocity contribution attributable to each respective component; MFV_0 and RAP_0 represent the average values over the 30 s leading up to the postural transition; and Δp, Δcp, Δr and ΔMFV, represent the change in BP_{MCA}, CrCP, RAP and MFV at each time point from their average values in the 30 s prior to postural transition.

Equation 5-1. Set of equations used to estimate velocity contribution from critical closing pressure and resistance area product.

Of the 68 older adults with successful TCD signals, technical difficulties during dynamic postural transitions (*i.e.*, loss of CBF velocity signal or finger-cuff blood pressure signal) resulted in valid dCA data for only 31 participants during the supine-to-sit transition and 44 participants during the sit-to-stand transition. Sorting of CVR tertile was based on resting bilateral ICA blood flow and BP_{MCA} on the entire cohort, and was not reassigned to account for participant dropout during the dynamic tests.

Statistical Analysis

Data were presented as mean ± standard deviation. Initial analysis in the young cohort included an examination between men and women using Student's *t*-test; however, as a result of few impressive differences between them relating to the posture change, men and women were pooled for the remaining analyses. In the young cohort, differences between arteries across SUP, SIT and STA postures were examined using a mixed linear, repeated measures analysis of variance (ANOVA) model (posture as repeated factor), incorporating an autoregressive

covariance structure. In the older cohort, differences between SUP and SIT were assessed by paired *t*-test. Relationships between blood flow and BP_{MCA}, and between blood flow and end-tidal carbon dioxide ($P_{ET}CO_2$), across the three postures (two postures for older adults) were assessed by linear regression for each participant. Linear regression coefficients from flow (velocity)-pressure relationship were taken to reflect sCA and were assessed between the ICA, ECA and MCA by a one-way ANOVA. The two-component model of dCA was assessed by a mixed linear, repeated measures ANOVA model (CVR tertile as a group factor and supine-to-sit vs. sit-to-stand as a repeated factor). In the three-component model of dCA, differences in the contribution of ABP, CrCP and RAP between groups (CVR tertile) were assessed using a similar model with time as the repeated factor. Time points relative to the initiation of the sit-to-stand posture change at 120, 123, 126, 129, 132, 140 and 180 s were chosen *a priori* for comparison. Post hoc comparison of repeated measures ANOVAs were performed by a least squares method. Significance was determined at α level $P < 0.05$. Statistical tests were performed using SAS v9.2.

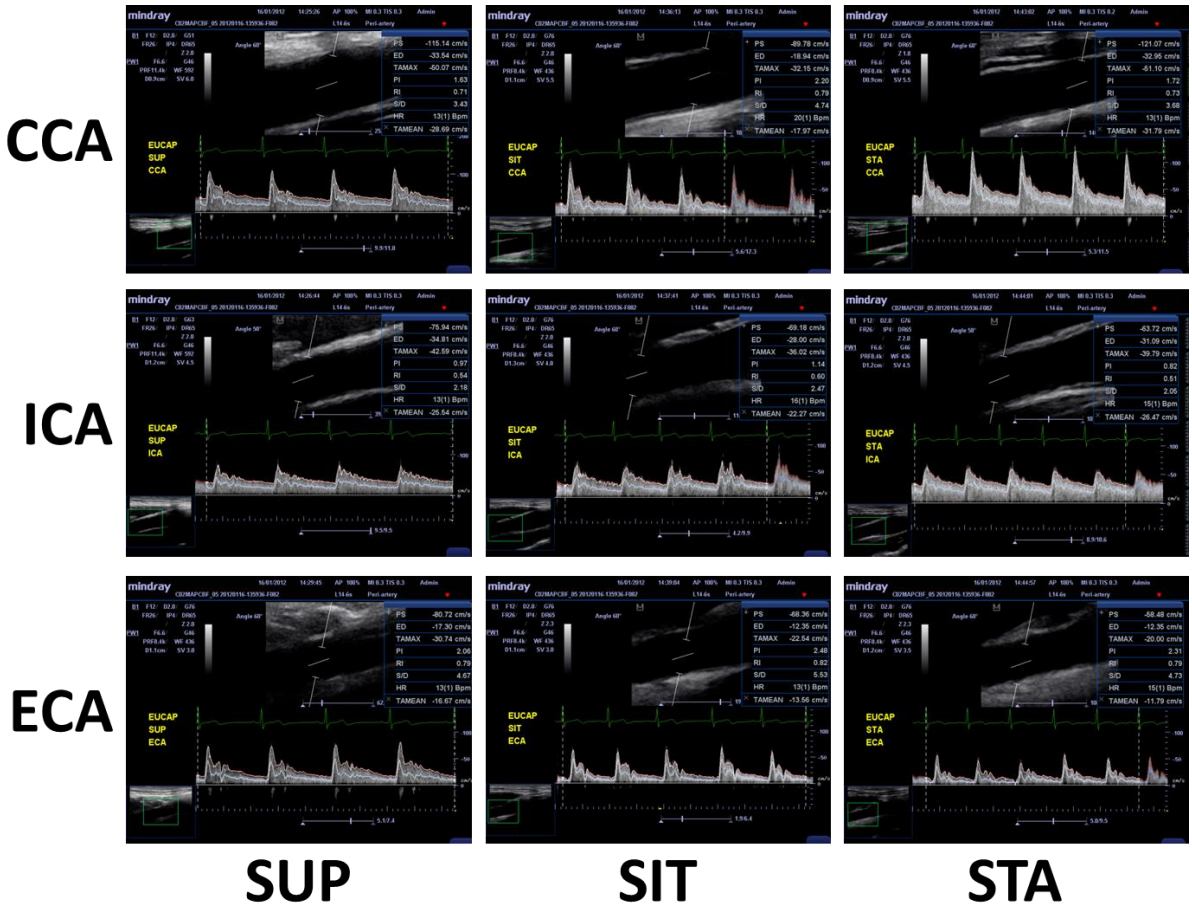

Figure 5-1. Representative ultrasound image captures of carotid artery in supine lying, sitting and standing.

Common carotid (CCA; upper panel), external carotid (ECA; middle panel) and internal carotid (ICA; lower panel) artery diameter and velocity in supine lying (SUP), sitting (SIT), and standing (STA) postures.

Results

Across the sample of young participants, men and women had similar resting heart rate (men: 58.7 ± 10.0 vs. women: 62.4 ± 10.4 bpm; t_{27} = -0.97, P = 0.202), MAP (84.1 ± 5.9 vs. 82.9 ± 6.2 mmHg; t_{27} = 0.52, P = 0.606), CO (6.2 ± 2.2 vs. 5.4 ± 1.9 L/min; t_{27} = 1.11 P = 0.276), and $P_{ET}CO_2$ (43.3 ± 3.8 vs. 41.3 ± 3.4 mmHg; t_{27} = 1.51, P = 0.76). Further, posture-induced changes in central cardiovascular characteristics were similar between men and women, except for heart rate, where men showed a smaller increase between sitting and standing than women (10.7 ± 6.6 vs. 17.2 ± 7.2 bpm; t_{27} = -2.51, P = 0.018). Given the similarity in the postural responses, as well as the focus on aging for the current study, men and women were pooled for analysis.

Steady state CBF in upright posture – influence of central hemodynamics and PCO_2

In young adults, upright postures were associated with reductions in mean blood flow (BF) through the extracranial carotid arteries (Figure 5-2, top; CCA_{BF}: $F_{2,54}$ = 17.29, P < 0.001; ECA_{BF}: $F_{2,53}$ = 27.13, P < 0.001; ICA_{BF}: $F_{2,56}$ = 4.45, P = 0.016). Post-hoc analysis with Tukey-Kramer adjustment for multiple comparisons showed that CCA_{BF} and ECA_{BF} were both significantly lower in sitting and standing compared to lying supine, with a trend for ECA_{BF} to be further decreased in standing compared to sitting. There was a smaller effect on ICA_{BF}, which only showed a difference between standing and lying supine. The mean flow through the CCA was less than the sum of flow through the ICA and ECA (difference: 57 ± 91 mL/min; t_{28}= 3.4, P = 0.002); however, the measured and summed CCA_{BF} were significantly correlated ($r_{(n=29)}$ = 0.47, P = 0.009)

Table 5-1 shows the reduction in CCA_{BF} and ECA_{BF} was a function of both reduced arterial diameter and MFV; while, ICA_{BF} was primarily affected by velocity. The relative maintenance of ICA_{BF} and CCA_{BF} compared to ECA_{BF} appeared to be facilitated by a decrease in vascular resistance in upright postures (Figure 5-2, bottom). Interestingly, in the carotid

arteries, CVR was noted to decrease in upright postures, but RAP was stable (Table 5-2). In addition, significant reduction in CrCP in all vessels was observed. The CCA and MCA showed ~70 % decreases, whereas the ECA showed a smaller ~30 % drop. The ICA CrCP was negative in all postures, which was difficult to interpret, but still showed a large reduction in upright postures.

To examine the local changes in the carotid arteries with respect to systemic cardiovascular factors, indices of blood flow were assessed as a function of posture-induced changes in CO. Further, blood flow through the extracranial carotid arteries and MFV through the intracranial MCA were assessed as a function of changes in MAP at the level of the MCA (BP_{MCA}) and $P_{ET}CO_2$. There was a relationship between posture and CO, such that CO was lower in standing than when lying supine (Figure 5-3, top; SUP: 5.69 ± 0.39, SIT: 5.46 ± 0.34, STA: 5.19 ± 0.38 L/min; $F_{2,56} = 10.57$, $P < 0.001$). Notably, ICA_{BF} as a proportion of CO was unchanged across postures (SUP: 6.0 ± 0.6, SIT: 5.6 ± 0.6, STA: 6.2 ± 0.9 %; $F_{2,56} = 1.35$, $P = 0.269$), but ECA_{BF} was reduced (SUP: 2.7 ± 0.3, SIT: 2.2 ± 0.2, STA: 2.1 ± 0.2 %; $F_{2,53} = 11.45$, $P < 0.001$). The distribution of flow through the carotid artery to intracranial and extracranial vascular beds was characterized by examining the change in the ICA_{BF}:ECA_{BF} ratio across the three postures. Moving from the supine lying to standing posture, this ratio gradually increased (SUP: 2.5 ± 1.1, SIT: 2.8 ± 1.4, STA: 3.0 ± 1.3; $F_{2,53} = 7.27$, $P = 0.002$; Figure 5-3, bottom).

Individual autoregulatory responses between extracranial blood flow (or MCA MFV) and BP_{MCA} were assessed by linear regression using values from supine lying, sitting and standing postures (Figure 5-4, left panel). The regressions for each vessel had $r^2 \geq 0.5$ in ~70 % of participants. A one-way analysis of variance indicated differences between vessels of

interest ($F_{172,3} = 7.94$, $P < 0.001$). In post hoc testing with Tukey-Kramer adjustment, the slope of the ECA_{BF}-BP_{MCA} relationship was steeper than both ICA_{BF}-BP_{MCA} ($P < 0.001$) and MCA_{MVF}-BP_{MCA} ($P = 0.009$). The ICA_{BF}-BP_{MCA} and MCA_{MVF}-BP_{MCA} mean slopes were not different ($P = 0.367$). Paralleling the reduction in BP_{MCA} with upright posture in young participants, there was a drop in $P_{ET}CO_2$ (Figure 5-4, right panel). Independently, distinguishing whether blood flow was reduced due to decreased perfusion pressure or increased vascular resistance, pursuant to the decrease in $P_{ET}CO_2$, was not possible. Figure 5-5 plots the slope of the pressure-flow (or velocity) relationships from Figure 5-4 against the change in $P_{ET}CO_2$ observed across the three postures. An inverse relationship is seen in the ICA, ECA and MCA, indicating that individuals with a greater drop in $P_{ET}CO_2$ had a steeper slope in the pressure-velocity relationship, whereas individuals with less of a drop in $P_{ET}CO_2$ had a flatter slope.

Older Adults

In most of the older adults, MAP at the heart either returned near to, or surpassed its supine resting value after 2 minutes in upright posture. Of note, 11 older adults exhibited signs of orthostatic hypotension as indicated by sustained reduction in SBP > 20 mmHg (n = 3) or DBP > 10 mmHg (n = 9) after 2 minutes of upright posture. These individuals appeared to be evenly distributed across tertiles of CVR, so they were included in the overall analysis. Older adults showed posture-related differences in the anterior but not posterior cerebral circulation (Figure 5-6). A main effect of posture was observed in anterior CBF ($F_{1,58} = 24.93$, $P < 0.001$) that saw blood flow drop in upright posture. When flow was examined as a function of CVR in the anterior circulation, the anterior CBF showed the expected relationship that the highest CVR tertile had the lowest anterior CBF. Interestingly, posterior CBF was not different across

tertiles of anterior CVR. Examination of the interaction effect of posture and CVR tertiles found a trend for a smaller posture-related drop in anterior CBF in the participants with the highest CVR ($F_{2,58} = 2.51$, $P = 0.090$).

The drop in anterior CBF was mediated by decreases in both ICA diameter and velocity, whereas VA showed no change in either (Table 5-2). Both ICA and VA showed decreases in CVR when sitting, as opposed to the ECA CVR, which was unchanged. In contrast to CVR, RAP was similar in supine and sitting for the ICA and VA, whereas CrCP was reduced. In the MCA, MFV was reduced in sitting, along with CVRi. CrCP and RAP had contrasting effects as CrCP was reduced, but unexpectedly, RAP was increased.

While resting supine, older adults had a smaller proportion of CO going to both the ICA and ECA, compared to the younger cohort. There was not a noticeable drop in CO in sitting (note: a drop in the young group was only significant between standing and supine lying), and no changes in ICA_{BF} and ECA_{BF} when considered as a proportion of CO were observed (Table 5-3). However, older adults in the highest tertile of CVR did tend to have a lower proportional ECA_{BF} at rest than those with lower resistance.

Linear regression analysis between supine and sitting (for ICA, ECA and VA) and across supine lying, sitting and standing for MCA MFV for assessment of sCA in older adults (Table 5-4) showed that the pressure-flow relationship was most passive in the ECA, similar to the young group. There was no difference across the CVR tertiles. When these slopes were examined as a function of the drop in $P_{ET}CO_2$ associated with posture change (Figure 5-7), there was less of an association than noted in the young. Of note, the older adults had an attenuated drop in $P_{ET}CO_2$ compared to the young.

Dynamic cerebral autoregulation in older adults

Figure 5-8 (top panel) shows continuous data of the dynamic response of BP_{MCA} (left) and MFV (right) during supine-to-sit and sit-to-stand transitions. The point at which BP_{MCA} reaches a nadir during the sit-to-stand transition is highlighted in the bottom panel of the figure. The magnitude of the drop in MFV for the HIGH CVR group was less than either the LOW or MID groups, despite a similar drop in BP_{MCA}, suggesting intact or even superior dCA. Table 5-5 summarizes key dCA markers in both the supine-to-sit transition and the sit-to-stand transition. No difference across CVR tertiles was noted during the supine-to-sit transition; however, during the sit-to-stand transition, the HIGH CVR group had a smaller change in mean and diastolic velocity, contributing to a lower dCA index. The change in $P_{ET}CO_2$ from baseline at BP_{MCA} nadir during the transition was not different from zero ($\Delta P_{ET}CO_2$: SUP-to-SIT = -0.6 ± 3.1 mmHg, P = 0.852; SIT-to-STAND = -0.1 ± 2.6 mmHg, P = 0.350). There were no differences between the dCA index calculated from the supine-to-sit transition and from the sit-to-stand transition.

In the three-component model, a comparison between the measured MFV and the sum of the components was done to assess how well the model predicted the actual response (Figure 5-9, Top). Except for the period of transition, the modeled flow velocity closely tracked the change in MFV. During the transition phase, the modeled change in flow underestimated the actual drop in flow (Figure 5-9, Bottom). Between LOW and HIGH tertiles, differences in the response of V_CrCP and V_RAP are apparent (Figure 5-10). During the transition, the change in V_ABP followed MAP, while the changes in V_RAP and V_CrCP were opposite that of RAP and CrCP. That is, a reduction in RAP and CrCP led to increases their respective contribution to the velocity signal. Overall, the rapid drop in BP_{MCA} that

occurred during posture change was quickly countered by a drop in RAP (increase in V_RAP). This served to attenuate the immediate drop in MFV. After a brief delay, V_CrCP was noted to increase, contributing to an overshoot in MFV ~ 10 s after the beginning of transition. The influence of CrCP was most notable in the HIGH CVR group ($F_{2,34} = 2.88$, $P = 0.069$). A trend for a greater influence of CrCP in older adults with high CVR was noted as soon as 9 s following the transition (Table 5-6). No significant differences between the RAP response was noted across tertiles of CVR ($F_{2,34} = 0.36$, $P = 0.700$).

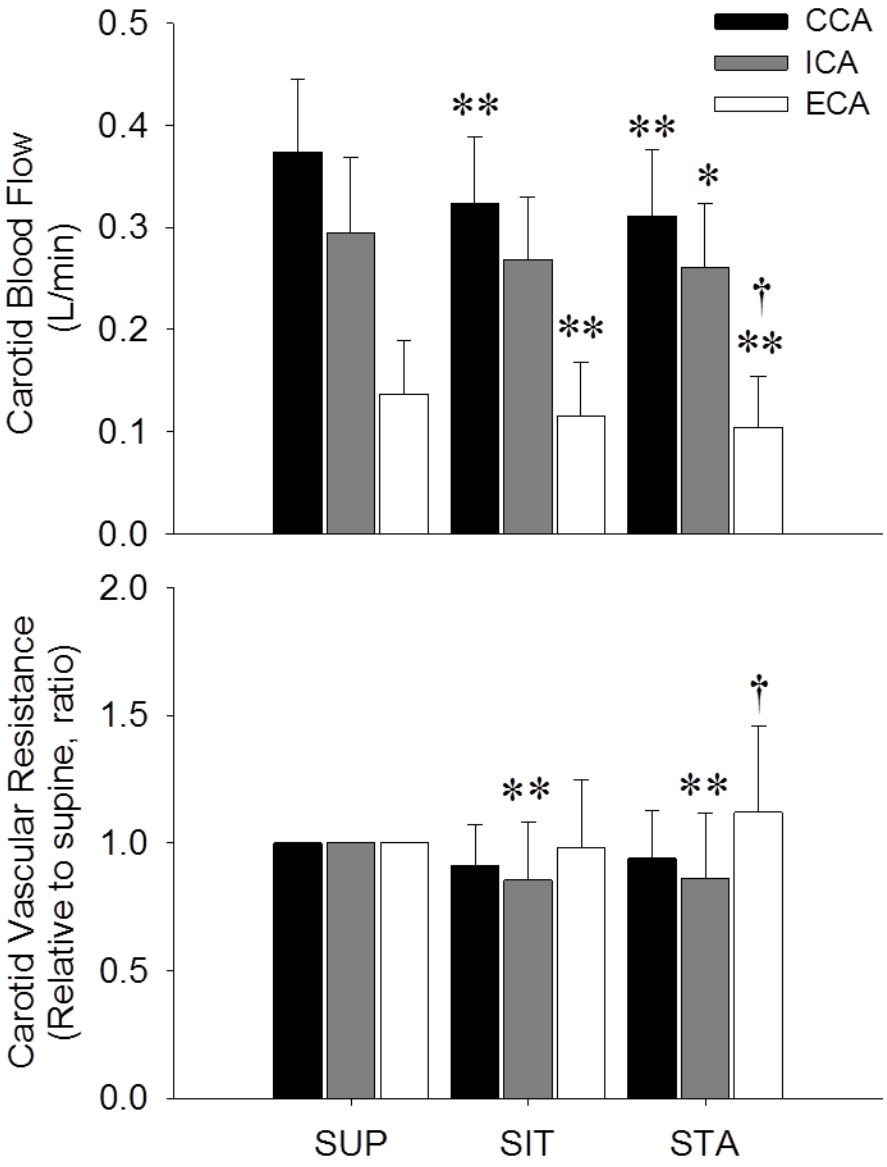

Figure 5-2. Carotid blood flow and resistance in supine lying, sitting, and standing postures in young adults.

Mean blood flow (top) and the relative change in vascular resistance (bottom) in supine (SUP), sitting (SIT) and standing (STA) postures for the common (black bars), internal (grey), and external (white) carotid arteries. Vessels were imaged in sequential fashion 5-10 minutes following postural transition. Data were presented as mean + SD; n = 29 (26 for ECA).

* different from SUP at $P < 0.05$
** different from SUP at $P < 0.01$
† different from SIT at $P < 0.05$

Table 5-1. Posture-related changes in the carotid and middle cerebral arteries of young adults

Artery	Variable	Posture			*P* value
		SUPINE	SIT	STAND	
Common Carotid	Diameter, cm	0.55 ± 0.05	0.52 ± 0.05†	0.53 ± 0.05†	**<0.001**
	Mean Velocity, cm/s	26.1 ± 5.6	24.7 ± 5.7	23.0 ± 4.8†	**0.015**
	CVR, mmHg/mL/min	0.24 ± 0.06	0.21 ± 0.05	0.21 ± 0.06	**0.048**
	Pulsatility Index	1.81 ± 0.56	1.99 ± 0.62	1.82 ± 0.57	0.257
	CrCP, mmHg	19.9 ± 15.4	6.1 ± 9.6†	5.9 ± 13.4†	**0.008**
	RAP, mmHg/cm/s	1.77 ± 0.52	1.62 ± 0.36	1.66 ± 0.51	0.669
External Carotid	Diameter, cm	0.41 ± 0.06	0.39 ± 0.06	0.39 ± 0.06†	**<0.001**
	Mean Velocity, cm/s	16.5 ± 4.0	14.5 ± 3.9†	13.4 ± 3.5†	**<0.001**
	CVR, mmHg/mL/min	0.71 ± 0.26	0.70 ± 0.31	0.77 ± 0.36	**0.041**
	Pulsatility Index	2.20 ± 0.41	2.66 ± 0.72†	2.47 ± 0.63	**0.032**
	CrCP, mmHg	30.4 ± 13.1	19.3 ± 7.8†	20.7 ± 12.8†	**0.018**
	RAP, mmHg/cm/s	2.12 ± 0.42	1.97 ± 0.87	2.04 ± 0.56	0.817
Internal Carotid	Diameter, cm	0.48 ± 0.05	0.47 ± 0.05	0.48 ± 0.05	0.686
	Mean Velocity, cm/s	26.5 ± 5.6	24.9 ± 4.0	23.9 ± 4.8†	**0.022**
	CVR, mmHg/mL/min	0.31 ± 0.10	0.26 ± 0.09†	0.26 ± 0.06†	**<0.001**
	Pulsatility Index	1.01 ± 0.19	1.12 ± 0.25	0.94 ± 0.22‡	**0.025**
	CrCP, mmHg	-5.3 ± 28.6	-30.6 ± 34.3	-32.4 ± 50.8	0.196
	RAP, mmHg/cm/s	2.68 ± 1.40	2.71 ± 1.03	2.69 ± 1.06	0.997
Middle Cerebral	Mean Velocity, cm/s	69.7 ± 14.3	62.1 ± 11.9†	59.3 ± 10.7†	**<0.001**
	CVRi, mmHg/cm/s	1.25 ± 0.33	1.06 ± 0.24†	1.07 ± 0.25†	**<0.001**
	Pulsatility Index	0.84 ± 0.16	0.88 ± 0.19	0.84 ± 0.26	0.229
	CrCP, mmHg	25.7 ± 12.6	8.3 ± 14.0†	7.7 ± 15.7†	**<0.001**
	RAP, mmHg/cm/s	0.87 ± 0.31	0.92 ± 0.32	0.94 ± 0.33†	**0.010**

Abbreviations: CVR – vascular resistance; CrCP – critical closing pressure; RAP – rate area product.

Data were presented as mean ± SD; n = 29 (26 for ECA), except ICA, ECA and CCA Pulsatility Index, CrCP and RAP, where n = 12. *P* values represent effect of posture determined by one-way repeated measures ANOVA.

Tukey's post-hoc test was used to examine differences between each posture:
† different from supine at $P < 0.05$
‡ different from sitting at $P < 0.05$.

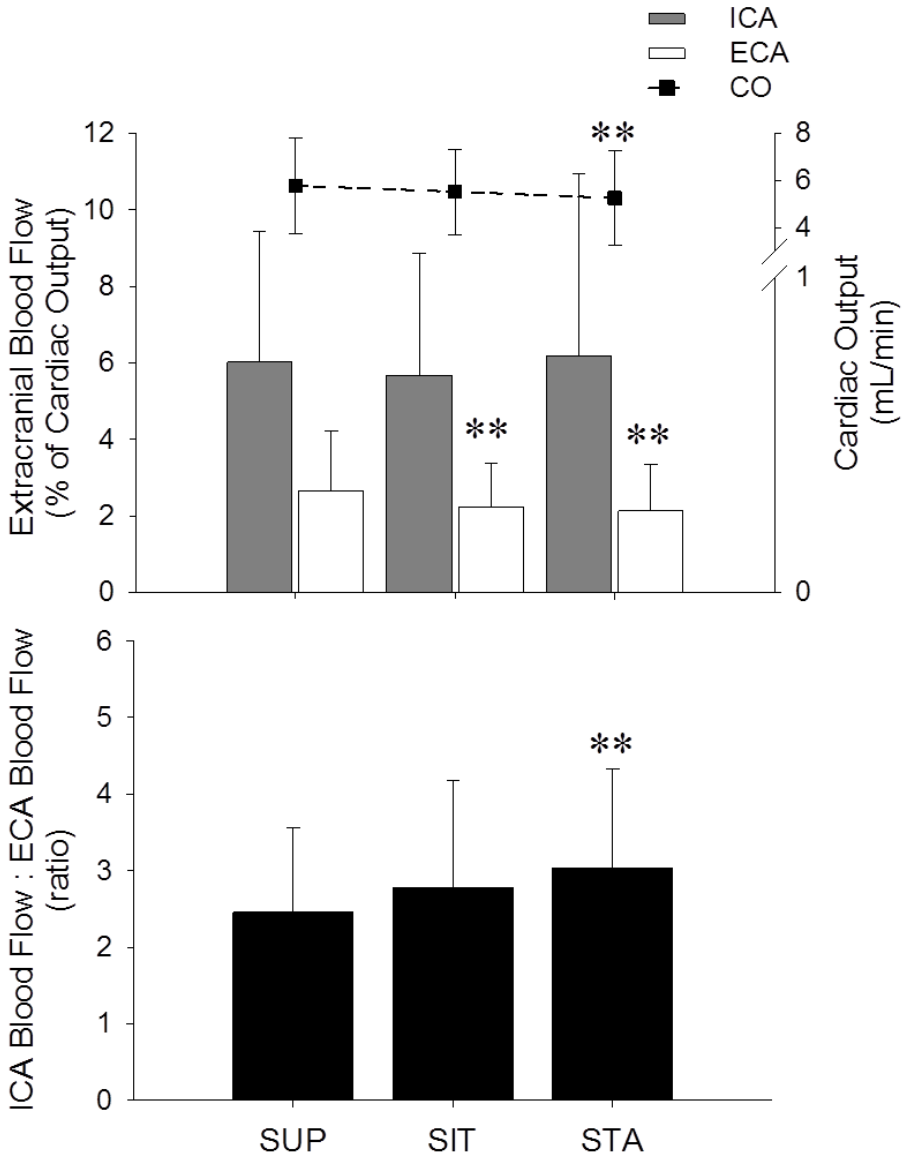

Figure 5-3. Proportional change in internal and external carotid artery blood flow of young adults in upright posture.
Top: Internal (ICA; grey bars) and external (ECA; white) carotid artery blood flows are represented as a proportion of cardiac output (CO) in supine lying (SUP), sitting (SIT) and standing (STA) postures. The dashed line plot represents absolute changes in CO. **Bottom:** The change in the ratio of ICA and ECA blood flow as a function of posture. Data were presented as mean ± SD, n = 26.

** different from SUP at $P < 0.05$.

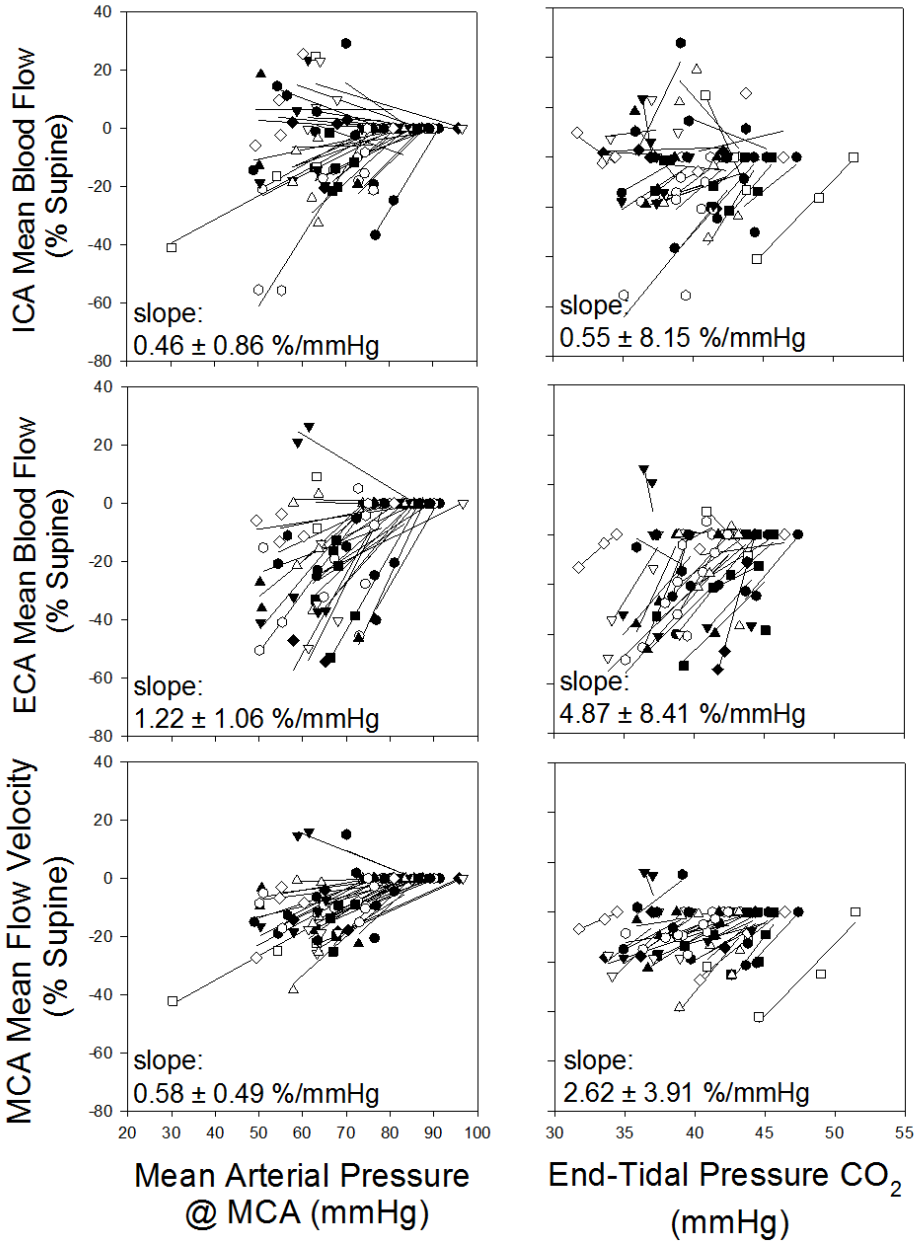

Figure 5-4. The relationships of blood flow with blood pressure and PCO_2 in supine lying, sitting and standing postures in young adults.

Individual participants' blood flow (velocity) in the internal (ICA, top) and external carotid artery (ECA, middle) and middle cerebral artery (MCA, bottom). Relative changes in blood flow from supine are expressed as a function of the change in mean arterial pressure (left panel) and end-tidal pressure of carbon dioxide (right panel) between supine lying, sitting and standing postures. Individual data presented, n = 29 (26 for ECA; note white squares missing from middle panel).

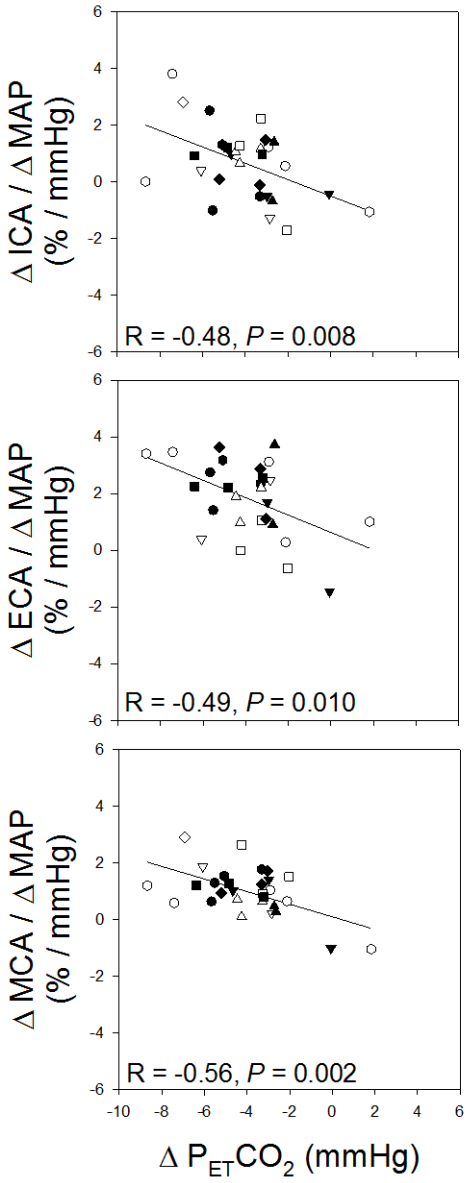

Figure 5-5. The relationship between changes in blood pressure and blood flow with postural transition is modulated by the change in $P_{ET}CO_2$ in young adults.
Each data point represents an individual line (slope) from Figure 5-4 (left panel). A more positive slope represents a greater influence of MAP on CBF. The negative correlation in each vascular bed indicates that regulation of $P_{ET}CO_2$ might play a permissive role in the relationship between pressure and CBF, such that controlling the drop in $P_{ET}CO_2$ in upright posture, might mitigate the pressure induced drop in CBF. Individual data presented, n = 29 (26 for ECA).

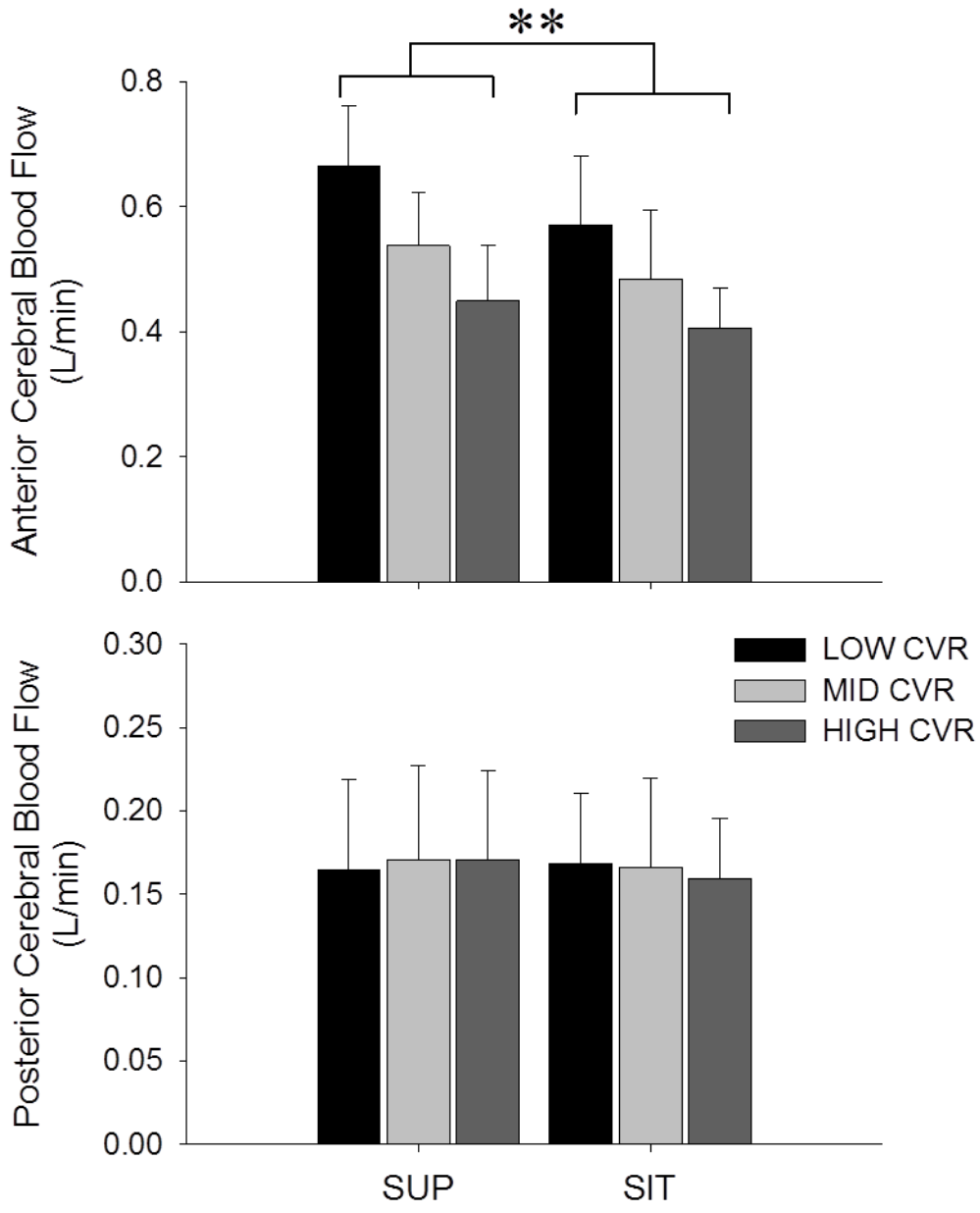

Figure 5-6. Anterior and posterior cerebral blood flow in supine lying and sitting postures in older adults.

Bilateral ICA (top) and VA (bottom) blood flow in supine lying and seated positions. Blood flow is stratified by sex-adjusted cerebrovascular resistance (CVR) tertiles. Data presented as mean ± SD; Anterior CBF: n = 61; Posterior CBF: n = 24. A two-way repeated-measures ANOVA (posture x CVR) was performed.

** Main posture effect, $P < 0.001$

Table 5-2. Carotid, vertebral and middle cerebral characteristics of older adults while supine and sitting.

Artery	Variable	n	Posture		*P* value
			SUPINE	SIT	
External Carotid	Diameter, cm	24	0.39 ± 0.08	0.38 ± 0.07	0.087
	Mean Velocity, cm/s	24	15.6 ± 4.6	11.5 ± 3.0	**<0.001**
	CVR, mmHg/mL/min	19	1.00 ± 0.55	0.99 ± 0.50	0.883
	Pulsatility Index	22	2.52 ± 0.58	3.77 ± 0.81	**<0.001**
	CrCP, mmHg	19	44.7 ± 13.8	34.7 ± 9.4	**<0.003**
	RAP, mmHg/cm/s	19	2.02 ± 0.61	1.77 ± 0.70	**0.009**
Internal Carotid	Diameter, cm	65	0.50 ± 0.09	0.49 ± 0.09	**0.042**
	Mean Velocity, cm/s	65	23.0 ± 6.4	21.9 ± 6.3	**0.030**
	CVR, mmHg/mL/min	54	0.38 ± 0.11	0.30 ± 0.12	**<0.001**
	Pulsatility Index	35	1.19 ± 0.26	1.34 ± 0.28	**<0.001**
	CrCP, mmHg	29	18.7 ± 13.0	-2.4 ± 12.4	**<0.001**
	RAP, mmHg/cm/s	29	2.33 ± 0.83	2.21 ± 0.84	0.295
Vertebral	Diameter, cm	44	0.36 ± 0.05	0.36 ± 0.05	0.668
	Mean Velocity, cm/s	44	12.7 ± 4.5	12.3 ± 5.0	0.331
	CVR, mmHg/mL/min	37	1.53 ± 0.94	1.16 ± 0.91	**0.002**
	Pulsatility Index	22	1.56 ± 0.44	1.87 ± 0.60	**0.003**
	CrCP, mmHg	19	31.7 ± 17.3	5.54 ± 15.1	**<0.001**
	RAP, mmHg/cm/s	19	3.00 ± 1.13	3.07 ± 1.30	0.804
Middle Cerebral	Mean Velocity, cm/s	66	56.3 ± 14.3	51.9 ± 11.8	**<0.001**
	CVRi, mmHg/cm/s	65	1.80 ± 0.49	1.36 ± 0.44	**<0.001**
	Pulsatility Index	66	0.90 ± 0.16	0.98 ± 0.17	**<0.001**
	CrCP, mmHg	66	32.1 ± 11.5	5.2 ± 12.5	**<0.001**
	RAP, mmHg/cm/s	65	1.19 ± 0.37	1.25 ± 0.43	**0.022**

Abbreviations: CVR(i) – vascular resistance (index); CrCP – critical closing pressure; RAP – resistance area product. Values for internal and external carotid, and vertebral arteries are from the right side.

Data were presented as mean ± SD. Data taken from right hemisphere vessels.

Table 5-3. Posture-related change in extracranial and intracranial blood flow as a function of cardiac output and cerebral vascular resistance in older adults

Variable	Tertile of Cerebrovascular Resistance (CVR)			*P* value
	LOW (n=11)	MID (n=3)	HIGH (n=5)	
ICA Flow, % CO	4.9 ± 1.8	3.8 ± 0.4	4.5 ± 1.8	0.107
Δ ICA Flow, % CO	0.0 ± 1.1	-0.2 ± 0.9	-0.4 ± 0.9	0.791
ECA Flow, % CO	1.8 ± 0.9	2.2 ± 0.8	1.5 ± 0.5	**0.049**
Δ ECA Flow, % CO	-0.6 ± 0.8	-0.6 ± 0.5	-0.2 ± 0.2	0.597
Cardiac Output, L/min	6.8 ± 1.1	5.0 ± 0.3	5.7 ± 2.2	0.122
Δ Cardiac Output, L/min	-0.8 ± 1.3	-0.3 ± 0.8	0.0 ± 1.0	0.499
Heart Rate, beat/min	61.2 ± 5.2	67.7 ± 9.4	65.6 ± 4.4	0.191
Δ Heart Rate, beat/min	+1.1 ± 5.3	+0.3 ± 2.0	+0.6 ± 1.3	0.907
BP_{MCA}, mmHg	89.5 ± 8.0†	88.0 ± 5.8†	100.3 ± 4.0	**0.013**
Δ BP_{MCA}, mmHg	-28.7 ± 6.5‡	-23.4 ± 4.3‡	-31.4 ± 3.3‡	0.735
$P_{ET}CO_2$, mmHg	37.4 ± 4.1	36.4 ± 4.5	33.2 ± 3.3	0.063
Δ $P_{ET}CO_2$, mmHg	-0.6 ± 1.9	-0.1 ± 2.7	-0.7 ± 2.0	0.987

Abbreviations: ICA – internal carotid artery; ECA – external carotid artery; CO – cardiac output; MCA – middle cerebral artery; MAP – mean arterial pressure; $P_{ET}CO_2$ – end tidal partial pressure of carbon dioxide. Δ represents change between steady state values in supine lying and sitting. ICA, ECA and MCA were from the right side.

Data were presented as mean ± SD. Only includes older participants who had both ICA and ECA measures.

† different from HIGH at $P < 0.05$

‡ reflects difference from zero at $P < 0.05$ (*i.e.*, implying a significant difference between supine and sitting postures).

Table 5-4. The relative change blood flow as a function of mean arterial pressure between supine lying and sitting postures in older adults.

Artery	n	All	Cerebrovascular Resistance Tertiles			*P* value
			LOW	MID	HIGH	
ICA, % flow/mmHg	20/15/15	0.41 (0.90)	0.43 (0.84)	0.34 (0.46)	0.44 (1.44)	0.799
VA, % flow/mmHg	14/12/7	0.32 (0.94)	0.23 (0.48)	0.28 (1.01)	-0.14 (3.41)	0.472
MCA, % velocity/mmHg	21/18/18	0.25 (0.29)	0.20 (0.16)	0.33 (0.55)	0.27 (0.30)	0.505
ECA, % flow/mmHg	10/2/5	1.00 (1.24)	1.60 (1.32)	1.27 (1.21)	0.72 (1.09)	0.286

Abbreviations: ICA – internal carotid artery; VA – vertebral artery; MCA – middle cerebral artery; ECA – external carotid artery.

Data were presented as median (interquartile range). *P* values represent effect of baseline cerebrovascular resistance assessed by non-parametric Kruskal-Wallis test.

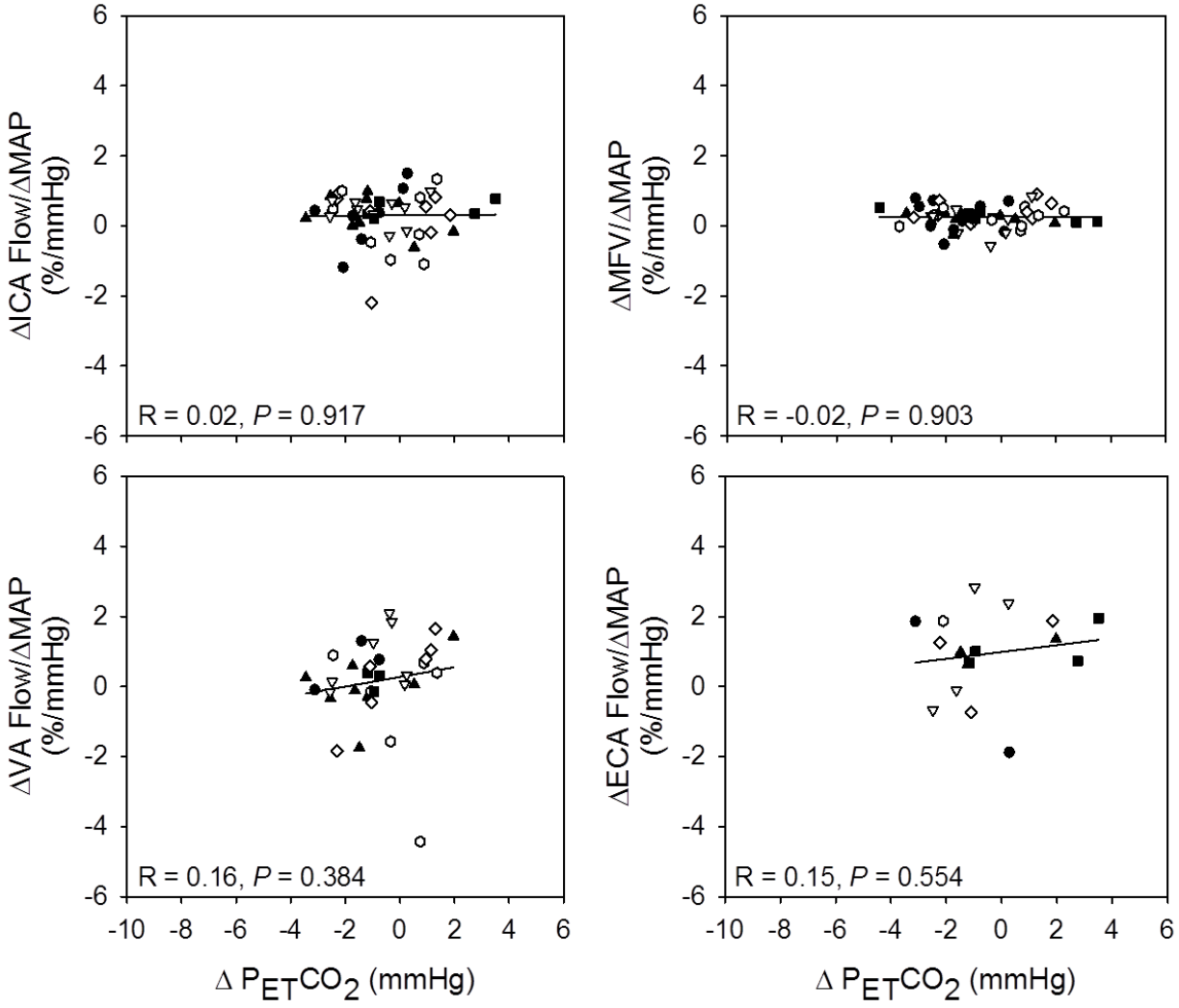

Figure 5-7. The relationship between changes in blood pressure and blood flow with postural transition is not modulated by the change in $P_{ET}CO_2$ in older adults.
The slope of the relationship between blood flow and blood pressure between supine lying and sitting (Table 5-4) is plotted against the change in $P_{ET}CO_2$ that was observed.

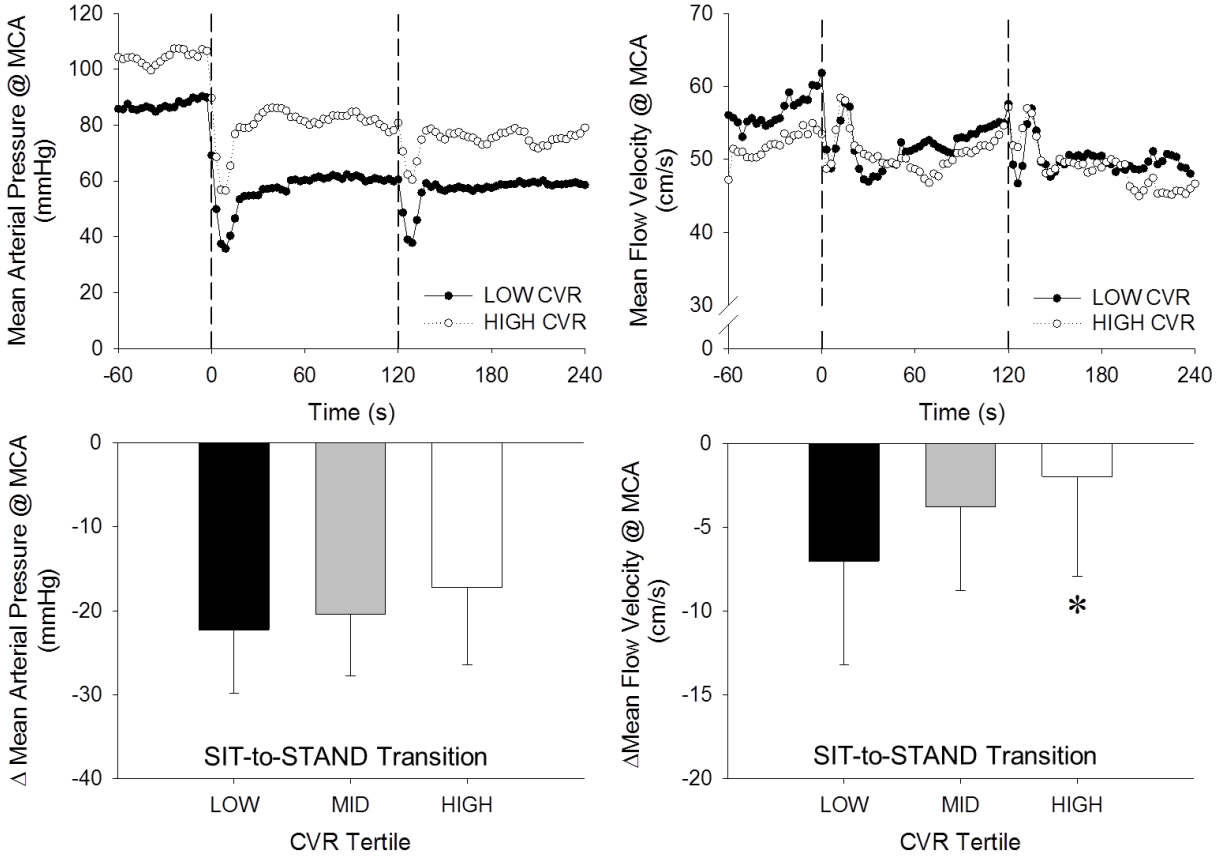

Figure 5-8. Dynamic relationship between blood pressure and blood flow velocity across postural transitions in older adults stratified by baseline cerebrovascular resistance.
Continuous mean arterial pressure (BP_{MCA}; top left) and mean flow velocity (MFV; top right) at the middle cerebral artery (MCA) across supine lying, sitting, and standing postures as a function of cerebrovascular resistance (CVR) tertile. Supine-to-sit (0 s) and sit-to-stand (120 s) transitions are marked by the dashed lines. For clarity, only LOW (closed circles) and HIGH (open circles) tertiles are shown; standard deviation is reported in the Table 5-5. Dynamic changes during the sit-to-stand transition as a function of tertiles of CVR are shown for BP_{MCA} (bottom left) and MFV (bottom right). Values represent BP_{MCA} and MFV at the BP_{MCA} nadir following the posture change. Data were presented as mean ± SD; n for each LOW, MID and HIGH tertile are 12, 9, and 10, respectively.

* different from LOW CVR at $P < 0.05$

Table 5-5. Dynamic cerebral autoregulation in older adults assessed by supine-to-sit and sit-to-stand postural transitions

Hemodynamic Index	Cerebrovascular Resistance Tertile			*P* value
	LOW (n = 12)	**MID (n = 9)**	**HIGH (n=10)**	
SUPINE to SIT				
dCA, ratio	0.64 ± 0.45	0.42 ± 0.44	0.25 ± 0.36	0.109
Time to MAP nadir, s	8.5 ± 1.6	7.0 ± 1.5	7.6 ± 0.8	0.058
Δ BP_{MCA}, mmHg	-53.8 ± 10.5	-49.6 ± 12.8	-56.2 ± 9.0	0.413
Δ MFV, cm/s	-9.2 ± 6.8	-5.5 ± 5.5	-4.4 ± 4.2	0.132
Δ DFV, cm/s	-9.7 ± 5.3	-5.8 ± 6.0	-5.3 ± 4.7	0.133
SIT to STAND				
dCA, ratio	0.50 ± 0.48	0.30 ± 0.44	-0.01 ± 1.06‡	**0.040**
Time to MAP nadir, s	7.6 ± 1.2	8.1 ± 1.8	8.2 ± 1.8	0.565
Δ BP_{MCA}, mmHg	-22.3 ± 7.6†	-20.4 ± 7.4†	-17.2 ± 9.2†	0.270
Δ MFV, cm/s	-7.0 ± 6.2	-3.8 ± 5.0	-2.0 ± 6.0‡	**0.003**
Δ DFV, cm/s	-7.6 ± 4.9	-4.6 ± 4.6	-2.3 ± 4.9†‡	**<0.001**

Abbreviations: dCA – dynamic cerebral autoregulation; MAP – mean arterial pressure; BP_{MCA} – MAP at the level of the middle cerebral artery; MFV – mean flow velocity; DFV – diastolic flow velocity.

Data were presented as mean ± SD. *P* value represents group effect in two-way repeated-measures ANOVA [resistance x transition (repeated)]

† different from supine-to-sit transition at $P < 0.05$
‡ different from LOW CVR at $P < 0.05$

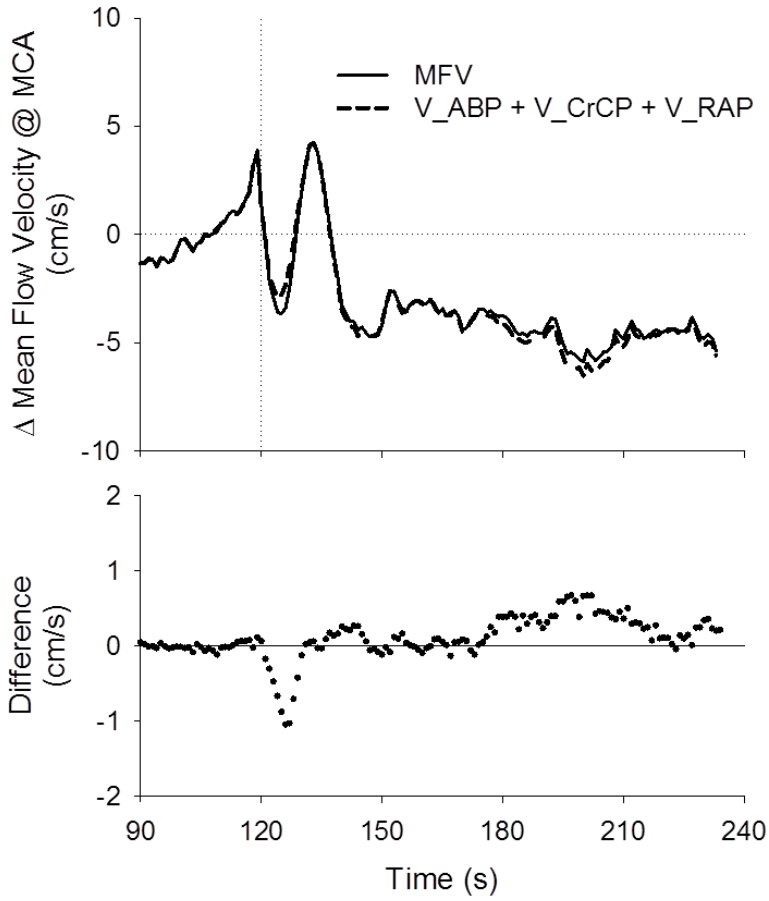

Figure 5-9. Comparison of summed component velocities to relative mean flow velocity during sit-to-stand transition in older adults.

TOP: The change in mean blood flow velocity (MFV; solid line) and the sum of calculated velocity components (dotted line) through middle cerebral artery (MCA) during the sit-to-stand transition (at 120 s) for older adults. MFV normalized to the average value over the final 30 s of sitting baseline. V_ABP, V_CrCP and V_RAP represent portion of velocity accounted for by changes in mean arterial pressure, critical closing pressure and resistance area product, respectively. BOTTOM: Residual plot of the difference between the MFV signal and the summed components throughout the sit-to-stand transition. Mean residual is 0.11 cm/s, n = 44.

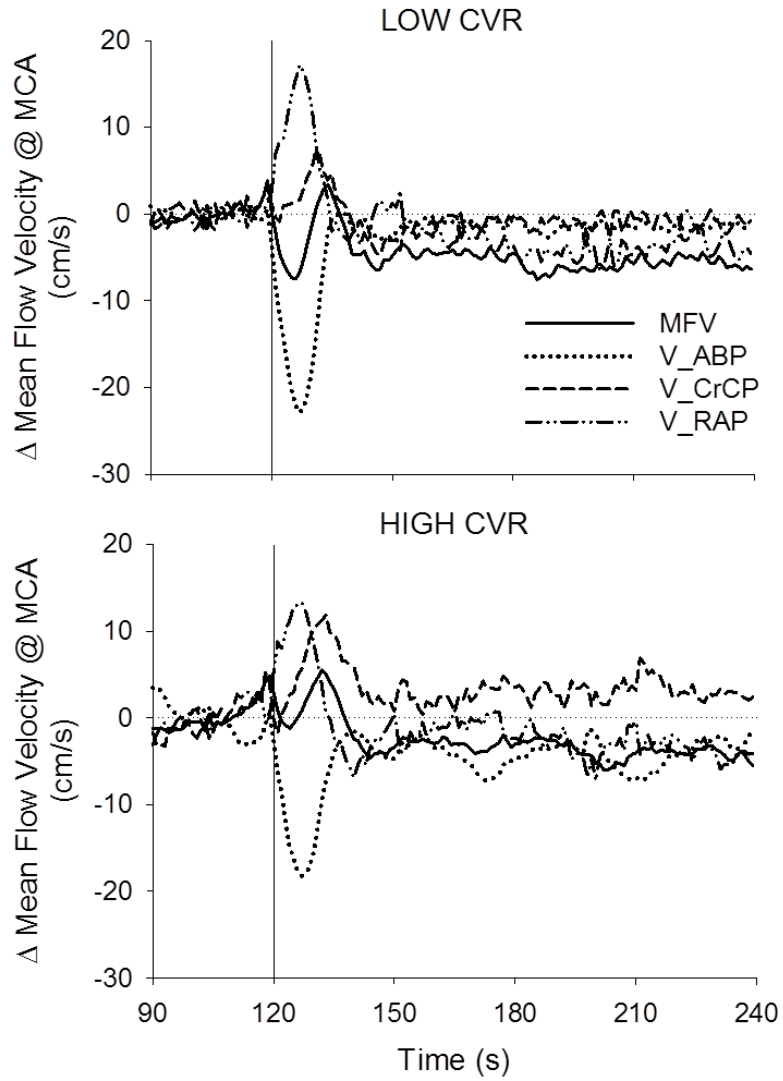

Figure 5-10. Multi-component influence on dynamic cerebral autoregulation.
Change in the measured mean blood flow velocity (MFV; solid line) and the individual contributions of calculated velocity components (see legend) through middle cerebral artery (MCA) during the sit-to-stand transition (at 120 s) for older adults in the lowest (TOP) and highest (BOTTOM) tertile of CVR. MFV was normalized to the final 30 s of sitting baseline. V_ABP, V_CrCP and V_RAP represent portion of velocity accounted for by changes in mean arterial pressure, critical closing pressure and resistance area product, respectively. Data were presented as means; n = 16 for LOW CVR and 15 for HIGH CVR. The variance in the model is excluded for clarity; see Table 5-6 for mean ± SD values at selected time points.[2]

[2] To assess the robustness of this observation, two additional comparisons were explored. First, a subset of participants (n=5) from the LOW and HIGH CVR groups were chosen at random and compared. Second, LOW and HIGH CVR was assessed by a median cut-off, rather than tertiles, to include a larger sample (n = 44). Similar results were observed.

Table 5-6. Dynamic change in velocity subcomponents during sit-to-stand transition across low and high tertiles of cerebrovascular resistance in older adults.

Time, s #	Blood Flow Velocity Components, cm/s							
	LOW CVR (n = 16)				HIGH CVR (n=15)			
	ΔMFV	V_ABP	V_CrCP	V_RAP	ΔMFV**	V_ABP	V_CrCP*	V_RAP
90-120	-0.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	-0.1 ± 0.2	-0.2 ± 0.3	-0.1 ± 0.4	0.1 ± 0.3
123	-4.6 ± 5.6	-11.4 ± 7.1	-0.9 ± 7.2	8.4 ± 4.9	-0.3 ± 5.0†	-5.5 ± 4.7	0.8 ± 6.3	4.5 ± 5.6
126	-7.3 ± 5.3	-20.4 ± 8.8	1.2 ± 5.8	13.7 ± 5.9	-1.0 ± 5.2†	-12.6 ± 10.5	3.9 ± 6.0	8.1 ± 4.0
129	-5.2 ± 7.1	-22.4 ± 13.0	3.0 ± 5.4	16.4 ± 11.6	1.0 ± 4.7†	-15.4 ± 11.6	6.9 ± 8.8‡	9.7 ± 6.1
132	0.6 ± 6.7	-14.3 ± 11.4	7.2 ± 7.1	8.1 ± 8.9	3.5 ± 4.6†	-11.0 ± 9.9	8.7 ± 6.8	5.7 ± 5.4
140	-2.8 ± 3.9	-0.4 ± 5.9	0.5 ± 5.1	-3.1 ± 7.5	-1.4 ± 3.4	-0.3 ± 7.8	4.3 ± 5.7‡	-5.5 ± 6.1
180-240	-5.8 ± 3.5	-1.5 ± 4.5	-1.0 ± 3.6	-3.9 ± 8.4	-3.6 ± 2.3	-3.4 ± 5.9	3.1 ± 7.0‡	-3.6 ± 4.5

Abbreviations: CVR – tertile of cerebrovascular resistance (middle tertile not shown); ΔMFV – change in middle cerebral artery mean flow velocity from the average of the 30 s prior to posture change; V_ABP, V_CrCP and V_RAP – the change in velocity estimated from change in mean arterial pressure, critical closing pressure and resistance area product, respectively.

Data were presented as mean ± SD. Middle CVR tertile was included in the statistical model, but not shown here. A two-way repeated-measures ANOVA (Time x CVR) was performed. Post hoc analyses using a least squares method and adjusting for multiple comparisons examined velocity components between CVR tertiles

\# main effect of time was observed for each variable ($P < 0.001$)
** main effect of CVR tertile on ΔMFV, $P = 0.004$
* trend for main effect of CVR tertile on ΔV_CrCP, $P = 0.069$
† different ΔMFV between groups at the corresponding time points, $P < 0.05$
‡ different ΔV_CrCP between groups at the corresponding time point, $P < 0.10$

Discussion

This comprehensive analysis of extracerebral and intracerebral blood flow in upright posture has contributed novel insight to the field of CA. First, comparison of intracerebral and extracerebral circulations allowed for a pairwise comparison of vascular territories with and without proficient autoregulatory characteristics. In older adults, sCA was maintained across tertiles of CVR, with similar responses measured in intracranial and extracranial vessels. Dynamic CA was also assessed by tertile of CVR. For dCA, traditional models of regulation involving MAP and CVRi indicated that MFV was more tightly regulated in the group of older adults with the highest CVR. Importantly, the use of a 3-component model involving MAP, RAP and CrCP, which relates to the instantaneous pressure-flow characteristics of the cerebral circulation, revealed distinct differences in the mechanisms used to regulation MFV between the lowest and highest tertile of CVR.

The confirmation of effective sCA in older adults from quantitative measurements in the ICA extends knowledge from previous observations obtained from MFV in the MCA (Eames *et al.,* 2003). In both younger and older adults, upright posture induced greater decreases in ECA_{BF} than ICA_{BF}. These differences between intracerebral and extracerebral vascular beds were consistent with prior reports of the peripheral response to an orthostatic challenge. Qualitative differences between cerebrovascular and peripheral vascular responses to orthostatic stress have been demonstrated using Doppler ultrasound of the MCA and brachial artery, respectively, during lower body negative pressure (Levine *et al.*, 1994). Additionally, using a combination of head-up tilt and lower body negative pressure, Arbeille *et al.* (2012) reported increased vascular resistance in the extracerebral temporal artery prior to changes in the MCA. The current observation of quantitative differences in the change of

ICA_{BF} and ECA_{BF} from supine to upright postures supports these earlier qualitative findings suggesting a redistribution of CO away from the ECA and towards the cerebral circulation. In addition, differences in systemic factors between healthy younger and older adults were identified that are consistent with a focus towards preservation of cerebral perfusion, including smaller upright posture-related drops in both CO and PCO_2 in older adults.

Dynamic CA, which was only assessed in older adults, appeared to be maintained within normal limits (Sorond *et al.*, 2009). In fact, MFV was more tightly regulated in older adults with higher CVR suggesting improved dCA. This observation using MFV and MAP characteristics is consistent with the literature in healthy aging (Lipsitz *et al.*, 2000; Deegan *et al.*, 2011). Notably, examination of the dynamic sit-to-stand response using a model that considered instantaneous contributions of CrCP and RAP (Panerai *et al.*, 2012) revealed differences in the mechanisms through which autoregulation was achieved. The smaller changes in MFV in the sit-to-stand transition in older adults with high CVR could be attributed to a more effective change in CrCP. Individuals with low CVR relied primarily on changes in RAP during the dynamic response. Thus, although older adults with changes in vascular health consistent with a higher CVR display autoregulation during posture change, the local regulatory mechanisms, through which this is achieved, vary.

Cerebral Blood Flow in Upright Posture

A reduction in CBF when moving to an upright posture, as evidenced by both MCA MFV and ICA_{BF}, was observed in both younger and older adults. The relative magnitude of the fall (Supine → Sitting: YOUNG – MCA: 10.7 %; YOUNG – ICA: 6.5 %; OLD – MCA: 6.6 %; OLD – ICA: 6.6 %) is in line with the literature, which has reported mean reductions in MFV and CBF between supine and upright postures ranging from 4 to 14 % (Savin *et al.*, 1995;

Ouchi *et al.*, 2001; Alperin *et al.*, 2005b; Sato *et al.*, 2012). The factor most responsible for this reduction in blood flow is a reduced perfusion pressure. BP_{MCA} is primarily reduced in upright postures by the introduction of an orthostatic gradient between the brain and the heart. The transition to an upright posture involves an initial central hemodynamic challenge including reduced SV, CO, and MAP consequent to a transient reduction in venous return (Romero-Ortuno *et al.*, 2010). However, over time, baroreflex-mediated responses help to establish a new resting MAP. In 11 older adults (14 %), ABP at the heart remained depressed after 2 minutes in the upright posture. As a group, BP_{MCA} remained lower when upright as compared to supine by ~ 20 mmHg in young adults and ~30 mmHg in older adults. In addition, small reductions in $P_{ET}CO_2$ and CO in the upright posture might have contributed to lower CBF in younger adults. The exact relationship between CO and CBF is unclear. Dynamic regulation of MFV and CO were shown to be uncoupled in the hypotensive response to bilateral leg cuff deflations (Deegan *et al.*, 2010b); however, there is evidence suggesting a direct relationship between CO and CBF during the steady state, independent of MAP (Harms *et al.*, 2010). Interestingly, when ICA blood flow was viewed as a proportion of CO, it was stable across postures (~ 6 % of CO in younger and ~ 4.5 % in older adults). This is consistent with a previous study that found a linear relationship between CO and MCA_{MFV} at rest that was independent of CA (Ogoh *et al.*, 2005). In contrast, the proportion of CO flowing to the ECA was reduced in upright postures (discussed in more detail below). Thus, posture-related drops in CBF in young adults might not be a sign of inefficient sCA (*i.e.*, pressure-flow regulation), but rather a consequence of reduced bulk flow (*i.e.*, CO), in addition to a potential contribution of reduced PCO_2.

Anterior vs. Posterior Cerebral Circulation

In the older adults, a comparison of the anterior and posterior cerebral circulation revealed distinct differences pointing to a more stable posterior circulation. The vertebral artery blood flow is the primary supplier of the brain stem including the circulatory and respiratory centers located in the medulla oblongata (Tatu *et al.*, 1996) and thus might involve specialized mechanisms to facilitate tighter regulation. The stability of the posterior circulation reported here is consistent with a recent report by Sato *et al.* (2012) that healthy young men had stable VA blood flow in head-up tilt, despite reduced ICA blood flow. While the current data are in agreement, suggesting that static CA is more robust in the posterior circulation contributing to greater stability in perfusion during orthostasis, the literature surrounding dynamic regulation is still contradictory. dCA in the posterior circulation has been shown to be either similar to (Deegan *et al.*, 2010a) or less effective than (Haubrich *et al.*, 2004) the anterior circulation. Sato *et al.* (2012) proposed that increased conductance through the vertebral circulatory bed led to the ability to maintain blood flow. This was consistent with the current study which observed a drop in CVR (*i.e.*, MAP/flow); however, examination of the passive pressure-velocity relationship demonstrated that this change was secondary to a decrease in CrCP and no change in the RAP in the vertebral circulation when upright. Importantly, reducing CrCP can increase effective perfusion pressure and flow in the face of stable resistance (Equation 1-5).

External carotid artery circulation

In both young and older adults, CBF maintenance was, in part, a function of the redistribution of cardiac output away from the ECA and towards the ICA vascular bed. In younger adults, this was observed between supine and upright postures when CO was reduced and the

proportion of CO directed towards the ECA was also reduced. In older adults, this was observed when comparing ICA_{BF} and ECA_{BF} between tertile of CVR. Those with the highest CVR also had a smaller proportion of CO directed towards the ECA. The finding of autoregulatory differences between cerebral and extracerebral hemodynamics are consistent with reported differences between the MCA and superficial temporal artery responses to upright posture (Savin *et al.*, 1995; Arbeille *et al.*, 2012). Savin *et al.* (1995) reported drops in MFV through the MCA and temporal artery of 14% and 55%, respectively, after 3 minutes of standing. In young adults in the current study, the decrease in flow between supine lying and standing through the ECA (23 %) was greater than through the ICA and MCA (10 % and 15 %). The observed drop in ECA_{BF} was half of that reported in the temporal artery by Savin *et al.* (1995). This discrepancy might be a function of non-linearities in the relationship between velocity and flow; however, this explanation alone would depend on a significant dilation of the temporal artery in response to standing. Given the observed reduction in the diameter of the ECA, a dilation of downstream vasculature, although not ruled out, seems unlikely. An alternate hypothesis is that the temporal artery might reflect a more pure extra-cerebral vessel compared to the proximal ECA, which is not completely isolated from the influence of cerebral circulatory networks. The maxillary branch of the ECA gives rise to the middle meningeal artery, which has demonstrated autoregulatory characteristics in an animal model (Michalicek *et al.*, 1996). It is plausible that cerebral branches from the ECA might have blunted the response in the current study; however, the cerebral contribution of extracranial-to-intracranial collateral pathways is minor unless obstructive disease mechanisms limit the primary ICA pathway (van Laar *et al.*, 2008). Future studies examining the relationship between ECA flow and temporal flow velocity will help clarify this issue.

These findings suggest that the ECA is the conduit to a more pressure-passive vascular bed than either the ICA or MCA. This theory would suggest that as perfusion pressure drops, flow is diverted towards the route of least resistance – namely, the cerebral circulation. Alternatively, the reduction in ECA blood flow could be the result of an active increase in ECA resistance during standing to divert blood flow toward the ICA. In an isolated canine carotid artery, infusion of norepinephrine and epinephrine or controlled bleeding (lowering blood volume) resulted in redistribution of blood from the ECA to the ICA (Kawai *et al.*, 1984). Preferential sympathetic sensitivity of the ECA vascular bed might actively contribute to the redistribution of blood flow towards cerebral circulation coordinated through the adrenergic response to upright posture (Kimmerly & Shoemaker, 2002). Interestingly, the reduction in MCA MFV is greater in patients with sympathetic failure (Harms *et al.*, 2000) when this active redistribution mechanism moving flow from the extracerebral to the cerebral circulation might be impaired. However, this latter result could also be mediated through a greater MAP reduction in sympathetic dysfunction. The hypothesis of active blood flow redistribution from the ECA to ICA through vasoconstriction of the extracerebral circulation is supported by Arbeille *et al.* (2012), who reported an increased resistance profile in the superficial temporal artery velocity waveform in the minutes leading up to syncope. As well, the redistribution of blood from other peripheral vascular beds (*e.g.*, skin) has been reported to assist in the maintenance of MFV during head-up tilt (Wilson *et al.*, 2002).

Static Cerebral Autoregulation

Static CA evaluates CBF during steady state conditions after a change in pressure and provides a measure of the overall efficiency of the cerebral vessels in maintaining perfusion (Tiecks *et al.*, 1995). Traditionally, static CA impairment has been defined as a non-zero slope for the

regression relating perfusion pressure to CBF (Paulson *et al.*, 1990). The current observation of posture-induced reductions in CBF in young and old adults add to other reports of mild pressure-passive characteristics of the steady state cerebral circulation (Ouchi *et al.*, 2001; Alperin *et al.*, 2005b). Rather than being viewed as a dichotomous phenomenon (*i.e.*, normal or impaired), the notion that CA works on a continuous scale, influenced by the endothelial, myogenic, and metabolic milieu is gaining favour (Panerai, 1998; van Beek *et al.*, 2008). Regression analysis, used in the current study to assess static CA sensitivity, provided similar flow-pressure slopes for the ICA and MCA in younger and older adults (~ 0.25 – 0.60 % /mmHg), which were significantly less than those observed in the more passive ECA (~ 0.90 – 1.20 % /mmHg).

Of note, in young adults there appeared to be a direct relationship between the autoregulatory slope and the reduction in $P_{ET}CO_2$. The similar relationships for the ICA, ECA and MCA in Figure 5-6, at first, infer similar regulation in response to posture change, but might in fact be a consequence of different mechanisms. The direct influence of PCO_2 on the cerebral vasoconstriction is well described (Ide *et al.*, 2003), and thus in the ICA and MCA, greater reductions in PCO_2 might override autoregulatory changes in resistance and give the illusion of a pressure-passive system. Based on a hypocapnic cerebrovascular reactivity of 2.5 %/mmHg (Ide *et al.*, 2003), the expected drop in CBF for the changes in $P_{ET}CO_2$ reported here (-4.0 ± 2.1 mmHg) is ~ 10 %. Young adults in the current study had reductions of 10 % and 15 % in the ICA and MCA, respectively, when moving from a supine lying to a standing posture, suggesting that the change was predominantly due to changes in $P_{ET}CO_2$ and that autoregulatory mechanisms were able to account for changes in pressure. In the ECA, however, the drop in PCO_2 might have no influence on vascular regulation (*i.e.*, the observed

drop in ECA_{BF} is the result of a simpler pressure-passive system), or might indirectly contribute to vasoconstriction in the ECA territory through increased sympathetic nerve activity (Shoemaker *et al.*, 2001). In a feedback mechanism, the reduction in $P_{ET}CO_2$ might trigger sympathetically-mediated constriction of the ECA vascular bed, redistributing blood toward the cerebral circulation. This relationship was not present in older adults who, notably, had an attenuated drop in $P_{ET}CO_2$ between supine lying and upright postures.

Suggested thresholds for impaired CA range from 0.5 to 4 %/mmHg change in blood pressure (Panerai, 1998). These values have stemmed from studies on newborns, emphasizing the need for further research surrounding appropriate methodology and consensus threshold values for adult populations. Although the mean cerebrovascular slopes in the current study were below reported thresholds, a large range of values was noted with frequent cases above 0.5 %/mmHg; the coefficient of variation approached 100 % in the MCA and 300 % in the ICA. Such variation has to be reduced before it is feasible to introduce widespread use of sCA into the clinical setting. Perhaps some of the individual differences were the result of sequential rather than simultaneous measurement of internal and external blood flow and the non-stationarities of CBF (Panerai *et al.*, 2000; Mitsis *et al.*, 2004). Although conclusions are difficult due to high standard deviations, it appears that no differences in sCA were observed between younger and older adults. Further, no difference was noted in the ICA or MCA with respect to the static autoregulatory slopes across tertiles of CVR. Observations of intact sCA and impaired dCA in stroke have led some to suggest that dCA is a more sensitive measure of regulatory capacity (Dawson *et al.*, 2003).

Dynamic Cerebral Autoregulation

Although a line of evidence suggests that dCA is unaffected by healthy aging (Carey *et al.*, 2000; Carey *et al.*, 2003; Serrador *et al.*, 2005; Deegan *et al.*, 2011), it was hypothesized that a sub-group of the aging population who exhibit elevated CVR, perhaps due to structural changes associated with arterial stiffening and/or vascular wall hypertrophy, would demonstrate impairment. Using a posture change protocol to assess dCA in older adults, participants in the HIGH CVR group were noted to have enhanced capacity to regulate MFV in response to fluctuations in blood pressure. This was a function of a smaller drop in MFV despite a similar drop in BP_{MCA}, compared to their LOW CVR counterparts. The HIGH CVR group exhibited lower baseline CBF, so the clinical importance of intact autoregulation, to prevent hypoperfusion, is noteworthy. dCA has been shown to be intact in older hypertensive adults (Lipsitz *et al.*, 2000; Serrador *et al.*, 2005). Indeed, elevated cerebrovascular tone in older and hypertensive adults might prove to be an advantageous adaptation for autoregulation. Increased tone, induced by hypocapnia, was associated with an enhanced autoregulatory response (Newell *et al.*, 1996; Edwards *et al.*, 2004). Older adults in the current study had a lower resting $P_{ET}CO_2$ than the younger adults, which may be a systematic compensatory mechanism to favour maintained intact CA. and might have contributed to the sensitivity of the response in this group of older adults. A recent report in young adults observed dCA in response to leg-cuff deflation to be attenuated in head-up tilt, when vascular tone would theoretically be lower (Sato *et al.*, 2012). In contrast, the current study found no differences in dCA between the supine-to-sit and the sit-to-stand transitions for older adults after stratification by CVR tertiles. In head-up tilt vs. active upright posture, CO is maintained at a lower level secondary to reduced venous return. Therefore, observations of differences in

thigh-cuff release during supine vs. head-up tilt are not a direct comparison to differences between supine-to-sit and sit-to-stand transitions.

The above observation of intact dCA relied on a two-component model (MAP and CVRi). The wide use of this model has led to a greater understanding in both dCA (Aaslid *et al.*, 1989) and sCA (Tiecks *et al.*, 1995), yet it is limited in the amount of physiological understanding that can be gained. Panerai *et al.* (2005) first used the three component model as a method to discriminate between the mechanisms contributing to changes in CVR. They examined V_ABP, V_CrCP and V_RAP in an evaluation of spontaneous MFV fluctuations and the response to cognitive stimulation (Panerai *et al.*, 2005). The current study has reported novel findings by incorporating this paradigm into the examination of dynamic vascular response following the transition from sitting to standing. In prolonged upright posture, increased CrCP has been proposed as a basis for the 'paradoxical vasoconstriction' as individuals approach syncope (Carey *et al.*, 2001; Zuj *et al.*, 2013); however, the incorporation of Panerai's model in the current study allowed characterization of dynamic autoregulatory fluctuations using CrCP and RAP.

When the dCA data set was examined by the three-component model, the mechanisms of autoregulation were different between the low and high CVR groups. In the low CVR group, the sudden drop in MAP upon standing was associated with competing influences on MFV – MAP contributing to a reduction in velocity, and a rapid RAP response as compensation. V_RAP peaked just prior to the BP_{MCA} nadir, and is consistent with the onset of MFV recovery. This time point is also consistent with the onset of change in V_CrCP, which helped to drive MFV upwards. The dynamic nature of the responses occurs over the first 15 s following the initiation of the posture change, after which these independent components were

stable. In the HIGH CVR group, however, the V_CrCP response played a larger role in the dynamic response. Compared to the LOW CVR group, the dynamic response in the HIGH CVR group involved similar rapid, divergent influences of V_ABP and V_RAP, but the onset time and magnitude of the V_CrCP response was exaggerated, surpassing the V_RAP response after ~ 9 s.

CrCP appears to be regulated primarily by vasomotor tone (Weyland *et al.*, 2000; Panerai, 2003). Functional regulation of CrCP to modify CBF has been demonstrated in response to changes in cognitive stimulation (Panerai *et al.*, 2012), spontaneous fluctuations in MAP (Panerai *et al.*, 2005), and exercise (Ogoh *et al.*, 2010); however, it has yet to be explored in depth during postural change (Carey *et al.*, 2001). RAP, while influenced to some degree by cognitive stimulation, is largely affected by changes in MAP (Panerai *et al.*, 2012; Zuj *et al.*, 2013). Given this understanding, CrCP and RAP might be thought of as general, although not pure, measures of metabolic and myogenic regulation, respectively (Panerai *et al.*, 2012). Relying on this assumption, the current data would support the notion that myogenic regulation is primarily responsible for the immediate rapid response during dCA (Paulson *et al.*, 1990). In individuals with baseline CBF closer to an under-perfused threshold, as might be the case in the high CVR tertile, brief hypoperfusion during posture change might trigger metabolic mechanisms to assist in maintaining CBF, through a CrCP-related mechanism. Results from an anesthetized cat model suggested segmental heterogeneity in dCA, such that the larger vessels respond to changes in pressure and smaller pial vessels respond to changes in flow through hypoxic stimuli (Kontos & Wei, 1985). Alternatively, CrCP might have been influenced by a transient increase in PCO_2 at the level of the tissue (Panerai *et al.*, 1999). Any transient hypoperfusion could have created a mismatch between oxygen delivery and cerebral

metabolism creating local increases in tissue PCO_2 that could have driven the response. The MFV response to a step change in P_aCO_2 manifests after ~ 6-10 s (Poulin *et al.*, 1996), which is similar to the CrCP lag time observed following the postural transition. The current study did not assess cerebral metabolic state, so the notion of hypoperfusion at baseline or during the hypotensive stimulus is only speculative based on the responses observed. The differential interaction of myogenic and metabolic mechanisms might contribute to the conflicting reports regarding the role of nitric oxide in dCA in humans (White *et al.*, 2000; Zhang *et al.*, 2004).

Methodological Considerations

A few limitations need to be considered. In the residual plot comparing the real MFV and summed component velocity traces (Figure 5-9, Bottom), a distinct deviation from zero is noted following the point of transition. The locality of this deviation introduces concern for bias in the analysis. Four potential reasons for this deviation were considered: (1) low signal-to-noise; (2) change in diameter of the MCA; (3) change in diameter of the finger artery; and/or (4) non-linear pressure-velocity relationships. First, the calculation of CrCP relies on extrapolation outside of the limits of normal physiological variability and subsequently suffers from a low signal-to-noise ratio (Panerai *et al.*, 2011). The current approach used the mean-to-diastolic method to estimate CrCP and RAP. Although this method was shown by Panerai *et al.* (2011) to be adequate for estimation of CrCP for both static and dynamic analyses, a first harmonics approach might provide a more accurate estimation that is less susceptible to high frequency noise during the transition. Repeated trials would also increase signal-to-noise and should be encouraged in future work. Alternatively, if the MCA dilated immediately after transition, the drop in MFV would have overestimated the actual drop in CBF. In addition, dilation would have had independent consequences for RAP and CrCP (Panerai, 2003).

Dilation would have reduced RAP leading to an elevated V_RAP, increasing the modeled MFV response more than the real MFV, as was observed. The discrepancy between the measured and modeled response starts to appear immediately after the transition, so any vasodilation would have had to occur rapidly, perhaps implying involvement of myogenic or intrinsic properties of the cerebral arterial system. A third possibility could have involved a change in the finger pulse wave secondary to peripheral vasoconstriction, independent of changes in the cerebral circulation. In some older adults, the transition to a standing position resulted in a gradual decay in the blood pressure signal, despite no signs of orthostatic hypotension (confirmed by auscultation). This was believed to be secondary to peripheral vasoconstriction that prevented the Finometer from obtaining an accurate pulse wave. Individuals who demonstrated this signal decay were not included in these particular analyses; however the possibility exists that others, who were included, might have had more subtle changes in the finger pulse waves secondary to peripheral vasoconstriction. Such vasoconstriction was not expected to occur in the cerebral arteries, so this change in the periphery could have contributed to error in the calculation of RAP and CrCP. To limit the impact of morphological differences between the finger and the cerebral arteries (O'Rourke & Hashimoto, 2007), the model used in the current study involved only the mean and diastolic portions of the waveform. Unlike the systolic peak of the arterial pulse, mean and diastolic pressures are relatively stable from the aorta until just proximal to the resistance arterioles (O'Rourke *et al.*, 2001). Thus, this model should have effectively filtered out bias related to differences in arterial stiffness or small changes in constriction between the finger artery and the MCA. A final consideration for the residual observed in Figure 5-9 was the likelihood of non-linearities in the pressure-velocity relationship (Mitsis *et al.*, 2006). The model considered

here remains a relatively simple linear model yet, as has been demonstrated, may provide unique insight into the mechanisms used in autoregulation and how they are altered in aging.

Another methodological consideration that merits further discussion involves the use of $P_{ET}CO_2$ in this study as a proxy for P_aCO_2. Although the use of $P_{ET}CO_2$ as a non-invasive measure of its arterial counterpart is prevalent in the field, there is evidence that the relationship between arterial and end-tidal gases might be altered by posture (Immink *et al.*, 2006; Serrador *et al.*, 2006). Upon movement to upright posture, blood flow through the alveoli is redistributed lower in the lungs altering the alveolar ventilation/perfusion ratio such that air coming from the upper lung might more closely reflect ambient air and air from the lower lung might more closely reflect arterial PCO_2. This redistribution, in concert with increased breathing frequency, suggests $P_{ET}CO_2$ underestimates true P_aCO_2 in upright postures (Immink *et al.*, 2006; Serrador *et al.*, 2006). In the current study, no dynamic fluctuation in $P_{ET}CO_2$ was observed; however, this measure was limited to breath-by-breath temporal resolution (~ 4-5 s), and might not be indicative of P_aCO_2 during periods of transition (Immink *et al.*, 2009). The present observation that CrCP regulatory pathways, which might involve metabolic mechanisms, are recruited during dynamic CBF responses to posture change suggest that tissue PCO_2 is a driving factor, and therefore even P_aCO_2 might not provide the sensitivity necessary for assessing its true impact.

The participants involved in the current study did not undergo radiological tests to confirm the absence of any cerebrovascular pathology. The presence of white matter hyperintensities has been related to impaired sCA during tilt table testing (Matsushita *et al.*, 1994). It is reasonable to assume that the participants in the current study most likely to have early evidence of pathology are those in the HIGH CVR group. This is consistent with the

hypothesis that there is a continuum of cerebrovascular health in older adults with those at the far right of the continuum being most susceptible to impairment in cerebrovascular regulation.

Perspectives and Significance

This study has provided unique insight into the interaction of RAP and CrCP in the static and dynamic regulation of CBF. While changes in CrCP were primarily responsible for mitigating the drop in perfusion pressure associated with a step decrease in ABP, RAP was observed to regulate CBF against the dynamic MAP fluctuation during postural transitions. Further, older adults with high baseline CVR were able to maintain adequate dCA, in part by the influence of transient changes in CrCP, which was less apparent in those with lower baseline CVR. Blood pressure varies spontaneously throughout the day and in response to daily activities. The ability to regulate CBF against these fluctuations through myogenic regulation alone might theoretically preserve metabolic regulatory reserves to match necessary changes in perfusion associated with neuronal activity. If, however, metabolic regulatory reserves are recruited to guard against fluctuations in blood pressure, individuals might be more susceptible to impaired perfusion-activity matching. Of note, the older adults in the current study demonstrated robust cerebrovascular reactivity to CO_2 (see Chapter 3), which has been related to CrCP regulation (Carey *et al.*, 2001). This efficiency might have related to their ability to adapt to the dynamic postural demands through a lowering of CrCP. Individuals with high cerebrovascular tone, who also exhibit impaired reactivity, might be at greater risk for hypoperfusion during posture change.

CHAPTER 6. INCREASED CEREBROVASCULAR TONE IS INDEPENDENTLY ASSOCIATED WITH SLOW GAIT SPEED IN COMMUNITY-LIVING OLDER ADULTS

Introduction and Rationale

Slow gait is a robust predictor of functional dependence in community-living older adults (Guralnik *et al.*, 2000; Cesari *et al.*, 2005), and declining gait speed is associated with an increased likelihood of future disability, cognitive impairment, falls, institutionalization and mortality (Abellan van Kan *et al.*, 2009). The presence of cardiovascular risk factors is linked with gait difficulties, which suggests a vascular origin (Rosano *et al.*, 2011) , yet a physiological mechanism remains unclear. Aging is associated with a reduction in cerebral blood flow (CBF) (Scheel *et al.*, 2000b); CBF is lower still in the upright position (see Chapter 5; also Alperin *et al.*, 2005a) placing the brain at a greater risk for potential mismatches between cerebral perfusion and metabolism that might contribute to functional impairment.

In a comparison of initial orthostatic hypotension[3] vs. clinical orthostatic hypotension[4], Romero-Ortuno *et al.* (2011) reported that the presence of initial hypotension was more closely associated with reports of dizziness, faintness or light-headedness, than clinical hypotension. This initial hypotension during the dynamic phase of the posture change was also associated with an increased incidence of falls and slow gait speed. The ability to regulate CBF in response to dynamic changes in ABP likely plays a significant role in the manifestation of orthostatic intolerant symptoms (e.g., dizziness); however, CBF was not measured in this study.

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[3] A transient blood pressure drop within the first 15 seconds after standing of ≥ 40 mmHg in systolic blood pressure or ≥ 20 mmHg in diastolic blood pressure with symptoms of cerebral hypoperfusion.
[4] A drop in blood pressure within the first 3 minutes after standing of ≥ 20 mmHg in systolic blood pressure or ≥ 10 mmHg in diastolic blood pressure.

Sorond and her colleagues reported slower gait in community-living older adults who exhibited impaired neurovascular response to cognitive activation (Sorond *et al.*, 2011) and lower cerebrovascular reactivity to carbon dioxide (Sorond *et al.*, 2010). However, it is unclear how cerebral autoregulation to dynamic changes in blood pressure (dCA) is related to these impairments. While dCA appears to be maintained in healthy aging (Lipsitz *et al.*, 2000; Deegan *et al.*, 2011; Dineen *et al.*, 2011), signs of impairment are noted in individuals with clinical vascular disease (White & Markus, 1997; Aoi *et al.*, 2012). Impairment appears most evident in patients with severe carotid stenosis (White & Markus, 1997) and ischemic stroke (Aoi *et al.*, 2012). Further, an association between dCA and gait speed was found in stroke survivors, but not age-matched controls (Aoi *et al.*, 2012). The complexity of the cerebrovascular network is a model for contingency in that multiple regulatory mechanisms, including metabolic, myogenic and neurogenic pathways, contribute to ensure sufficient perfusion (Panerai, 2003). These same characteristics increase the difficulty of quantifying regulatory control. Classical measurement of dCA examines mean flow velocity (MFV) or resistance, averaged over the cardiac cycle, in response to induced or spontaneous fluctuations in ABP (Aaslid *et al.*, 1989; Levine *et al.*, 1994; Lipsitz *et al.*, 2000; Zhang *et al.*, 2000; Hughson *et al.*, 2001). This model does not consider the pulsatile characteristics within the cardiac cycle that result from the combination of passive and active vascular control.

Recently, Panerai *et al.* (2005) reported on dynamic changes in the critical closing pressure (CrCP) and resistance area product (RAP) that were sensitive to subtle, but physiologically relevant, changes in cerebrovascular tone. These measures relate to the passive relationship between ABP and CBF velocity within a cardiac cycle (Panerai, 2003). In using this model to quantify the contribution of RAP and CrCP to fluctuations in MFV, the dynamic

response to posture change was observed to differ according to baseline cerebrovascular resistance (Chapter 5). Importantly, although no differences were observed in the middle cerebral artery (MCA) MFV response during the sit-to-stand transition, the relative velocity components related to changes in CrCP (V_CrCP) and RAP (V_RAP) were enhanced and attenuated, respectively, in individuals with elevated baseline tone. V_CrCP has been partially attributed to characteristics of metabolic regulation (Panerai *et al.*, 2012), suggesting that these individuals might have relied on cerebrovascular reserve pools closer to the level of the tissue during the posture change.

The purpose of the current study was to assess the cross-sectional relationship between CBF regulation, gait speed and falls in a group of community-living older adults. Executive function and grip strength were explored as potential confounding factors related to the extent of frailty. It was hypothesized that slow gait speed (< 1.0m/s) and history of falls (in the past year) would be associated with lower upright CBF, greater cerebrovascular resistance (RAP), and a smaller dynamic autoregulatory response of the velocity component attributed to changes in RAP (V_RAP) during a sit-to-stand transition.

Methods

This chapter discusses the cohort and cerebrovascular assessment described in Chapter 2. The maximum response of V_RAP (Maximum V_RAP) and V_CrCP (Maximum V_CrCP) during sit-to-stand posture change was used to characterize the autoregulatory response (Figure 6-1). Methodological limitations noted in Chapter 2 resulted in only a partial data set being appropriate for this analysis. Principal analysis involved relationships among 61 older adults (for upright CBF) and 43 older adults (for dCA responses during the sit-to-stand transition). Sample sizes are noted for each analysis in the results.

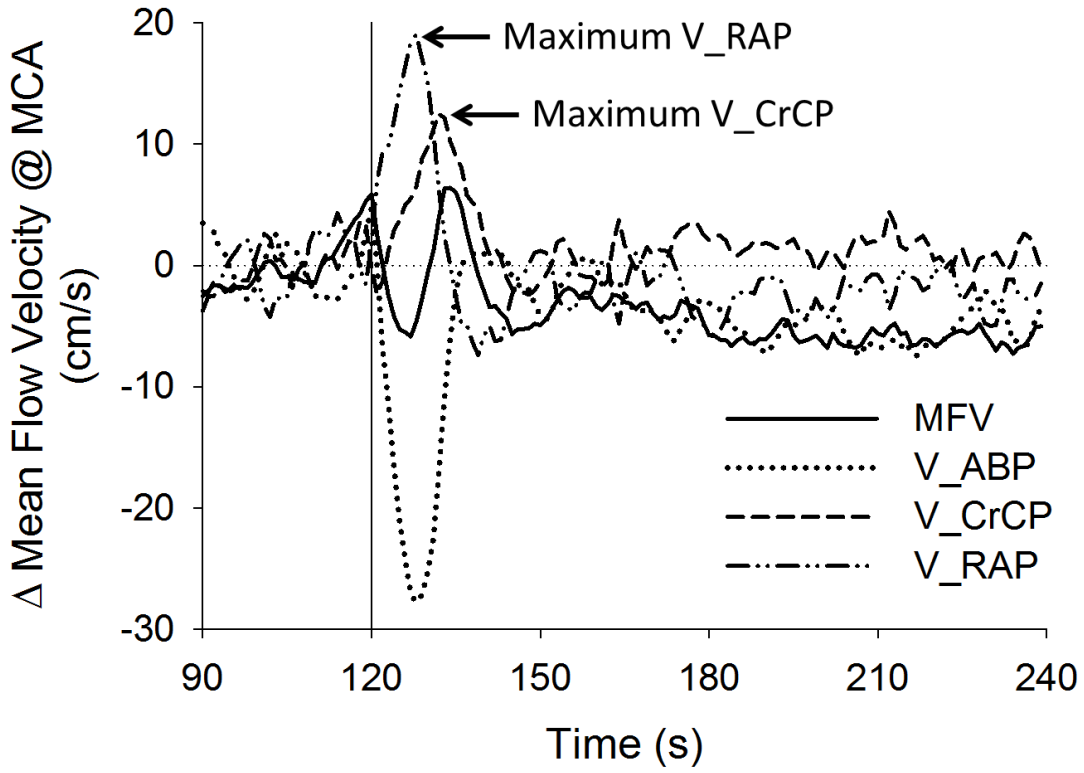

Figure 6-1. Dynamic cerebral autoregulatory response during the sit-to-stand transition. Component velocity responses related to changes in arterial blood pressure (V_ABP), critical closing pressure (V_CrCP) and resistance area product (V_RAP) following a posture change from sitting-to-standing (vertical line). Data were presented as a percent change from sitting. The solid line reflects the middle cerebral artery (MCA) mean blood flow velocity (MFV) during the response and also represents the sum of the 3 component velocities. Maximum V_RAP and V_CrCP values were used to quantify the individual autoregulatory response.

Functional Assessment

Gait speed was assessed by instructing participants to "walk at your usual pace" over 8 meters. Walking time was measured on a manual stopwatch sensitive to 0.1s and the average time of two consecutive trials was used for analysis. Only 5 of 82 participants had a difference of more than 1 s between trials. A threshold of 1 m/s was used to score slow gait (Cesari *et al.*, 2005). Grip strength was measured using a custom-made pneumatic device with a rubber bulb attached to a manometer. The softer rubber handle was ideal for testing older adults as it reduced stress on weaker and/or more painful joints allowing for a more reliable effort across the entire study sample (Roberts *et al.*, 2011). Participants performed the task while seated, with their shoulder adducted and neutrally rotated, and their elbow flexed at 90°, in line with recommendations of the American Society of Hand Therapists (Roberts *et al.*, 2011). The average of the 2nd and 3rd trials, following appropriate rest, was used for analysis. The disadvantage of using this method is the relative scarcity of normative data relative to the standard dynamometer (Desrosiers *et al.*, 1995; Bautmans *et al.*, 2007). Fall history, grip strength and gait speed were used to describe the participants functional health. Participants were asked to report the number of times they had fallen within the past year, and any injuries sustained as a result (see Health Questionnaire in Appendix A). Performances on the Montreal Cognitive Assessment (MoCA; Nasreddine *et al.*, 2005) and the Trail Making Test (Trails A and B) were used to explore prefrontal cognition as a confounding variable for the relationship between cerebrovascular health and gait speed (Strauss *et al.*, 2006; Rosano *et al.*, 2012).

Statistical Analysis

Participant characteristics and cerebrovascular hemodynamics were separated by gait speed (threshold 1.0 m/s) and compared using unpaired *t*-tests and chi-square analyses, for continuous and categorical variables, respectively. The influences of cerebrovascular

hemodynamics on the dependent measures of gait speed, grip strength and number of falls were assessed by multiple linear regressions. Parallel models to examine the influences of separate cerebrovascular characteristics were assessed, including that of steady state (anterior CBF in upright sitting and RAP in standing) and dynamic (maximum V_RAP during the sit-to-stand transition) factors. Maximum V_RAP was chosen over maximum V_CrCP to characterize the dynamic response because CrCP is extrapolated from the velocity-pressure relationship, whereas RAP is a measured variable and has better reproducibility (Panerai *et al.*, 2011). All final models were adjusted for age, sex, height, cognitive function (MoCA score), metabolic syndrome (yes/no) and C-reactive protein (CRP). Continuous variables of interest were examined for normality. Participant age, gait speed, and RAP underwent natural log transformation, and grip strength underwent square root transformation prior to for linear regression analyses. Non-transformed means are presented in the results for clarity. Regression diagnostic tests were used to assess fully-adjusted models for residual assumptions and collinearity. Further, dichotomous measures of slow gait (< 1.0 m/s) and incidence of falls were assessed using logistic regression techniques. All of the covariates indicated above were entered into the model and were sequentially removed through backwards elimination until a final model was obtained. An $\alpha = 0.15$ was used as an inclusion criteria (Tyas *et al.*, 2000). Data were presented as mean ± SD, except where noted. Significance was inferred at $P < 0.05$. All statistical analyses were completed using Statistical Analysis Software v9.2 (SAS Institute, Cary NC, USA).

Results

Eighty-one community-living older adults underwent a comprehensive geriatric assessment of their medical history, cognitive function, functional performance and vascular health. Fifteen

(18.3 %) exhibited slow gait speed (<1 m/s). Participants with slow gait were older, more likely to be women, and tended to score lower on the MoCA and perform poorer on the Trails test (see Table 6-1). There was no difference between the two groups for body mass index (BMI); however, there was a trend for reduced waist:hip ratio (WHR) in slow walkers. Faster gait speed was associated with a more active lifestyle, as these individuals took more steps per day and tended to expend more energy each day. Also, individuals who reported falling at least once within the past year had a slower gait (1.08 ± 0.16 vs. 1.20 ± 0.20 m/s; t_{79} = 2.57, P = 0.019). However, when using 1.0 m/s as a risk threshold for gait speed, the occurrence of falls was not different between slow and normal gait.

Table 6-2 provides details on the cerebrovascular characteristics of fast and slow walkers. Successful transcranial Doppler ultrasound tracking of the right MCA was obtained in only 64 participants in the standing posture. No difference in walking speed or fall history over the past year was noted between those with and without TCD data (Gait Speed, P = 0.422; Falls, P = 1.000). Subsequent analysis refers to this smaller group. No differences were noted in CBF indices between the fast and slow walkers, although in the upright, seated posture, vascular resistance in the anterior cerebral circulation did tend to be greater in the group exhibiting slow gait (t_{59} = 1.87, P = 0.067). The dynamic changes in BP_{MCA} during the sit-to-stand transition were similar between groups (slow gait: -27 ± 16 vs. normal gait: -34 ± 15 %; t_{47} = -1.16, P = 0.251), as were the dynamic cerebrovascular changes (Table 6-2). Although, structural markers of arterial health, including IMT and cf-PWV, were not different, CRP, a blood borne marker of inflammation associated with vascular aging, was elevated in slow walkers.

When walking speed was assessed as a continuous variable, the relationship between cerebrovascular health and gait became more apparent. This was particularly the case for cerebrovascular indicators during standing, as well as in the transition between sitting and standing (Table 6-3). Multiple linear regression showed that elevated RAP in standing was associated with slower gait (n = 64, $P = 0.001$). This relationship was constant after adjusting for age, sex, height, cognitive function, metabolic syndrome, and inflammatory markers (Table 6-4). A relationship between RAP and grip strength, on the other hand, disappeared after adjusting for age and sex, as well as the other covariates. The dynamic response of V_RAP during the sit-to-stand transition tended to be related to gait speed in bivariate and multivariate models ($P < 0.10$), but the strength of the relationship was less than that of steady state standing RAP. Maximum V_RAP also tended to be associated with increased grip strength. When relationships were assessed with slow gait speed as a binary variable, logistic regression identified a final model that included steady state RAP-standing [odds ratio (OR) 4.02 (95% confidence intervals (CI): 0.86, 18.72), $P = 0.076$] and female sex [OR 8.99, 95% CI (0.91, 89.06), $P = 0.061$] as main contributing factors. When the dynamic response was assessed, a final model including Maximum V_RAP [OR 0.69 (95% CI: 0.47, 1.01), $P = 0.059$] and CRP [OR 2.48 (95% CI: 1.16, 5.28), $P = 0.019$] was observed.

The frequency of falls in the past year was related to the dynamic Maximum V_RAP only in a fully-adjusted linear model (Table 6-4). Participants who reported falling did not demonstrate a greater BP_{MCA} fluctuation during the dynamic transition (fall: -27 ± 15 vs. no fall: -35 ± 15 %; $t_{47} = -1.59$, $P = 0.120$). The likelihood of falls within the past year was increased with female sex [OR 19.82 (95% CI: 2, 219), $P = 0.015$] and greater height [OR 1.14

(95% CI: 1.01, 1.28), *P* = 0.033], and tended to be reduced with higher MoCA score [OR 0.61 (95% CI: 0.36, 1.04), *P* = 0.072], but was not related to any cerebrovascular parameter.

Table 6-1. Clinical characteristics of older adults with slow gait speed (<1 m/s)

Variable	Normal Gait (n= 66)	Slow Gait (n = 15)	*P* value
Age, years	73.5 ± 5.5	77.5 ± 7.6	**0.021**
Sex, % women (n)	56.7 (38)	86.7 (13)	**0.022**
Height, cm	167.8 ± 9.4	161.2 ± 9.9	**0.016**
Weight, kg	76.1 ± 14.2	72.3 ± 17.5	0.377
Education, years	15.0 ± 3.9	15.0 ± 4.3	0.968
MoCA, score of 30	28.4 ± 1.2	27.3 ± 2.0	0.064
GDS, score of 15	0.7 ± 1.2	0.9 ± 0.8	0.682
Body Mass Index, kg/m^2	27.0 ± 3.8	27.6 ± 4.9	0.580
Waist:Hip, ratio	0.90 ± 0.09	0.86 ± 0.10	0.094
TMT A, s	28.8 ± 7.3	35.0 ± 12.9	0.091
TMT B, s	69.8 ± 30.8	93.4 ± 50.2	0.099
TMT B-A, s	41.0 ± 27.5	58.4 ± 43.5	0.155
Dom. Grip Pressure, kPa	53 ± 23	37 ±16	**0.011**
Dom. Finger Tapping, count (n = 57/12)	42 ± 8	38 ± 9	0.138
Usual Walking Speed, m/s	1.22 ± 0.17	0.92 ± 0.08	**<0.001**
Steps, count/day	7500 ± 2950	5290 ± 2210	**0.010**
Physical Activity, kcal/day	970 ± 690	580 ± 570	0.053
Falls in past year, % yes (n)	22.4 (15)	33.3 (5)	0.167
Falls in past year, count	0.3 ± 0.6	1.0 ± 1.7	0.146

Abbreviations: MoCA – Montreal Cognitive Assessment; GDS – Geriatric Depression Scale; TMT – trail making test; Dom – dominant hand (n = 5 where dominant is left).

Data were presented as mean ± SD for continuous variables; percent (count) for categorical variables.

Significance determined by unpaired t-test (continuous) and Fisher exact test (categorical).

Table 6-2. Cerebrovascular characteristics associated with slow gait speed (<1 m/s)

Variable	n (normal/slow)	Normal Gait	Slow Gait	*P* value
Supine CBF Characteristics				
Total, ml/min	48/11	730 ± 150	670 ± 150	0.206
Anterior, ml/min	59/14	560 ± 130	520 ± 120	0.254
Posterior, ml/min	51/12	170 ± 60	160 ± 50	0.514
Resistance (Anterior), mmHg/L/min	59/14	180 ± 50	200 ± 60	0.126
Seated CBF Characteristics				
Total , ml/min	29/5	680 ± 110	610 ± 60	0.180
Anterior, ml/min	52/9	500 ± 120	440 ± 100	0.159
Posterior, ml/min	29/5	170 ± 50	170 ± 20	0.986
Resistance (Anterior), mmHg/L/min	52/9	140 ± 50	180 ± 70	0.067
Standing CBF Characteristics				
MCA MFV, cm/s	57/11	50 ± 12	46 ± 9	0.318
MAP, mmHg	56/9	96 ± 11	99 ± 14	0.344
CVRi, mmHg/cm/s	56/9	1.4 ± 0.4	1.8 ± 0.8	0.186
$P_{ET}CO_2$, mmHg	57/11	37.1 ± 3.9	37.4 ± 2.7	0.844
CrCP, mmHg	55/9	7 ± 13	8 ± 7	0.710
RAP, mmHg/cm/s	55/9	1.2 ± 0.4	1.6 ± 0.7	0.200
Cerebrovascular Dynamics				
CR_{CO2}, %/mmHg	54/10	3.6 ± 1.2	3.6 ± 1.2	0.950
sCA (Sit-Stand), cm/s/mmHg x 10^{-3}	54/9	0.07 ± 0.67	-1.15 ± 3.04	0.267
dCA (Sit-Stand), cm/s/mmHg	43/6	0.23 ± 0.45	0.06 ± 0.51	0.398
Maximum V_CrCP, cm/s	38/5	15.9 ± 12.9	11.2 ± 10.0	0.442
Maximum V_RAP, cm/s	38/5	18.4 ± 9.6	11.7 ± 5.9	0.139
Arterial Structure/Function				
cPP, mmHg	65/15	52 ± 14	59 ± 15	0.089
cf-PWV, m/s	67/15	8.9 ± 1.6	9.7 ± 2.3	0.247
ba-PWV, m/s	67/15	11.1 ± 1.6	11.3 ± 1.7	0.705
IMT, mm	65/15	0.78 ± 0.21	0.77 ± 0.13	0.883
CRP, mg/L	63/15	1.95 ± 1.85	3.42 ± 2.62	**0.014**
Creatinine, μmol/L	62/15	95 ± 24	82 ± 17	**0.045**

Abbreviations: CBF – cerebral blood flow; MCA MFV – middle cerebral artery mean flow velocity; MAP – mean arterial pressure (brachial); CVRi – cerebrovascular resistance index; $P_{ET}CO_2$ – partial pressure of end-tidal carbon dioxide; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; CA – cerebral autoregulation (s, static; d, dynamic); cPP – carotid pulse pressure; PWV – pulse wave velocity (cf, carotid-femoral; ba, brachial-ankle); mIMT – mean intima-media thickness; CRP – C-reactive protein.

Data were presented as mean ± SD. Significance determined by unpaired *t*-test.

Table 6-3. Partial correlations between cerebrovascular characteristics and gait speed

Variable	n	Gait Speed §
Supine		
Anterior CBF	73	-0.07 (0.545)
Posterior CBF	63	0.12 (0.363)
CVR (anterior)	73	0.00 (0.986)
Sitting		
Anterior CBF	61	0.01 (0.957)
Posterior CBF	34	0.00 (0.993)
CVR (anterior)	61	-0.09 (0.483)
Standing		
MFV §	67	0.12 (0.305)
CVRi §	65	**-0.32 (0.010)**
CrCP	64	0.15 (0.231)
RAP §	64	**-0.41 (0.001)**
Dynamic Response		
CR_{CO2}	63	-0.12 (0.355)
sCA (Sit-Stand)	63	**0.29 (0.022)**
dCA (Sit-Stand)	49	0.14 (0.365)
Maximum V_CrCP	43	0.15 (0.355)
Maximum V_RAP §	43	0.28 (0.082)

Abbreviations: CBF – cerebral blood flow (a, anterior; p, posterior); CVR – cerebral vascular resistance; MFV – middle cerebral artery mean flow velocity; CVRi – cerebrovascular resistance index; CrCP – critical closing pressure; RAP – resistance area product; CR_{CO2} – cerebrovascular reactivity to carbon dioxide; CA – cerebral autoregulation (s, static; d, dynamic); V_CrCP and V_RAP – the peak velocity component during the sit-to-stand transition from changes in CrCP and RAP, respectively.

Data were presented as Pearson correlation coefficient (*P*-value) after adjustment for age and sex.

§ RAP, V_RAP, gait speed, age and C-reactive protein underwent natural log transformation, and grip strength underwent square root transformation, prior to analysis.

Table 6-4. Associations between cerebral hemodynamics (per 1SD increment) and physical function with adjustment for covariates

	Anterior CBF – sitting (n = 61)			RAP - standing § (n = 63)			Maximum V_RAP § (n = 43)		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Gait Speed §	0.18	0.01	-0.04	**-0.45‡**	**-0.40‡**	**-0.32****	**0.29***	**0.27***	**0.30***
Model R^2	0.03	0.35	0.52	0.21	0.31	0.34	0.08	0.14	0.32
Grip Strength §	0.15	-0.02	-0.02	**-0.25****	-0.14	-0.13	**0.27***	**0.22****	**0.21****
Model R^2	0.02	0.55	0.65	0.06	0.55	0.66	0.07	0.71	0.72
Falls	0.02	0.05	0.12	0.11	0.15	0.08	-0.18	-0.25	**-0.33***
Model R^2	0.00	0.02	0.24	0.01	0.03	0.23	0.03	0.15	0.24

Abbreviations: CBF – cerebral blood flow; RAP – resistance area product; Maximum V_RAP – the peak velocity component related to changes in RAP during the sit-to-stand transition.

Data were presented as standardized regression coefficients.

§ RAP, V_RAP, gait speed, age and C-reactive protein underwent natural log transformation, and grip strength underwent square root transformation, prior to analysis.

Model 1: Unadjusted.
Model 2: Adjusted for age and sex.
Model 3: Adjusted form age, sex, height, MoCA score, metabolic syndrome, and C-reactive protein.

Standardized parameter estimate significance level is defined by the following symbols:
* $P < 0.10$
** $P < 0.05$
† $P < 0.01$
‡ $P < 0.001$

Discussion

The major novel finding of this study was that steady-state cerebrovascular resistance and the dynamic cerebrovascular response to posture change were associated with gait speed in community-living older adults. Elevated RAP, an indicator of the passive resistance in cerebral vessels, was independently associated with slow gait speed, after adjustment for age, sex, cognitive function, metabolic health and systemic inflammatory markers. In addition, the magnitude of the dynamic MFV response attributed to changes in RAP (Maximum V_RAP) during a sit-to-stand transition was directly related to gait speed. This autoregulatory variable reflects the immediate drop in cerebrovascular resistance in response to a drop in ABP, which minimizes the change in MFV and facilitates a faster recovery of MFV toward baseline values. A smaller Maximum V_RAP (*i.e.,* less effective dCA) was associated with slower gait, as well as a higher occurrence of falls over the past year after adjustment for confounding variables.

Most studies indicate that dCA is maintained with aging (Deegan *et al.,* 2011; Dineen *et al.,* 2011). Impaired autoregulation has been linked to impaired physical function only in the presence of overt cerebrovascular disease (Aoi *et al.,* 2012). The current study used a novel approach to examine dCA incorporating the passive relationship between pressure and CBF (Panerai *et al.,* 2005). This approach involved examining the pressure-CBF velocity relationship within each cardiac cycle across the posture change to obtain measures of CrCP and RAP. CrCP is an estimate of the pressure at which CBF is zero and reflects effective downstream pressure against which ABP is working (Weyland *et al.,* 2000). RAP is calculated as the inverse of the slope between pressure and velocity within a cardiac cycle and therefore reflects the passive resistance of the system. Modulation of CrCP and RAP has been shown to independently influence MFV (with their independent effects being expressed as V_CrCP and

V_RAP, respectively), and might be indicative of separate regulatory pathways (Panerai *et al.*, 2005). Preliminary evidence during cognitive stimulation suggests that V_CrCP more closely reflects metabolic influences at the tissue level, whereas V_RAP reflects myogenic pathways in resistance vessels (Panerai *et al.*, 2012).

RAP is a characteristic of the instantaneous cerebrovascular resistance, and might be more sensitive to physiological changes than the more common parameters used to assess autoregulation (*i.e.*, mean beat-to-beat measures). In particular, RAP has been proposed as a sensitive marker of cerebral autoregulation (Panerai *et al.*, 1996). The relationship to gait speed was stronger for RAP than for CrCP. RAP is believed to relate to the myogenic characteristics of the cerebral vessels, while CrCP might relate more to metabolic regulatory pathways (Panerai *et al.*, 2005). CrCP did not demonstrate any relationship in the current study; however, future work might consider the involvement of dual task paradigms in conjunction with gait, which might highlight greater metabolic impairments in similar populations (Nadkarni *et al.*, 2012).

The V_RAP response was more closely associated with number of falls in the past year than either upright CBF or RAP in standing. It has been suggested that individuals might be at the greatest risk for falls during transitions in posture, where dips in ABP might transiently compromise CBF (Wieling *et al.*, 2007). Romero-Ortuno *et al.* (2011) reported that initial orthostatic hypotension was related to slow gait speed and increased number of falls within the past 6 months. Their study did not measure CBF, and therefore could not comment on the participants' ability to regulate CBF during the postural transition. The current study did not observe any differences in relative or absolute drop in ABP between participants who reported at least 1 fall within the past year and those who did not, or in slow walkers. However, a

bivariate relationship between dCA and gait speed was noted, suggesting that individuals who have a smaller MFV drop relative to the initial drop in MAP had better functional performance.

In addition to gait speed, RAP was found to have a strong bivariate relationship with grip strength raising the possibility this was the expression of a general frailty phenotype (Fried *et al.*, 2001). However, the association between RAP and grip strength was lost after adjusting for confounding variables, while the association between RAP and gait speed was retained. This infers a characteristic inherent within the cerebral vessels that influences gait. Gait speed has more cognitive underpinnings than grip strength (Holtzer *et al.*, 2006), thus impaired cerebrovascular regulation might be a precursor to both cognitive and functional impairment (Rosano *et al.*, 2005; Rosano *et al.*, 2012). A cognitive hypothesis proposes that cerebrovascular small vessel disease contributes to changes in executive function that might be expressed in a geriatric phenotype, including slow gait (Hajjar *et al.*, 2009; Srikanth *et al.*, 2009). White matter lesions – a clinical indicator of small vessel cerebrovascular disease – are associated with impaired gait in older adults (Rosano *et al.*, 2005; Rosano *et al.*, 2007; Srikanth *et al.*, 2009). In the current study, trends for lower performance on the MoCA, and Trails A and B tests were noted in individuals with slow gait. The MoCA is a screening test that has been shown to be particularly sensitive to cognitive concerns from a vascular origin (Sikaroodi *et al.*, 2013). It is noteworthy that both RAP and V_RAP remained significant covariates in a fully-adjusted model, including MoCA score, suggesting that vascular characteristics might impact functional performance independent of their cognitive effects. In particular, the observation of a trend for the dynamic response of RAP during posture change might suggest that autoregulatory factors play a role in the aging-related slowing of gait. Interestingly, and in contrast with the proposed cognitive link to slow gait speed, Rosano *et al.* (2011) have recently

suggested that the impact of hypertension on gait speed over ~15 years did not appear to be mediated through end-organ damage and might instead by the result of peripheral neuropathy or other, unaccounted for age-related variables.

Inflammatory Markers

The finding of elevated CRP in slow gait is consistent with the known relationship between inflammatory markers and functional performance in older adults (Cesari *et al.*, 2004; Brinkley *et al.*, 2009). An association between elevated creatinine and physical frailty has also been demonstrated (Shlipak *et al.*, 2004). In older adults, elevated serum creatinine as a marker of kidney function has been related to cardiovascular events and mortality (Fried *et al.*, 2003). A relationship between kidney function and cerebral small vessel disease, highlighted in the Rotterdam Scan Study (Ikram *et al.*, 2008), likely reflects parallel pathways in the kidney and brain – two organs that are particularly susceptible to accelerated vascular aging (O'Rourke & Safar, 2005). Surprisingly, the current analysis found individuals with slow gait to have lower serum creatinine levels. The cohort described in this thesis was relatively healthy, with only 2 individuals reporting overt cardiovascular disease (both cases of myocardial infarction). Therefore, the age-related changes in vascular structure and function were likely not sufficient to contribute to the end-organ damage necessary to effect changes in serum creatinine. Instead, the differences observed might have been due to biological heterogeneity or an unknown confounding factor.

Thresholds of Risk

In the current study, a cut-point of 1.0 m/s was used to determine slow walkers from fast walkers. Many cut-points have been used in the literature to assess health status or future outcomes. In a review of 27 related articles, 0.8 m/s was proposed as a cut-point for risk of

adverse outcomes, while those with gait speed above 1.0 m/s generally have lower risk of health-related events and improved survival (Abellan van Kan *et al.*, 2009). The Health, Aging and Body Composition (Health ABC) Study demonstrated that a cut-point of 1.0 m/s identifies persons at elevated risk of health-related outcomes in high-functioning older adults (Cesari *et al.*, 2005). In the current study, gait speed < 1.0 m/s was observed in 18% (n = 15) of the population, while only one individual exhibited a speed slower than 0.8 m/s. The low prevalence of slow gait speed in this healthy population of community-dwelling elders likely contributed to the limited strength and wide confidence intervals of the odds ratios for slow gait speed and falls.

Limitations

The current study has several limitations. Due to the cross-sectional nature of this investigation, it was not possible to establish that slow gait is a consequence of changes in cerebrovascular health. Indeed, it is plausible that other impairments, musculoskeletal or cognitive in origin, prevent individuals from habitual physical activity, which places them at a greater risk for progressive changes in vascular structure and function. Future longitudinal investigations are required to confirm the hypothesis that cerebrovascular factors lead to gait and balance impairments. In addition, missing data reduced sample sizes for various cerebrovascular measures. No difference in certain key variables was observed for individuals with missing data; however, the missing data must be assumed to be at random for many of the findings to hold true across the entire sample. This small sample, in addition to the fact that this group of community-living older adults reflected a relatively healthy, educated subgroup of the population, limits the generalizability of the current findings to a wider population. Future

work should compare community-living older adults with those in assistive-care and nursing-home settings to obtain a greater spectrum of human aging.

Conclusions

This study examined the association between indices of cerebrovascular aging and gait speed. In estimating measures of passive resistance from the instantaneous relationship between CBF and ABP, an independent relationship between elevated resistance and slow walking speed was found. These relationships remained after accounting for differences in executive function and processing speed, suggesting the contribution of a factor separate from cognitive state. Notably, slow walking speed was associated with attenuated dynamic responses of RAP during postural transitions from sitting to standing, which might indicate that age-related impairments in cerebrovascular autoregulation contributed to slower gait.

CHAPTER 7. GENERAL DISCUSSION

The preservation of cognitive health is paramount to successful aging. Recent evidence has linked subclinical vascular disease to the continuum of cognitive impairment (Luchsinger *et al.*, 2009; Wiederkehr *et al.*, 2009; Viticchi *et al.*, 2012), yet the mechanisms through which central vascular risk affects cerebral function is still poorly understood. This thesis examined cerebrovascular hemodynamics in high-functioning older adults from combined epidemiological and physiological vantage points. A strong component of this thesis is the breadth with which it examined vascular aging, including the impact of physical activity and sleeping habits, clinical vascular function and physiological mechanisms, as well as their impact on functional outcomes. In this final chapter, a brief summary of the important findings in this thesis were reviewed. Particular emphasis was placed on their physiological implications with an eye toward cerebrovascular health and cognitive impairment. As well, practical implications that lend themselves to appropriate knowledge translation themes in the continuum of care involving older adults were put forth. Finally, limitations of the overall body of work were considered, and ideas for the future extension of this work were proposed.

Summary of Findings

The use of passive pressure-flow models to investigate how the constructs of critical closing pressure (CrCP) and resistance-area product (RAP) contribute to cerebrovascular regulation offered novel insights. In Chapter 5, older adults, who were characterized by elevated cerebrovascular resistance (CVR), engaged different regulatory pathways to achieve effective dynamic cerebral autoregulation. Briefly, individuals with elevated CVR were found to recruit relatively more CBF reserve through a lowering of CrCP, while individuals with lower resting CVR were able to modulate perfusion primarily through changes in RAP. In Chapter 6, an

association between individuals who demonstrated lower dynamic RAP responses during posture change, as well as higher steady state CVR in upright postures, and evidence of early functional decline was noted. Functional decline associated with this altered autoregulatory function included a higher prevalence of falls over the past year and slower gait speed. The elevated CVR, that was associated with these aforementioned characteristics, was primarily related to greater intima-media thickness (IMT), as shown in Chapter 4, suggesting that processes within the vascular wall contributed to this functional change. Finally, an examination of lifestyle characteristics revealed that sedentary behaviour and short sleep duration have modest effects on cerebrovascular health. In Chapter 3, low levels of physical activity and short sleep duration were associated with lower CBF and lower cerebrovascular reactivity to carbon dioxide (CR_{CO2}). Contrary to the hypothesis, these relationships were not related to metabolic syndrome or a pro-inflammatory state.

Physiological Significance / End-Organ Damage

Recently, the impact of age-related changes in central vascular health on the brain's blood supply has received significant attention. The cerebral circulation is marked by high compliance and thus might be more susceptible to increased pulsatile forces associated with arterial stiffening (O'Rourke & Safar, 2005; Mitchell *et al.*, 2011; Webb *et al.*, 2012). Changes to central artery structure and function might allow pulsatile stresses to make their way further downstream into the microcirculation, where they can impact smaller, more fragile vessels and contribute to pro-inflammatory conditions (Mattace-Raso *et al.*, 2004; van Bussel *et al.*, 2011). In a mouse model of small vessel disease (NOTCH3 mutation), observed vascular changes included myogenic dysfunction, reduced vessel diameter and impaired autoregulation. In these animals, microcirculatory rarefaction lead to cerebral hypoperfusion prior to the development

of white matter damage (Joutel *et al.*, 2010), suggesting a temporal path through which vessel impairment leads to cerebral pathology. However, this was a genetic model of small vessel disease, so no direct link can be made to observations from the thesis.

Among the multiple clinical vascular characteristics measured as part of this study, IMT was the strongest correlate of low CBF and was related to higher CVR. A link between IMT and covert ischemia has been observed in other high flow vascular beds, such as the coronary circulation (Nagai *et al.*, 1998; Tzortzis *et al.*, 2010). The observation of a greater CrCP involvement during the dynamic phase of cerebral autoregulation in individuals with elevated baseline CVR is noteworthy considering preliminary associations between CrCP and cerebral metabolic regulation during cognitive tasks (Panerai *et al.*, 2012). A potential implication is that increased reliance on cerebrovascular reserves closer to the metabolically-sensitive microcirculation is needed to maintain sufficient flow in upright postures. This involvement could be at the cost of a reduced capacity to further increase flow if necessitated by subsequent increases in neural activity during standing. Recently, Baker *et al.* (2013) reported evidence in a rat model that showed the CBF response to functional stimulation became more attenuated with increasing levels of global ischemia. In addition, the modulation of RAP and CrCP in response to a reading task was shown to be sensitive to upright posture in healthy adults (Castro *et al.*, 2012). These observations are consistent with the theory that lower CBF puts greater strain on reserve pools within the cerebral circulation to regulate perfusion during increased neural activity; however, this was not answered by this thesis and remains speculation for future research.

Practical Significance / Public Health

The aging society is at a stage where preventative and early detection of modifiable and reversible vascular dysfunction can help prevent future long term pressures on the health care system. Preventative health strategies remain an important tool to promote behaviours that might help stave off the development of vascular-related cerebral impairment. Chapter 3 of this thesis highlighted potentially modifiable risk factors (physical inactivity and poor sleep hygiene) that can be addressed through public and/or private programs.

Sleep Quality

Sleep disturbances are common in community-living and assisted-living older populations. Although ~80 % of the current sample reported adequate sleep [between 7 and 8 hours per night (Table 3-2)], population-wide studies estimate prevalence of inadequate sleep to be closer to 50 % and 69 % in the community and assisted-living homes, respectively (Foley *et al.*, 1995; Rao *et al.*, 2005). The current study found a relationship between low sleep duration and lower CR_{CO2}, which is consistent with known associations between sleep disturbances and poorer overall health (*e.g.*, greater number of comorbidities and poorer functional status) (Fung *et al.*, 2012). Recognition of sufficient sleep as an important health behaviour will need to be a focus of public health promotion moving forward (Redline & Pack, 2006). With respect to older adults, assisted-living centres are an important area to focus knowledge translation efforts and improve conditions of sleep hygiene that might contribute to the health benefits (Martin *et al.*, 2010). Proven methods to improve self-reported sleep quality include exercise training and sleep hygiene education (Reid *et al.*, 2010; Yang *et al.*, 2012).

Physical Activity

Physical inactivity is a well-documented modifiable risk factor for cardiovascular and cerebrovascular disease (Heckman & McKelvie, 2008). Consequently, exercise is recommended as a preventative tool to reduce the incidence of events. There exists strong evidence that habitual physical activity can prevent or reverse age-related changes in peripheral vascular structure and function (Tanaka *et al.*, 1998; Desouza *et al.*, 2000; Pierce *et al.*, 2011), which might extend into the cerebrovascular domain (Lange-Asschenfeldt & Kojda, 2008). In this thesis, increased levels of habitual physical activity were related to higher CBF, independent of other cardiovascular risk factors.

The American Heart Association guidelines for the prevention of stroke suggest a daily minimum of 30 minutes of moderate-intensity activity (Goldstein *et al.*, 2006). In the current sample, 66 % of participants reported engaging in at least 20 minutes of moderate physical activity at least 3 times per week, while 43 % of the participants reported being part of a fitness group or club (Table 3-2). This compares more favourably than the Canadian Community Health Survey, which found that less than 50 % of older Canadians were getting sufficient exercise. Stessman *et al.* (2009) have demonstrated that physical activity is protective against mortality even when active lifestyle habits are adopted after 70 years of age. Taking these findings as a whole, public health agencies should be encouraged to strengthen their efforts to promote physical activity options for older adults that include lifestyle changes rather than structured interventions to maximize adherence (Opdenacker *et al.*, 2011), as well as gender-specific programming to maximize engagement in older men and women (Brach *et al.*, 2004; Chipperfield, 2008).

Limitations

The cross-sectional design of this thesis prevents any causal relationships from being established with respect to the influence of physical activity and sleep duration, as well as the impact on functional outcomes that were explored. It is possible to conceive that the findings reported in Chapter 3 might be interpreted such that low CBF or low CR_{CO2} are reflective of comorbidities that are barriers to engagement in physical activity or obtainment of restful sleep, rather than the view that a lack of activity or sufficient sleep leads to cerebrovascular dysfunction. Considering the relative health of this sample, with no cognitive impairment or depressive symptoms, this study targeted early health deficits that would not, on their own, outwardly dissuade physical activity or disturb sleep patterns. Rather, I believe these findings set the foundation for future prospective and interventional studies to understand the influence of lifestyle on mechanisms of cerebrovascular regulation and the functional consequences thereof.

Consequent to the heavy weighting of recruitment on a self-selected elderly database and relatively strict exclusion criteria (see Chapter 2), the findings reflect a highly-educated, Caucasian sample with relatively good health. Therefore, applying these results to the general older adult population should only be done with caution. Although the focus was intentionally geared towards healthy older adults, the range of vascular health observed was unexpectedly narrow. For example, only 7 of 79 older adults exhibited IMT and carotid-femoral pulse wave velocity values above established risk levels, set at ~ 75^{th} percentile of the general population. Despite this healthy, low-risk population, observations of structural and functional variations were noted that have potential for long-term impact on health. Thus, this thesis has shown that these cerebrovascular changes occur even at early stages of central arterial dysfunction.

From a physiological perspective, this study was limited to examination of large cerebral arteries, including the extracranial internal carotid (ICA) and vertebral arteries (VA), and the basal middle cerebral artery (MCA). As such, the discussion surrounding the metabolic regulation and microvasculature are only inferences made to spur discussion and generate future research. Of a similar note, the absence of brain imaging limits the findings from firmly distinguishing whether differences in CBF across the sample were secondary to underlying vascular health or differences in brain volume. Given the general health of the participants and the absence of mild cognitive impairment, atrophic changes were not anticipated to significantly confound the relationships which were observed. Estimating tissue perfusion based on published equations for brain mass in health adults, which consider age and sex (Lemaitre *et al.*, 2005), did not influence CBF across the sample.

Finally, results from steady state and dynamic measures emphasize the necessary caution needed when using CBF velocity as an index of flow. Noted in Table 4-1, MCA MFV was higher in women than men, yet men had elevated anterior CBF as determined through bilateral ICA measurement. Based on these differences, women's MCA is estimated to be ~15 % smaller than men's. Thus, when relying solely on TCD to estimate blood flow, men and women should be considered separately. In the current analysis, participants were stratified into tertiles of CVR based on sex, so that equal proportions of men and women would be included in each grouping for analysis. Populations of older adults, who might have variable subclinical changes in vascular structure, also require some caution when evaluating the validity of TCD measurements as CBF (Ozdogmus *et al.*, 2008). The correlation between MCA MFV and ICA MFV in the current study was modest (Pearson's correlation coefficient = 0.44, $P < 0.001$).

Future Directions

This work used a model of hemodynamics that considered independent influences of CrCP and RAP to uncover early changes in cerebrovascular autoregulation that have fundamental impact for everyday tasks of independent living, including posture change and gait. Since these variables consider the passive characteristics of the cerebral vessels, future work should examine if they provide a more sensitive marker of changes in resistance than the more pervasive cerebrovascular resistance index (CVRi). Although a model considering separate influences of ABP, CrCP and RAP might reveal differences in cerebral regulation associated with very early changes in vascular structure and function, a greater understanding of the physiological underpinnings of RAP and CrCP is needed. Preliminary evidence has linked RAP and CrCP to the interplay of myogenic and metabolic influences (Panerai *et al.*, 2012; Zuj *et al.*, 2013). Given the anatomical separation and mechanistic differences in where and how myogenic and metabolic regulatory systems are controlled, autonomic and nitric oxide blockade studies might help to further elucidate differences between the RAP and CrCP. Further, the evidence established here will need to be extended to populations at different stages of the aging continuum. Even in a sample population with relatively low-risk vascular health, the current study found evidence of mild changes in cerebral autoregulatory function that were associated with slower gait speed and increased occurrence of falls. It will be important to extend the framework to middle age and the oldest old populations, as well as pathological states, and comparisons between independent and dependent older adults.

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APPENDICES

APPENDIX A – AGING HEALTH QUESTIONNAIRE

Partial Health History, Part I:

Please complete and bring with you for review

1. Your Background

1. People living in Canada come from many different cultural and racial backgrounds. Are you? *(Check (√) all that apply)*

□ Aboriginal: □ 1st Nation □ Inuit □ Métis
□ White □ Black □ Southeast Asian (*i.e.,* Vietnamese)
□ Filipino □ Chinese □ South Asian (*i.e.,* East Indian/Pakistan)
□ Latin American □ Other: _____

2. What language did **you** first learn to speak as a child? _____

3. In what languages can **you** conduct a conversation? _____

4. What is **your** birthplace?
City/Town_____ Province_____
If born outside Canada, Country _____ Years in Canada: _____

5. What is **your mother's** birthplace?
City/Town _____ Province_____
If born outside Canada, Country _____

6. What is **your father's** birthplace?
City/Town_____ Province_____
If born outside Canada, Country _____

2. Your Marital Status

□ never married
□ married or living with a partner
□ widowed, not currently married
□ divorced, not currently married
□ separated

3. Your Education

1. What are your total years of formal education (includes grades 1 and higher):
_____years

2. What diplomas, certificates or degrees have you obtained?
(Check (√) all that apply)

□ None
□ High School
□ Trade certificate/diploma
□ Community college diploma
□ University undergraduate degree
□ University graduate degree

4. Your Occupation

1. What kind of work did you do for most of your life?
_____

2. If you did not work for pay, what did your spouse do for most of their life?
_____

3. Are you working for pay now?
If yes, please check (√): □ casual
□ part time
□ full time
If no, year last worked _____ or □ never worked

5. Handedness

Waterloo Handedness Questionnaire – Revised

INSTRUCTIONS: Please indicate your hand preference for the following activities by **circling** the appropriate response. Think about each of the questions. You might try imagining yourself performing the task in question. Take your time.

- **If you use one hand 95% or more of the time to perform the described activity, then circle right always or left always as your response.**
- **If you use one hand about 75% of the time, then circle right usually or left usually.**
- **If you use both hands roughly the same amount of time, then circle equally.**

1. With which hand would you use a paintbrush to paint a wall?
   Left Always Left Usually Equally Right Usually Right Always

2. With which hand would you use a spoon to eat soup?
   Left Always Left Usually Equally Right Usually Right Always

3. With which hand would you use the eraser on the end of a pencil?
   Left Always Left Usually Equally Right Usually Right Always

4. Which hand would you use to draw a picture?
   Left Always Left Usually Equally Right Usually Right Always

5. Which hand would you use to hammer a nail?
   Left Always Left Usually Equally Right Usually Right Always

6. Which hand would you use to turn the pages of a book?
   Left Always Left Usually Equally Right Usually Right Always

7. With which hand would you use a pair of tweezers?
   Left Always Left Usually Equally Right Usually Right Always

8. Which hand would you use to insert a plug into an electrical outlet?
   Left Always Left Usually Equally Right Usually Right Always

9. Which hand would you use to throw a baseball?
   Left Always Left Usually Equally Right Usually Right Always

10. Which hand do you use for writing?
    Left Always Left Usually Equally Right Usually Right Always

11. Which hand would you use to pick up a piece of paper?
Left Always Left Usually Equally Right Usually Right Always

12. Which hand would you use to saw a piece of wood with a hand saw?
Left Always Left Usually Equally Right Usually Right Always

13. In which hand would you hold a needle while sewing?
Left Always Left Usually Equally Right Usually Right Always

14. Which hand would you use to turn on a light switch?
Left Always Left Usually Equally Right Usually Right Always

15. Which hand would you use to open a drawer?
Left Always Left Usually Equally Right Usually Right Always

16a) Is there any reason (*e.g.*, injury) why you have changed your hand preference for any of the above activities?

No____
Yes____

16b) Have you been given special training or encouragement to use a particular hand for certain activities?

No____
Yes____

16c) If you stated Yes to either 16a and/or 16b, please explain:

_____

6. Your Related Chronic Health Checklist (Check (√) all that apply to you)

	Cardiovascular (heart) risk factors:		Gastrointestinal (stomach/intestine):
□	Hypertension (high blood pressure)	□	Irritable bowel syndrome
□	Hyperlipidemia (high cholesterol)	□	Heart burn/esophageal reflux disease (GERD)
□	Diabetes mellitus	□	Peptic ulcer disease
		□	Gall bladder
		□	Other: _____
	Pulmonary (lung):		Musculoskeletal(muscles/bones):
□	Chronic bronchitis	□	Osteoporosis
□	Emphysema	□	Low intensity trauma fracture
□	Asthma		□ Hip
□	Other lung disease:_____		□ Vertebral
□	Sleep apnea		□ Wrist
□	Other sleep disorders:_____	□	Osteoarthritis
		□	Other: _____
	Neurological (Brain)/Psychiatric (Mood):		Anemia (blood disorders):
□	Seizure disorder	□	Sickle cell anemia
□	Migraine	□	Hypercoaguable (blood clotting) states or related conditions:
□	Depression		□ DVT - deep vein thrombosis
□	Schizophrenia		□ Pulmonary emboli - blood clot in the lung
□	Manic depression/bipolar		
□	Anxiety disorder (generalized, panic, phobia, etc.)		
□	Cancer: Specify type and location: _____	□	Color blind

7. Your Family Health History

Please make a **checkmark (√)** for family members (**blood relations only**) who have any of these conditions or has passed away from them.

CONDITION	Mother	Father	Brothers	Sisters	Sons	Daughters
Heart Attack						
Angina						
High Blood Pressure						
Peripheral Vascular Disease						
Stroke						
Dementia						

8. Your Surgeries

Date (month and year)	**Surgery (type of surgery, area of body)**

9. Your Medications (including supplements) taken within the last week.

Medication Name	Strength (mg)	Route (*e.g.*, by mouth, on skin, inhaled)	Frequency (*e.g.*, 1x/day, 4x/day)

10. Medication Adherence

Thinking of the medications PRESCRIBED to you by your doctor(s), please **circle** the most appropriate response for each question.

1. Do you ever forget to take your medications?

   Never Rarely Sometimes Often Always

2. Are you careless at times about taking your medications?

   Never Rarely Sometimes Often Always

3. When you feel better, do you sometimes stop taking your medications?

   Never Rarely Sometimes Often Always

4. Sometimes if you feel worse when you take your medications, do you stop taking them?

   Never Rarely Sometimes Often Always

5. Many people have trouble taking their medications exactly as prescribed by their doctor. Thinking back to the last time you didn't take your medication(s) as prescribed, can you describe why?

_____
_____
_____
_____

End of Questionnaire Partial Health History Part I.
Partial Health History Part II will be given to you at the end of Session I.
Thank you for your time

Partial Health History, Part II

Please complete and bring with you for review

1. Falls

A fall is a sudden, unintentional change in position causing an individual to land at a lower level, on an object, the floor, or the ground, ***other*** than as a consequence of a sudden onset of paralysis, epileptic seizure, or overwhelming external force.

1. In the **last year**, have you fallen?

   No____ (*go to **1. Falls - Question 2***)

   Yes____ (*please go to **1. Falls - Question 1a***)

   a) How many times have you fallen this year? _____

   b) What injuries did you sustain from the fall(s)?

   *(Check all that apply - this will summarize injuries from **all** falls)*

   Fractures (broken bones)

   - □ skull
   - □ neck or back
   - □ arm
   - □ hip
   - □ leg
   - □ other: _____

   Open wounds

   - □ head
   - □ arm
   - □ leg
   - □ other: _____

   - □ Dislocations
   - □ Sprains/strains
   - □ Intracranial bleeding (bleeding inside the head)
   - □ Superficial injuries (small cuts with minor bleeding, abrasions)
   - □ Contusions (bruises)
   - □ Other: _____

2. In your **entire life**, have you ever experienced a head injury(s)?

   No____

   Yes____, *if so, please complete the following chart*

	Loss of Consciousness? No	Yes	*If yes, number of minutes*
1			
2			
3			

Enter additional head injuries on the back of this paper.

*End of **Falls** questions.* → *Go to **2. Sleep Hygiene** questions.*

2. Sleep Hygiene

1. How many hours do you sleep within a **24-hour** period of time? Please exclude time awake overnight but include daytime naps.

   (Check (√) which time most frequently applies to you)

   ___6 hours or less

   ___7 hours

   ___8 hours

   ___9 hours or more

2. Have you had any of the following symptoms within the last **30 days**?

   Sleeping disorders or insomnia Yes____ No____

   Fatigue and tiredness Yes____ No____

*End of **Sleep Hygiene** questions.* → *Go to **3. Alcohol Use** questions.*

3. Alcohol Use

The next 6 questions are about drinking alcoholic beverages. Included are liquor (such as whiskey or gin), beer, wine, wine coolers and any other type of alcoholic beverage.

1 drink	= 12 oz. of beer
	= 5 oz. of wine
	= 1 ½ oz. of

1. In **any one year**, have you had at least 12 drinks of any type of alcoholic beverage?

   □ Yes (*go to question **4. Alcohol Use – Question 3.***)
   □ No

2. In your **entire life**, have you had at least 12 drinks of any type of alcoholic beverage?

   □ Yes
   □ No *(end of alcohol questions → Go to **5. Tobacco Use** questions.)*

3. In the **past 12 months**, how **often** did you drink any type of alcoholic beverage? (That is, how many days per week, per month or per year did you drink?)

   Number of days: _____ per *(check one)* □ week
   □ month
   □ year

4. In the **past 12 months**, on those days that you drank alcoholic beverages, on average**, how many drinks** did you have?

   Number of drinks: _____

5. In the **past 12 months**, on how many days did you have **5 or more drinks** of any alcoholic beverage?
   (That is, how many days per week, per month or per year did you have 5 or more drinks in a single day?)

   Number of days: _____ per *(check one)* □ week
   □ month

□ year

6. Was there **ever a time or times in your life** when you drank **5 or more drinks** of any kind of alcoholic beverage **almost every day**?
   □ Yes
   □ No

End of alcohol questions. → *Go to **4. Tobacco Use** questions.*

3. Tobacco Use

Section A is about **cigarette** smoking. Section B is about cigar, pipe and other tobacco use.

Section A

1. Have you smoked at least 100 **cigarettes** in your **entire life**?
   □ Yes
   □ No *(go to **5. Tobacco Use – Section B – Question 1**)*
2. How **old** were you when you first started to smoke cigarettes fairly regularly?
   _____ age (in years)
   □ never smoked cigarettes fairly regularly
3. Do you **now** smoke cigarettes? (check one)
   □ every day (*go to **Question 6.***)
   □ some days (*go to **Question 6.***)
   □ not at all (*go to **Question 4.***)
4. How **old** were you when you **last smoked cigarettes fairly regularly**?
   _____age (in years)
   If you quit smoking regularly in this past year, how many weeks has it been? _____
5. At **that time**, how many cigarettes did you **usually** smoke per day?

1 pack = 20 cigarettes

Number of cigarettes/day_____
or
Number of packs/day_____

*(If you no longer smoke, skip **Questions 6 & 7** → Please go to **Section B, Question 1.**)*

6. During the **past 30 days**, on how many days did you smoke cigarettes?

Number of days_____

7. During the **past 30 days**, on the days that you smoked, how many cigarettes did you smoke per day?

1 pack = 20 cigarettes

Number of cigarettes/day_____
or
Number of packs/day_____

Section B

Cigars include cheroots and cigarillos. Smokeless tobacco includes chewing tobacco or snuff.

1. Have you **ever** smoked a pipe, smoked a cigar or used smokeless tobacco?
   Pipe □ No □ Yes
   Cigar □ No □ Yes
   Smokeless tobacco □ No □ Yes
   *If 'no' to all → go to **6. Physical Activity** questions.*

2. How **old** were you when you first started to smoke a pipe/cigar/smokeless tobacco regularly?
   _____ Age (in years)
   □ never smoked regularly

3. Do you **currently** smoke a pipe, smoke a cigar or use smokeless tobacco?
   Pipe □ No □ Yes
   Cigar □ No *go to **Question 4*** □ Yes *go to **Question 5***
   Smokeless tobacco □ No □ Yes

4. How **old** were you when you **last smoked** a pipe/cigar/smokeless tobacco regularly?
   _____age (in years)
   If you quit smoking regularly in this past year, how many weeks has it been? _____
   *Go to **6. Physical Activity** questions*

5. On average, how **many times a week** do you smoke a pipe, smoke a cigar or use smokeless tobacco?

Pipe	___ (times per week)
Cigar	___ (times per week)
Smokeless tobacco	___ (times per week)

6. On **average**:
   How many pipes full of tobacco do you smoke each week? _____
   How many cigars do you smoke each week? _____
   How much smokeless tobacco do you use each week? _____

End of tobacco questionnaire → *Go to **5. Physical Activity** questions.*

5. Physical Activity

Physical activity is any form of body movement that requires effort, but does not include routine activities of daily living such as self-care and cooking. Physical activity can be required for work or transportation, or for pleasure.

The **intensity** of physical activity refers to the amount of effort you put into the activity. It can be judged on a 10-point scale, where '0' is sitting and '10' is all out effort, or it can be described in terms of how much you are sweating and breathing. To help us group activities together, we split intensity into 3 categories: **LIGHT, MODERATE** and **HARD.**

LIGHT:	2-4 on a scale from 0-10. No sweating, but faster breathing, *e.g.* walking.
MODERATE:	5-6 on a scale from 0-10. Some sweating and deeper breathing, but still able to talk comfortably, *e.g.* brisk walking or biking.
HARD:	7-8 on a scale from 0-10. Heavy sweating and heavy breathing with difficulty talking, *e.g.* running or swimming.

1. This question asks you to list specific activities that you have regularly performed **during the past 4 months**. List any regular activity from gardening to running. Circle the appropriate **Frequency** and **Duration** for each **Activity** you list. **Intensity** might vary within the activity. Please indicate the percentage of time you spend at each **Intensity** for all the **Activities** you list. An example listing has been completed in the first row.

Activity Description	**Frequency** (# of sessions per week)	**Duration** (minutes per session)	**Intensity** (described above)
E.g. Cycling	1-2 (3-4) 5+	up to 20 (20-30) 30-60 60+	Light Moderate Hard 50% 50% 0%
	1-2 3-4 5+	up to 20 20-30 30-60 60+	Light Moderate Hard ___% ___% ___%
	1-2 3-4 5+	up to 20 20-30 30-60 60+	Light Moderate Hard ___% ___% ___%
	1-2 3-4 5+	up to 20 20-30 30-60 60+	Light Moderate Hard ___% ___% ___%
	1-2 3-4 5+	up to 20 20-30 30-60 60+	Light Moderate Hard ___% ___% ___%
	1-2 3-4 5+	up to 20 20-30 30-60 60+	Light Moderate Hard ___% ___% ___%

2. This question asks you about the amount of regular physical activity you perform. Only consider activities you perform for at least 20 continuous minutes at a time. Please check one box in each intensity category that describes the frequency of your average physical activity habits **during the past year.**

a) I have engaged in **LIGHT** physical activity:

□ No days per week
□ 1 to 4 days per week
□ At least 5 days per week

b) I have engaged in **MODERATE** physical activity:

□ No days per week
□ 1 or 2 days per week
□ 3 or 4 days per week
□ At least 5 days per week

c) I have engaged in **HARD** physical activity:

□ No days per week
□ 1 or 2 days per week
□ 3 days per week
□ At least 4 days per week

3. Next, we would like to know about your average Physical Activity level at specific periods in your **entire adult life**. For each time period, please indicate the how often you participated in LIGHT, MODERATE and HARD physical activity.

Your age, in years	**Physical Activity**		
	Light *(Check one)*	**Moderate** *(Check one)*	**Hard** *(Check one)*
64 years to present (minus the year you already answered in Question 2	□ No days per week □ 1 to 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 or 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 days per week □ At least 4 days per week
51-64 years	□ No days per week □ 1 to 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 or 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 days per week □ At least 4 days per week
31 – 50 years	□ No days per week □ 1 to 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 or 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 days per week □ At least 4 days per week
18-30 years	□ No days per week □ 1 to 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 or 4 days per week □ At least 5 days per week	□ No days per week □ 1 or 2 days per week □ 3 days per week □ At least 4 days per week

4. These next questions are about strength training (weight lifting).
   a) In the **last year,** I have been lifting weights, on <u>average</u> (*check one):*
   □ Twice or more a week □ Once a week □ Less than once a week □ Never
   b) In my **entire adult life,** I have been lifting weights, on <u>average</u> (*check one):*

   □ Twice or more a week □ Once a week □ Less than once a week □ Never

Comments:

_____

End of Physical Activity questionnaire. → *Go to* ***<u>7. Physical Activity and Environment</u>*** *questions.*

<u>Fitness Centers/Clubs</u> (excerpt from original questionnaire for purposes of thesis)

1. Do you belong to a fitness group or club (*i.e.,* in your building or in and around your neighbourhood)?

__Yes
__No

End of Partial Health History Questionnaire Part II.
Thank you for your time.

APPENDIX B – SCORING OF SELF-REPORTED PHYSICAL ACTIVITY

(Refers to Appendix A Health Questionnaire: Question II-5-2)

Answer Combinations			Scores			Total Score	Categories
Light	Mod	Hard	Light	Mod	Hard		
no	no	no	0	0	0	0	Sedentary
1-4days	no	no	1	0	0	1	
no	1-2days	no	0	2	0	2	
5+days	no	no	2	0	0	2	
no	no	1-2days	0	0	3	3	Active
no	3-4days	no	0	3	0	3	
1-4days	1-2days	no	1	2	0	3	
1-4days	no	1-2days	1	0	3	4	
1-4days	3-4days	no	1	3	0	4	
5+days	1-2days	no	2	2	0	4	
no	1-2days	1-2days	0	2	3	5	
no	5+days	no	0	5	0	5	
5+days	no	1-2days	2	0	3	5	
5+days	3-4days	no	2	3	0	5	
no	3-4days	1-2days	0	3	3	6	
1-4days	1-2days	1-2days	1	2	3	6	
1-4days	5+days	no	1	5	0	6	
1-4days	3-4days	1-2days	1	3	3	7	Highly Active
5+days	1-2days	1-2days	2	2	3	7	
5+days	5+days	no	2	5	0	7	
no	no	3days	0	0	7	7	
no	5+days	1-2days	0	5	3	8	
5+days	3-4days	1-2days	2	3	3	8	
1-4days	no	3days	1	0	7	8	
no	no	4+days	0	0	9	9	
1-4days	5+days	1-2days	1	5	3	9	
no	1-2days	3days	0	2	7	9	
5+days	no	3days	2	0	7	9	
1-4days	no	4+days	1	0	9	10	
5+days	5+days	1-2days	2	5	3	10	
no	3-4days	3days	0	3	7	10	
1-4days	1-2days	3days	1	2	7	10	
no	1-2days	4+days	0	2	9	11	
5+days	no	4+days	2	0	9	11	
1-4days	3-4days	3days	1	3	7	11	
5+days	1-2days	3days	2	2	7	11	
no	3-4days	4+days	0	3	9	12	
1-4days	1-2days	4+days	1	2	9	12	
no	5+days	3days	0	5	7	12	
5+days	3-4days	3days	2	3	7	12	
1-4days	3-4days	4+days	1	3	9	13	
5+days	1-2days	4+days	2	2	9	13	
1-4days	5+days	3days	1	5	7	13	
no	5+days	4+days	0	5	9	14	
5+days	3-4days	4+days	2	3	9	14	
5+days	5+days	3days	2	5	7	14	
1-4days	5+days	4+days	1	5	9	15	
5+days	5+days	4+days	2	5	9	16	