The Role of Activities of Daily Living in Healthy Vascular Aging

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Doctor of Philosophy

in

Kinesiology

Waterloo, Ontario, Canada, 2021

Author’s Declaration

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

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

Abstract

Normal aging leads to endothelial dysfunction and arterial stiffening implicated in cognitive decline. Cerebral blood flow also declines with aging. Elevated blood flow during exercise produces laminar shear stress which is known to enhance endothelial function, slowing age-related arterial stiffness. Exercise in young adults (YA) is associated with a reduced risk of cognitive impairment later in life. Most young and older adults (OA) do not engage in regular exercise. It is not known whether activities of daily living (ADL), including usual pace walking, are strenuous enough to elicit cerebrovascular responses that may elicit shear-induced improvements in endothelial function. Therefore, a primary purpose of this study was to determine the response of blood velocity in the middle cerebral artery (MCAV) to ADL. Participants performed ADL in the lab. Usual pace and fast walking were strenuous enough to increase MCAV, oxygen uptake (VO_2), mean arterial pressure (MAP), and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) above resting values in young and OA (Chapter 2). Even slow walking elicited these responses in older but not YA. There was no difference in MCAV between usual, slow and fast walking speeds (Chapter 2). The remaining ADL, vacuuming, descending and ascending stairs, and carrying and shelving groceries, were tested in OA only and also elevated MCAV, VO_2, and MAP (Chapter 3). On average, ADL were performed at moderate intensities. MCAV was shown to increase during ADL but the dynamic response of MCAV to exercise onset is not well understood, nor are the mechanisms responsible for that increase. Literature has described this response as a first order system; however, our data show the increase in MCAV with exercise was not

adequately described by a single exponential equation as demonstrated by residuals that were not random and uniformly scattered (Chapter 4). The mechanisms regulating this exercise response of MCAV were investigated during hypocapnia, hypercapnia, and normocapnia conditions. Results demonstrated that the change in MCAV with exercise was adequately predicted by both $PaCO_2$ and MAP (Chapter 4). Together these studies further the understanding of cerebrovascular responses to ambulatory ADL.

Acknowledgements

First and foremost, I thank God for qualities that made this experience desirable and the goal achievable.

John, my dear husband, our wedding vows said nothing about a mid-life PhD so I especially appreciate your understanding, patience, and so many accommodations. Thanks for being the best sounding board, for your solid and reasonable perspectives, and for picking up the slack when I could do nothing else but this. I could not have done this in this short time without you.

To my children, Lauren, Brian, and Megan, and Dan. Thank you for your understanding and support. Being with you is always grounding and puts everything in perspective. I promise not to talk about graphs or brain blood flow for at least a year, at home, when you're around.

Redeemer University, and most especially Dean Kyle Sypksma, thank you for your support and understanding and for decisions that promoted success in achieving this goal. I look forward to having both feet firmly planted at Redeemer. Ask me to volunteer anytime.

My friends who provided much encouragement and so kindly asked about this work and listened to my answers. Thank you for the fun and laughter that was so often the vacation I needed.

Dr. Duncan MacDougall and Dr. Digby Sale, who introduced me to research in my undergrad years that changed the course of my career. Thank you. In addition, Dr. Ira Jacobs, Dr. Manny Radomski, and Dr. Fred Buick, in working with you all I experienced research as exciting, important, meticulous, team work and ever so much fun! It was these experiences that led me to a PhD. Thank you all for your mentorship.

My committee, Laura Middleton, John Mielke, and Rich Hughson, it's been a long road. Thank you for your expertise and feedback at proposal and defense. I've learned some lessons I might not have learned elsewhere. Rich, thank you also for including me in some of the Space research. Laura, thank you for your willingness to discuss my questions, provide feedback and discuss interesting topics and research possibilities that were valuable to the learning experience.

Lab mates, Ikdip Brar, Thomas Beltrame, thank you for your research assistance. Kathryn Zuj, Andrew Robertson, Jackie Thibert, and Laura Fitzgibbon-Collins, thank you for your assistance and friendship.

I am grateful to participants for their time and effort in these experiments as well as their cheerful demeanor that made data collection even more enjoyable.

Dedication

It is with deepest gratitude and love that I dedicate this work to my mom and dad, Carl and Peggy Hinch, who passed away during this doctorate program. Thank you for everything!

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Chapter 1

Review of Literature

1.1 Introduction

By 2050, there will be more people aged 60 years or older worldwide than those between 10 and 24 years (2.1 billion versus 2.0 billion, respectively) (United Nations, 2017). The combination of rising lifespan and declining fertility imposes a rise in the number of older adults (OA) who have fewer children potentially for support and increases the 'total dependency ratio', a metric indicating the number of dependents for each person of working age (United Nations, 2017). Cerebrovascular disease presents a growing challenge as the population ages. For example, stroke and dementia are the second and seventh leading causes of death from disease worldwide and a major cause of dependency among older adults (Murray & Lopez, 1996, World Health Organization, 2021). These statistics emphasize the urgent need for interventions that reduce the risk of cerebrovascular disease and neurodegeneration and allow OA to live independently in the community for as long as possible. One of these interventions, perhaps with the greatest overall efficacy, is physical activity. Exercise has demonstrated pleiotropic effects on brain health and disease prevention (Ahlskog et al., 2011; Ahmed et al., 2012; Booth et al., 2012; Deslandes et al., 2009; Mikkelsen et al., 2017). The effect of exercise in slowing vascular aging is well documented, particularly as it pertains to reducing or even reversing arterial stiffening (Desouza et al., 2000; Seals et al., 2008; Tanaka et al., 2000; Tomoto et al., 2015). Conversely, sedentary behavior is associated with mortality and morbidity (Biswas et al., 2015). Increased blood flow during exercise elicits hyperemia-induced shear stress on vascular walls leading to

improved endothelial function in cerebral arteries (Smith et al., 2017). Furthermore, exercise increases brain derived neurotrophic factor (BDNF) (Berchtold et al., 2005; Boyne et al., 2019), vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) (Trejo et al., 2001) which together promote angiogenesis, neurogenesis, and neural plasticity (reviewed by (Lucas et al., 2015)). Increases in hippocampal perfusion following exercise training were associated with increased hippocampal volume and improved memory (Maass et al., 2015). Exercise has also demonstrated anti-inflammatory effects (reviewed by (Cotman et al., 2007)). However, most OA don't engage in regular, intentional exercise (Statistics Canada, 2015b). On the other hand, community living OA regularly engage in activities of daily living (ADL) such as shopping, cleaning, and walking at their usual pace as they run errands, walk the dog, and walk for pleasure. It is not known whether ADL are strenuous enough to stimulate increases in cerebral blood flow (CBF). This query is investigated in Chapters two, three, and six (Supplement) of this thesis.

Brain blood flow increases with exercise from onset to steady state however, that dynamic response has not been well researched nor have the mechanisms responsible for this increase. Understanding the kinetic response of CBF to acute exercise and the regulatory mechanisms that drive it may provide a greater understanding of compromised blood flow in disease and aging. For example, in Alzheimer's disease, the responsiveness of cerebral vascular resistance to changes in $PaCO_2$ (CR_{CO2}) is reportedly attenuated (Gongora-Rivera et al., 2018). A decreased CR_{CO2} was associated with lower scores on the mini-mental state examination while resting MCAV was not (Kim et al., 2021). Understanding the extent of the

role of CO_2 in the regulation of MCAV dynamics may elucidate the importance of vasomotor responses in this disease and the use of CR_{CO2} in prediction of disease risk. Furthermore, ADL vary in duration and intensity and understanding the kinetic response of MCAV to exercise onset may provide some insight into how MCAV responds to ADL and thereby define the value of ADL. For example, a fast rate of rise of MCAV may impose a greater shear stress that would impart endothelial benefits in a short duration. This could be valuable in short ADL that are repeated frequently such as short bouts of walking within a home or stair climbing. Chapter four of this thesis assessed the kinetic response of brain blood flow to exercise and the effect of changes in carbon dioxide, a significant dilator of cerebral vasculature, and mean arterial pressure, the driving force behind blood flow.

The ensuing review of literature will provide a foundation of knowledge for further discussion in subsequent chapters. An overview of the anatomy of cerebral arteries is followed by mechanisms responsible for the regulation of CBF with particular focus on carbon dioxide and cerebral autoregulation. The sequential cardiovascular and cerebrovascular responses to exercise will be reviewed with specific attention to the regulation of CBF to exercise onset providing foundational information for Chapter four. The effect of aging on CBF and the cerebrovascular benefits of exercise will provide justification and background information for Chapter two.

1.2 Anatomy of Cerebral Vasculature

Four extracranial conduit arteries carry blood from the heart to the cranium. In the anterior hemisphere, bilateral common carotid arteries stem from the aorta (left) or the brachiocephalic artery (right) and each bifurcates into two large extracranial arteries: the external carotid artery and the internal carotid artery (Chandra, Li, Stone, Geng, & Ding, 2017). The external carotid artery (ECA) supplies primarily the face and anterior neck. Internal carotid arteries carrying about 72% of the total CBF to the Circle of Willis, supply the intracranial middle cerebral arteries (MCA) and anterior cerebral arteries (ACA). In the posterior, bilateral vertebral arteries (VA), supplying roughly 29% of the total CBF, extend through the cervical spine and converge to form the basilar artery, which supplies blood to arteries to the brainstem, midbrain, and cerebellum, as it extends upward to converge with the Circle of Willis at the posterior cerebral arteries (PCA) (Zarrinkoob et al., 2015) . The Circle of Willis is completed by the anterior and posterior communicating arteries. MCA and ACA supply primarily the two cerebral hemispheres while the PCA supplies the occipital and medial temporal cortices. The MCA, the artery investigated in this thesis, supplies the dorsolateral frontal, temporal and parietal cortices and subcortical nuclei (Nagata et al., 2016; Chandra et al., 2017).

The large arteries (ACA, MCA, and PCA) branch extensively providing blanket vascularization to the brain's pia mater (the innermost layer of three membranes (collectively called the meninges) that cover the brain and spinal cord. Pial arteries protrude deeper into the parenchyma, containing neural grey and white matter, and form parenchymal arterioles

enveloped by astrocytic end-feet, capillaries, and venules which regulate blood flow according to neural activity (MacVicar & Newman, 2015). Flow through the capillary network that lies between the parenchymal arterioles and the venules ranges from 0.3 mm/s to 3.2 mm/s, regulated by vasoactivity, both contraction and dilation, of precapillary arterioles and postcapillary venules (Chandra et al., 2017). This microcirculation allows tight regulation of flow to meet neural metabolic demand.

The cerebral venous system consists of a communicating network of vessels and sinuses, organized in two groups; superficial cortical vein and deep or central veins. Superficial cortical veins in the pia matter drain the cerebral cortex and subcortical white matter. The deep veins drain the deep white and grey matter and converge at the superior sagittal sinus. Via numerous interconnecting sinuses, the venous outflow from the superior sagittal sinus and superficial cortical veins empties to the sigmoid sinuses and jugular veins. Jugular veins carry outflow parallel to the common carotid to the superior vena cava (Cipolla, 2009).

1.3 Regulation of Cerebral Blood Flow

At rest, the brain requires about 12 to 15% of cardiac output despite its relatively small mass of about 2% of body weight (Rowell, 1993). While regional and total blood flow may increase according to neural activation and metabolism, the rigid encasement of the brain prevents significant changes in brain blood volume. Therefore, cerebral perfusion is

regulated in conjunction with intracranial volume (including cerebral arterial vasculature, large veins and the production and reabsorption of cerebrospinal fluid). CBF is regulated according to Ohm's Law whereby CBF is directly related to cerebral perfusion pressure (CPP) and inversely related to cerebrovascular resistance. CPP is the difference between mean arterial blood pressure (MAP) and intracranial pressure (ICP) (or central venous pressure if it is greater than ICP (Cipolla, 2009). Intracranial pressure is determined by the volumes of primarily brain parenchyma, but also cerebrospinal fluid, and blood (Ainslie & Duffin, 2009). Intracranial pressure is assumed to be constant and low enough (5-15 mmHg) to be considered insignificant in healthy populations (Aaslid et al., 1989). Therefore, MAP becomes the surrogate for CPP. Regional variation in CBF required to match neural activation is achieved through complex and redundant mechanisms that regulate cerebral perfusion. Several factors regulate MCAV including autonomic innervation, blood gasses, cerebral autoregulation, myogenic, and endothelial mechanisms.

1.3.1 Autonomic Regulation of Cerebral Blood Flow.

Both large pial intracranial arteries and smaller arterioles regulate CBF. A network of perivascular nerves originating from the superior cervical ganglia (SCG), the sphenopalatine ganglia (SPG), the optic ganglia (OG), and the trigeminal ganglia (TG) of the peripheral nervous system innervate the large and small pial arteries and arterioles within their adventitial layer. The density of the network of extrinsic nerves is greatly reduced in the Virchow-Robin space and disappears at the level of the parenchyma. Extrinsic innervation

constitutes both autonomic and sensory ganglia including sympathetic (SNA), parasympathetic (PNA) and trigeminal (TG) nervous system activity (reviewed by (Cipolla, 2009)).

The primary neurotransmitter of SNA is norepinephrine (NE). Norepinephrine activates post-synaptic alpha1-adrenoceptors in arterial smooth muscle mediating vasoconstriction and pre-synaptic alpha-2 adrenoceptors mediating vasodilation, both of which are prevalent in extra and intracranial arteries and disappear in parenchymal vasculature. Beta-adrenoceptors, also innervated by NE, are negligible in larger arteries but most dense in parenchymal arterioles and induce vasodilation (Cipolla, 2009).

The role of SNA in the tonic regulation of CBF remains controversial (Brassard, Tymko, & Ainslie, 2017). One common theory is that during normal conditions, SNA has little influence on cerebrovascular blood flow but in challenging conditions, SNA influences CBF. For example, in acute hypertension leading to autoregulatory breakthrough, vasoconstriction from SNA reduces blood flow in order to protect the brain from edema and disruption of the blood brain barrier or alternatively, during cerebral vasospasm SNA may be reduced to restore flow (ter vanDijk, Elting, Stall, and Absalom, 2013). In contrast, support exists for SNA-induced vasoconstriction of extracranial and intracranial arteries in response to transient hypotension during lower body negative pressure and head up tilt independent of $PaCO_2$ (Brassard et al., 2017).

PNA originates in the OG and SPG with neurotransmitters acetycholine (ACh) and vasoactive intestinal peptide (VIP), and nitric oxide (NO) resulting in vasodilation and

increased CBF. TG, the only cerebrovascular sensory afferent, does not appear to play a role in the regulation of CBF except in extenuating circumstances such as during vasospasm, when TG would elicit vasodilation and increased flow for protection against ischemia (Cipolla, 2009).

1.3.2 Neurovascular Coupling

In the microvasculature, neurovascular coupling, the matching of CBF to meet the demands of neural activity, is maintained by the neurovascular unit. Astrocytes, specialized glial cells, encase almost the entire parenchymal arterioles and capillaries regulating blood flow, releasing lactate into interstitial fluid, and upregulating neurotransmitters (Sofroniew & Vinters, 2010). Together, vascular smooth muscle cells, neuron terminal or varicosities, astrocytes, and pericytes make up the neurovascular unit. The primary role of the neurovascular unit is to match local blood flow with local neural activity. Pericytes lie between the capillaries and the astrocytes and neurons (Kisler et al., 2017). Rodent experiments demonstrated that pericytes control capillary recruitment and diameter (Hall et al., 2014; Hartmann et al., 2021). Neurons with cell bodies release neurotransmitters (ACh, NE, 5-HT) that stimulate receptors on astrocytes primarily but also on endothelial cells and smooth muscle cells, regulating blood flow according to neural demand (Cipolla, 2009).

1.3.3 Myogenic Mechanisms

Myogenic regulation refers to the intrinsic property of smooth muscle in large arteries and resistance arterioles to respond to changes in intravascular pressure with vasodilation in response to low pressure and vasoconstriction in response to high pressure. This is a truly myogenic response of smooth muscle however, its effects may be influenced by other vasoactive regulators such as the endothelium or local metabolites (Cipolla, 2009; Cipolla et al., 2014). There are two types of myogenic regulation: myogenic tone and myogenic reactivity. Myogenic tone refers to the relatively constant vasoconstriction at rest. Myogenic reactivity refers to additional vasoconstriction in response to pressure once myogenic tone is established (Schubert et al., 2008). Increased intravascular pressure causes depolarization of the smooth muscle cell membrane opening voltage-operated calcium channels. Calcium increases myosin light-chain phosphorylation and polymerization of G-actin monomers resulting in vasoconstriction. The event ceases with the elimination of intracellular calcium. In contrast, during myogenic reactivity, calcium sensitivity is increased with little or no change in the quantity of smooth muscle calcium (Osol et al., 2002).

1.3.4 Endothelial Regulation of Vascular Tone

The endothelial single layer of vascular cells is a dynamic organ that plays a significant role in regulating vascular tone primarily via four chemical pathways; nitric oxide (NO), endothelium-derived hyperpolarization factor, prostacyclin, and endothelin. Of these, NO, produced in the endothelium by endothelial nitric oxide synthase (eNOS) is the most potent.

eNOS activity is primarily regulated by continuous shear stress exerted by the sliding motion of blood along endothelial cells. Laminar shear stress is transduced by the integrins that anchor the endothelial cells to the basal lamina of the basement membrane. Transduction elicits phosphorylation of protein kinase B, increasing endothelial NO synthase (eNOS) activity (D. L. Smith & Fernhall, 2010). In addition to shear stress, eNOS activity is elevated by receptor-binding agonists acetylcholine, bradykinin, calcium, and thrombin which increase endothelial intracellular calcium, therefore calcium-calmodulin complexes which stimulate eNOS activity.

NO is produced when it is cleaved from from L-arginine by eNOS. NO easily diffuses from endothelial cells to the smooth muscle cells where it activates guanylate cyclase which synthesizes cyclic guanosine monophosphate (cGMP). In turn, cGMP activates protein kinase G which leads to decreases in calcium concentration via reduced calcium channel activity or activation of potassium channels which elicits endothelial hyperpolarization and resultant closure of voltage-gated calcium channels (B. E. Robertson et al., 1993). Oxidative stress reduces NO bioavailability as superoxide radicals react with eNOS reducing NO production. For a thorough review see (Toda et al., 2009).

Endothelin, most prominently endothelin-1 (ET-1) is a potent vasoconstrictor that is released via angiotensin II, thrombin, reactive oxygen species, and also by shear stress-induced integrin stimulation. ET-1 and NO tightly regulate vascular smooth muscle tone by coordinated interactions that are complex and reciprocal (Andresen et al., 2006).

1.3.5 Cerebral Autoregulation

Cerebral autoregulation (CA) is the modulation of CBF during fluctuating arterial blood pressure (ABP). Static autoregulation refers to the relationship between CBF and ABP during steady state over long periods of time, even hours. Dynamic CA, on the other hand, refers to the transient response of CBF to dynamic changes in ABP over seconds. An intact dynamic CA is defined as a rapid return of CBF to baseline after a short reaction to the sudden change in ABP (Tiecks, Lam, Aaslid, & Newell, 1995). CA is typically measured as %change in CBF/%change in MAP.

In 1959 Lassen introduced the classic CA curve with a CBF plateau of about 50 ml /100g/min over a range of 60 and 160 mmHg MAP and a fall in CBF below the lower limit. Later Paulson described a climb in CBF above ABP upper limit (Lassen, 1959; Paulson et al., 1990) and curves of concomitant changes in arteriolar diameter and cerebrovascular resistance with changes in the physiological range of ABP. However, research over the years often conflicted with the concept of a concrete plateau and more recently the CA curve or relationship between ABP and MCAV has been revised. Numan and colleagues analyzed the results of over 20 experiments that measured the change in CBF during decreases and increases in MAP and presented a linear relationship between changes in ABP and changes in CBF. Average slope was 0.82%CBF/%MAP for the decreased MAP and 0.21%CBF/%MAP for increased MAP (Numan et al., 2014) . In 2021, Brassard et al. updated the Numan study with added experiments and applied a third-degree polynomial to the data resulting in a more similar curve to Lassen but using changes in the variables rather

than absolute values, recognizing between subject variability (Brassard et al., 2021). In this most recent model, CBF is relatively stable (mean increase of about 5%) over only a small span of ABP of ±10% and only in those experiments without pharmacological intervention. It has also become clearer that CA is more responsive to transient increases in ABP compared to decreases (Numan et al., 2014; Brassard et al., 2021). Another point of interest in the contemporary view of CA is that dynamic CA is considered a continuation of static CA according to rate of change in ABP (Tzeng & Ainslie, 2014) . Thus, static CA would include transitions in ABP where oscillation frequency is 0-0.02 Hz, taking a period of over 50 sec to complete a single oscillation. Dynamic CA would include all transitions of higher frequency, above 0.02 Hz and up to 0.20 Hz. CA has been referred to as a high pass filter which dampens CBF in lower frequency oscillations of ABP less than 0.2Hz, but allows frequencies greater than 0.2 Hz through cerebral circulation (Smirl, Hoffman, Tzeng, Hansen, & Ainslie, 2015).

CA mechanisms are primarily controlled by vasoactive resistance vessels. Although exact mechanisms and their relative contribution to CA are still unknown, the integration of four mechanisms is proposed most: neurogenic (primarily intrinsic), myogenic (local control), metabolic, and endothelial. Of these, it is believed that myogenic mechanisms are dominant (Armstead, 2016; Panerai, 2008; Paulson et al., 1990). CA appears to remain intact with aging (Carey et al., 2000; Edgell et al., 2012; Lipsitz et al., 2000).

1.3.6 Cardiac Output

The impact of cardiac output on CBF has been investigated using lower body negative pressure, orthostatic challenge, beta-adrenergic block, albumin, and saline infusion. In a recent review, Meng et al. (2015) provided convincing evidence that perturbations to cardiac output (Q) that were independent of MAP and $PaCO_2$ (which remained stable) led to respective changes in CBF. Furthermore, a beta-blockade attenuated both Q and CBF. The authors concluded that a one percent change in Q resulted in a 0.35% change in CBF (Meng, Hou, Chui, Han, & Gelb, 2015). Brassard subsequently argued that manipulations that alter Q, reflexively alter ABP and/or $PaCO_2$ which also impact CBF (Brassard et al., 2017). Experimental designs that investigate CBF responses to exercise intervention show changes in CBF, ABP, $PaCO_2$, and Q, making it impossible to tease out and quantify the role of Q per se on changes in CBF. Further research is needed to clarify the role of Q per se on CBF during exercise.

1.3.7 Carbon Dioxide

Cerebral vasculature is highly sensitive to the partial pressure of carbon dioxide ($PaCO_2$) in comparison to the less sensitive peripheral vasculature (Lennox & Gibbs, 1932). Arterial CO_2 that easily crosses the brain blood barrier to enter cerebrospinal fluid and interstitial fluid reacts with water to produce carbonic acid (H_2CO_3) which then dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+) (McConnell, 2013). The purpose of CR_{CO2} is to regulate cerebral acid-base balance, pH, to prevent neural damage. Extracellular acidosis may activate

potassium channels directly (Brian, Jr., 1998). Most studies report that vasculature responds to high concentration of H+ with vasodilation and low concentrations with vasoconstriction (Battisti-Charbonney et al., 2011; Fluck et al., 2014; K. J. Smith & Ainslie, 2017). A very recent study challenges this conventional theory to provide evidence that $PaCO_2$ rather than pH/H^+ regulates CBF and that bicarbonate (HCO_3) may have a direct effect on cerebrovasculature (Caldwell et al., 2021). In a well-designed study, Caldwell compared CO_2 reactivity to two stages each of hypocapnia and hypercapnia with and without sodium bicarbonate infusion. ICA and VA blood flow were measured using duplex ultrasound and arterial concentrations of HCO_3^- and H^+ and pH were measured directly with an arterial catheter. The authors reported that the infusion of sodium bicarbonate did not alter CBF during each level of $PaCO_2$, although pH was elevated (Caldwell et al., 2021). During isocapnic breathing following infusion of sodium bicarbonate, Caldwell and colleagues (2021) found CBF was higher suggesting that bicarbonate had a direct effect on cerebrovascular tone, which was independent of $PaCO_2$. Caldwell also reported that when indexed for concentrations of H^+ in trials following bicarbonate infusion, relative CR_{CO2} was elevated in hypocapnia and lowered in hypercapnia indicating a difference in the buffering influence on H^+ or pH and $PaCO_2$ in hypercapnia. It was suggested that these findings may indicate that cerebrovascular vasodilation mediated by shear stress increased NO bioavailability concomitant with the vasodilatory effects of arterial CO_2 (Drapeau et al., 2021). Evidence suggests NO-induced decreases in vascular smooth muscle cell (VSMC) calcium concentration play a primary role in CR_{CO2} (reviewed by (Ainslie & Duffin, 2009;

Brian, 1998). NO is likely not the only mediating vasodilator because during high levels of hypercapnia, NO inhibitors reduce but do not eliminate the vasodilatory response.

Studies investigating the role of CO_2 in MCAV response to exercise have typically increased $P_{ET}CO_2$ levels with inhalation of high CO_2 gas mixtures (CO_2 supplement) or kept $P_{ET}CO_2$ at lower levels than normal using regulated hyperventilation and/or an end tidal forced air apparatus (CO_2 clamp). Central chemoreceptors in the medulla in the retrotrapezoid nucleus within the blood-brain barrier respond to changes in CSF pH brought about by changes in CO_2 and H+ at the medulla. Elevated CSF CO_2 above some threshold stimulates central chemoreceptors leading to increased ventilation to reduce $PaCO_2$. On the other hand, hypocapnia, or lower than usual $PaCO_2$, slows ventilation and therefore the expiration of CO_2 (Ainslie & Duffin, 2009). Supplementation is usually in the form of inhalation of hypercapnic mixed gas containing higher than room air CO_2 (5-15% CO_2) with room air or higher O_2, and balance nitrogen. The high CO_2 sensitivity of cerebral vasculature was reported in an early study where inhalation of 5%CO_2 caused a 50% increase in CBF and 7% CO_2 caused a 100% increase in CBF (Kety & Schmidt, 1948). In a recent study, inhalation of high CO_2 significantly influenced CBF during rest in the absence of change in other regulatory factors such as cerebral activation and metabolic drive or blood pressure. Participants were asked to take two breaths of 9, 11, or 13%CO_2 (in room air) during hypocapnic (8 mmHg below baseline $P_{ET}CO_2$), normocapnic (baseline $P_{ET}CO_2$), or hypercapnic (8 mmHg above baseline $P_{ET}CO_2$) trials, respectively, in order to increase $PaCO_2$ by about 8-10 mmHg during rest (Edwards et al., 2004). Compared to normoxia

where MCAV was 60.6 ± 8 cm/s and cerebrovascular resistance index (MAP/MCAV, CVRi) was 1.02 ± 0.34 mmHg/cm/s, hypercapnia elevated MCAV to 82.3 ± 14.5 (SD) cm/s and decreased CVRi to 1.0 ± 0.24 mmHg/cm/s while hypocapnia had the opposite effect reducing MCAV to 47.5 ± 7.8 cm/s and increasing CVRi to 1.6 ± 0.44 mmHg/cm/s. Two breaths of hypercapnic gas significantly increased CBF velocity and decreased CVRi in all conditions. A small change in BP at the level of MCA (BP_{MCA}) was not significant between conditions ($p<0.098$). CVRi response to changes in BP and $PaCO_2$ with the two breaths were attenuated during hypercapnia compared to hypocapnia. In a review paper, Smith and Ainslie (2017) quantified the CR_{CO2} response as approximately 3-5% increase in CBF per mmHg increase in $PaCO_2$ above rest. Conversely, hypocapnia decreases CBF at the reported rate of about 1-3% with each mmHg drop in $PaCO_2$ (Smith & Ainslie, 2017). These results suggest a difference in CR_{CO2} between hypocania and hypercapnic conditions however, when $P_{ET}CO_2$ was elevated (supplemental CO_2 (5%)) or reduced (hyperventilation) to the same extent, CR_{CO2} was not different between resting hypercapnia (1.46 ± 0.6 cm/s/mmHg) and resting hypocapnia (1.43± 0.5 cm/s/mmHg) (Murrell et al., 2013).

An important distinction in CR_{CO2} literature lies between partial pressures of arterial CO_2 ($PaCO_2$) and end-tidal CO_2 ($P_{ET}CO_2$). Measurement of arterial gasses is invasive and impractical for most studies; even more so for exercise research. Thus, most studies prefer the more convenient measure of $P_{ET}CO_2$. $P_{ET}CO_2$ represents peak alveolar CO_2. The difference between $P_{ET}CO_2$ and $PaCO_2$ is directly proportional to physiologic dead space. Physiologic (total) dead space includes not only the respiratory bronchioles and alveolar duct

and sac, but also the alveolar space which is not included in gas exchange and not perfused with CO_2. This dead space dilutes alveolar CO_2 making $P_{ET}CO_2$ about 2-5 mmHg lower than $PaCO_2$ in healthy humans (Satoh et al., 2015). The partial pressure of alveolar CO_2 (P_ACO_2) reflects the concentration of CO_2 at the alveoli, which is typically a little lower than $PaCO_2$.

Using $P_{ET}CO_2$ as a surrogate for $PaCO_2$ may underestimate $PaCO_2$, particularly in disease conditions where dead space is increased. During exercise, dead space is decreased with increasing workload due to greater perfusion of the functional dead space. With increasing tidal volume, the ratio of dead space to tidal volume decreases to minimal levels (about 0.1) by about 50%Wmax (Plowman & Smith, 2017). During exercise, the relationship between $P_{ET}CO_2$ and $PaCO_2$ was explored at two workrates (Jones et al., 1979). It was determined that the $P_{ET}CO_2$ to $PaCO_2$ difference increased with increasing tidal volume and volume of expired CO_2, and decreased with increasing breathing frequency. Stepwise multiple regression analyses of several variables defined the final relationship (Equation 1.1) between $P_{ET}CO_2$ and $PaCO_2$ for use during exercise (Jones et al., 1979) and additional variables did not increase precision of the regression equation. This equation was validated in at least two studies discussed in Jones et al. (1979) and has been applied in exercise studies since (Fluck et al., 2014). Another equation (Equation 1.2) was more recently developed for use at rest using only $P_{ET}CO_2$ (Peebles et al., 2007) and additional variables such as ventilation, breathing frequency, and tidal volume did not increase the precision of the equation.

$$\text{Estimated } PaCO_2 = 5.5 + 0.9 \times P_{ET}CO_2 - 0.0021 \times V_T. \quad \text{(Equation 1.1)}$$

$$\text{Estimated } PaCO_2 = 2.367 + 0.884 \times P_{ET}CO_2 \qquad \text{(Equation 1.2)}$$

The main difference between these two equations is their application. The addition of tidal volume in the Jones equation improved the validity of the equation during exercise (Jones et al., 1979) where tidal volume can increase more than four-fold by 60%Wmax before decreasing with the onset of hyperventilation and elevated breathing frequency (Plowman & Smith, 2017). The Peebles equation (Equation 1.2) is designed for resting conditions where breathing frequency and tidal volume are relatively stable. Neither of these equations reflect brain tissue PCO_2 which has shown a greater correlation to CBF responses to alterations in CO_2 (Ainslie & Duffin, 2009). In summary, CO_2 has a clear and significant role in the regulation of CBF and therefore, must be measured whenever CBF is a measurement of interest.

CR_{CO2} is often measured to assess the vasomotor responses to aging, an intervention such as exercise, or brain trauma or disease. Two common methods of measuring CR_{CO2} are transcranial Doppler ultrasound (TCD) and magnetic resonance imaging (MRI), both of which represent CBF, the variable of interest in CR_{CO2}. TCD measures blood velocity and assumes constant blood vessel diameter however, the stability in MCAV diameter with hypercapnia has been recently questioned (Coverdale, Badrov, & Shoemaker, 2017; Verbree et al., 2014). There are several different MRI techniques. Blood oxygen level dependent (BOLD) MRI signal reflects factors that influence deoxyhemoglobin that include blood volume and cerebral metabolic oxygen consumption in addition to CBF, and is measured on

the venous side although its dependence on blood volume includes both venous and arterial blood. Arterial spin labelling (ASL) is another MRI technique that assesses CBF in whole brain grey matter. Finally, phase contrast angiography (PCA) measures blood velocity in specifically targeted vessels e.g., MCA, and therefore, is likely the best comparator for TCD. MRI is vulnerable to background noise (Redpath, 1998) due in part to factors such as matrix size, slice thickness, and measurement signal parameters (Welvaert & Rosseel, 2013) which reduce signal to noise ratio. The impact of these methods on the assessment of CR_{CO2} was recently investigated in young (24 y) and older (66 y) fit and unfit adults who inhaled 5%CO_2 for 4 min with 4 min of supine rest before and after (Burley et al., 2021). CR_{CO2} was assessed with TCD (MCA), and BOLD, PCA, or ASL (MCA) MRI. Although there was agreement between all four measurement modalities in resting CBF measures, this was not true of CR_{CO2}. Discrepancies in CR_{CO2} existed between the four measurement modalities (Burley et al., 2021) perhaps associated with different parts of the vascular tree that have been shown to respond differently to CO_2 challenge (Sato et al., 2012; Willie et al., 2012) according to ultrasound which may be hampered by changes in vessel diameters (Burley et al., 2021). BOLD MRI is affected by blood volume while TCD and PCA MRI are not. Where measurements occurred within the same vessel (PCA and TCD) agreement was hampered by poor day to day repeatability in the TCD (Burley et al., 2021). The aforementioned emphasizes the importance of similarity in measurement modalities and day to day repeatability especially in TCD where the signal depends on the operator expertise and similar measurement techniques. Ideally, repeated measures testing should be conducted on the same day.

Research has produced conflicting results regarding the effect of aging on CR_{CO2}. Studies have reported that in aging, CR_{CO2} is lower (Bailey et al., 2013; Leoni et al., 2017), preserved (Coverdale et al., 2017; Nowak-Flück et al., 2018; Oudegeest-Sander et al., 2013) or even higher and faster (Thomas et al., 2014). Comparisons between studies are confounded by factors such as measurement modalities, repeatability, vessel type, varying population ages and pathologies, and statistical analyses with group comparisons or regression. Further work is required to elucidate the effect of aging on CR_{CO2}. Age-related arterial stiffening due to both functional (endothelial function) and structural (arterial remodelling) changes would theoretically support a reduced capacity for vasodilation in response to challenge such as CO_2. However, there is little evidence that arterial stiffening is associated with changes in CR_{CO2}. A gap in the literature exists in relating peripheral FMD and cerebral reactivity. Further work is needed to describe this relationship. If NO was the mechanism of vasoreactivity then reduced endothelial dysfunction would logically be expected to reduce CR_{CO2}, yet a recent study provides evidence that bicarbonate or $PaCO_2$ is driving vasodilation (Caldwell et al., 2021). While more research is required, mounting evidence suggests that CR_{CO2} is preserved in aging.

1.4 The Effect of Sex on Cerebral Blood Flow

Females have significantly higher MCAV than males (Edgell, Robertson, & Hughson, 2012; Labrecque et al., 2019; Tarumi et al., 2014; Vriens, Kraaier, Musbach, Wieneke, & van Huffelen, 1989). The smaller diameter MCA in females compared to males (Shatri et al.,

2017) could explain a higher MCAV however, CBF measured by ASL MRI was higher in women than men in white and gray matter in the whole brain as well as regional areas in frontal, parietal, and temporal lobes partially supplied by the MCAV (Alisch et al., 2021). Resting mean, systolic and diastolic MCAV were higher and resistance index (systolic MCAV – diastolic MCAV/systolic MCAV (RI)), CVRi, and pulsatility index (systolic MCAV – diastolic MCAV/mean MCAV (PI)) were lower in females compared to males of similar ages (Edgell et al., 2012).

Sex differences in CBF have been primarily attributed to estrogen (Nevo, Soustiel, & Thaler, 2007). In 12 young females enrolled in in vitro fertilization treatment cycles, ICA blood flow was significantly elevated in late follicular and midluteal phases by 22.2% and 32% compared to ovarian suppression. When late follicular phase was compared to ovarian suppression, mean blood velocity was higher and cerebral vascular resistance lower (Nevo et al., 2007). The role of estrogen in higher CBF in females led to the recommendation for standardization of CBF measurements early within the follicular phase of the menstrual cycle (Burma et al.2020), which would be most pertinent in studies where repeated measures take place on separate days. However, recent study found no difference in resting MCAV, ACA velocity, cerebrovascular resistance, or cerebrovascular response to squat-stand or sit-stand, or CO_2 reactivity of CBF between measurements in early or late follicular or midluteal phases of the menstrual cycle (Favre 2019).

Sex differences in MCAV have been reported up to the fifth decade (Vriens et al., 1989) the seventh decade (Alwatban et al., 2021) and even the ninth decade (Bakker et al.,

2004). Data of 524 participants from previous studies were reanalyzed for resting MCAV and PI in males and females over the lifespan and within 5 age groups. Over the lifespan, MCAV was greater in females than males. More specifically, MCAV was higher in female YA (18-30 y) and adults aged 51-60 and 61-70 y, but not those aged 31-50 y (possibly due to a low sample size) or 71 - 90 y. PI was lower in young females 18-30 y only (Alwatban et al., 2021). Edgell et al. (2012) also found that females had a smaller increase in PI and showed a tendency toward a smaller increase in RI during postural transitions. Other studies found resting pulsatile flow was higher in females compared to age matched males (Lefferts, DeBlois, Augustine, Keller, & Heffernan, 2020; Tarumi et al., 2014). The reason for the differing results isn't clear, however methodology may have played a role. The former studies (Edgell et al.,2012; Alwatban et al., 2021) compared two discrete groups, younger and older, using ANOVA whereas the latter studies (Lefferts et al., 2020; Tarumi et al., 2014) applied linear regression to a range of ages and the number of participants per decade is unclear.

The effect of sex on CBF during postural changes show conflicting results. Studies show no sex difference (Burma et al., 2020; Edgell et al., 2012), females showing better CA response (Favre & Serrador, 2019) or attenuated response (Labrecque et al., 2019). Edgell et al. (2012) investigated the cerebrovascular and cardiovascular responses to supine-sit-stand postural changes in young males and females under 30 years and older males and females over 50 y. Women had higher MCAV at rest but there was no effect of sex on the change in MCAV with posture changes Burma 2020 also found no difference in dynamic CA response

to sit-stand when females were tested in the early follicular phase. In contrast, Favre and Serrador (2019) used transfer function analyses to show that cerebral autoregulation was better in women according to a lower gain in MCAV (indicating greater dampening of blood pressure effect on CBF) during repeated squat -to-stand in the very low frequency range and during spontaneous fluctuations in BP while standing in the low and very low frequency range. $P_{ET}CO_2$ was lower in women which might induce vasoconstriction, a mechanism of CA, however, results in this study provide evidence that CO_2 may not be related to greater vasoreactivity. First, there was no sex difference in cerebrovascular resistance in spite of the lower $P_{ET}CO_2$ in women. Furthermore, improved CA was observed in women during standing although there was no sex difference in $P_{ET}CO_2$. Finally, CR_{CO2} was similar between sexes. Labrecque et al. (2019) found that fit young females showed an attenuated response to sit to stand posture change albeit without syncope, compared to fit young men. The decrease in MCAV was greater and the vascular conductance response was delayed in females compared to males (Labrecque et al., 2019).

Recent studies find no difference in CR_{CO2} between males and females (Favre & Serrador, 2019; Miller et al., 2018; Peltonen et al., 2015) (Miller et al., 2018). Peltonen and colleagues found the change in MCAV during hypercapnia at 10 mmHg above $P_{ET}CO_2$ baseline was similar for males (19 cm/s) compared to females (23 cm/s). Indomethacin, a cyclooxygenase inhibiter, elicited a large decrease in vasodilation during hypercapnia to the same extent in both sexes (Peltonen et al., 2015). Furthermore, no sex differences in MCAV

response were observed during hypercapnic gas inhalation or hypocapnic hyperventilation in 13 young men and women (Favre & Serrador, 2019).

1.5 Assessment of Cerebral Blood Flow

The primary variable of interest in this thesis is CBF. The need for portability in studies investigating CBF during ambulatory activities of daily living necessitated the choice of two instruments for the assessment of CBF; TCD, using a portable device, the TCD-X or near infra-red (NIR) spectroscopy (NIRS).

NIRS device has a NIR light transmitter and two NIR light receiver optodes providing a continuous measure of cerebral tissue oxygenation, inferred from light absorbing properties of hemoglobin (Perrey, 2008). Oxygenated, deoxygenated, and total hemoglobin as well as tissue oxygen saturation, which correlates with arterial oxygen saturation, are continuously measured (Nicklin, Hassan, Wickramasinghe, & Spencer, 2003). NIRS and TCD have been shown to provide similar responses to ABP manipulations determined by comparison of transfer function analyses during squat-stand and passive oscillatory lower-body negative pressure maneuvers (Smirl et al., 2015). The use of the NIRS technology has expanded greatly over the last decade. Functional NIRS is currently used for neuroimaging partially because of its resistance to movement artifact and portability (Condy et al., 2021). A drawback of NIRS was its reported sensitivity to changes in forehead skin blood flow (Miyazawa et al., 2013). Any activity that raises or lowers skin temperature may distort the

NIRS assessment of CBF. Another drawback is that NIR light emitting source penetrates superficial tissue such as the scalp, skull, and blood vessels which may distort light absorption and scattering, impacting the estimation of oxygenated hemoglobin assumed to represent cortical blood flow (Condy et al., 2021). Another limitation of NIRS is its dependence on blood volume (Pham et al., 2019), which is decreased with aerobic exercise onset with the greatest decline during the first five minutes due to fluid shifts that reduce plasma volume (Fortney et al., 1981). Finally, the transit time of blood from arterial to venous side of microvasculature creates a time delay that is unaccounted for in the measurement of CBF. Therefore, NIRS was not used in this thesis because the effect of changes in blood volume and the time delay would confound kinetic analysis of CBF.

TCD applies an ultrasound wave which penetrates the skull, tissues, and vessels to an area of the head according to the vessel of interest. TCD software allows the operator to select a given vessel and optimize the signal based on depth, angle of probe, and signal acoustical pitch, shape and amplitude (Panerai, 2009). Sound waves are reflected by moving red blood cells within the vessel. The velocity of the red blood cells is determined by the Doppler Shift Equation $V = f * C / 2f_0 * \cos\theta$, where V is the velocity of the red blood cells, f is the Doppler shift frequency, (C) and (f_0) are the velocity and frequency of the incident ultrasound wave, and θ is the angle of insonation. MCAV has been shown to be a reliable surrogate for middle cerebral artery blood flow (Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1992; Bishop, Powell, Rutt, & Browse, 1986). However, the relationship is dependent on a constant arterial diameter. The effect of various perturbations to ABP and arterial CO_2 on

cerebral arterial diameter is under debate (Brothers & Zhang, 2016; Hoiland & Ainslie, 2016). There is no established threshold of $PaCO_2$, above or below which MCAV will change. However, after reviewing research using high-field MRI (Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014; Verbree et al., 2014), Hoiland and Ainslie concluded that MCAV may remain stable over changes in $PaCO_2$ up to a magnitude of ±5 mmHg but recommend that MCAV responses to hypo-and hypercapnia are not assumed to be accurate. The measurement of MCAV with TCD requires operator expertise in locating and optimizing signals from vessels of interest resulting in poor day to day repeatability. Furthermore, approximate 10 to 15% of OA, particularly women, have inadequate acoustic windows deeming insonation of arteries impossible (Purkayastha & Sorond, 2012),

An important advantage of the TCD methodology is the ability to synchronize ABP and MCAV as well as assess signal quality and detect and correct signal artifacts (Claassen, Meel-van den Abeelen AS, Simpson, & Panerai, 2016). ABP and MCAV waveforms can be synchronized over each cardiac cycle, ensuring precise synchronization of analysis in each variable. In addition, signal quality is assessed based on shape and regularity of waveforms. Artifacts are easily identified such as in the case of a Finometer autocalibration or a distorted waveform (Figure 1.1). This capability was crucial for assessing mechanisms regulating CBF during exercise in Chapter 3. TCD was suitable method of assessing CBF for this thesis because of its portability, ability to synchronize variables over cardiac cycles, and testing could be done in one day to avoid day to day variability.

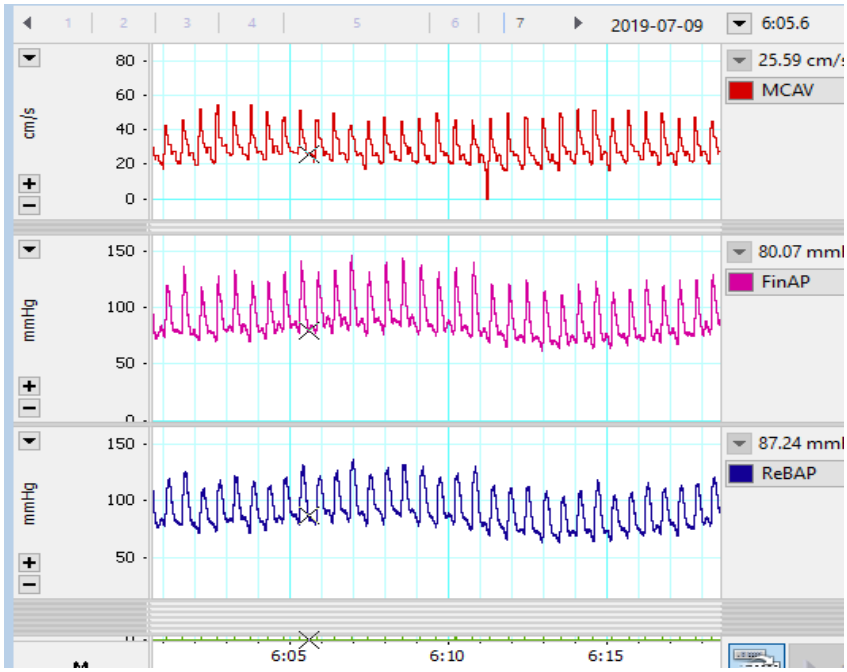

Figure 1-1. Real time traces of MCAV (top), finger arterial pressure (centre) and reconstructed brachial arterial pressure (bottom). Note the synchronization of beat by beat cycles for direct comparisons. Also note the clear artifact in the MCAV trace where diastolic drops to zero.

1.6 Cardiovascular and Cerebrovascular Responses to Exercise

For the purpose of this thesis, physical activity is defined as physical movement while exercise is intentional activity with fitness goals. Therefore, exercise is a category of activity. The role of cardiovascular regulation during activity is to provide adequate perfusion and substrate to meet the demand of working tissues and thermoregulation, while maintaining adequate supply to vital organs such as the brain. In a recent study using positron emission tomography (PET) and indwelling arterial transducer at heart level, regional CBF increased by 43% during the first 3 min of light exercise (30%HRR) primarily in the brain stem, and regions supplied by the MCA, insular cortex and basal ganglia (Hiura et al., 2018); regions

possibly involved in central command. Compared to rest, during light to moderate exercise (40-60%VO_2max) blood flow in CCA, ICA, VA, MCA, and ECA increased with no change in conductance, however, with heavy exercise (80%VO_2max) CBF in ICA and MCA returned to resting levels due to decreased conductance while CBF increased in CCA, VA, and ECA (Sato et al., 2011). VA and ICA did not change conductance throughout the range of exercise intensities. From rest to 80%VO_2max, the relative contribution of CCA blood flow increased to ECA and decreased to ICA. Furthermore, the relative contribution of VA to global CBF increased while that of ICA decreased a compensatory amount (Sato et al., 2011). The shift in flow from ICA and MCA to VA and ECA may contribute to thermoregulation. Increased ECA blood flow and conductance were related to forehead skin blood flow during exercise which may reflect vasodilation for the purpose of thermoregulation.

CBF increases with exercise in a well-established dose-dependent pattern (Fluck et al., 2014; Murrell et al., 2013), rising with increasing exercise load until about 60% VO_2max and then decreasing toward resting levels (Hellstrom et al., 1996; Sato et al., 2009; K. J. Smith & Ainslie, 2017). The inverted U relationship between MCAV and exercise intensity has been attributed to vasoconstriction elicited by hyperventilation-induced decreases in $PaCO_2$ (Hellstrom et al., 1996). While BP, SNA, and cerebral activation continue to increase with increasing exercise intensities, both $P_{ET}CO_2$ and MCAV decrease after ventilatory threshold (VT, about 60%VO_2max) (reviewed by (Smith & Ainslie, 2017)). A higher CR_{CO2} in the internal carotid circulation compared to the vertebral-basilar circulation may be

partially responsible for the shift in blood flow to the ECA from the MCA with increasing exercise intensity (Sato et al., 2012). Two studies found that inhalation of hypercapnic gas mixture during incremental exercise that kept $P_{ET}CO_2$ above 40 mmHg (Fluck et al., 2014) or 50 mmHg (Olin et al., 2011) obliterated the decrease in MCAV at workloads above VT. In 13 YA who breathed 5%CO_2 (in room air) during cycling, hypercapnic CR_{CO2} almost doubled at 30 and 70% HRR (2.7 ± 0.1 and 2.6 ± 0.6 cm/s/mmHg, respectively) compared to rest (1.46 ± 0.6 cm/s/mmHg) (Murrell et al., 2013). These studies verify the dose-dependent relationship between exercise intensity and CBF and implicate CO_2 as a significant regulating mechanism.

1.6.1 Regulation of Cerebral Blood Flow from Exercise Onset to Steady State

Research consistently identifies cerebral neural activation, $PaCO_2$, and blood pressure as three important regulatory mechanisms during steady state exercise (Braz & Fisher, 2016; Ogoh & Ainslie, 2009; Querido & Sheel, 2007; K. J. Smith & Ainslie, 2017; Willie et al., 2015; Wolf, 2015). Teasing out the relative and temporal influence of each as MCAV transitions to steady state from exercise onset has received very little attention. However, known cardiovascular and cerebrovascular responses to exercise permit some hypothesis of relevant mechanisms. At the onset of exercise, neural activation is increased. The motor cortex initiates movement and activates central command (CC). Central command is a feed-forward mechanism with signals arising from the forebrain or midbrain that activate descending motor neurons and concomitantly, stimulate the cardiovascular control centre

(CCC) within the medulla oblongata in the brain stem. The activation of the CCC is dose dependent with respect to both muscle mass engaged and exercise intensity (Asahara et al., 2018). Almost in parallel with increases in central command, deformation of contracting muscles stimulates mechanoreceptors (type III afferents) and later, accumulating metabolites activate metaboreceptors (type IV afferents), both of which activate CCC. Type III and IV afferents have been shown to increase CBF (Braz et al., 2014; Jorgensen et al., 1993).

Activation of CCC (by CC and type III afferents) elicits a rapid withdrawal of parasympathetic nervous system activity (PNA) which increases heart rate to about 100 bpm. A slightly slower increase in sympathetic nervous system activity (SNA) increases heart rate (HR) and stroke volume (SV), and therefore, Q. It is unlikely that SNA causes vasoconstriction via alpha1-adrenoceptors in the MCA during moderate exercise; however, the distal parenchymal arterioles contain beta-adrenoceptors causing vasodilation and reduced cerebrovascular resistance with elevated SNA. With exercise onset, CCC also resets the baroreceptors set point to a new blood pressure consistent with exercise intensity (reviewed by (Murphy et al., 2011)).

Neurovascular activity is elevated in regions of the brain involved in movement including sensorimotor, premotor and supplementary motor regions, cerebellum, and insular cortex. At the onset of exercise, CC generates descending signals that rapidly elevate common carotid artery flow and middle cerebral artery velocity in conjunction with increased CO; a response attributed to rapid cardiovascular adjustments in a feed forward process that lasted only 15 s after the onset of exercise (Sato et al., 2009).

During light to moderate exercise, blood pressure increases to support CBF to areas requiring increased oxygen supply. Hiura et al. (2018) found significant positive correlations between CBF and MAP in brain regions that overlapped with the central autonomic network, including higher brain regions, the brain stem, and the cerebellum (which may relay signals between the cortex and brainstem) suggesting the integration of these regions in the regulation of cardiovascular response to exercise. In healthy individuals blood pressures generally remain within the regulatory range and CA is maintained during exercise (Bronzwaer et al., 2017), blunting significant increases in CBF. With increasing exercise intensities above 60%VO_2max, steady state blood pressure continues to rise and MCAV decreases due to hyperventilation-induced vasoconstriction indicating a limited role for BP in CBF regulation during exercise in healthy individuals.

Two recent studies by the same research group (Billinger et al., 2017; Ward et al., 2018) investigated cerebrovascular dynamic response to exercise onset. In both studies, YA performed recumbent stepping exercise at 45 – 55%HRR. The kinetic response of MCAV to exercise was quantified using an exponential model:

$$MCAV(t) = BL + Amp(1-e^{-(t-TD/\tau)}) \qquad \text{(Equation 1.3)}$$

Where MCAV(t) is MCAV at any point in time, BL is the baseline MCAV prior to exercise onset, Amp is the peak amplitude, TD (time delay) is the time preceding a rise in MCAV,

and τ represents the time constant (i.e., time to reach 63% of peak amplitude). The authors concluded that a single exponential equation adequately described MCAV kinetics based on R^2 values, p significance values, and curve-fitting residuals. However, R^2 and p-values are not considered valid descriptors in a non-linear relationship and the residuals shown in their examples did not appear randomly or uniformly scattered about zero. Billinger concluded that neither $PaCO_2$ (a variable recorded but not reported) nor workrate were correlated to kinetic parameters. Both Ward and Billinger also reported that changes in MAP were not significantly correlated with changes in MCAV. In contrast, Steventon et al. (2018) applied a 12 min bout of hypercapnia (7%CO_2) at rest and after 20 min of moderate cycling exercise while measuring MCAV, posterior cerebral artery velocity (PCAV) and $P_{ET}CO_2$. Linear regressions were applied to time series individual MCAV responses for the exercise and hypercapnia with and without $P_{ET}CO_2$ in the model. ANOVA applied to regression coefficients showed that without $P_{ET}CO_2$ in the model, exercise significantly affected MCAV and PCAV but after including $P_{ET}CO_2$ in the model, the effect of exercise was no longer significant while $P_{ET}CO_2$ significantly increased MCAV.CR_{CO2} was similar before and after exercise. These results support a significant role of CO_2 in MCAV regulation, and a diminished role of exercise per se (Steventon et al., 2018). These aforementioned studies applied different approaches to describe the MCAV kinetic response to exercise (single exponential or linear regression) and differ in their conclusions regarding the role of $PaCO_2$. It seems unlikely that either linear regression or single exponential curve would represent the complex mechanisms affecting MCAV response to exercise. The role of CO_2 was not illustrated in the Billinger paper and Ward discussed CO_2 primarily in context of the

difference in MCAV response between YA and OA. In contrast, Steventon investigated the role of $PaCO_2$ in the MCAV response to hypercapnia at rest and during or after exercise and results support a primary role of $PaCO_2$ in MCAV kinetics.

Time delays in the exponential equations reported by both Billinger et al. (2017) and Ward et al. (2018) were 47 s and 53 s, respectively while TD reported by Ogoh et al (2009) was 5 s. A short TD is more consistent with expectation and may reflect the few cardiac cycles required for transit time from lungs to the MCA. A long TD in MCAV would suggest a lag between exercise onset and brain blood flow and a temporary neurovascular uncoupling such that demand from neural activation was not met by blood supply. As discussed previously, increased brain activation must occur before muscle contraction. The brain receives 15% of cardiac output and utilizes about 20% of total body oxygen consumption at rest. Although cerebral energy consumption is high, the brain contains very low intracellular energy stores. As a result, neurovascular coupling is precisely regulated. For this reason, a time delay in the dynamic response of MCAV to exercise beyond a few cardiac cycles is not expected. There may be some time of cerebrovascular adjustment as cerebrovascular autoregulation stabilizes, for example. Billinger and Ward both applied workload in three incremental 10 s stages. If the 30 s time period of incremental workload applied in the Billinger and Ward studies is subtracted from the average TD in these studies, the remaining TD of 17 s and 23 s, respectively, is more reasonable.

In conclusion, several physiological mechanisms initiate and adjust to exercise onset to steady state including neurovascular coupling, autonomic nervous system, and changes in

CO_2 and MAP which may confound the MCAV dynamic response to exercise and eliminate a single exponential description of the MCAV response. Currently, there are conflicting reports regarding mechanisms that drive MCAV dynamics.

1.7 Effect of Aging on CBF

CBF decreases with age (Bronzwaer et al., 2017; Tarumi et al., 2014; Xing et al., 2017; T. Y. Xu et al., 2012) at a reported rate of about 0.5% per year (Leenders et al., 1990). Lower diastolic and systolic MCAV (29% and 12%, respectively) have been observed in OA compared to YA (Xing et al., 2017). Cross-sectional studies comparing OA with YA by group analyses or correlation regularly show an inverse relationship between CBF and age, particularly in the cerebral cortex and basal forebrain (reviewed in (Nagata et al., 2016)). While this decline in CBF is partially explained by brain atrophy with aging (Poels et al., 2008), lower CBF in OA compared to YA has been observed even independent of regional brain atrophy (Chen et al., 2011). Both human and animal studies have shown that hypoperfusion precedes biomarkers for brain abnormalities. Cerebral hypoperfusion preceded biomarkers for Alzheimer's Disease abnormalities, such as hypometabolism and cerebral spinal fluid amyloid in humans and impaired neuronal protein synthesis in animals which could lead to accumulation of neuronal toxins such as amyloid-B proteins (discussed in (Tarumi & Zhang, 2017). Numerous animal studies where cerebral hypoperfusion was imposed show that cerebral hypoperfusion is a common underlying factor in cerebral hyperintensities leading to dementia and cognitive decline (for a thorough review see

(Duncombe et al., 2017). A lower than age-associated norm in CBF has been identified as the most important risk for vascular cognitive impairment and dementia (Sabayan et al., 2012; Scheel et al., 1999). Mechanisms reducing CBF with aging may include a reduced CR_{CO2} and endothelial dysfunction (Mayhan et al., 2008), or as related to the development of atherosclerosis (Yamamoto et al., 1980).

Normal aging leads to vascular structural and functional changes that promote vascular stiffness in conduit and resistance arteries leading to reduced CBF. Research with peripheral conduit arteries has shown structural changes including remodeling of vascular extracellular matrix by increased fragmentation and thinning of the elastin lamellae, collagen and collagen cross-linking, fibronectin, vascular smooth muscle cell (VSMC) hypertrophy and hyperplasia, and calcium accumulation in the tunica media (reviewed by (Atkinson, 2008; Greenwald, 2007; Lakatta, 2008; Seals et al., 2008; Sherratt, 2009). Animal models have found stiffening in both the large cerebral arteries and parenchymal arteries in mice due to arterial remodeling that increases both the lumen and outer arterial wall diameters (Diaz-Otero et al., 2016). Functional changes to vasculature with aging also leads to arterial stiffening via endothelial dysfunction (ED) due mostly to reduced bioavailability of nitric oxide (NO) in peripheral (reviewed by (Seals et al., 2008, 2009)) and cerebral (Modrick et al., 2009) conduit arteries. NO is the chief vasodilator of arteries regulating blood flow. Acetylcholine induced NO-mediated vasodilation in the basilar arteries of excised brains was greater in younger compared to older mice and was progressively worse with aging. Furthermore, the administration of tempol, a scavenger of superoxide which therefore

prevents superoxide from reacting with NO, improved endothelial function to that of young mice. The authors concluded that endothelial dysfunction by decreased NO bioavailability in aging was mediated by oxidative stress (Modrick et al., 2009). In addition to its primary role in endothelial dependent dilation, NO is also involved in the suppression of VSMC proliferation, cell adhesion, inflammation, and endothelial cell death, all of which promote hardening of the arteries with age-related reductions in NO (reviewed by (Higashi et al., 2009; Seals et al., 2008, 2009; Thijssen et al., 2011)). In summary, age related reductions in NO oxide bioavailability as well as structural changes leads to arterial stiffening in conduit and resistance arteries. Stiffening of the arteries increases the risk of CBF pulsatility and vascular resistance.

Vascular stiffening of the conduit arteries transmits pulsatile flow from proximal extra-cranial arteries (e.g., ICA) to distal arteries (e.g., MCA). Zarrinkoob et al. (2016) used phase-contrast magnetic resonance imaging to measure blood flow pulsatility in the extra-cranial arteries and the cerebral arteries that they feed in YA and OA. Results showed that 86% of pulsatility was transferred from the ICA to the distal MCA in young people while in OA it was 94%. ICA pulsatility that transferred even further downstream in the MCA beyond the MCA bifurcation was 88% in OA and 78% in YA. In every major cerebral artery, there was higher pulsatility transferred from its feeder conduit artery (VA or ICA) in OA compared to YA. The authors reported that with aging, the dampening factor (defined as the proximal artery pulsatility index/distal artery pulsatility index) in 21 cerebral and extra-cerebral arteries was reduced (Zarrinkoob et al., 2016). As conduit arteries become stiffer with age,

elevated pulse pressure of the incident wave overcoming aortic stiffness is transferred along the arterial system. Stiffer arteries along the arterial tree, unable to dampen the incident wave, transfer pulsatility to distal arteries. Pulsatile flow transferred to microvasculature has been implicated in the development of white matter lesions and cognitive impairment (Purkayastha et al., 2014). In response to increased pulsatility, resistance arterioles constrict to protect microvasculature from repeated hemodynamic insult, which may enhance the pulsatility of the reflected waveform. In stiffer arteries, this reflective wave travels faster, augmenting the systolic portion of the incident wave in OA, rather than the diastolic portion as in YA (Tarumi et al., 2014). Therefore, CBF pulsatility would be increased by the greater incident wave pulse pressure upstream and greater resistance downstream. Tarumi et al. (2014) studied 83 adults aged 22 to 84 years (37 males) and found aging was associated with higher cerebral flow pulsatility and lower CBF.

Arterial stiffness in OA has been strongly associated with elevated cerebrovascular resistance independent of blood pressure (A. D. Robertson et al., 2010). Brachial to ankle pulse wave velocity, a measure of conduit arterial stiffness, was positively correlated to CVRi, which was inversely related to anterior CBF. It was hypothesized that higher resistance due to arterial stiffness resulted in lower CBF. This relationship between arterial stiffness and CBF was reported in 35 middle-aged adults (Tarumi et al., 2011). Central arterial stiffness measured by carotid femoral pulse wave velocity (cfPWV) was associated with reduced perfusion in frontal white matter. In fact, arterial stiffness explained 11% of the

variability in frontal white matter perfusion, independent of age, sex, race, blood pressure, and medication.

Aging has been shown to impact the cardiovascular and cerebrovascular responses to exercise. Fisher and colleagues (2013) investigated CBF and metabolism during rest and various exercise intensities (25, 50, 75, and 100% of maximal workload, Wmax) in YA (aged 22 ± 1 y) and OA (aged 66 ± 2 y). At rest, MAP was higher and systemic vascular compliance (SVC) lower in OA compared to YA, while Q and HR were not different between the two age groups. During exercise, MAP, HR, SVC, and Q increased with exercise intensity in both groups however, MAP was greater while Q, HR, and SVC were lower in the OA compared to YA at each workload. MCAV, $PaCO_2$, and cerebrovascular conductance index (CVCi, MCAV/MAP) were lower in OA compared to YA at rest and every workload. In spite of these effects of aging, brain metabolism, assessed by O_2-carbohydrate index, (O_2 / (glucose + ½ lactate)) was similar at rest and exercise between age-groups.

In conclusion, normal age-related changes lead to arterial stiffening in the arterial tree from conduit arteries to arterioles resulting in reduced CBF, increased MAP and cerebrovascular resistance, decreased compliance and increased pulsatility, all of which are associated with increased risk of brain damage and cognitive impairment. Endothelial dysfunction in large arteries and arterioles, mediated by oxidative stress, reduces the bioavailability and synthesis of NO promoting vascular stiffness and reducing the pleiotropic

vascular protective effects of NO. Exercise is gaining widespread acclamation for its preventative effect on vascular aging and cognitive impairment.

1.8 Effect of Fitness on Cerebral Blood Flow

High aerobic fitness may attenuate the age-related decline in MCAV and increase in cerebrovascular resistance (Ainslie et al., 2008; Bailey et al., 2013; Barnes et al., 2013; Miller et al., 2018). Ainslie et al (2008) found that the rate of decline in MCAV in 153 males aged 18-79 y was independent of training status although MCAV was ~17% (~9 cm/s) higher in trained versus sedentary men throughout the age range. Weekly recreational aerobic activity attenuated age-related decrease in VO_2max, MCAV, CVCi, and CR_{CO2} and increase in CVRi (Bailey et al., 2013). Furthermore, linear relationships between VO_2max and both MCAV and CR_{CO2} were significant ($r=0.58$-0.77, $P<0.05$). In the same year, Barnes et al. (2013) affirmed the benefit of fitness in cerebral hemodynamics especially in OA. MCAV was measured during rest and hypercapnia (at 2%, 4%, and 6% inspired CO_2) before and after ingestion of cyclooxygenase inhibitor (indomethacin) in 16 young (26 y) and 13 older (65 y) adults. CR_{CO2} was greater in YA compared to OA with no effect of fitness over all subjects. MCAV CR_{CO2} was correlated to VO_2max in OA, and not YA. VO_2max also correlated to the difference in MCAV reactivity between the control and indomethacin condition in OA but not YA. Higher fitness was associated with greater cerebrovascular dilatory responses in OA. CVCi reactivity ($CVCi/P_{ET}CO_2$) was not related to VO_2max. Also of note, there was no relationship between VO_2max and resting MCAV or CVCi. In contrast

to the aforementioned studies, Zhu et al. (2013) found that a life time of endurance training did not influence cerebral vasomotor reactivity in response to changes in $P_{ET}CO_2$. Eleven young sedentary (YS, 27 y), 10 older sedentary (OS, 72 y), and 11 Masters athletes (MA, 72 y) underwent hyperventilation-induced hypocapnia and hypercapnic rebreathing. Resting MCAV was lower in OS and MA than in YS with no effect of training in the OA. During hypocapnia, vasomotor reactivity was lowest in MA, and MA and OS were lower than YS. In hypercapnia, OS and MA showed greater cerebral vasomotor reactivity than YS with no difference between OS and MA (Zhu et al., 2013). The authors concluded that lifelong exercise did not significantly impact age-related cerebral vasomotor reactivity. However, a limitation of this study is that there may not have been a meaningful difference in the fitness levels between the sedentary participants and MA. MA selected were runners with weekly running, swimming, and/or cycling mileage of 20 to 50 miles for more than 15 years. Sedentary participants were only excluded if they exercised for more than 30 min, three times a week. Depending on exercise intensity which was not provided in the study, the sedentary groups may have good fitness levels. A limitation of this study is that there was no measure of fitness such as VO_2max.

In conclusion, there is convincing evidence that superior aerobic fitness slows the age-related decline in cerebral hemodynamics.

1.9 Cerebrovascular Health Benefits of Exercise Training

Previous sections of this review have discussed the cardiovascular and cerebrovascular responses to acute exercise. Regular repeated bouts of exercise, exercise training, are known to produce physiological adaptations that improve fitness (VO_2max) and reduce the risk of disease and cognitive impairment. The activity in this thesis is ADL which can be performed at light to moderate intensity, and even short bouts of high intensity as in climbing stairs. The purpose of this section is to review the known cerebrovascular health promoting adaptations with regular exercise and discuss the evidence for similar adaptations with activities of daily living.

A primary benefit of exercise is slowing or reversing age-related endothelial dysfunction (Aarsland, Sardahaee, Anderssen, & Ballard, 2010; Ahlskog et al., 2011; Niebauer & Cooke, 1996; Nishijima, Torres-Aleman, & Soya, 2016; Tarumi & Zhang, 2017; Yung et al., 2009). It is well understood that laminar shear stress associated with exercise improves endothelial function in peripheral conduit arteries primarily due to enhanced nitric oxide bioavailability and synthesis (Katusic & Austin, 2014; Leung et al., 2008; Seals et al., 2008, 2009). Nitric oxide may also be responsible for reduced pro-atherogenic factors. When a monolayer of endothelial cells of the aorta were previously exposed to laminar shear stress in vitro, incubation with cytokine tumor necrosis factor-alpha (TNF-α) or oxidized low density lipoprotein (LDL) resulted in lower superoxide production, and reduced pro-atherogenic VCAM-1 expression and monocyte adhesion. Effects were clearly due to NO

since the presence of NO inhibitor (Nitro-L-arginine) obliterated these effects (Tsao et al., 1996).

There is increasing evidence that elevated CBF during exercise improves cerebrovascular health particularly as it increases shear stress-induced release of NO and vaso-protective properties. Recently, duplex ultrasound has been used to show shear-mediated vasodilation in the internal carotid (Hoiland et al., 2016; Iwamoto et al., 2018) and vertebral arteries in response to hypercapnia and exercise (Smith et al., 2017). In YA, cycling exercise at 60% HRR provided sufficient stimulus to elicit shear-mediated vasodilation in the ICA and VA and increase CBF velocity by 28% in the MCA and 20% in the PCA as well as elevate $P_{ET}CO_2$ by 7.5% (Smith et al., 2017). Walking is a part of many ADL and is, therefore, a primary interest in this thesis. Several studies have investigated the effect of exercise including walking on cerebrovascular responses. A preliminary study in this thesis showed that treadmill walking at usual pace increased MCAV to the same extent as walking at 60%HRR in YA (Moroz & Hughson, 2013). Walking at moderate to high intensities (at or above 65%HRR or 72%HRmax) has been found to reduce carotid arterial stiffness in OA (Moreau et al., 2003; Tanaka et al., 2000). A 12-week multi-modal training program including walking that progressed to 40-50 min, 4 times per week at 65-80%HRR found improvements in CR_{CO2} (Murrell et al., 2013). A 3-month program of primarily walking 42 min an average of 5.5 d/wk at 72%HRmax decreased common carotid arterial stiffness by 20% and increased compliance by 25% unrelated to body composition, blood pressure, cholesterol, or fitness (Tanaka et al., 2000). The exercise intensity in these studies

was prescribed at or above 60% HRR, at intensities known to increase cardiorespiratory fitness and MCAV. However, also at these intensities, the onset of hyperventilation may attenuate MCAV although not likely to resting levels. In addition to shear stress, exercise has been shown to improve brain health through metabolic (e.g., NO), humoral (vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1)), and molecular (e.g., brain derived neurotrophic factor (BDNF)) mechanisms that promote neurogenesis, angiogenesis, and synaptic plasticity (for reviews see (Lucas et al., 2015; Voss et al., 2010). BDNF, a mediator of neurogenesis and synaptic plasticity, was increased in humans after acute bouts of both high intensity and moderate continuous exercise (Boyne et al., 2019). In rodents, BDNF gene and protein levels increased progressively during two months of daily or bi-daily wheel-running, remained high for up to three days after exercise cessation, and following two weeks of de-training, were rapidly elevated with levels of exercise that were below the threshold for BDNF rise in previously untrained animals and would require two weeks of training to achieve in the untrained rodent (Berchtold et al., 2005) Exercise-induced shear stress has been identified as a mechanism responsible for the elevated BDNF response to physical training in rodents (Banoujaafar et al., 2014). In this study, the rise in CBF during exercise was attenuated by unilateral common carotid artery occlusion and reduced exercise workload, both of which significantly reduced or eliminated BDNF levels. Furthermore, manipulations to block NO response to shear stress by genetic hypertension (spontaneously hypertensive rats) or administration of N-nitro-L-arginine methyl ester successfully dampened the BDNF response. Moreover, BDNF levels were higher after high compared to low intensity exercise and lowest in the sedentary. Together, the results of this study provide

convincing evidence of a role for shear-stress induced elevations in BDNF primarily via NO production (Banoujaafar et al., 2014).

IGF-1 supports survival of new neurons. When IGF-1 was blocked from the brain in rodents, the exercise-induced increase in the number of new neurons in the hippocampus was inhibited and when the block was removed, normal increases in new neurons returned (Trejo et al., 2001). VEGF is a protein that stimulates angiogenesis. BDNF, VEGF, and IGF-1 appear to act together to mediate exercise-induced brain plasticity and neurogenesis (reviewed by (Cotman et al., 2007). Oxidative stress has been shown to impair endothelial function, however free radicals, at lower concentrations, also serve the purpose of mediating and upregulating the growth factors, BDNF, VEGF, and IGF-1 therefore, promoting neurogenesis (Lucas et al., 2015). The production of anti-inflammatory myokine, interleukin-6 (IL-6), which increases with exercise in a dose dependent fashion, stimulates angiogenesis in addition to inhibiting pro-inflammatory cytokines (Fiuza-Luces et al., 2013).

Exercise has also been shown to increase brain volume, particularly in the hippocampus, a structure rich with high-affinity BDNF receptors, tropomyosin receptor kinase. In a highly references article, Erickson et al. (2011) showed that a walking training program 40 min/wk, 65-75%HRR that increased VO_2max by 8%, increased hippocampal volume by 2% in 69 participants (aged 68 y). In contrast, in an age-equivalent stretching control group, hippocampal volume decreased by about 1.4% and higher fitness levels at baseline attenuated the decline providing further support of the protective effect of moderate intensity continuous exercise. Furthermore, there was a positive correlation between

increases in VO_2 and hippocampal volume and also between increases in BDNF and hippocampal volume (Erickson et al., 2011).

In conclusion, there are numerous cerebrovascular benefits to regular exercise training, some of which are related to shear stress imposed by increased MCAV. The question addressed in this thesis is will activities of daily living be strenuous enough to elicit increases in MCAV.

The role of habitual activity and inactivity in vascular aging and health outcomes has been the focus of recent research (Colley RC et al., 2011; Dobkin & Dorsch, 2011; Gando et al., 2010; Halloway et al., 2018). Home activity monitoring using accelerometers in 538 healthy men and women for 14 days determined that when amounts of vigorous and moderate activity were similar, more time spent in light activity (1.1 to 2.9 metabolic equivalents, METS) compared to less time, was associated with lower arterial stiffening (cfPWV) in the OA (>60 y) but not in middle aged or YA (Gando et al., 2010). Also in the OA, amount of light activity was inversely related to arterial stiffness only in unfit versus fit OA. These results suggest that even light activity may be strenuous enough to reduce arterial stiffening in unfit OA. In the middle age group, cfPWV was inversely related to amount of moderate and vigorous PA but not light. In the young group, there was no relationship between habitual activity and arterial stiffness. The authors found that 3 METs required a relative oxygen uptake ($\%VO_{2peak}$) of 29%, 35%, and 38%, in the young, middle-aged, and OA, respectively, underscoring that light exercise may not be strenuous enough to elicit changes in arterial stiffness in middle-age or YA. In a recent study, 262 OA without

cognitive impairment wore wrist accelerometers for 10 days and underwent magnetic resonance imaging and cognitive testing (Halloway et al., 2018). The authors found that higher daily activity was associated with larger gray matter volumes but with no relation to cognitive function. Over the last decade, there is increasing concern and evidence for the role of sedentary behavior in morbidity and mortality rates. Sedentary behavior has been defined as a metabolic rate between 1 and 1.5 times resting level during sitting or supine postures (Pate et al., 2008) or 1- 2 times resting level (Statistics Canada, 2015a), %HRR less than 20, or low accelerometer counts (<100) using an Actical® accelerometer (Colley & Tremblay, 2011; Jago et al., 2007; Statistics Canada, 2015a). Sedentary time is an independent predictor of mortality and morbidity. Therefore, even in active individuals, more sedentary time is an important health risk. Time spent in sedentary time is associated with greater risk of all-cause mortality, cancer incidence and mortality, cardiovascular disease incidence and mortality, incidence of type-II diabetes and cerebrovascular consequences (Biswas et al., 2015; Bouchard et al., 2015; Bronas et al., 2019; Koster et al., 2012; Maasakkers et al., 2021; Zlatar et al., 2019). One of the roles of ADL may be simply reducing time spent in sedentary behaviour. Sedentary behaviour is associated with lower CBF (Launer et al., 2015; Zlatar et al., 2019), lower regional or total brain volume (Launer et al., 2015), and white matter hyperintensities (Bronas et al., 2019). Total medial temporal lobe thickness and thickness of subregions of the medial temporal lobe were inversely related to hours of sedentary time over a range of 2 to 15 hours per day (Siddarth et al., 2018). Sedentary behaviour was associated with lower total brain volume and gray matter CBF (Launer et al., 2015). Zlatar et al. (2019) found that sedentary time measured by accelerometer for at least 600 minutes per day for

four to 12 consecutive days and adjusted for light or moderate intensity exercise, was inversely related to CBF in medial and lateral frontal regions CBF, whereas more time spent at either light activity or moderate to vigorous activity was associated with higher CBF in medial and lateral frontal regions (Zlatar et al., 2019).

Sedentary behavior has been associated with increased mortality rate independent of participation in moderate-to intense exercise (Koster et al., 2012). In fact, meeting the recommended dosage of moderate to intense exercise does not necessarily reflect the amount of sedentary time (Craft et al., 2012). However, the effects of sedentary behavior were modified by PA such that all-cause mortality risk was 30% lower in those participating in high levels (duration and intensity) of PA compared low levels (Biswas et al., 2015). Sedentary behavior is associated with increased arterial stiffening in adults. A systematic review of 12 studies in adults over 18 years of age found that time spent in sedentary behavior was significantly related to arterial stiffness (higher cfPWV), while time spent in moderate or even light activity was related to lower cfPWV (Germano-Soares et al., 2018). The aforementioned suggest that at the very least, activities of daily living may improve cerebrovascular function and mortality by reducing sedentary time.

1.10 Summary

Exercise that is strenuous enough to increase CBF has been shown to slow age-related arterial stiffness with vascular aging and the pulsatile flow and vascular resistance associated

with stiffening arteries. Furthermore, while government and health organizations set recommendations for moderate to vigorous weekly exercise, most OA fall short of meeting those targets. However, walking as part of ADL for locomotion or leisure activities and other ADL such as cleaning or shopping are a prominent part of independent living. It is not known whether ADL are strenuous enough to elicit an increase in CBF although some support exists in the literature. The kinetics of MCAV response to exercise are not well understood but may inform the value of ADL with respect to blood flow, especially for activities such as stair climbing that may be too short to elicit significant increases in blood flow. Understanding the mechanisms for this response may explain the dynamic response and inform cognitive impairment and techniques for treating hypoperfusion. The dynamic increase in MCAV with activity has been described in the literature by a single exponential equation. The various mechanisms that have been shown to contribute to MCAV make it unlikely that it meets the properties of a first order system. For example, $PaCO_2$ is a potent dilator of cerebral vasculature and MAP drives blood flow, making them likely contributors to the dynamic response that together, would not meet the requirements of a first order system.

1.11 Thesis Purpose

The purpose of this thesis is twofold.

1. To determine whether ambulatory ADL are strenuous enough to elicit cerebrovascular responses, particularly MCAV.
2. To assess the adequacy of a single exponential equation to describe the dynamic MCAV response to exercise and to assess how changes in $PaCO_2$ and MAP influence the changes in MCAV.

1.12 Thesis Questions

1. Is over-ground walking at usual pace and a pace faster or slower than usual strenuous enough to elicit cerebrovascular responses, in particular, MCAV, in YA and OA? Will ambulatory ADL elevate MCAV in OA adults?
2. Will a single exponential equation describe the kinetic response of MCAV to exercise? Will changes in $PaCO_2$ and/or MAP adequately predict the increase in MCAV with exercise? How will hypocapnia and hypercapnia influence the MCAV response to exercise?

1.13 Hypothesis

1. Walking at usual pace and faster will be strenuous enough to elicit cerebrovascular responses in particular, an increased MCAV, in OA and YA. ADL will be strenuous enough to elicit increases in MCAV in OA.

2. A single exponential equation will not adequately describe the MCAV kinetic response to exercise. Changes in $PaCO_2$ and MAP will adequately predict the MCAV response to exercise in control conditions. Hypocapnia will attenuate the MCAV increase during exercise while hypercapnia will augment the response.

Chapter 2

Cerebrovascular Response to Over-ground Walking in Young Adults and Community Dwelling Older Adults

2.1 Abstract

Elevated blood flow during exercise produces laminar shear stress which is known to enhance endothelial function, slowing age-related arterial stiffness – a major factor in the development of cognitive impairment. Exercise at moderate intensity has been shown to increase CBF and shear stress improving endothelial function. At least 80% of Canadian YA and OA fail to meet World Health Organization recommendations for this intensity of exercise. In contrast, the prevalence of walking for leisure and locomotion among those over 18 years is increasing. It is not known whether walking at usual pace, or slower or faster than usual will also increase CBF. The purpose of this study was to determine whether over-ground walking at various speeds is strenuous enough to elicit cerebrovascular as well as hemodynamic and metabolic responses in YA and OA. Eleven young (22.7 ± 3.2 y, 7 males) and 11 older (71.2 ± 2.8 y, 4 males) adults walked four minutes at three speeds: usual pace, slow, and fast. While usual pace was self-selected, a metronome set slow pace at 75 steps per min for both age groups and fast pace at 115 (seniors) or 135 (young) steps per min. Mean middle cerebral artery blood velocity (MCAVm) measured using a portable Doppler ultrasound device, mean arterial pressure (MAP) using continuous finger-cuff

plethysmography, heart rate (HR), oxygen uptake (VO_2) and end tidal carbon dioxide ($P_{ET}CO_2$) were measured continuously. VO_2 and HR increased sequentially with walking speed ($p<0.00001$) in both age groups. Walking elicited higher MCAV, $P_{ET}CO_2$ and MAP compared to rest ($p<0.00001$) with similar levels between speeds. In conclusion, usual pace and fast walking were strenuous enough to increase MCAV in YA and OA. Even slow walking educed MCAV higher than rest in OA.

New and Noteworthy

Over-ground walking at usual pace or fast pace is strenuous enough to increase MCAV in both YA and OA. In OA, even walking slower than usual elicited cerebrovascular responses.

In spite of graded increases in absolute and relative workloads, there was no difference in MCAV between walking speeds likely due to similar $P_{ET}CO_2$ and MAP.

Key words: CBFV, age, activities of daily living, exercise, middle cerebral artery, oxygen uptake.

2.2 Introduction

Normal aging leads to endothelial dysfunction and vascular stiffening of conduit arteries (Thijssen et al., 2016) implicated in the development of white matter lesions and cognitive

impairment (Purkayastha et al., 2014). Elevated CBF in response to moderate exercise reportedly increases flow-mediated shear stress on conduit artery walls that improves endothelial function in the internal cerebral and vertebral arteries (Smith et al., 2017). CBF decreases with aging. A growing body of research suggests that moderate cardiorespiratory exercise in middle age and OA may reduce the risk of cognitive impairment (Ahlskog et al., 2011; Akazawa et al., 2012; Asl et al., 2008; Bailey et al., 2013; Blumenthal et al., 2019; Uemura et al., 2012; Williamson et al., 2009). Furthermore, activity early in life reportedly delays the onset and slows the progress of dementia later in susceptible adults (L. E. Middleton et al., 2010; Nyberg et al., 2014; Stern et al., 2019). Only about 16% of Canadian older and younger adults met the recommended 150 minutes of moderate-to-vigorous physical activity per week by the Canadian Physical Activity Guidelines (Statistics Canada, 2019). However, the prevalence of walking for locomotion and leisure increased from 2005 to 2015 by almost 10% in adults 18 years and older (Ussery et al., 2018).

It is unknown whether usual pace walking would be strenuous enough to elicit an increase in CBF in YA and/or OA. Usual or comfortable walking speeds and fast or maximal walking speeds are 71% to 97% slower in OA compared to YA (Moroz & Hughson, 2013; Steffen et al., 2002) In our pilot study with YA, usual pace treadmill walking that elevated heart rate to 20 %HRR, raised MCAVm from 47 ± 7 cm/sat rest to 55 ± 9 cm/s, which was statistically similar to that during walking at 60 %HRR (56 ± 14 cm/s) (Moroz & Hughson, 2013) (see Chapter 6 of this thesis). Murrell et al. (2013) found that MCAV was elevated above rest and increased during cycling similarly at 30 and 70 %HRR in both YA (7 and 9

cm/s, respectively) and OA (2 and 2 cm/s, respectively). These results are similar to our pilot study that found treadmill walking at just 22%HRR elevated MCAV and increases were similar between 22 and 60%HRR (Chapter 6, Supplement). CBF is reportedly higher in YA compared to OA during both rest and exercise (Fluck et al., 2014; Murrell et al., 2013. It isn't known whether ambulatory walking at usual pace or faster is strenuous enough to elicit increases in CBF in this young age group. If so, regular usual pace, slow, or fast walking as a part of ADL may constitute a similar cerebrovascular challenge and vascular adaptation as regular intentional exercise session, but with more frequent daily bouts.

The primary purpose of this research was to determine whether over-ground walking at usual pace will elicit metabolic, hemodynamic, and cerebrovascular responses in OA and even YA. If so, it would be interesting to know if walking a little slower, perhaps with a slower partner, was also strenuous enough. If not, perhaps walking faster would elicit these physiological responses.

We hypothesized that walking at usual pace and faster will elicit physiological changes in older and YA while walking slower may not. We also hypothesize that YA will select a faster usual pace, and longer stride lengths during Slow and Fast walking, increasing those walking speeds and enhancing the resultant physiological responses.

2.3 Methods

Participants were recruited by posters and through the Waterloo Research in Aging Participant pool, a database of community dwelling adults who have identified themselves as being interested in participating in research by responding to university advertisements. Inclusion criteria were the ability to perform the tasks without the use of eye glasses and aged 18-25 or over 60 y. (The ultrasound probe is attached to clear eye glasses.) Exclusion criteria included any conditions that limited mobility (e.g., neuromuscular, neurological), diabetes, stroke, medications that affect heart rate, uncontrolled hypertension, or heart disease. Study procedures were compliant with the Declaration of Helsinki and approved by the Office of Research Ethics at the University of Waterloo (ORE 17714). Eleven YA and 11 OA participated in this study after providing informed consent.

Study procedure

Participants made one visit to the lab and were instructed to avoid moderate to strenuous levels of exercise 24 hours prior to testing and to refrain from consuming alcohol or caffeine within four hours of testing. Upon arrival, height and weight were measured. Participants sat quietly during the 30 to 40 min of instrumentation. After donning instrumentation, participants rested until respiratory exchange ratio (RER) was below 0.9 or as low as possible and steady, usually about 10 to 15 min. Seated resting measurements were taken over the subsequent 5 min (Sit). Participants walked a clear hallway for four minutes at each of three speeds beginning with usual pace (Usual), then a slow pace (Slow) and finally a fast pace

(Fast). For Usual, participants were asked to "walk at their usual pace as though walking from their car, unhurried" allowing them to self-select both stride rate and length. During Slow and Fast, participants walked at a pre-determined pace standardized using a metronome, however, stride length was self-selected. Slow pace was 75 steps per min for both YA and OA and the Fast pace was slower for OA (115 steps per min) than YA (135 steps per min). Speed was selected using data showing that females in their 60s and 70s and males in the same age groups walked at a comfortable pace for 7.62 m at about 1.28 and 1.34 m/s, respectively. Maximal pace was approximately 1.76 and 2.0 m/s for females and males, respectively (Bohannon, 1997). We reasoned those speeds should be slightly less in our study since participants were walking 4 min, compared to 20 sec, and the fast pace in our study was to be sustainable for several minutes to reflect fast walking in ADL such as hurrying to class or work, and not the fastest possible walk for 20s as in Bohannon's study. Participants stood between walking for at least two minutes to reduce HR to resting levels. Walking speeds were ordered so that Fast walking was last, in case a longer recovery for MCAV was required for harder work. The TCD-X unit offers no way to see data as it is collected during ambulatory activities. Walking distance was recorded to determine walking speed.

Measurements were taken in the last minute of each activity.

Instrumentation and measurements

The a priori sample size was calculated using the equation $n = 2 * (Z_{(0.025)} * Z\beta)^2 *$ standard deviation ^{2}/Acceptable margin of error^2, where β is power and $Z_{(0.025)}$ is the Z score for a two-tailed test with α equal to 0.05. Using resting MCAV data for YA and OA from Fluck et al. 2014, sample size was estimated at 3 for a power of 80% and 5 for a power of 97%. Thus, our sample size of 11 YA and 11 OA was acceptable to compare age groups. MCAV data from rest to 25% Wmax was used to predict sample size for comparison between ADL. Sample size was predicted to be 24, well within the 27 participants in the second study but well above the 11 participants in the first study. Sample size of 9 to 11 is typical in studies comparing groups and/or interventions (Fluck et al., 2014; Murrell et al., 2011, 2013)

Instrumentation common to both Chapters 2 and 3 is described in detail below.

Middle cerebral artery blood velocity (MCAV)

The right middle cerebral artery was insonated using a 2-MHz Transcranial Doppler Device; the TCD-X (TCD-X; Atys Medical, Soucieu en Jarrest France). The advantage of the TCD-X is its portability, which was necessary for over-ground activities of daily living. The 2-MHz Doppler probe is held in place on the arm of eye glasses on the TCD-X. The probe was maneuvered along the temporal window with slightly forward orientation to locate the MCA at a depth close to 50 mm. The MCA was distinguished from other arteries by outer envelope, strength of signal, depth, and auditory pitch. The signal was optimized using standard procedures (Aaslid, Markwalder, & Nornes, 1982). Angle of insonation was below

60°. TCD assumes an angle of zero with the ultrasound wave emitted in the direction of flow. Error in the measurement of velocity occurs with angle deviation from zero and increases exponentially at angles above 60° (Logason et al., 2001). MCAV outer envelope waveform was analyzed for mean, systolic, and diastolic middle cerebral artery velocities, (MCAVm, MCAVsys, and MCAVdia, respectively).

Cardiovascular Hemodynamics

Arterial blood pressure was measured using continuous finger-cuff plethysmography (Portapres, Finapres Medical Systems, Enschede, The Netherlands). The advantage of the Portapres is its portability. A finger cuff is place on the middle finger just below the proximal interphalangeal joint and a hydrostatic column is used to correct the pressure values to brachial artery, heart level values. The waveforms are reconstructed to brachial arterial pressure waveforms. The Portapres uses proprietary external software (Beatscope 1.1a, Finapres Medical Systems, Amsterdam Netherlands) to reconstruct the arterial pressure wave, correct for heart level, and calculate stroke volume (SV). Beat to beat SV is estimated using a validated proprietary algorithm called Modelflow (Wesseling, Jansen, Settels, & Schreuder, 1993). This algorithm also calculates Q and TPR based on the blood pressure and stroke volume.

Metabolic measurements

HR, VO_2, and $P_{ET}CO_2$ were measured using a Polar heart rate monitor coupled with a COSMED portable metabolic system (K4b2, COSMED, Agrate Brianza, Italy). The COSMED consists of carbon dioxide and oxygen analyzers, turbine ventilation monitor, temperature and barometric pressure sensors and software/hardware to acquire, measure, and calculate numerous metabolically related variables.

All data were stored on individual devices and downloaded to Microsoft® Excel® spreadsheets for management and analyses. Measurements were averaged over the last min of each activity. Resting measures were averaged over the minute most representative of rest based on the lowest respiratory exchange ratio, VO_2, and HR. ADL were compared to seated rest rather than standing for three reasons. First, sitting is a highly engaged behaviour and has been associated with disease risk and mortality as discussed in the review of literature in this thesis. Second, a pilot study showed no significant difference in MCAV between sitting and standing for four minutes. Third, about 20 min was required to achieve a resting state in participants donning full measurement apparatus. Standing for this period would be a challenge for OA and may not achieve resting state.

Calculations

Percent heart rate reserve (%HRR) was calculated using the Karvonen formula (Karvonen, Kentala.E., & Mustalo.O., 1957) where maximum HR was calculated as 208 – (0.7 x age).

CVRi) was estimated using the equation MAPmca/MCAVm where MAP (mean arterial pressure) was corrected to the level of the TCD probe over the MCA using the equation MAPmca = MAP – (distance above heart in cm*0.78). Resistance index (RI) was calculated as (MCAVsys – MCAVdia)/ MCAVsys. Pulsatile CBF was evaluated using Gosling's pulsatility index (PI) as (MCAVsys – MCAVdia)/ MCAVm. Pulse pressure (PP) was calculated as Systolic blood pressure minus diastolic blood pressure and pulsatile flow velocity (PFV) was calculated by MCAVsys-MCAVdia. Total peripheral resistance (TPR) was calculated as MAP/Q. Effect size was determined using Cohen's d and was calculated to indicate effect size for activities comparing Groups: Effect $Size_{Group} = (M_{young} - M_{older})/\sqrt{((SD_{Young}^2 + SD_{Older}^2)/2)}$. Cohen's d was also calculated to indicate effect size for activities compared to Sit: Effect $Size_{Sit} = (M_{Activity} - M_{Sit})/\sqrt{((SD_{Activity}^2 + SD_{Sitr}^2)/2)}$ where M is mean and SD is standard deviation. Commonly, effect sizes are interpreted as small (d = 0.2 to .49), medium (d = 0.5 to .79), and large (d > 0.8) according to suggestions (Cohen, 1988).

Statistical analyses

The effect of age and activity on each variable was investigated using a two-way ANOVA, with one between (Age) and one repeated measure (Activity) factors. Activity had four levels (no activity (Sit), usual pace (Usual) and slower (Slow) and faster (Fast) than usual pace). Significance was established at alpha = 0.05. Significant interactions were investigated

using Tukey post-hoc tests. Statistical analyses were administered using Statistica 7 (Statsoft, Inc. Tulsa, OK).

2.4 Results

Subject Characteristics

Eleven YA (7 males) and 11 OA (4 males) were recruited for this study. Subject characteristics are provided in **Table 2.1**. There was no significant difference in height ($F_{(1,21)} = 1.21$, $p = 0.297$) or weight ($F_{(1,21)} = 1.77$, $p = 0.213$), between YA and OA.

Comparisons in the following results focus on the effect of activity compared to Sit on measured variables between the two age groups. Furthermore, walking speeds will be compared to Usual in both age groups.

Workload and Metabolic

An Age by Activity interaction ($F_{(2,40)} = 10.33$, $p = 0.0002$) showed that walking speeds increased from Slow to Usual to Fast in YA. In OA Usual speed was faster than Slow but not different from Fast. YA self-selected a usual pace (1.25 ± 0.17 m/s) that was similar to OA (1.23 ± 0.16 m/s). Slow speed was also similar between YA (0.75 ± 0.10 m/s) and OA (0.74 ± 0.14 m/s) suggesting age groups adopted a similar stride length. However, Fast was greater

in YA (1.68 ± 0.13 m/s) than OA (1.36 ± 0.21 m/s) reflecting the faster metronome-set pace in YA during that trial.

A main effect of Activity showed that VO_2 ($F_{(3,60)} = 322.79$, $p < 0.0001$), HR ($F_{(3,60)} = 190.79$, $p < 0.0001$), and $P_{ET}CO_2$ ($F_{(3,60)} = 54.9$, $p < 0.0001$) were greater than Sit during all walking speeds with no effect of Age ($p>0.14$) (**Figure 2.1**). VO_2 and HR were lower in Slow and greater in Fast compared to Usual (**Figure 2.1A and C**). $P_{ET}CO_2$ was lower in Slow but similar to Fast (**Figure 2.1B)**. %HRR showed a main effect of Age ($F_{(2,20)} = 13.65$, $p = 0.0014$) and Activity ($F_{(2,40)} = 97.77$, $p < 0.0001$). %HRR was lower in young (22.0 ± 5.6%) compared to OA (27.7 ± 6.61%) and increased sequentially from Slow to Usual to Fast (**Figure 2.1D**).

Hemodynamics

MAP showed a main effect of Activity ($F_{(3,60)} = 34.14$, $p < 0.00001$) and an Age by Activity interaction ($F_{(3,60)} = 4.95$, $p = 0.0038$). MAP was different from Sit only during Slow and Fast in YA, but was different from Sit in all walking speeds in OA (**Figure 2.2A**). Effect $Size_{Sit}$ were considered small in YA (Cohen's d 0.13 to 0.29) and large in OA (Cohen's d 1.7 to 2.0) (**Table 2.2**). MAP during Usual was not different than other walking speeds in YA or OA. There was a main effect of Age ($F_{(1,20)} = 15.48$, $p = 0.0008$), Activity ($F_{(3,60)} = 53.56$, $p < 0.0001$), and an Age by Activity interaction in SBP ($F_{(3,60)} = 9.37$, $p < 0.0001$). In YA, SBP at Sit was similar to Usual but lower than Slow and Fast (**Figure 2.2B**) while in OA, SBP

was lower during Sit than all other activities. In OA, SBP during Usual was greater than Slow but not different from Fast whereas in YA Usual was similar to Slow but lower than Fast. There was a main effect of Activity ($F_{(3,60)} = 16.88$, $p < 0.0001$) in DBP. DBP was higher during walking than Sit but similar between walking speeds (**Table 2.2C**).

Q showed a main effect of Age ($F_{(1,19)} = 8.14$, $p = 0.010$), Activity ($F_{(3,57)} = 103.07$, $p < 0.0001$) and an Age by Activity interaction ($F_{(3,57)} = 4.61$, $p = 0.0059$). All walking speeds increased Q beyond that at Sit in both age groups (**Figure 2.2D**). Q was greater in YA (10.1 ± 1.98) compared to OA (8.0 ± 1.62) (Effect $Size_{Group}$: Cohen's d = 0.73 to 1.93) however, there was no difference between age groups at any given activity. Q during Usual was lower than during Fast in YA, while in OA there was no difference between walking speeds. Total peripheral resistance (TPR) was higher in older (14.2±3.39) compared to younger adults (10.8 ± 2.31) ($F_{(1,19)} = 8.93$, $p = 0.0076$) (**Figure 2.2E**). A main effect of Activity showed that while TPR was lower at all walking speeds compared to Sit, Usual was not significantly different from Fast ($F_{(3,57)} = 58.49$, $p < 0.0001$). There was a main effect of Age ($F_{(1,20)} = 37.2$, $p = 0.0001$), Activity ($F_{(3,60)} = 71.54$, $p = 0.0001$) and an Age by Activity interaction in PP ($F_{(3,60)} = 14.83$, $p = 0.0001$). In both YA and OA, PP increased from Sit during all walking speeds. In YA PP during Usual was similar to Slow but lower than Fast. In contrast, in OA, PP during Usual was similar to Fast but greater than slow (**Figure 2.2F**). PP in YA was lower than OA at Usual and Fast, but not Sit or Slow.

Cerebrovascular

MCAVm was greater in YA (55.0 ± 9.0 $cm \cdot s^{-1}$) compared to OA (44.3 ± 10.50 $cm \cdot s^{-1}$) ($F_{(1,20)}$ = 7.25, p = 0.014) however, Age by Activity interaction ($F_{(3,60)}$ = 3.70, p = 0.016) showed no difference between young and OA at any activity. In OA, MCAVm was greater during all walking speeds compared to Sit while in younger adults, MCAVm was greater than Sit during Usual and Fast but not Slow (**Figure 2.3A**). Effect $Size_{Sit}$ in MCAVm at Usual was larger in OA (Cohen's d = 0.94) than in YA (Cohen's d = 0.54). In OA, even Slow showed medium Effect $Size_{Sit}$ (Cohen's d = 0.62) (**Table 2.2**). There was no difference in MCAVm between the three walking speeds in either age group. Although MCAVsys was greater in younger (92.8 ± 15.52 $cm \cdot s^{-1}$) compared to older (77.3 ± 17.27 $cm \cdot s^{-1}$) adults ($F_{(1,20)}$ = 5.14, p = 0.035), there was no difference between age groups at any activity (Age by Activity interaction, $F_{(3,60)}$ = 4.87, p = 0.004). All walking speeds elicited a greater MCAVsys compared to rest in both age groups (**Figure 2.3B**). MCAVsys during Usual walking was greater than Slow but similar to Fast in YA. In OA, MCAVsys was similar during all walking speeds. Diastolic MCAV (MCAVdia) was higher in young (33.1 ± 6.25 $cm \cdot s^{-1}$) compared to older (23.1 ± 7.02 $cm \cdot s^{-1}$) adults ($F_{(1,20)}$ = 14.59, p = 0.001) (**Figure 2.3C**). There was no effect of Activity on MCAVdia.

CVRi was lower in young (1.3 ± 0.26 cm/s/mmHg) than older (1.7 ± 0.42) ($F_{(1,20)}$ = 7.96, p = 0.011) adults however, there was no difference between YA and OA at any given Activity (Age by Activity interaction, $F_{(3,60)}$ = 2.95, p = 0.040) (**Figure 2.3D**). In YA, there was no difference between Sit and any walking speed. However, in OA, CVRi during Sit was

similar to Slow but lower than Usual and Fast. There was no significant difference in CVRi between the three walking speeds in either age group. RI showed a main effect of Age ($F_{(1,20)}$ = 7.72, p = 0.012) and of Activity ($F_{(3,60)}$ = 46.5, p = 0.0001) with no interaction. RI was lower in YA (0.64±0.05) than OA (0.69 ± 0.05) (**Figures 2.3E**). PI showed a main effect of Activity only ($F_{(3,60)}$ = 35.49, p < 0.0001) (**Figures 2.3F**). For both RI and PI, Sit was lower than all walking speeds but there was no difference between the walking speeds. There was a main effect of Activity ($F_{(3,60)}$ = 81.15, p < 0.0001) on PFV, which was lower during Sit (43.9 ± 10.41 cm/s), compared to Slow (56.5 ± 13.27 cm/s), Usual (62.3 ± 13.27 cm/s) and Fast (65.2 ± 14.12 cm/s). Usual pace was greater than Slow but not different from Fast.

2.5 Discussion

The objective of this research was to determine whether over-ground walking at usual pace was strenuous enough to elicit metabolic, hemodynamic, and cerebrovascular responses in OA and even YA or whether individuals needed to walk faster or could walk slower to elicit responses. The primary variable of interest was MCAV, a surrogate of CBF.

Walking compared to sitting

To our knowledge, this is the first study to investigate MCAV responses to over-ground walking. A unique finding in this study is that over-ground walking at usual pace that raised %HRR to 20% in YA and 30% in OA was strenuous enough to elevate MCAVm by a mean

of 11% in YA and 27% in OA. Furthermore, in OA, even walking significantly slower than usual pace evoked increases in MCAVm. This was in contrast to YA who required a walking pace of usual or faster in order to elicit increases in MCAVm. In both age groups, even slow walking was strenuous enough to elicit metabolic (VO_2 and $P_{ET}CO_2$,) and hemodynamic (Q, HR, MAP, SBP, TPR, PP) responses different from resting levels. MAP and $P_{ET}CO_2$, both important regulators of MCAV during exercise, increased during walking in both age groups. There is also increasing evidence for the role of Q in the regulation of MCAVm, especially in OA (Bronzwaer et al., 2017; Meng et al., 2015). In this study, Q was elevated above seated rest during all walking speeds in both age groups.

Usual pace compared to Slow and Fast walking

As expected, there was a sequential rise in absolute (HR, VO_2) and relative (%HRR) workload with increasing walking speeds in YA but also in OA despite the similarity in walking speeds during Fast and Usual. There was no difference between walking speeds in OA or YA suggesting similar cerebrovascular challenges and possible benefits (discussed later) even at paces slower than Usual.

Young versus OA

MCAVm was lower in OA compared to YA, consistent with previous literature (Bronzwaer et al., 2017; Tarumi et al., 2014). A higher cerebrovascular resistance with aging has been

previously reported (Robertson et al., 2010) and is supported in this study by the greater CVRi and RI in OA compared to YA. In addition, MCAVdia was greater in YA than OA. Elevated pulsatility in flow has also been reported in OA (Purkayastha et al., 2014; Tarumi et al., 2014). There was no effect of Age on pulsatility or CVRi at Sit or any given walking speed suggesting YA and OA in this study experienced similar resistance and pulsatility during rest and each walking speed. Older participants were recruited from a group of adults who volunteer for research in aging and may not reflect the average older adult. Almost all older participants walked faster than 1 m/s with the mean speed of 1.23 m/s during usual pace. Only one of the OA walked slower than 1 m/s (0.85 m/s). These walking speeds are indicative of lower risk of cardiovascular adverse events (A. Middleton et al., 2015) and reflect the healthy population of OA who volunteered to participate in this study.

This study is unique in that the speed of walking was self-selected. Interestingly, and in contrast to our hypothesis, YA and OA chose similar usual walking paces. Furthermore, both age groups selected similar stride lengths during Slow resulting in the same speed. This explains the similar VO_2 and HR between YA and OA, although relative load (%HRR) was greater for OA. Where there was a significant Age by Activity interaction (MAP, CO, MCAVm, MCAVsys, CVRi, PP) there was no significant age difference at any of the three walking speeds for any of the variables with the exception of PP, which was greater in OA during both Slow and Fast, and SBP, which was greater in OA compared to YA during Usual.

Possible benefits of walking to cerebrovascular health in young and older adults

In our study, even slow walking elicited Light activity (2.6 METS) while Usual and Fast would be classified as Moderate activity (3.6 to 5 METS) in both YA and OA. Walking and light activity have been shown to reduce the risk of cognitive decline. For example, walking was shown to be protective even very late in life (Wang et al., 2014). Women over age 85 (n = 1249) self-reported number of blocks walked per week. Five years later, the highest tertile of walking were at lower risk of developing dementia and also performed better on tests for global cognition, category fluency, and executive function compared to the lowest tertile of walking but there was no effect of activity on risk of cognitive impairment. Cerebrovascular function improved with Light activity in OA and Moderate activity in Middle aged. Gando et al. (2010) assessed time spent in various categories of activity from accelerometry monitoring for two weeks in 538 adults and administered a carotid-femoral pulse wave velocity (cfPWV) test to estimate arterial stiffness. Results showed that arterial stiffness was lower in older unfit adults who spent more time in Light activity (1.1 to 2.9 METS) compared to less time OA In middle aged adults, cfPWV was associated with Moderate and Vigorous activity, not Light (Gando et al., 2010). The current study showed that in YA Slow did not elevate MCAV but that Usual pace, the most common pace of ADL, did elicit cerebrovascular response. Recent research supports a positive influence of physical activity in youth on brain health later in life (Nyberg et al., 2014; Stern et al., 2019; Z. Xu et al., 2020; Zabriskie & Heath, 2019). Physical activity in teenage has been shown to significantly affect the risk of cognitive impairment later in life (L. E. Middleton et al., 2010). Almost

10,000 women completed a modified Paffenbarger activity questionnaire regarding activity frequencies of low, moderate, and high-intensity activity in teenage, age 30, age 50, and current late life. They also completed a Mini-Mental State Examination (MMSE) to determine current cognitive impairment. When activity was categorized as either Active or Inactive (no regular activity) at each age, MMSE score was slightly greater for the Active versus Inactive at every age point. Also of relevance, those who were inactive in teenage reduced their risk of cognitive impairment later by engaging in activity at any of the other ages, but for those who were active in teenage, activity in other ages did not relate to rates of cognitive impairment. This study highlights the importance of activity in teenage to reduce the risk of cognitive impairment later in life. Our study shows that this may be achieved by simply walking at usual pace. Together these studies show that activity levels that equate to those of ADL contribute to cerebrovascular health in both YA and OA.

One of the mechanisms contributing to improved brain health with exercise training may be increased CBF that elicits shear stress-mediated improvement in endothelial function and reduced arterial stiffening (Ballermann et al., 1998; Hoiland et al., 2017). Stationary cycling at 60% HRR that increased shear stress, MCA flow, and MCAVm about 28% resulted in a 6% increase in ICA diameter (K. J. Smith et al., 2017). By comparison, in our study, relative work rate for usual pace walking was 17 to 53 %HRR in OA and 13 to 25 %HRR in YA. Fast walking was performed between 28 to 63 %HRR in OA and 26 to 53 %HRR in YA. MCAVm during usual pace walking increased an average of 26% with a range of 9.6 to 47%, (a variability in CBF response to intervention that has been previously

reported (Borle et al., 2017). These results suggest that at least some participants in our study experienced increases in blood flow that have been shown to elicit shear-induced flow mediated dilation.

In summary, light to moderate activity levels represented in our study by walking, elevated MCAV to levels that may enhance shear mediated endothelial function. Walking at usual pace or faster evoked a metabolic and cardiovascular response which, for those who live otherwise inactive lives, may provide some cardiorespiratory training stimulus. Furthermore, hemodynamic and cerebrovascular responses were similar whether an individual walked at their usual pace, faster, or even slower. This is an important message for OA who may walk with a slower partner or experience temporary injury or illness that reduces exercise intensity and also YA whose primary form of exercise is walking at usual pace or faster for locomotion.

2.6 Limitations

1. A graded exercise test to fatigue was not administered therefore, there are no measures of VO_2 or HR. Relative intensity of ADL expressed as $\%VO_2max$ or %HRmax would have provided more information about the physiological demand of the exercise for comparison between individuals, ADL, and existing literature. Instead, absolute values of VO_2 were converted to METs for the purpose of comparison and %HRR was calculated to represent relative workload.

2. HRmax was estimated, not measured. The equations for maximum HR used in the calculation of %HRR, even when adapted for older populations have an error greater than ± 10% (Heyward & Gibson, 2014). Furthermore, RHR was measured while participants were fully instrumented and therefore, breathing through a face mask which is not conducive to true resting conditions. Using the formula, $\%HRR_{ADL} = (HR_{ADL} - RHR)/(HRmax - RHR) \times 100$, where $\%HRR_{ADL}$, HR_{ADL} are values during the last minute of any given ADL, an elevated RHR would lower the $\%HRR_{ADL}$, and overestimate reported relative workloads. To reduce the risk of elevated RHR, resting conditions were quiet and participants were asked to try to relax. Participants rested until RER was stable and lower than 0.9.
3. The use of the metronome is a cognitive task that may have slowed walking speed (E. Smith et al., 2016) or increased MCAV (Gatouillat et al., 2015) are discussed thoroughly in the discussion of this chapter.

Table 2-1. Participant characteristics showing mean, standard deviation (SD) and range (minimum and maximum values).

		Age (y)	Weight (kg)	Height (cm)
Young n=11	mean	22.7	73.2	173.3
	SD	3.2	11.1	6.0
Seniors n=11	mean	71.2	78.2	168.6
	SD	2.8	8.6	10.5

Table 2-2. Effect size (Cohen's d) calculated between young and older adults (Effect $Size_{Group}$), and in walking speeds compared to Sit (Effect $Size_{Sit}$) in young and older adults.

Variable	Cohen's d (Young vs Older Adults) (Effect $Size_{Group}$)				Cohen's d compared to Sit (Young adults) (Effect $Size_{Sit}$)			Cohen's d compared to Sit (Older adults) (Effect $Size_{Sit}$)		
	Sit	Usual	Slow	Fast	Usual	Slow	Fast	Usual	Slow	Fast
MCAV cm/s	1.51	0.83	0.86	1.23	0.54	0.09	0.53	0.94	0.62	0.69
MCAVsys mmHg	1.45	0.73	0.66	0.98	0.89	0.41	1.05	1.36	1.00	1.34
MCAVdia mmHg	1.77	1.31	1.40	1.51	-0.30	-0.45	-0.42	0.11	-0.01	-0.29
$P_{ET}CO_2$ mmHg	0.69	0.66	0.51	0.86	1.49	1.06	1.51	1.44	1.33	1.20
VO_2 ml/kg/min	1.01	-0.04	0.17	1.14	6.28	5.99	8.26	5.39	4.46	5.96
HR bpm	0.96	0.14	0.37	0.56	1.99	1.12	3.25	4.14	3.41	4.38
MAP mmHg	0.63	-0.93	-0.08	-0.37	0.13	0.19	0.29	1.91	2.03	1.74
SBP mmHg	-0.33	-2.30	-1.28	-1.13	1.46	1.86	3.10	3.18	2.72	2.50
DBP mmHg	0.80	-0.29	0.55	0.00	0.61	1.05	1.29	1.27	1.13	1.24
Q L/min	1.93	0.73	0.86	1.33	2.42	1.66	3.13	3.24	2.23	2.82
TPR mmHg/L/min	-1.49	-1.24	-0.80	-1.19	-1.97	-0.86	-2.43	-1.65	-1.27	-1.40
CVRi mmHg/cm/s	-0.96	-1.35	-0.79	-1.18	0.18	0.79	0.83	0.64	0.58	0.99
Ri	-0.86	-1.03	-1.26	-0.96	0.36	0.89	0.58	1.53	1.47	2.18
Pi	-0.49	-0.59	-0.79	-0.67	1.21	0.93	1.35	1.15	1.21	1.76

Mean, systolic, and diastolic middle cerebral artery velocities, (MCAVm, MCAVsys, and MCAVdia, respectively, partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), oxygen uptake (VO_2), heart rate (HR), percent heart rate reserve (%HRR), mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac output (CO), total peripheral resistance (TPR), cerebrovascular resistance index (CVRi), pulsatility index (PI), and resistance index (RI), pulse pressure (PP).

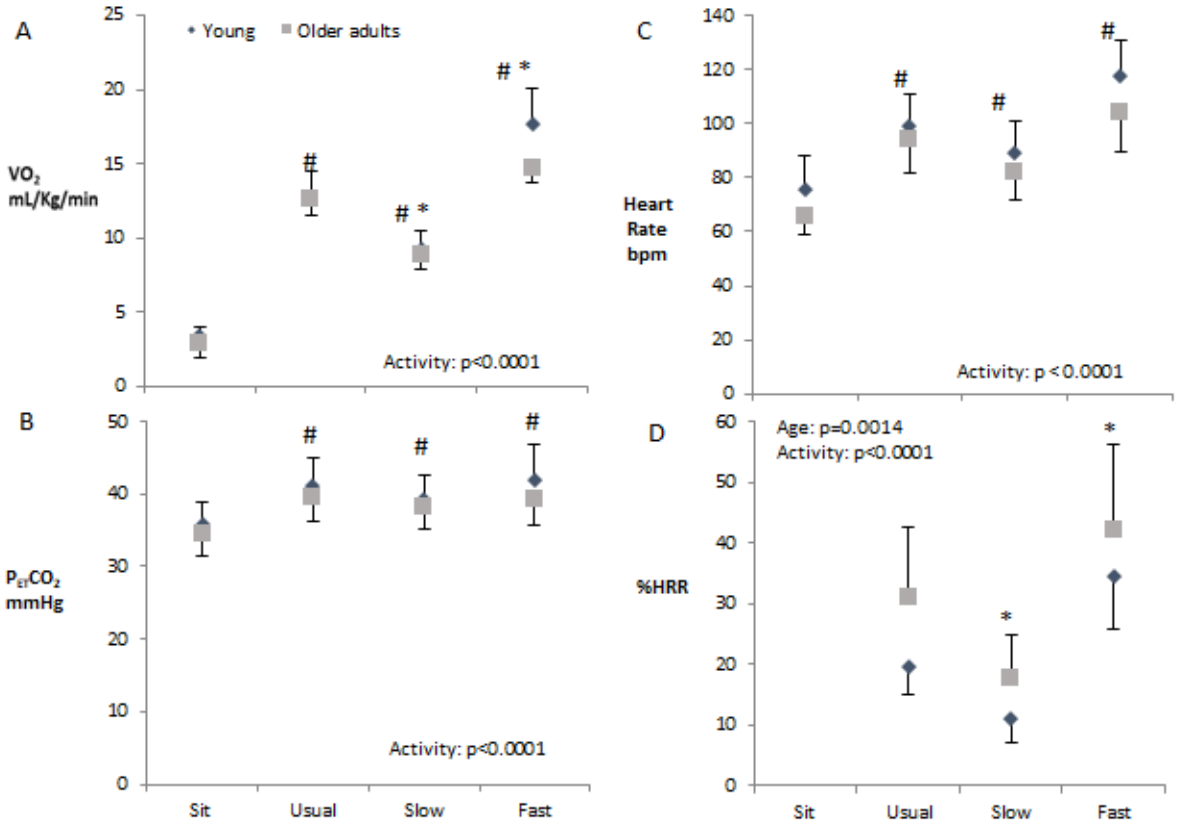

Figure 2-1. Work rate and metabolic responses to activities in 11 older and 11 young adults.

Percent heart rate reserve (%HRR), oxygen uptake (VO_2), end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) in young (diamonds) and older (squares) adults during 4 Activities: seated rest (Sit) and usual pace (Usual), slow, and fast walking. Values are mean ± SD. # indicates difference from Sit. * indicates walking speed difference from Usual.

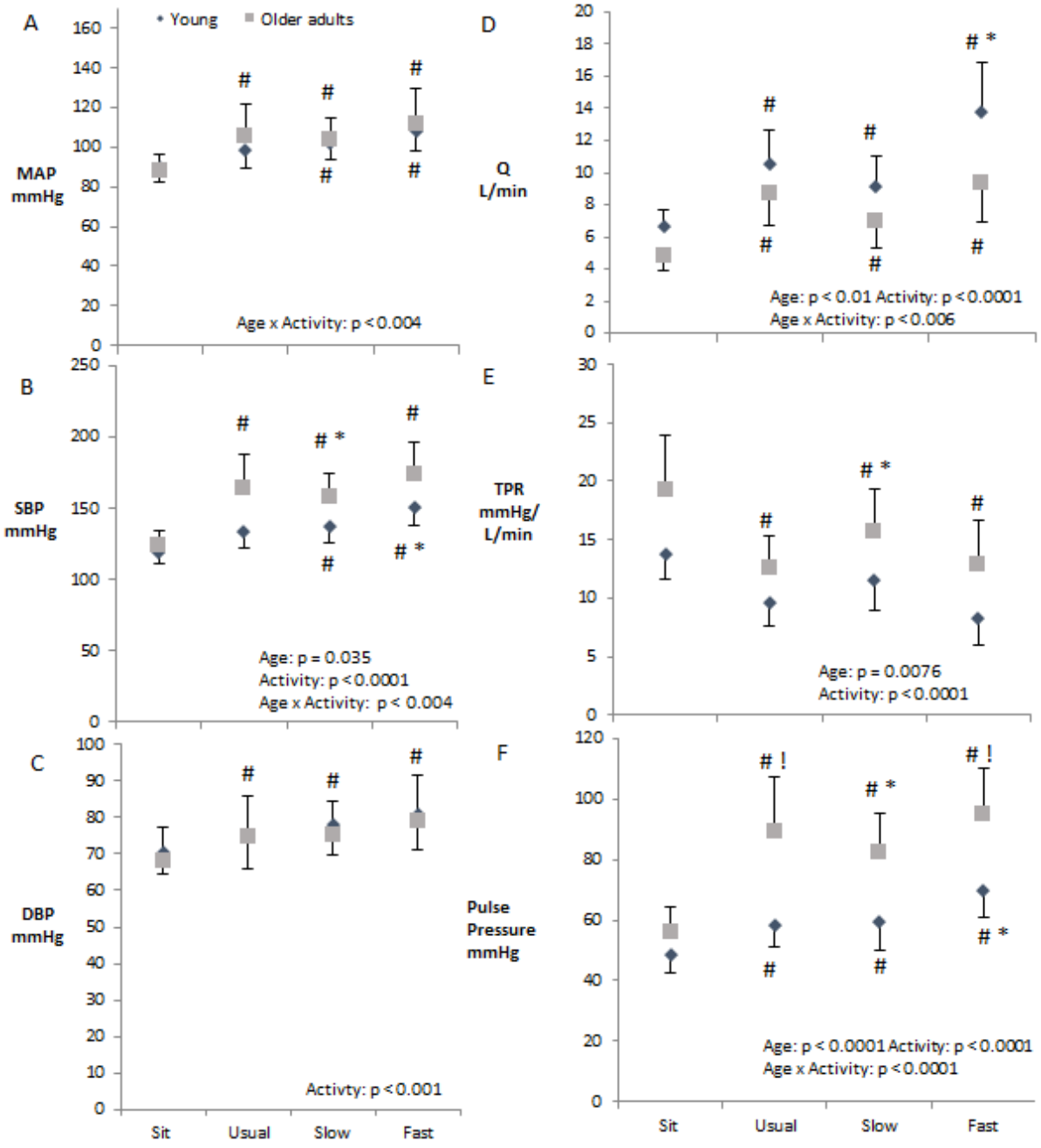

Figure 2-2. Hemodynamic responses to activities in 11older and 11 young adults.

Mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac output (Q), total peripheral resistance (TPR), and pulse pressure (PP) in young (diamonds) and older (squares) adults during 4 Activities: seated rest (Sit) and usual pace (Usual), slow and fast walking.

Values are mean ± SD. # indicates difference from Sit. * indicates walking speed difference from Usual. ! indicate difference between young and older adults at a given Activity.

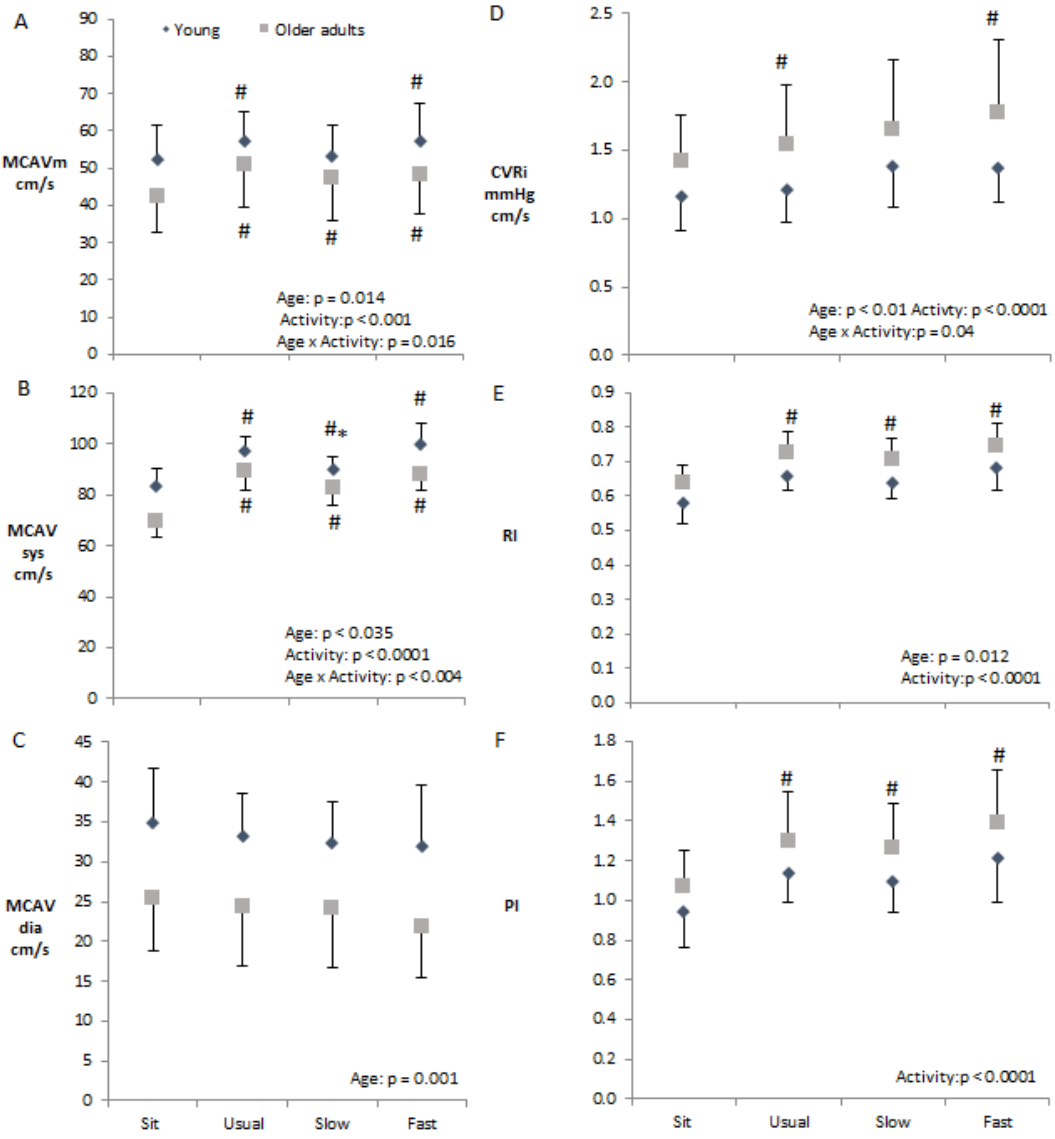

Figure 2-3. Cerebrovascular responses to activities in 11older and 11 young adults.

Mean, systolic, and diastolic middle cerebral artery velocities, (MCAVm, MCAVsys, and MCAVdia, respectively, cerebrovascular resistance index (CVRi), pulsatility index (PI), and resistance index (RI) in young (diamonds) and older adults (squares) during 4 Activities: seated rest (Sit) and usual pace (Usual), slow and fast walking. Values are mean ± SD. # indicates difference from Sit. * indicates walking speed difference from Usual.

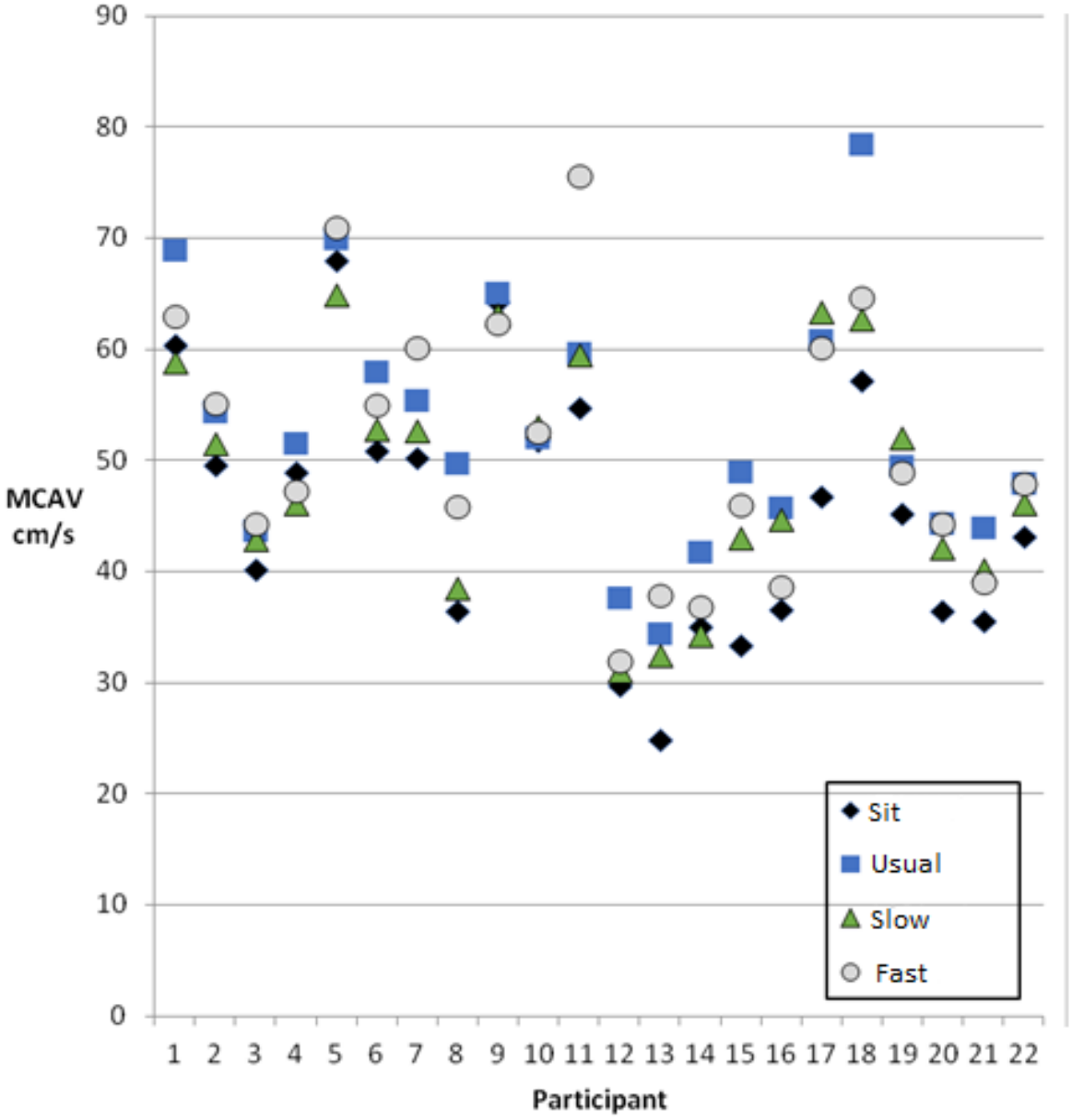

Figure 2-4. Individual values of MCAV for all participants. Participants 1 to 11 are young adults and participants 12 to 22 are older adults.

ADL are represented by black diamond (Sit), blue square (Usual), green triangle (Slow) and grey circle (Fast).

Chapter 3

Cerebrovascular Response to Ambulatory Activities of Daily Living in Community Dwelling Older Adults

3.1 Abstract

CBF is reduced during aging and endothelial dysfunction is increased. Endothelial dysfunction is a factor in arterial stiffening, atherosclerosis, and cognitive impairment. A primary benefit of elevated CBF is laminar shear stress leading to enhanced endothelial function. Exercise at moderate intensity increases CBF in OA. However, it is not known whether ADL also increase CBF. Males and females may perform ADL at different intensities given differences in body size and muscle composition. The purpose of this study was to determine whether ambulatory ADL are strenuous enough to elicit cerebrovascular responses, particularly elevation in MCAV, as well as metabolic and hemodynamic responses and whether there are sex differences. Twenty-seven OA (69.8±3.8 y, 11 males) walked overground for four minutes each at three speeds: usual pace, slow, and fast, then ascended and descended stairs for 2 min, vacuumed for four minutes and finally, simulated carrying and putting away groceries for four min, in that order each with appropriate rest between. MCAV measured using a portable Doppler ultrasound device with a 2MHz probe, HR, VO_2 and $P_{ET}CO_2$ were measured continuously. MAP was measured by continuous finger-cuff plethysmography. VO_2, HR, MAP, Q, and MCAV was greater than rest during all

ADL ($p<0.00001$). End tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) was also greater during all ADL except during groceries. In conclusion, ambulatory ADL in this study evoked metabolic, hemodynamic, and cerebrovascular responses.

New and Noteworthy

Over-ground, ambulatory, ADL including usual pace walking and regular household chores were vigorous enough to elicit cerebrovascular responses most notably, increased MCAV.

Key words: cerebral blood flow, middle cerebral artery velocity, exercise, older adults, sex, activities of daily living, cerebrovascular, oxygen uptake, metabolic, hemodynamic, blood pressure.

3.2 Introduction

Normal aging leads to endothelial dysfunction resulting in vascular stiffening of conduit arteries (Thijssen et al., 2016). The ensuing pulsatile flow is then transmitted from proximal extra-cranial arteries (e.g., ICA) to distal arteries (e.g., MCA and distal MCA) (Zarrinkoob et al., 2016) and has been implicated in the development of white matter lesions (Purkayastha et al., 2014). An abundance of research has shown that cardiorespiratory training improves endothelial function via flow-mediated shear stress on peripheral conduit artery walls (reviewed by (Di Francescomarino S. et al., 2009; Thijssen et al., 2016)). Shear-mediated vasodilation in response to hypercapnia and moderate exercise has been reported in the internal carotid arteries (Hoiland et al., 2017; K. J. Smith et al., 2017) and vertebral arteries (K. J. Smith et al., 2017) OA 20% of Canadian adults and fewer OA met the recommended 150 minutes of moderate-to-vigorous exercise per week set out by the Canadian Physical Activity Guidelines (Statistics Canada, 2015b) and World Health Organization (World Health Organization, 2018) Participation in activity in OA decreased with age (Centre for Disease Control and Prevention, 2016). In another report, 30% of adults over age 65 met the guidelines for 150 min of moderate-to-vigorous exercise per week accumulated in bouts of 10 min. When activities of daily living (ADL) were included and accumulated by the minute rather than 10 min bouts, 79% of this age group met the guidelines measured by acceleration counts (Zenko et al., 2019). ADL such as cleaning and grocery shopping may be performed in a way that elevates CBF. Walking at usual pace is not only an integral part of many ADL but it is also is a preferred intentional exercise for OA (Szanton et al., 2015). In general,

walking speeds slow with aging (Steffen et al., 2002). It is unknown whether ADL would be strenuous enough to elicit an increase in CBF. Support for elevated CBF during ADL is found in studies that have shown elevated MCAV above resting levels even during light exercise such as cycling at 30%HRR (Murrell et al., 2013) and 25%Workrate max (Fluck et al., 2014) in both YA and OA.

Higher resting CBF in females compared to males has been shown in those up to 70 y old (Grolimund & Seiler, 1988; Vriens et al., 1989). It is unknown whether sex differences in CBF may be confounded by a self-selected walking pace or whether intensity of activities of daily living are different enough between males and females to elicit differences in CBF. In a study where OA were asked to walk at their usual pace or as fast as they could for six minutes each, walking speeds were reportedly not different for males and females 60-89 y old at either usual or maximal pace (Lusardi et al., 2003). However, in that study, the group of 60- to 69-year-olds consisted of only 1 male and 6 females. Steffen et al. (2002) found that males walked faster over six minutes than females. In a sample of 84 seniors, 15 were considered slow walkers during an 8 m usual pace test (<1 mps) and these participants tended to be older and female (A. D. Robertson, 2013). Walking pace was linearly correlated to age, height, and lower extremity dynamic muscle strength (Bohannon, 1997) providing support for slower walking speeds in females.

The main objective of this research was to determine whether ambulatory ADL elicit cerebrovascular responses, most importantly MCAV, and if so, whether these responses differ between male and female OA. We hypothesized that ADL will be strenuous enough to

elicit cerebrovascular responses including elevated MCAV. Second, we hypothesize that females will walk slower than males and perform ADL with a lower intensity, eliciting a lower metabolic rate and MCAV.

3.3 Materials and Methods

Participants were recruited by posters and through the Waterloo Research in Aging Participant pool, a database of community dwelling adults who responded to university advertisements to agree to be contacted for potential participation in various research studies. Inclusion criteria was the ability to perform activities without the use of eye glasses and age 60 y or older. Exclusion criteria included any conditions that limited mobility (e.g., neuromuscular, neurological), diabetes, stroke, medications that affect heart rate, uncontrolled hypertension, or heart disease. Study procedures were in accordance with the Declaration of Helsinki and approved by the Office of Research Ethics at the University of Waterloo (ORE 17714) and Research Ethics Review Committee at Redeemer University. Twenty-seven OA participated in this study after providing informed consent.

Study procedure

Participants made one visit to the lab and were instructed to avoid moderate to strenuous exercise 24 hours prior to testing and to refrain from consuming alcohol or caffeine within four hours of testing. Participants ate a light breakfast at least two hours before arrival. Upon

arrival, height and weight were measured. Participants were seated during instrumentation and remained seated after instrumentation until RER was below 0.9 or as low as possible and steady (about 10 to 15 min). Seated resting measurements were taken over the subsequent 5-10 min (Sit). Participants walked for four minutes at each of three speeds first with usual pace (Usual), second at slow speed (Slow) and finally at fast speed (Fast) with two or more minutes between speeds. The order of the trials was the same for all participants. For Usual, participants were instructed to "walk at their usual pace as though walking from their car, unhurried", self-selecting both stride rate and length. Slow and Fast paces was standardized using a metronome, with self-selected stride length. The Slow pace was 75 steps per min and Fast pace was 115 steps per min. Walking speed was determined by recording the time and distance of walking. Following walking activities, participants ascended and descended a staircase at a pace of their choice for two minutes (Stairs). After a rest to return metabolic rate to resting levels (about 10 to 20 min), walking and shelving groceries was simulated by carrying a 5-pound bag of groceries at usual pace for 65 m (about 1 min), placing the bag on a counter where another bag of groceries was sitting, and then proceeding to take an item at a time from the bags and place it on a nearby shelf, in a refrigerator, or cupboard for the remainder of the four minutes (Grocery). In the final activity, using a light-weight, upright vacuum participants were asked to vacuum a carpet area at least 1.7 x 2.7 m for four minutes "as they would at home" (Vacuum). Crumbs were dropped on the carpet to improve realism. These activities were performed at self-selected intensities. Stair climbing was performed for only two minutes since a pilot project showed some participants found three minutes uncomfortably tiring.

All data were analyzed in the last minute of each activity except with the grocery activity. Because it included both carrying the groceries and putting them away, measurements were averaged over the entire four-minute activity. Since ascending and descending stairs was performed for only two minutes and assessments were made in the last minute, it must be noted that analyses began after just one minute of performing that task, and began and ended at the same point of a climb-descend cycle to ensure one aspect was not over-represented in the average. Usually, a minute included three full cycles of ascending and descending stairs. VO_2 has often been expressed in METS (defined as 3.5 ml/kg/min for each MET), used to categorize exercise intensity as Light (2 to <3 METS), Moderate (3-<6 METS), and Vigorous (≥ 6 METS) (Statistics Canada, 2015b).

Instrumentation and measurements

Instrumentation in this study is the same as that used in Chapter 2 and are discussed in detail in Section 2.2 *Instrumentation and measurements*. A brief outline is provided below.

CBF was measured using a portable transcranial Doppler ultrasound (TCD-X; Atys medical, Soucieu en Jarrest, France). This unit consists of a 2-MHz Doppler probe attached to the arm of clear eye glasses. Mean, systolic, and diastolic middle cerebral artery velocities, (MCAVm, MCAVsys, and MCAVdia, respectively) were determined by analysis of the CBF velocity outer envelope waveform. Heart rate (HR), oxygen uptake (VO_2) and end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) were measured using a COSMED portable

metabolic system (K4b2, COSMED, Italy) coupled with a Polar heart rate monitor. Continuous arterial blood pressure was measured with portable continuous finger-cuff plethysmography (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands). Beat to beat cardiac output (Q) was estimated using the Modelflow algorithm (Beatscope 1.1a, Finapres Medical Systems, Amsterdam, The Netherlands). Activity start times were identified by a triaxial accelerometer (16g or 8 g accelerometer data logger. x16-mini or X8m-3, respectively, Gulf Coast Data Concepts, LLC, Waveland, MS, USA) worn on the hip. Cohen's d was also calculated to indicate effect size for activities compared to Sit: Effect Size = $(M_{Activity} - M_{Sit})/\sqrt{((SD_{Activity}^2 + SD_{Sit}^2)/2)}$ where M is mean and SD is standard deviation. Commonly, effect sizes are interpreted as small (d = 0.2 to 0.49), medium (d = 0.5 to 0.79), and large (d > 0.8) (Cohen, 1988). VO_2max was predicted using a single stage model where $VO_2max = VO_{2SM}$ x (HRmax –RHR)/ HR_{SM} – RHR) where SM is submaximal and RHR is resting HR.

Statistical analyses

The effect of sex and ADL on each variable was investigated using a two-way ANOVA, with one between (Sex) and one repeated measure (ADL) factors. ADL had seven levels (no activity (Sit), Usual, Slow, and Fast walking, Stairs, Grocery, and Vacuum). Significance was established at alpha = 0.05. Significant interactions were investigated using Tukey post-hoc tests. Statistical analyses were administered using Statistica 7 (Statsoft, Inc. Tulsa, OK).

Data analysis revealed a large inter-individual variability in MCAV, therefore, post-hoc analyses were performed to investigate possible explanations. Single linear regressions with dependent variable, MCAV at Usual, and independent variables of fitness or vascular health (predicted VO_2max, Usual walking speed), submaximal workload (%HRR, HR, and VO_2 at Usual) and variables modulating MCAV at Usual (MAP and $P_{ET}CO_2$ at Usual) were conducted. Furthermore, individuals were assigned to a High Fit or Low Fit group. High Fit was defined as a predicted VO_2max with an excellent or superior rating and Low Fit was with a poor, fair, or good rating (Heyward & Gibson, 2014). A one-factor ANOVA with two levels, High Fit and Low Fit, were conducted on MCAV. Usual pace was selected for analyses because of its relevance to ADL, large variability between individuals, and the work was performed under VT as indicated by respiratory exchange ratio less than one.

3.4 Results

Thirty adults participated in this study. In three participants finger-cuff plethysmography failed to provide reliable hemodynamic results. Therefore, twenty-seven adults (69.8 ± 3.8 y, 11 males) were included in the analyses for this study. Participant characteristics are provided in **Table 3.3.**

Ultrasound was not reliable during vacuuming in two participants (S26, S33) so ANOVA for cerebrovascular measurements or calculations included only 25 participants. In

addition, Q and TPR were unavailable for one participant (S25) therefore, results including Q and TPR would have only 26 participants.

There were only main effects of Sex with one exception of a Sex by ADL interaction in walking speed discussed later. Therefore, main effects of Sex on all variables are presented in one table in order to simplify interpretation of results.

Workload and Metabolic

Absolute workload (Walking speeds, HR), relative workload (%HRR), and metabolic rate (VO_2, $P_{ET}CO_2$) increased progressively from Slow to Usual to Fast pace (**Table 3.4**). All ADL elicited greater VO_2, HR, and %HRR compared to Sit. $P_{ET}CO_2$ was elevated from rest during all ADL except groceries. Average exercise intensity was Light during Usual, Moderate during Grocery, Vacuum, and Fast, and Vigorous during Stairs, consistent with a compendium of physical activity (Ainsworth et al., 2011).

HR and %HRR during Grocery and Vacuum were not different from Usual indicating a similar absolute and relative workload (**Table 3.4**). VO_2 during Usual was similar to Vacuum, but higher than Grocery, although VO_2 was not significantly different between Grocery and Vacuum (**Table 3.4**). Stairs was most strenuous, indicated by greater VO_2, HR, and %HRR than any other ADL. $P_{ET}CO_2$ during Usual was similar to Slow, Fast and Stairs but significantly lower during Sit, Vacuum, and Grocery (**Table 3.4**).

Hemodynamics

All ADL were strenuous enough to elicit changes in hemodynamic variables. MAP, Q, SBP, DBP, PP were greater and TPR lower during all ADL compared to Sit (**Table 3.5**). Effect size was high for all hemodynamic variables in comparisons with Sit, except in DBP where effect size was moderate to high (**Table 3.5**). MAP was similar between walking speeds, Grocery, and Vacuum but significantly higher during Stairs than any ADL except Fast. Q during Usual was different than all ADL except Fast. Q was similar between Slow, Grocery, and Vacuum. TPR during Usual was similar to Fast, Stairs, and Grocery and lower than during Vacuum and Slow.

Cerebrovascular

All ADL were strenuous enough to elevate MCAVm above Sit (**Table 3.6**). The average increase in MCAVm from Sit was 12.7% (SD 15.8) for Slow, 15% (SD 16.8) for Fast, 17.4% (SD 16.4) for Stairs, 10.6% (SD 13.2) for Grocery and 11.3% (SD 17.8) for Vacuum. Cohen's D effect size was calculated in comparison to Sit and was 0.84 for Usual, 0.45 for Slow, 0.53 for Fast, 0.64 for Stairs, 0.38 for Grocery and 0.46 for Vacuum, which are considered medium to large according to standards of assessment determined by Cohen (1988).

Figure 3.4 illustrates individual MCAVm values for each ADL for each of the 27 participants, including the two who did not have MCAVm values for Vacuum. **Figure 3.5**

illustrates the percent change in MCAVm compared to Sit for each individual during each ADL. Both figures highlight the person-to-person variability in MCAV response to performance of ADL. During Usual, MCAVm was 5.5 to 68.9% higher than during Sit (21.7 ± 14.3) with a median change of 19.1%. Fitness level was considered a possible explanation for some variability however, regression analysis showed no relationship between MCAV at Usual and VO_2max ($F_{(1,26)} = 0.983$, $p = 0.331$) or Usual walking speed ($F_{(1,26)} = 0.029$, $p = 0.87$). There was no difference in MCAV between High Fit and Low Fit groups ($F_{(1,26)} = 0.387$, $p = 0.540$). MCAV at Usual showed no significant linear relationship with Usual VO_2 ($F_{(1,26)} = 0.98$, $p = 0.331$), HR ($F_{(1,26)} = 1.033$, $p = 0.32$), MAP ($F_{(1,26)} = 1.134$, $p = 0.26$), or $P_{ET}CO_2$ ($F_{(1,26)} = 1.164$, $p = 0.29$).

MCAVsys was greater during all ADL compared to Sit (**Table 3.6**). MCAVdia was similar between all ADL except Fast, during which MCAVdia was significantly lower than all other ADL including Sit.

CVRi was elevated from Sit at every ADL except Usual and Grocery. Resistance Index and Pulsatility Index were higher than Sit at every other ADL.

Sex Differences

Females represented 16 of the 27 participants. All females were post-menopausal and without hormone replacement. **Table 3.7** lists the main effect of sex statistical results for each variable. Age was similar between males and females. Height was greater in males and

weight trended toward greater values in males (p=0.052). There was no difference in walking speeds between males and females. There was only one sex by ADL interaction in all the analyses, showing that females chose a usual walking speed that was similar to their fast speed (1.2 ± 0.17 m/s and 1.30 ± 0.16 m/s, respectively) whereas the usual speed chosen by males was slower (1.16 ± 0.2 m/s) than their fast walking (1.44 ± 0.12 m/s) (p = 0.026). Females had higher %HRR, HR, MAP, DBP and MCAVdia and a lower PI and RI compared to males. There was a tendency for higher MCAV in females (p = 0.056).

3.5 Discussion

Hemodynamic Responses to ADL

A unique finding in this study is that all ADL were strenuous enough to raise MCAV above seated rest values in most participants with no effect of sex. In fact, even the least strenuous activity (Slow) with average VO_2 less than 9 ml/kg/min and %HRR of 17, elevated MCAVm by an average of 12% above seated rest. Usual pace increased MCAVm in all participants. However, the range in responses is noteworthy. Some participants showed very little change in MCAVm with activities of daily living in comparison to others. For example, MCAVm increase from Sit ranged less than 3.0 cm/s in participant 30 across all ADL except Stairs where the increase was only about 4.5 cm/s. In contrast, MCAVm increased by more than 20 cm/s during every ADL in participant 31. In participant 30, MCAV did not change from Sit to Fast but $P_{ET}CO_2$ increased 7 mmHg with a small increase in MAP of 9 mmHg. In

participant 31, MCAV increased 20 cm/s from Sit to Fast, with increases in $P_{ET}CO_2$ and MAP of 6 and 33 mmHg, respectively. During Fast, participant 31 was working harder reflected by %HRR of 64 and VO_2 of 13.1 ml/kg/min compared to participant 30 with %HRR of 15 and VO_2 of 9 ml/kg/min. This single case comparison emphasizes the inter-individual differences in cerebrovascular sensitivity to stimuli investigated previously (Borle et al., 2017). Borle's research found that there was no consistency in the magnitude of cerebrovascular and respiratory chemoreflex response within or between individuals. Therefore, there was no individual phenotype that would dictate a similar response between organ systems or a similar response between individuals of the same phenotype. Furthermore, this study is unique in that the effort applied to each ADL was determined by the individual. Although step rate was assigned to Slow and Fast walking, stride length was self-selected, resulting in between-individual variation in walking speeds. Only three of the participants in this study walked slower than 1 m/s (0.6, 0.8, and 0.85 m/s). Almost all participants walked faster than 1 m/s with the mean speed of 1.2 m/s during usual pace and 1.37 mps during fast walking. All except three participants walked above 1 mps. These walking speeds are indicative of lower risk of cardiovascular adverse events (A. Middleton et al., 2015) and reflect the healthy population of OA who volunteered to participate in this study. Similar walking speeds were found in a study of 96 independent community-dwelling OA (61-89 years of age) where usual pace walking speeds ranged from 0.60 to 1.45 m/s and fast walking speeds from 0.85 to 2.1 m/s (Steffen et al., 2002). Post-hoc analyses showed that the variability in MCAV was not explained by walking speed, fitness, submaximal workload, or MAP or $P_{ET}CO_2$. In summary, there was a wide range in work rate and physiological

responses as reflected by standard deviations (see **Tables 3.4 to 3.6**). Of particular interest is inter-individual variability in MCAV that has not been well explained in this experiment or the literature.

Average MCAVm responses align with concomitant increases in average MAP and $P_{ET}CO_2$, both important regulators of MCAV during exercise. Increased Q in conjunction with only modest increases in CVRi may have contributed to the elevated MCAVm. There is increasing evidence for the role of Q in the regulation of MCAVm, especially in OA (Bronzwaer et al., 2017; Meng et al., 2015). The light to moderate activity levels represented in our study by walking and ADL evoked a typical cardiovascular response with elevations in HR, MAP, and Q, and lower TPR compared to rest.

Potential Benefits of ADL to Vascular Health

Most of the ADL in our study were performed at moderate or vigorous intensities (Usual, Fast, Grocery, and Vacuum at 3.6, 4.2, 3.0, and 3.3 METs, respectively, and Stairs at 6.1 METs), known to incur cardiovascular training effects such as increased VO_2max and lowered resting HR (ACSM, 1998; Wallace J, 2006). Aerobic fitness has been shown to attenuate age-related declines in MCAV (Ainslie et al., 2008), CR_{CO2}, (Bailey et al., 2013; Barnes et al., 2013; Miller et al., 2018), and CVCi (Bailey et al., 2013).

The increased MCAVm during ADL may provide cerebrovascular health benefits due to shear-stress mediated increases in nitric oxide bioavailability and ensuing improved

endothelial function, and reduced inflammation and oxidative stress in peripheral (Leung et al., 2008) and cerebral conduit arteries (for review see (Bolduc et al., 2013). Furthermore, reports show that laminar shear stress reduced pro-atherogenic factors in

human aorta ECs (Tsao et al., 1996), and increased several anti-atherogenic genes and reduced expression of various pro-atherogenic genes in human umbilical cord endothelial cells (Chiu et al., 2005). Recently, duplex ultrasound has been used to show shear-mediated vasodilation in the human ICA (Hoiland et al., 2017; Iwamoto et al., 2018; K. J. Smith et al., 2017). In response to cycling at 60% HRR, shear stress, ICA flow, and MCAV increased by 28% and ICA diameter by about 6% (Smith et al., 2017). Similar to peripheral conduit arteries, blood flow induced shear stress was the primary mediator of vasodilation in the ICA (Hoiland et al., 2017). In the current study, MCAVm during usual pace walking increased an average of 22% with a range of 5.5 to 69%. Even carrying and putting away groceries and vacuuming increased MCAVm up to 60 and 68% above Sit, respectively with median values of 10 and 12%, respectively. This suggests that at least some of the participants in our study were performing ADL at intensities that have been shown to enhance flow mediated dilation. Walking at moderate to high intensities (at or above 65 %HRR) more than three times per week for 3 months has been found to reduce cerebral arterial stiffness in in OA (Moreau et al., 2003; Murrell et al., 2013; Tanaka et al., 2000). Following a 3-month walking program at 65-80% HRmax, 4.5 days per week for 40-45 min, common carotid artery compliance, measured with simultaneous ultrasound imaging and applanation tonometry, increased by 40% in post-menopausal women bringing compliance to a level similar to pre-menopausal

women (Moreau et al., 2003) and by 25% in older sedentary males (Tanaka et al., 2000). However, these studies prescribed walking at a moderate to high intensity for the purpose of inducing cardiovascular and cerebrovascular challenge. In the present study, the range of intensities for usual pace walking was 9 to 56 %HRR. For fast walking, the intensity was higher, ranging between 15 to 77 %HRR suggesting few participants in the present study would be walking at the higher intensities associated with the aforementioned literature. However, it isn't clear that higher intensities are necessary in order to improve endothelial function. There is evidence in peripheral vasculature to support adaptations to relatively small stimulus that might suggest that even regular light activity, such as that performed by some participants during ADL in this study, is sufficient to elicit improved endothelial function over time. Since there is a paucity of research on cerebral endothelial function, much of our understanding is based on peripheral vasculature with cautious belief that cerebral vasculature may respond in similar ways. A recent study showed that even transient but regular exposure to shear stress, elevated resting blood flow and improved endothelial function in peripheral conduit arteries. Eleven young healthy males underwent shear stress via 5 s brachial occlusion every 15s for 30 min 5 times per week. Brachial artery resting and peak forearm blood flow, forearm vascular conductance, and brachial flow mediated dilation (FMD) were elevated after 3 weeks. FMD increased further after 6 weeks (Hodges et al., 2018). After 6 h of sitting, blood flow, shear rate, and endothelial reactivity were significantly reduced in lower leg macro- and microvasculature and forearm microvasculature. A subsequent ten min bout of walking fully restored lower but not upper limb endothelial function (Restaino et al., 2015). Only 3 hours of sitting decreased popliteal

artery mean shear rate by 45% in ten healthy young men (from 43-24 s^{-1}) with a concomitant decrease in flow-mediated dilation of 55% while an increase in blood flow due to limb heating obliterated the effect of sitting on endothelial function (Restaino et al., 2016). Fidgeting by heel tapping 250 times for 1 min every 5 min that elevated shear rate to 24 s^{-1} not only prevented the decrease in FMD with sitting but increased popliteal artery FMD by almost twice that at baseline before sitting. There was no effect of heel tapping on the contralateral leg (Morishima et al., 2016). Further research is needed to determine the effect of ADL on shear-mediated vasodilation in cerebral conduit arteries.

In addition to vascular compliance, other benefits of ADL to brain health include elevated BDNF (Berchtold et al., 2005; Boyne et al., 2019), VEGF (Lucas et al., 2015), IGF-1 (Trejo et al., 2001), as well as brain (hippocampal) volume (Erickson et al., 2011), and reduced inflammation (Fiuza-Luces et al., 2013).

ADL are an integral part of life especially for community dwelling OA who often live alone with the responsibility of home chores and upkeep and personal care. Three walking speeds were included in this study because of the prevalence of walking in intentional exercise, recreation, and as an integral part of ADL such as grocery shopping and dog walking, locomotion to and from the car, house, or workplace. A longitudinal pilot study monitored daily activity with accelerometry and questionnaires in 12 adults prior to and following a move to a retirement home, and found overall activity was lower and sedentary behaviour was elevated, likely due to reduced ADL (Regan et al., 2016). Engaging in ADL may slow cerebrovascular aging by reducing sedentary time.

The use of a metronome during walking may have introduced concurrent demands for attention and neurovascular resources (Gatouillat et al., 2015) which may have slowed walking speed (E. Smith et al., 2016) or increased MCAVm (Gatouillat et al., 2015). In a meta-analysis, Smith et al. (2016) found that in healthy community dwelling OA with gait speeds over 1 m/s, a cognitive task during walking reduced gait speed by 0.19 m/s from 1.2 ±0.13 m/s to 1.02±0.16 m/s. Several studies have shown that dual task walking changes gait characteristics but few have investigated the effect of dual task on CBF. Goutillat et al. (2015) had eleven YA walk at 2 mph on a treadmill as "thought free" as possible for 6 min followed by 6 min walking at the same speed but performing a backward counting task. This protocol was repeated one week later. CBF velocity was measured using transcranial Doppler on both the right and left MCA. MCAVm was 3% higher during the walk plus cognitive task trial compared to the walking alone trial. While that study differs from the current study in that their participants were walking on a treadmill and unable to change their gait, it does demonstrate that cognitive tasks during walking measurably increase CBF demand.

Contrary to our hypothesis, older males and females chose similar walking speeds. In contrast to males, females chose usual pace and fast walking speeds that were statistically similar. Activities of daily living were effective in raising CBF similarly in both sexes. Although absolute workload was similar between sexes as evidenced by walking speed and VO_2, females performed ADL at a higher relative workload (%HRR). Accordingly, MAP was also higher in females, driven by a higher DBP compared to males. Consistent with a higher DBP was a higher MCAVdia in females, which led to an insignificant rise in

MCAVm ($p = 0.056$). Research has reported greater CBF in females compared to males between the ages of 20 and 50 (Vriens et al., 1989), or 60 (Purkayastha & Sorond, 2012) or even 80 y (Grolimund & Seiler, 1988). Another study of 83 participants 22 to 80 y old (37 men) found that during rest MCAVdia, (as well as MCAVm and systolic) was higher and CVRi was lower in females compared to males (Tarumi et al., 2014). In our study, lower resistance in females was evidenced by a lower RI and higher MCAVdia, and possibly also reflected by lower PI which could be the result of less small vessel resistance or may be the cascade effect of higher DBP. CVRi was not lower since MCAVm and MAP were not different. In spite of higher MCAVdia in females, PFV was not different between sexes. The mechanisms responsible for the differences between sexes observed in this study are not clear. We considered the possibility that females, working at a greater % HRR may have been at or above their ventilatory threshold in some activities, reducing CO_2 and reducing MCAVm. However, there was no significant difference in respiratory exchange ratio between sexes (0.89 ± 0.02 and 0.82 ± 0.06 for males and females, respectively, $p<0.093$). It would seem that the higher DBP increased MCAVdia enough to influence the numerator of the indices of PI and RI, reducing them both. There is little support in the literature for sex differences in DBP or endothelial function in older adults.

3.6 Conclusion

In conclusion, walking at usual pace or faster or slower and common ADL elicit metabolic, hemodynamic, and cerebrovascular response in older males and females. The primary

variable of relevance in this study was MCAV. Although ADL were performed at moderate intensity (<3 METs) by participants, there was large variability in the MCAV response. Shear-mediated vasodilation due to increases in MCAV may stimulate improvements in endothelial function, slowing the cerebrovascular aging process. To date, there is no known threshold for MCAV, VO_2, or HR that will elicit shear-mediated changes to endothelial function. Further research is needed to elucidate a range in workload required to evoke shear-mediated vasodilation in cerebral conduit arteries.

3.7 Limitations

The limitations in this study overlap with those in Chapter 2. Please see section 2.5 of this thesis.

Analyses of stair climbing was not conducted during second minute and although it included the same number of ascending-descending cycles and started and ended at the same point in the cycle, the overall time of the analyses varied between individuals. Furthermore, it is not clear from these results how MCAV will respond to shorter bouts of stair activity, more typical of ADL. Participants took less than 10 sec to climb or descend the stair case of 15 steps. The results of the next study in Chapter 4 shed light on the response of MCAV during shorter activities.

Table 3-1. Subject Characteristics.

		Age (y)	Weight (kg)	Height (cm)
Females n=16	mean	71.0	79.0	176.8
	SD	4.5	12.3	7.7
Males n=11	mean	69.0	70.6	162.9
	SD	3.1	9.1	7.8

Table 3-2. Differences in workload and metabolic variables in ADL compared to Sit and Usual.

Variable		Activities of Daily Living							Main Effect
		Sit	Usual	Slow	Fast	Stairs	Grocery	Vacuum	ADL
Walking	mean		1.19*	0.74*	1.37*				$F_{(2, 50)}$= 288.8
Speed mps	SD		0.19	0.13	0.16				p<0.0001
%HRR	mean		31.15	17.63^	42.11^	67.29^	33.35	36.17	$F_{(5, 125)}$=118.2
	SD		11.49	7.27	14.25	15.25	12.78	14.51	p<0.0001
Heart Rate	mean	65.7	94.23*	81.85*^	104.20*^	127.64*^	96.10*	98.71*	$F_{(6, 150)}$=177.4
bpm	SD	6.95	12.74	9.81	15	14.54	14.36	15.67	p<0.0001
	Cohen's d		2.78	1.90	3.29	5.44	2.69	2.72	
VO_2	mean	2.88	12.57*	8.89*^	14.69*^	21.4*^	10.60*^	11.56*	$F_{(6, 150)}$=218.9
$ml \cdot kg^{-1} \cdot min^{-1}$	SD	0.65	2.52	1.72	2.84	3.71	2.05	3.25	p<0.0001
	Cohen's d		5.26	4.62	5.72	6.95	5.07	3.70	
$P_{ET}CO_2$	mean	34.62	39.46*	38.30*	39.20*	40.00*	35.24^	36.27*^	$F_{(6, 150)}$=51.1
mmHg	SD	3.16	3.31	3.03	3.55	4.29	3.46	3.66	p<0.0001
	Cohen's d		1.50	1.19	1.36	1.43	0.19	0.48	

ADL compared to Sit (*) and Usual (^). Percent heart rate reserve (%HRR), oxygen uptake (VO_2), end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$),

Table 3-3. Differences in hemodynamic variables in ADL compared to Sit and Usual.

Variable		Activities of Daily Living							Main Effect
		Sit	Usual	Slow	Fast	Stairs	Grocery	Vacuum	ADL
MAP	mean	87.78	105.22*	104.09*	112.00*	119.89*^	100.41*	106.10*	$F_{(6, 150)}$=24.4
mmHg	SD	8.72	16.18	10.65	17.52	18.72	11.07	12.1	p<0.0001
	Cohen's d		1.34	1.68	1.75	2.20	1.27	1.74	
SBP	mean	124.03	163.87*	157.72*	173.71*	187.25*^	148.60*^	155.21*	$F_{(6, 150)}$=37.8
mmHg	SD	10.04	23.47	16.72	23.13	24.53	14.48	16.6	p<0.0001
	Cohen's d		2.21	2.44	2.79	3.37	1.97	2.27	
DBP	mean	68.25	74.52*	75.38*	78.84*	85.93*	74.78*	79.33*	$F_{(6, 150)}$=15.5
mmHg	SD	9.06	11.1	8.83	12.75	13.15	9.24	10.2	p<0.0001
	Cohen's d		0.62	0.80	0.96	1.57	0.71	1.15	
Q	mean	4.78	8.72*	6.00*^	9.37*	10.58*^	7.36*^	7.04*^	$F_{(6, 144)}$=83.4
$L \cdot min^{-1}$	SD	0.91	2.01	1.65	2.44	2.92	1.71	1.6	p<0.0001
	Cohen's d		2.53	1.67	2.50	2.68	1.88	1.74	
PP	mean	55.79	89.34*	82.35*	94.87*	101.31*^	73.82*^	75.87*^	$F_{(6, 150)}$=44.0
mmHg	SD	8.71	17.75	13.11	15.56	17.7	9.94	11.21	p<0.0001
	Cohen's d		2.40	2.39	3.10	3.26	1.93	2.00	
TPR	mean	19.21	12.59*	15.63*^	12.85*	12.46*	14.50*	16.04*^	$F_{(6, 144)}$=21.9
$mmHg \cdot L^{-1} \cdot min^{-1}$	SD	4.77	2.72	3.66	3.84	4.57	3.77	4.47	p<0.0001
	Cohen's d		-1.70	-0.84	-1.47	-1.44	-1.10	-0.68	

ADL compared to Sit (*) and Usual (^). Mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac output (Q), pulse pressure (PP), total peripheral resistance (TPR). Q and TPR include 26 participants.

Table 3-4. Differences in cerebrovascular variables in ADL.

Variable		Activities of Daily Living							Main Effect
		Sit	Usual	Slow	Fast	Stairs	Grocery	Vacuum	ADL
MCAVm	mean	42.26	51.11*	47.51*^	48.14*^	49.29*	46.27*^	47.02*^	$F_{(6, 138)}$=13.8
cm/s	SD	9.38	11.68	11.62	10.49	11.38	9.6	11.21	p<0.0001
	Cohen's d		0.84	0.45	0.53	0.64	0.38	0.46	
MCAVsys	mean	69.87	89.56*	83.12*^	88.18*	90.96*	81.93*^	80.26*^	$F_{(6, 138)}$=36.1
cm/s	SD	13.69	17.79	18.06	17.91	16.43	16.18	17.48	p<0.0001
	Cohen's d		1.24	0.83	1.15	1.39	0.80	0.66	
MCAVdia	mean	25.33	24.32	24.17	21.89*^	23.17	24.94	27.02	$F_{(6, 138)}$=8.0
cm/s	SD	6.49	7.47	7.37	6.37	8.75	6.63	7.46	p<0.0001
	Cohen's d		-0.14	-0.17	-0.54	-0.28	-0.06	0.24	
CVRi	mean	1.42	1.54	1.65*	1.78*^	1.91*^	1.59	1.74*^	$F_{(6, 138)}$=11.1
cm/s/mmHg	SD	0.33	0.44	0.51	0.53	0.55	0.41	0.67	p<0.0001
	Cohen's d		0.29	0.53	0.80	1.08	0.44	0.60	
PI	mean	1.07	1.3*	1.26*	1.39*^	1.43*^	1.26*	1.15*^	$F_{(6, 138)}$=40.6
	SD	0.18	0.25	0.22	0.27	0.31	0.23	0.18	p<0.0001
	Cohen's d		1.03	0.93	1.39	1.40	0.88	0.44	
RI	mean	0.64	0.73*	0.71*	0.75*	0.74*	0.69*^	0.66*^	$F_{(6, 138)}$=56.9
	SD	0.05	0.06	0.06	0.06	0.08	0.07	0.05	p<0.0001
	Cohen's d		1.51	1.29	1.88	1.63	0.91	0.49	

ADL compared to Sit (*) and Usual (^). Mean middle cerebral artery blood velocity (MCAVm), systolic MCAV (MCAVsys), diastolic MCAV (MCAVdia), and cerebrovascular resistance index (CVRi), pulsatility index (PI), and resistance index (RI) for 25older adults.

Table 3-5. Statistical analysis results for main effect of sex across all ADL.

		Male	Female	P and F values
Age (y)	mean	71	69	$F_{(1, 25)} = 1.85$
	SD	4.5	3.1	$p = 0.186$
Height (cm)	mean	176.8	162.9	$F_{(1, 25)} = 21.0$
	SD	7.7	7.8	**$p<0.0001$**
Weight (kg)	mean	79.0	70.6	$F_{(1, 25)} = 0.4.15$
	SD	12.3	9.1	$p = 0.052$
Walking	mean	1.1	1.1	$F_{(1, 25)} = 0.75$
Speed (mps)	SD	0.04	0.03	$p = 0.394$
%HRR	mean	31.1	42.6	$F_{(1, 25)} = 10.0$,
	SD	9.3	9.2	**$p<0.004$**
Heart Rate	mean	88.1	100.6	$F_{(1, 25)} = 11.7$,
(bpm)	SD	2.7	3.6	**$p<0.002$**
VO_2	mean	11.3	12.2	$F_{(1, 25)} = 1.57$
($ml \cdot kg^{-1} \cdot min^{-1}$)	SD	1.0	1.2	$p = 0.222$
PetCO2	mean	37.6	37.6	$F_{(1, 25)} = 0.000$
mmHg	SD	0.4	0.6	$p = 1.00$
MAP	mean	99.7	108.7	$F_{(1, 25)} = 5.7$,
mmHg	SD	2.4	4.9	**$p<0.025$**
SBP	mean	155.6	160.7	$F_{(1, 25)} = 1.23$
mmHg	SD	4.1	7.2	$p = 0.279$
DBP	mean	72.1	79.9	$F_{(1, 25)} = 6.5$,
mmHg	SD	1.2	2.1	**$p<0.017$**
Q	mean	8.2	7.6	$F_{(1, 24)} = 0.82$
($l \cdot min^{-1}$)	SD	0.5	0.7	$p = 0.375$
TPR	mean	13.2	15.7	$F_{(1, 24)} = 4.13$
($mmHg \cdot l^{-1} \cdot min^{-1}$)	SD	0.5	0.8	$p = 0.053$
PP	mean	83.6	80.8	$F_{(1, 25)} = 0.64$
mmHg	SD	3.3	4.6	$p = 0.433$
MCAV mean	mean	42.8	50.5	$F_{(1, 23)} = 4.06$
($cm \cdot s^{-1}$)	SD	1.1	1.1	$p = 0.056$
MCAV sys	mean	82.6	82	$F_{(1, 23)} = 0.58$
($cm \cdot s^{-1}$)	SD	1.9	1.7	$p = 0.455$
MCAV dia	mean	19.9	27.4	$F_{(1, 23)} = 8.1$,
($cm \cdot s^{-1}$)	SD	0.9	0.8	**$p<0.009$**
CVRi	mean	1.71	1.63	$F_{(1, 23)} = 0.08$
$mmHg \cdot cm^{-1} \cdot s^{-1}$)	SD	0.16	0.11	$p = 0.781$
PI	mean	1.36	1.12	$F_{(1, 23)} = 16.7$,
	SD	0.04	0.16	**$p<0.001$**
RI	mean	0.75	0.66	$F_{(1, 23)} = 12.5$,
	SD	0.07	0.05	**$p<0.006$**
PFV	mean	61.7	57.2	$F_{(1, 23)} = 0.155$
	SD	3.0	1.9	$p = 0..697$

Percent heart rate reserve (%HRR), oxygen uptake (VO_2), end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), cardiac output (Q), pulse pressure (PP), total peripheral resistance (TPR), middle cerebral artery blood velocity (MCAV), systolic MCAV (MCAVsys), diastolic MCAV (MCAVdia), cerebrovascular resistance index (CVRi), pulsatility index (PI), resistance index (RI), and pulsatile flow velocity (PFV). Significant p values are bolded. Q and TPR are missing one participant and any variables with MCAV are missing two participants.

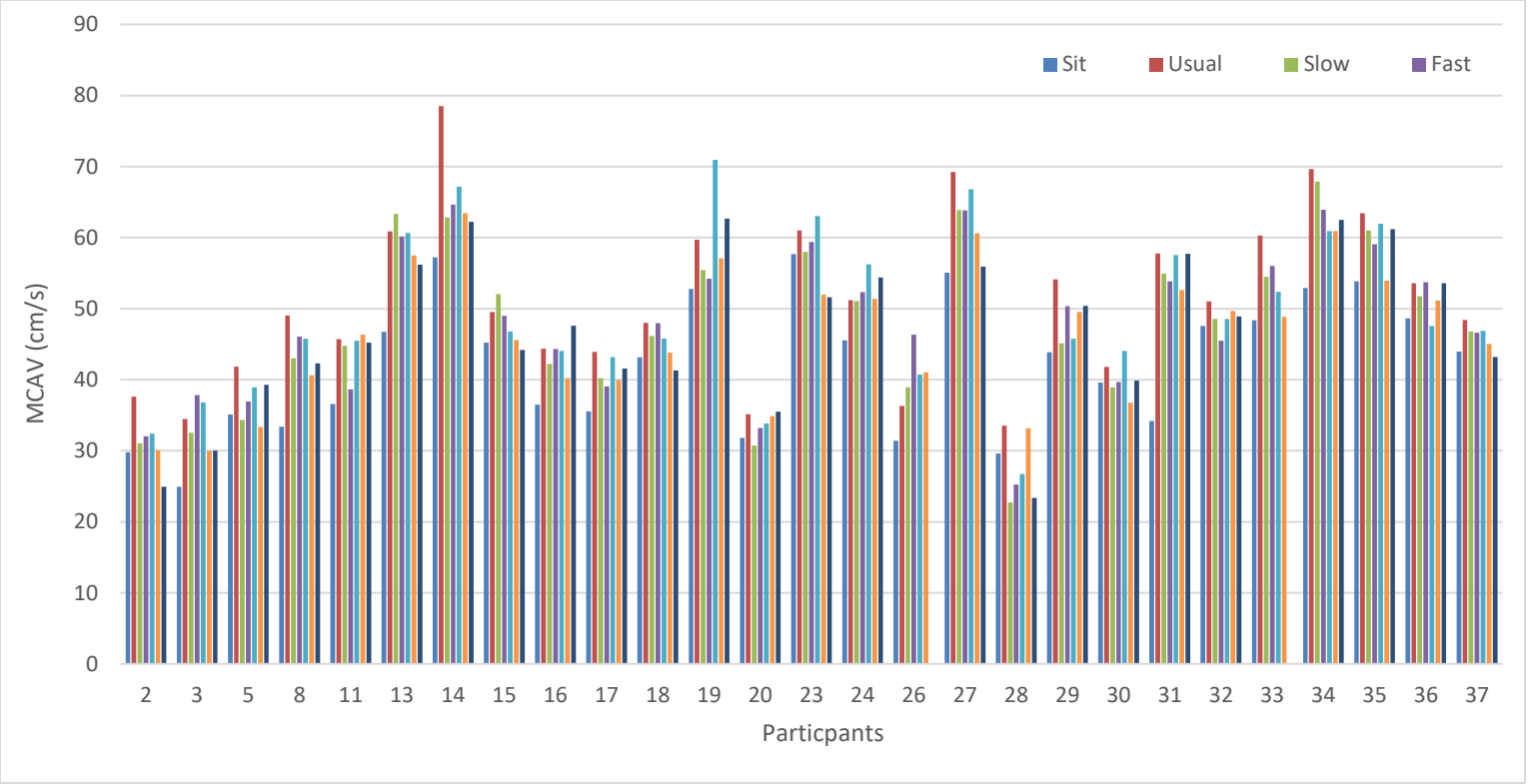

Figure 3-1. Individual middle cerebral artery velocity (MCAV) response to each ADL.

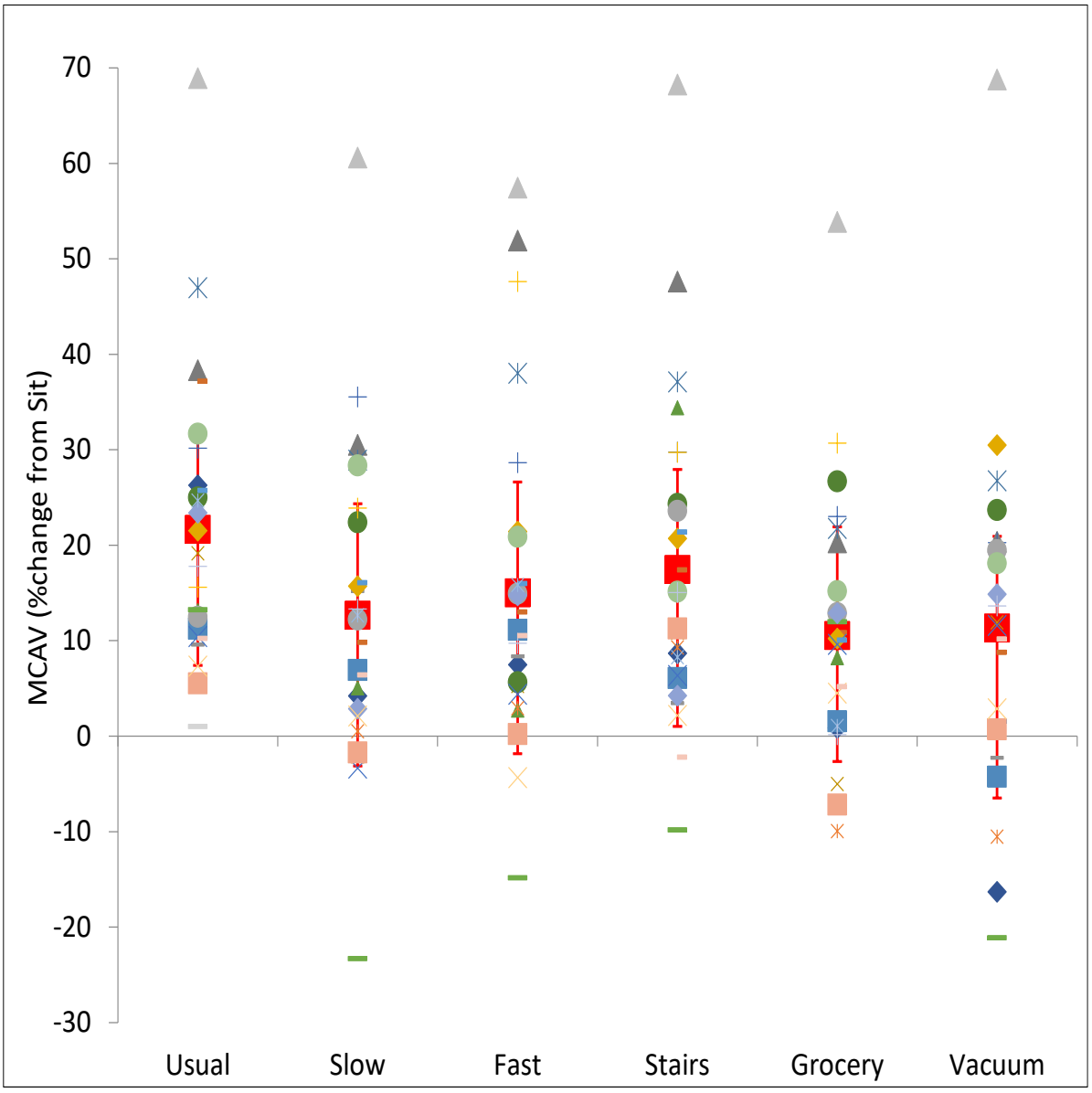

Figure 3-2. The percent change in MCAV from Sit to each ADL for each participant. Each symbol represents a different participant. Mean and SD MCAV is represented by the large red square and whiskers.

Chapter 4: Changes in estimated arterial carbon dioxide and mean arterial pressure account for the exercise-induced increase in middle cerebral artery velocity: effects of hypo- and hypercapnia.

4.1 Abstract

The dynamic response of mean middle cerebral artery velocity to exercise onset has been quantified in the literature by a single exponential equation. Given the complexity of the factors contributing to MCAV during exercise, it seems unlikely that the kinetic response would meet the principles of a first order system. Of these factors, arterial carbon dioxide ($PaCO_2$), is a potent vasodilator and MAP is the driving force for blood flow but their roles in MCAV kinetics are undefined. The purpose of this study was to assess the kinetic response of MCAV to exercise onset and to examine the effect of changes in $PaCO_2$ and MAP in this response. Ten males (aged 23 ±1.9 y) cycled for 5 min at 0 Watts followed by a step increase in load to 50% peak work rate maintained for another 5 min during three conditions: Control, Hypocapnia (controlled hyperventilation), and Hypercapnia (5% CO_2 inhalation). MCAV, mean arterial blood pressure (MAP), and end-tidal CO_2 ($P_{ET}CO_2$) were measured continuously. Single exponential equations were applied to MCAV from exercise onset to 5 min for each participant. A review of the residuals, coefficients, and assumption testing of the predicted exponential fit indicated the predicted equation did not represent the MCAV dynamic response to exercise. Linear regression showed that measured MCAV (MCAV) was best predicted by both $PaCO_2$ and MAP compared to $PaCO_2$ or MAP alone as determined by lower variability (SEE ($F_{(2,16)} = 17.21$, $p < 0.001$) and higher R^2 ($F_{(4,80)} = 4.95$, $p = 0.003$).

Three models were developed to reflect the change in MCAV due only to changes in $PaCO_2$ ($MCAV_{CO2)}$, MAP ($MCAV_{MAP}$), or both ($MCAV_{CO2MAP}$) during exercise. $MCAV_{CO2MAP}$ showed the least difference from MCAV in all conditions. $PaCO_2$ made a greater contribution to $MCAV_{CO2MAP}$ than MAP, at least in Control and Hypercapnia. These results suggest that the MCAV dynamic response to exercise is more complex than a first order system, greatly influenced by $PaCO_{2,}$ and subject to inter-individual variability as reflected by large standard deviations in curve-fitting and linear regression coefficients.

New and Noteworthy

In contrast to previous literature, the kinetic response of middle cerebral artery blood velocity to exercise load onset and transition for five minutes could not be adequately or reliably quantified by a single exponential equation.

Carbon dioxide is a primary mechanism contributing to the kinetic response of middle cerebral artery to exercise.

Key words: cerebral blood velocity, middle cerebral artery velocity, kinetics, exercise, carbon dioxide.

4.2 Introduction

It is well known that MCAV increases with exercise (Fluck et al., 2014; Murrell et al., 2013) in a dose-dependent fashion (Hellstrom et al., 1996). The inverted U response of steady state MCAV with increasing exercise intensity has primarily been attributed to the rise and fall in arterial partial pressure of CO_2 ($PaCO_2$) before and after ventilatory threshold (Hellstrom et al., 1996). Little is known about the kinetic response of MCAV to exercise, however, understanding this dynamic response may inform the value of ADL in vascular health. The previous work in this thesis showed that ADL were performed mostly at moderate intensities with some at the low end of vigorous, below VT. ADL are often performed in short bursts of activity such as stair climbing or walking quickly carrying groceries. A fast rate of rise in MCAV with exercise may induce greater shear stress over a short duration supporting the value of such short ADL. Very short episodes of shear stress have been shown to elevate vascular conductance and flow in the peripheral brachial artery (Hodges et al., 2018). Five-second forearm cuff inflation was sufficient to elevate shear rate and forearm blood flow over the following 10s, both of which subsequently decreased to baseline levels over that 10 s interval. These results lend support to the value of short duration ADL and, applied to cerebrovasculature, supports a short TD in MCAV kinetics. In contrast, Dyson et al. (2011) found that following 15 min of forearm occlusion, brachial artery dilation was only slightly elevated in a trial where hyperemia occurred for 20 s. In trials of 40 s or 60 s brachial artery diameter was elevated above baseline and related to total shear but not peak shear rate (Dyson et al., 2011). Applied to cerebrovasculature, these results suggest a longer TD in MCAV kinetics and could suggest a that a meaningful MCAV response would require that

ADL are performed for a longer duration. The authors reported an FMD response of 2.9 +/- 2.8% (SD) for the 20 s trial. While this represented a low response compared to longer trials, it may have clinical significance, albeit not for all participants given the high inter-individual variability. Yeboah et al. (2007) found that a 2.8% higher average baseline FMD was associated with significantly higher event-free survival rates of cardiovascular events including strokes in older adults aged 78 y (Yeboah et al., 2007). A direct comparison of these latter two studies is hindered by different age groups and different occlusion durations that influence the mechanisms of dilation. A 15-min occlusion (Dyson et al. 2011) employs primarily endothelium-derived hyperpolarizing factor and potassium channels compared to a 5-min occlusion (Yeboah et al., 2007) after which dilation is NO dependent. However, the latter may provide some perspective to the clinical value of small increases in FMD. In addition to a fast rate of rise in MCAV, a high magnitude of rise would likely impose a greater shear stress enhancing the effect on endothelial function.

While there is an abundance of research addressing the steady state response of MCAV to exercise, the kinetic response of MCAV to exercise onset and transition during the first several minutes has received little attention. Steventon et al. (2018) found a significant relationship between $P_{ET}CO_2$ and MCAV during and after 20 min of moderate cycling exercise. The authors fit a linear regression to individual MCAV responses to exercise and determined that $P_{ET}CO_2$ was a significant influence on MCAV compared to exercise per se, and accounted for 39% of the variability in MCAV overall. A hypercapnic (+7 mmHg) bout (applied for a duration required for $P_{ET}CO_2$ to reach steady state) during baseline and after

exercise showed similar cerebrovascular reactivity (CR_{CO2}) indicating that exercise per se did not influence the relationship between MCAV and $PaCO_2$. Two recent studies from the same lab (Billinger et al., 2017; Ward et al., 2018) investigated cerebrovascular dynamic response during moderate exercise finding MCAV kinetics were best fit by a single exponential regression equation as follows:

$$MCAV(t) = BL + Amp(1\text{-}e^{-(t\text{-}TD\ /\tau)}) \quad \text{(Equation 4.1)}$$

Where MCAV(t) is MCAV at any point in time, BL is the baseline MCAV averaged one minute before exercise onset, Amp is the peak amplitude, TD is the time prior to the rise in MCAV, and τ (Tau) represents the time constant (i.e. time to reach 63% of peak amplitude).

In both the Billinger and Ward studies, workload was applied in a multi-level, step-wise increase consisting of three 10 s intervals, therefore taking 30 s total to apply the target workload, a protocol that may have confounded the kinetic response to exercise onset. In a similar protocol, participants performed dynamic unloaded arm flexion exercise for 2 min following a 10 s count-down (Sato et al., 2009). Sato reported significant increases in Q, HR, and MCAV with a concomitant decrease in cerebrovascular resistance in the MCA immediately at the onset of volitional exercise and lasting 15 s or more. The authors concluded that the significant increase in MCAV at onset was likely mediated by the rapid cardiovascular adjustments in a feed-forward process (Sato et al., 2009). It is therefore likely that a protocol that increases the load in 10 s intervals would elicit cardiovascular and MCAV responses to both that would overlap from one 10 s load into the next 10 s load. Therefore, the dynamic response of MCAV from exercise onset to steady state in these

studies may have been confounded by the adjustment to three 10 s stepwise cerebrovascular and cardiovascular transitions. Furthermore, several mechanisms that influence MCAV including neurovascular coupling, carbon dioxide, blood pressure and neurohumoral activity, respond to exercise with varying magnitudes and timeframes. Taken together, the aforementioned provides reason to further investigate the use of the single exponential function to describe MCAV dynamic response to exercise.

Cerebral neural activation, arterial CO_2, and blood pressure are consistently identified as three important mechanisms regulating CBF during steady state exercise (reviews include (Braz & Fisher, 2016; Ogoh & Ainslie, 2009; Querido & Sheel, 2007; K. J. Smith & Ainslie, 2017; Willie et al., 2011; Wolf, 2015)), although the role of each and their relative contribution during steady state exercise is not clear. Even less is known about mechanisms regulating CBF during the transition from rest to exercise. $PaCO_2$ is a potent vasodilator in cerebral vasculature (Lennox & Gibbs, 1932). Brain blood flow is highly sensitive to changes in $PaCO_2$ during rest and exercise (Battisti-Charbonney et al., 2011; Fluck et al., 2014; K. J. Smith & Ainslie, 2017). Cerebrovascular sensitivity to CO_2 is greater during hypercapnia compared to hypocapnia at rest (Sato et al., 2012; Zhu et al., 2013) and is greater during exercise compared to rest (Ogoh et al., 2008; Rasmussen et al., 2006). Furthermore, cerebrovascular reactivity is greater in the region supplied by the internal carotid artery (e.g., MCA) compared to that supplied by the vertebral artery (Sato et al., 2012). Together, these observations drive the hypothesis that $PaCO_2$ is a regulating mechanism during the dynamic transition in MCAV at exercise onset.

The purpose of this study was twofold. First, to assess the adequacy of a first order response to describe the MCAV kinetic response to exercise and second, to assess how changes in $PaCO_2$ influence the kinetic response of MCAV to moderate, constant cycling exercise from onset to 5 min by manipulating $PaCO_2$ during exercise. We hypothesize that a single exponential equation will not adequately describe the dynamic response of MCAV to exercise and that changes in $PaCO_2$ and MAP will largely predict the MCAV response to exercise.

4.3 Materials and Methods

Experimental procedures were in accordance with the Declaration of Helsinki and received ethics clearance by the Office of Research Ethics at the University of Waterloo and Research Ethics Review Committee at Redeemer University. Each participant received a written and verbal explanation of the study prior to signing a consent form and data collection. A priori sample size calculations using mean and standard deviations of baseline and peak amplitude MCAV from a study of dynamic MCAV response to exercise (Billinger et al., 2017) was estimated to be 8 for a power of 90% and α equal to 0.05. A sample size of 9-11 is typical for investigations of MCAV response to exercise (Billinger et al. 2017**,** Ward et al. 2018) and gas inhalation (Ogoh et al., 2009). Accordingly, participants were 10 young, male, non-smokers (aged 23 ± 1.9 y) who were recreationally active but not athletes (VO_2max 42.9 ± 7.8 $ml \cdot kg^{-1} \cdot min^{-1}$, see Appendix Table 1). Inclusion criteria was males or females, 19 to 25 years old, who were able to cycle on a stationary bike. Participants were YA to compare with

results of Billinger et al. (2017). Exclusion criteria were failure to insonate the middle cerebral artery (determined in the first visit), uncontrolled hypertension or diabetes mellitus, history of heart disease, any contraindication to exercise such as respiratory disease or musculoskeletal disorder, or medications that influence heart rate or blood pressure.

Instrumentation and Measurements

All exercise was performed in an upright seated posture on an electrically-braked cycle ergometer (LODE, Groningen, The Netherlands). A programmable controller unit allowed pre-programmed session sequence so that participants had no warning that the exercise load would change, therefore reducing the likelihood of anticipation and cognitive preparation to load change.

Metabolic measurements were made using a metabolic cart (VistaMini-CPX, VacuMed, Ventura, CA, USA). The inspired side of the breathing valve was connected to a hose leading to a three-way Hans Rudolph valve that was manually controlled to turn in room air or the hypercapnic gas mixture from a Hans Rudolph gas bag (Series 600, Hans Rudolph).

Continuous BP was measured using finger-cuff plethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands). Modelflow software with a proprietary algorithm estimates stroke volume and calculates Q and HR. A 2-MHz Transcranial Doppler Device (TCD) (Compumedics DWL, Singen DE) was applied over the

right MCA to measure blood velocity. During each condition, $P_{ET}CO_2$ was measured using infra-red absorption technology (CapStar-100 Capnograph, CWE Inc.Ardmore, PA, USA).

BP, MCAV, and $P_{ET}CO_2$ were sampled at 1kHz using an analog to digital converter (PowerLab) interfaced with LabChart7Pro for signal calibration and real-time display (both from ADInstruments, Colorado Springs, CO, USA). HR (Polar monitor) and breath by breath metabolic variables were measured separately.

Procedures

Participants came to the lab twice.

Visit 1. The consent form, including all procedures, was explained to participants who were encouraged to ask questions before signing. Height and weight were measured. TCD was used to verify that the MCA could be successfully insonated. Participants then performed a graded exercise test to determine VO_2max and Wmax (255 ± 20.3 W, Appendix Table 4.5) on a LODE cycle ergometer (CE). Participants cycled at 100 W for 2 min with 30 W increments every 2 min until volitional fatigue. After some recovery, participants were familiarized with the hyperventilation technique (described below) that they would perform during the experiment sessions. Participants were coached to hyperventilate enough to lower $P_{ET}CO_2$ by 20% during 0 W cycling and maintain the $P_{ET}CO_2$ level during a transition to 110 W. Data from this familiarization were used to set the workload for the experiment (50%Wmax, 125 ± 13.5 W, Appendix Table 4.5) and ensure it was under ventilatory

threshold, above which MCAV is known to decrease. A workload of 50%Wmax was high enough to elicit elevations in MCAV in all participants but low enough to raise VO_2 to the range of 19 to 27 ml/kg/min, values in the moderate to low end of vigorous intensity that are similar to intensities performed during ADL in the previous studies of this thesis.

Visit 2: Experiment Sessions 1 and 2. At least one week after the first visit, participants returned to the lab and were instrumented for non-invasive measurements of MCAV, BP, and HR. Participants rested for 10 min. They then mounted the cycle ergometer, positioned the mouthpiece and nose clips, and began cycling at 0 W for 5 min. Thereafter, the condition (control, hyper-or hypocapnia) was applied and participants cycled another 5 min at 0W, followed by a step increase in load to 50% Wmax maintained for 5 min. Participants completed the combined 5 min loadless pedaling and step increases in work rate under three conditions within each of the two sessions: Control, Hypercapnia, and Hypocapnia, for a total of six zero load to 50%Wmax transitions in sessions 1 and 2.

Hypercapnia was achieved by breathing from a bag containing 5% CO_2, 21% O_2, balance N_2. For Hypocapnia, participants were coached to hyperventilate in time with a metronome and adjust breathing depth to decrease $P_{ET}CO_2$ by approximately 20% and maintain this level during exercise by increasing breathing frequency and tidal volume.

The order of the conditions within sessions was varied. Zero load cycling for 5 min with normal respiration followed each exercise bout, and participants were allowed to stand up and have a drink of water if they wished before commencing the next condition. Between sessions, participants dismounted the cycle ergometer for 20 min.

Data Analysis

All data were scrutinized for reasonable signal waveforms during data collection. Following data collection, beat by beat analyses of MCAV, MAP, CO, and HR (calculated from peak topeak intervals of MCAV waveforms) were conducted using LabChart macro protocols. Similarly, breath by breath analyses were conducted for $P_{ET}CO_2$. All data were time interpolated to 1 Hz (SigmaPlot, Systat Software Inc., Chicago, IL). Data were smoothed using binned averages of 3 seconds therefore 297 smoothed data points were included in the analyses. The two sessions were averaged, except where Finometer BP measurements were unreliable in a given session and condition. Two sessions were averaged to reduce the noise to signal ratio of the MCAV variable. Previous literature investigating MCAV kinetics employed one trial (Ogoh et al., 2009; Ward et al., 2018) although Billinger et al. (2017) used three trials to investigate test-retest repeatability. $PaCO_2$ was estimated from $P_{ET}CO_2$ using the formula (Jones et al., 1979): Estimated $PaCO_2 = 5.5 + 0.9 \times P_{ET}CO_2 - 0.0021 \times V_T$, where V_T is tidal volume .

To assess the validity of using a first order equation to describe MCAV kinetics, the predicted equation (Equation 4.1, $MCAV(t) = BL + Amp(1-e^{-(t-TD/\tau)})$) was applied to MCAV (y) and time (x) data of all three conditions, using the Dynamic Fit Wizard feature of SigmaPlot. Baseline was the average of the last min of cycling at 0 Watts under the condition. The equation fit amplitude, time delay (TD), and tau. TD is the point at which the fitted line intersects with the baseline. All 5 min of the exercise data were included in the fit. Residuals of the fit, were plotted and visually inspected for uniform, random scatter

(ungrouped), uniform width around the zero line (indicating equal variance), and uniform horizontal scatter (without slopes) which would indicate the model was not a good predictor of the data. Assumption testing included Durbin Watson test to determine if the residuals were independent from one another, constant variance test to determine if the model follows the pattern of the data, Shapiro-Wilk normality test to determine if the data represent a normal distribution.

In order to determine how a change in $PaCO_2$ and/or MAP affected MCAV from exercise onset to 5 min, three models were developed ($MCAV_{CO2}$, $MCAV_{MAP}$, and $MCAV_{CO2MAP}$) as follows:

$$MCAV_{CO2} = MCAV_{BL} + (PaCO_{2i} - PaCO_{2BL}) \times M_{CO2} \quad \text{(Equation 4.2)}$$

Where: $MCAV_{CO2}$ is the value of MCAV at any time after t=0 due only to changes in $PaCO_2$. $MCAV_{BL}$ and $PaCO_{2BL}$ were averaged during 1 minute at 0 W cycling prior to t=0. $PaCO_{2i}$ is $PaCO_2$ at any time after t=0. M_{CO2} *is the slope of the regression analyses of dependent variable, MCAV versus independent variable, $PaCO_2$.*

$$MCAV_{MAP} = MCAV_{BL} + (MAP_i - MAP_{BL}) \times M_{MAP} \quad \text{(Equation 4.3)}$$

Where: $MCAV_{MAP}$ is the value of MCAV at any time after T=0 due only to changes in MAP. MAP_i is MAP at any time after T=0 and MAP_{BL} is averaged during 1 minute at 0 W cycling prior to T=0. M_{MAP} *is the slope of the regression analyses of MCAV versus MAP.*

$$MCAV_{CO2MAP} = MCAV_{BL} + [(PaCO_{2i} - PaCO_{2BL}) \times Mi] + [(MAP_i - MAP_{BL}) \times Mii]$$

(Equation 4.4)

Where $MCAV_{CO2MAP}$ is the value of MCAV at any time after T=0 due to changes in both $PaCO_2$ and MAP. M_i *and Mii are the slopes for CO_2 and MAP, respectively from the multiple regression analyses of MCAV versus CO_2 and MAP.*

Statistical Analysis

Statistical analyses were administered with SigmaPlot 12.5 (Systat Software, Inc., San Jose California, USA). Significance was acknowledged at alpha =.05. Where required, post-hoc pairwise multiple comparison procedures used Holm-Sidak method.

To determine the effect of condition or session order, baseline BP, MCAV, and $P_{ET}CO_2$ measured during zero watt cycling while breathing room air was averaged over the last min before breathing intervention began. A two-way ANOVA with two factors within (session and condition) was applied to the data.

To determine the effectiveness of the breathing regimes in altering $PaCO_2$, the magnitude of change in $P_{ET}CO_2$ and P_aCO_2 from the last min of baseline to the last min of exercise load was calculated (delta$P_{ET}CO$, deltaP_aCO_2). Average $P_{ET}CO_2$ and $PaCO_2$ were calculated over the last min of baseline with condition and the last min of exercise to represent Baseline ($BLP_{ET}CO_2$, $BLPaCO_2$) and between four and five minutes of exercise ($SSP_{ET}CO_2$,

SS$PaCO_2$), respectively. A one-way ANOVA with one repeated-measures factor (condition) was applied to the data.

To determine the effect of condition on MCAV kinetics, a one-way repeated measures ANOVA was applied to the coefficients from curve fitting with the predicted equation (Equation 4.1) for each condition. Since MCAV response was suppressed in Hypocapnic condition and in several subjects did not reflect an exponential curve, separate similar ANOVA analyses were performed without Hypocapnia. Comparisons of coefficients of the best predicted fit during Hypercapnia and Hypocapnia were compared to Control, separately.

To determine the contribution of CO_2 and/or MAP in the dynamic response of MCAV to exercise load, several statistical analyses were applied to the data. Similar to delta and SS calculations for $P_{ET}CO_2$ and $PaCO_2$ described above, the magnitude of change in MCAV and MAP from the last min of baseline to the last min of exercise load was calculated (deltaMCAV and deltaMAP, respectively). Average MCAV and MAP were calculated over the last min of baseline with condition and the last min of exercise to represent Baseline (BLMCAV, BLMAP) and between four and five minutes (last minute) of exercise (SSMCAV , SSMAP), respectively. A one-factor (Condition) repeated measures ANOVA was applied to each of BLMCAV, BLMAP, deltaMCAV, deltaMAP, SSMCAV and SSMAP. Furthermore, single linear regression analyses with dependent variable MCAV and independent variable $PaCO_2$ or MAP were applied to the individual data for 5 min of

exercise in each condition. Multiple regression analysis was applied with dependent variable MCAV and independent variables $PaCO_2$ and MAP for each participant and each condition. A two-factor ANOVA with repeated measures on both factors: variable with three levels: $PaCO_2$, MAP, or $PaCO_2$ and MAP, and condition with three levels: Control, Hypocapnia, and Hypercapnia, was applied to the regression standard error of the estimate (SEE) and R^2, separately. To determine if there was a difference in slope of regressions between conditions for a given variable MAP or $PaCO_2$, a two-factor ANOVA with repeated measures on both factors: variables with two levels: $PaCO_2$ and MAP, and condition with three levels: Control, Hypocapnia, and Hypercapnia, was applied to the regression resultant slopes. Finally, to compare the methods of assessing gain in MCAV due to either $PaCO_2$ or MAP, the slope of the single linear regressions for MCAV versus $PaCO_2$ was compared to deltaMCAV/delta $PaCO_2$ and the slope of the linear regressions for MCAV versus MAP was compared to deltaMCAV/deltaMAP using paired t-tests for each condition separately.

In order to determine how the slope or gain in the linear regressions affected the relationship between MCAV and $PaCO_2$ and/or MAP, the three models ($MCAV_{CO2}$, $MCAV_{MAP}$, and $MCAV_{CO2MAP}$) were compared to measured MCAV overall and each sec by applying a two-way ANOVA with repeated measures on both factors: Time (297 s) and Model (MCAV, $MCAV_{CO2}$, $MCAV_{MAP}$, $MCAV_{CO2MAP}$) for each condition separately.

4.4 Results

There was no effect of condition or session order on MCAV ($F_{(2,34)}$ = 3.07, p=0.06; $F_{(1,16)}$ = 44, p=0.52, respectively), $P_{ET}CO_2$ ($F_{(2,32)}$ = 0.21, p=0.82; $F_{(1,16)}$ = 0.48, p=0.50, respectively), or MAP ($F_{(2,32)}$ = 2.24 p=0.12; $F_{(1,17)}$ = 0.53, p=0.48, respectively), during baseline cycling at 0 Watts without condition, suggesting that varying the order of conditions was effective and rest periods between conditions and sessions were sufficient. **Figure 4.1** illustrates the average response of measured variables to exercise during each condition. Participant 7 showed unreliable MAP measurements in the Control condition and was unable to perform adequate hyperventilation.

Effectiveness of the breathing regimes on $PaCO_2$ and MAP

$BLPaCO_2$ and $SSPaCO_2$ were greater during Hypercapnia and lower during Hypocapnia compared to Control ($F_{(2,28)}$ = 140.9, p<0.001 and $F_{(2,27)}$ = 326.3, p<0.001, respectively, **Table 4.1**). As expected, the magnitude of change in $PaCO_2$ (delta$PaCO_2$) from the last min of baseline to the last min of exercise load was greater in Hypercapnia and lower in Hypocapnia compared to Control ($F_{(2,28)}$ = 29.1, p<0.001, **Table 4.1**).

BLMAP and SSMAP was greater during Hypercapnia than Control ($F_{(2,28)}$ = 12.1, p<0.001,$F_{(2,27)}$ = 14.8, p<0.001, respectively, **Table 4.1**), with no difference in SSMAP between Control and Hypocapnia. There was no significant effect of condition on deltaMAP.

Exponential fitting of MCAV

The response of MCAV to exercise load from onset to five minutes was fit with a single exponential equation using Equation 4.1. The goodness of fit of the resultant predicted values was assessed using residuals and assumption testing. A sample of curve fitting results and the accompanying residual plots for each are provided for one participant for Control in **Figure 4.2A and B, respectively**. In addition, the time-based response in absolute values of MCAV, MAP, and $PaCO_2$ are provided in **Figure 4.2C** and the change in these variables from baseline is presented in **Figure 4.2D**. In all participants in Control, residuals of the function were not random, but instead were grouped and patterned, even showing slopes that clearly indicate that some factor other than randomness was contributing to the variation. At the onset of exercise MCAV decreased in every participant showing residuals above zero then fall below zero within 20 to 50 s (see example for one subject in **Figure 4.2B**). **Figures 4.2C** and **4.2D** provide visual support for the close relationship between changes in MCAV and changes in MAP and $PaCO_2$.The final assessment of goodness of fit was in assumption testing which showed that Durbin-Watson test failed in all participants, the constant variance test failed in 5/10 and Shapiro-Wilk normality test failed in 8/10 participants.

Group averaged coefficients of the predicted line of fit are provided in **Table 4.2** and individual coefficients for Control, Hypocapnia, and Hypercapnia in **Appendix Table 4.6**. TD ranged from -13.5 to 50 s (**Table 4.6**) but was less than 7 s in 6 of the 10 participants for the Control condition. In Hypocapnia, TD was less than 1 s in all individuals and in Hypercapnia only 1 participant showed a TD of greater than 18 s (**Table 4.6**). Mean

estimated tau for Hypocapnia was about 5 to 11 times greater than for Control or Hypercapnia and reflects a slow and dampened MCAV response to exercise (**Table 4.2**). ANOVA was applied to group averaged best fit coefficients to compare conditions (**Table 4.2**). Significant findings were only seen in the baseline coefficient ($F_{(2,28)} = 121.1$, $p < 0.001$). Post-hoc comparisons showed baseline in Control was lower than in Hypercapnia ($t_{(1,18)} = 14.9$, $p<0.001$) and higher than in Hypocapnia ($t_{(1,18)} = 4.2$, $p<0.001$).

The dampening effect of Hypocapnia on MCAV repressed ANOVA findings. When Hypocapnia was removed from the analyses, baseline, amplitude, and tau were greater during Hypercapnia than Control (Baseline: $F_{(1,19)} = 153.7$, $p < 0.001$; Amplitude $F_{(2,19)} = 23.6$, $p < 0.001$; Tau: $F_{(2,19)} = 5.8$, $p < 0.001$, **Table 4.2**). The finding that amplitude in Control was different only from Hypercapnia is consistent with the SSMCAV ($F_{(1,28)} = 154.8$, $p<0.001$) and the deltaMCAV ($F_{(2,28)} = 20.7$, $p<0.001$, **Table 4.1**), showing elevations during Hypercapnia compared to Control with no significant differences between Control and Hypocapnia. SEE was lower ($F_{(2,28)} = 7.6$, $p = 0.004$) in both Hypocapnia ($t_{(1,18)} = 3.8$, $p = 0.004$) and Hypercapnia ($t_{(1,18)} = 2.5$, $p = 0.046$) compared to Control.

The individual variability in the presence or absence of TD, and of the "exponential" increase in MCAV can be appreciated from the individual responses, shown without curve fits, in **Figures 4.3, 4.4, and 4.5**.

The relative contribution of $PaCO_2$ and/or MAP to MCAV during exercise

Linear Regressions

Two single linear regressions (MCAV vs. $PaCO_2$ and MCAV vs. MAP and one multiple linear regression (MCAV vs. $PaCO_2$ and MAP) were conducted for each individual (**Appendix Tables 4.7, 4.8, and 4.9)**. MCAV was best predicted by including both $PaCO_2$ and MAP in a regression compared to only $PaCO_2$ or MAP alone as determined by a main effect of SEE ($F_{(2,16)} = 17.21$, $p < 0.001$, **Table 4.3**). In Control, R^2 in MCAV vs. $PaCO_2$ and MAP (Model by Condition Interaction $F_{(4,80)} = 4.95$, $p = 0.003$) was greater than the other two regressions. However, in Hypocapnia, R^2 in MCAV vs. $PaCO_2$ and MAP was greater only than MCAV vs. $PaCO_2$ ($t_{(1,18)} = 3.2$, $p = 0.009$) and was similar to R^2 in MCAV vs. MAP. Conversely in Hypercapnia, the opposite was true with R^2 in MCAV vs. $PaCO_2$ and MAP greater than in MCAV vs. MAP ($t_{(1,18)} = 3.9$, $p < 0.001$) and similar to MCAV vs. $PaCO_2$ (**Table 4.3, Appendix Tables 4.7, 4.8, and 4.9**). R^2 in multiple regression analysis showed that $PaCO_2$ and MAP together explained less of the variation in MCAV during Hypocapnia compared to Control ($t_{(1,18)} = 4.5$, $p < 0.001$) and Hypercapnia ($t_{(1,18)} = 7.2$, $p < 0.001$). The standard coefficients for each of $PaCO_2$ and MAP for individual multiple regressions during each condition highlight the inter-individual variability (**Appendix Table 4.9**). Slopes of linear regressions applied to MCAV versus $PaCO_2$ during Control were greater than during Hypercapnia ($p<0.001$) and lower than during Hypocapnia ($p = 0.033$) (**Table 4.4**). Slopes of linear regressions applied to MCAV versus MAP was greater during

Control than during Hypocapnia ($p < 0.001$) (**Table 4.4**) with no difference between Control and Hypocapnia.

The use of slope to represent gain in our models is supported by the findings that slope of MCAV vs. $PaCO_2$ was similar to deltaMCAV/deltaPaCO_2 in all conditions. Furthermore, linear regression slope of MCAV vs. MAP and deltaMCAV/deltaMAP was also not different in Control and Hypocapnia but did however, show a significant difference in Hypercapnia ($t_{(1,18)} = -5.60$, $p < 0.001$).

Modeling: MCAV predicted from changes in $PaCO_2$ and/or MAP

Slopes from the MCAV versus $PaCO_2$ and/or MAP regressions were used to estimate the gain in the MCAV due solely to either or both $PaCO_2$ and MAP in the development of models $MCAV_{CO2}$, $MCAV_{MAP}$, and $MCAV_{CO2MAP}$ (see Equations 4.2, 4.3, and 4.4 in Methods). As described above, the R^2 values and SEE provide acceptable support for the use of these regression slopes. Measured MCAV (MCAV) was compared to MCAV predicted from changes in both $PaCO_2$ and MAP ($MCAV_{CO2MAP}$) for each participant in **Figure 4.3** for Control, **Figure 4.4** for Hypocapnia, and **4.5** for Hypercapnia. In Control conditions, it appears that the MCAV was well predicted by $MCAV_{CO2MAP}$. One participant (D9) shows a shift in $MCAV_{CO2MAP}$ due to a difference between the baseline MCAV (calculated over the last min of 0 W cycling with condition) and the MCAV at exercise onset. In Hypocapnia, there is greater discrepancy between measured and predicted $MCAV_{CO2MAP}$ due at least partially to baseline offset in two participants (D2, D4). $MCAV_{CO2MAP}$ underpredicted

measured MCAV in participants D2, D4 and D6 during most of the 5 min shown by predicted values well below measured. Underestimation of MCAV is also seen in participant D3 during the last 3 min of exercise following overestimation in the first 2 min. In three participants (D8, D10, D11) MCAV is seemingly well predicted by $MCAV_{CO2MAP}$. During Hypercapnia, the model more closely predicts measured MCAV, however overestimates MCAV within the first 150 s most notably in D8 and D9, but also in D1, D4 and D10.

The effectiveness of each of the three models ($MCAV_{CO2}$, $MCAV_{MAP}$, $MCAV_{CO2MAP}$) in predicting MCAV in all conditions is illustrated in **Figure 4.6**. The difference between MCAV and predicted MCAV by each of the models for each min of the exercise was analyzed using ANOVA. A significant main effect of MCAV ($F_{(3, 10727)} = 4.2$, $p = 0.017$) whereby MCAV was greater than $MCAV_{MAP}$ ($t_{(3, 294)} = 3.0$, $p=0.034$). There was a significant Time by Model interaction ($F_{(891, 10727)} = 1.96$, $p < 0.001$). MCAV was best predicted by $MCAV_{CO2MAP}$ in all conditions, illustrated by a more precise spread around zero and fewer time points with significant differences between measured and predicted MCAV. $PaCO_2$ made a greater contribution to $MCAV_{CO2MAP}$ than MAP, at least in Control and Hypercapnia where significant differences between MCAV and $MCAV_{CO2}$ (panel A) are also observed in $MCAV_{CO2MAP}$ (panel C) but significant differences between MCAV and $MCAV_{MAP}$ (panel B) are not. In Control, there are only 7 of the 297 s with significant difference between measured MCAV and $MCAV_{CO2MAP}$. These same 7 s time points also showed significant difference between MCAV and $MCAV_{CO2}$ but only one overlapped with $MCAV_{MAP}$. Of the 297 s, MCAV was significantly different from $MCAV_{CO2}$ at only 26 s and from MAP at 91

sec. In Hypocapnia, all models underestimated MCAV (as seen by the scatter of values above zero) and significant differences for most of the time between 90 and 297 s. In Hypercapnia, $MCAV_{CO2}$ significantly overestimated MCAV during the first 150 s but adequately predicted MCAV during the last 2-3 min. In contrast, $MCAV_{MAP}$ adequately predicted MCAV during the first 150 s with few minutes of significant difference from MCAV but consistently underpredicted MCAV in the last 2-3 min. $MCAV_{CO2MAP}$ showed the same trends as $MCAV_{CO2}$.

4.5 Discussion

This study demonstrates that the dynamic response of MCAV to constant moderate/low vigorous exercise is greatly influenced by arterial CO_2 and is not adequately described by a single exponential equation.

Evidence Against a Single Exponential Fitting of MCAV Kinetic Response to Exercise

Individual MCAV responses to exercise over time were fit with a single exponential equation that fit amplitude, time delay, and tau. Goodness of predicted curve fit was evaluated by visual assessment of residuals and assumption testing. Residuals were clearly clumped and patterned rather than uniform and random (for an example see Figure 4.2). There were clear slopes and peaks or valleys in the residuals indicating that other factors may have influenced the kinetic response. Furthermore, individual variability in the exponential fit was seen in the

wide range in amplitude and tau values. Finally, assumption testing of the curve fitting indicated mostly failed parameters of constant variance, residual independence, and normality. Therefore, consistent with our hypothesis, we conclude that MCAV kinetic response to exercise onset is not well described by a first order system. This finding is in contrast to previous literature. Billinger et al. (2017) concluded the increase in MCAV with moderate exercise was well fit by a time delay and exponential function, supported by R^2 values, p-value significance, and inspection of residuals. However, visual inspection of their displayed residuals provided for only one subject, reveal long periods (40 sec) where the residuals lie below zero and a period of well over 90 sec where they lie above zero. From the same lab, Ward et al. (2018) provided a figure of the group mean MCAV response during six min of moderate exercise with residuals that were similar to those of Billinger in their patterns and non-uniformity. Therefore, a single exponential equation to describe MCAV kinetic response to exercise was not supported by data in these studies.

Methodology to apply exercise workload was designed differently in the current study compared to Billinger et al (2017) and Ward et al. (2018), influencing the MCAV dynamic response to exercise. Both previous studies applied the workload in their experiments from rest in a multi-level, step-wise increase consisting of three 10 s intervals, therefore taking 30 s total to apply the target workload which is apparent in their figure of the MCAV response versus time. Billinger identified initial data "preceding a rise in MCAV" as a time delay and omitted it from curve fitting (Billinger et al., 2017). Accordingly, Billinger and Ward reported greater TD (44 s and 53 s, respectively) compared to our study where TD

was fit by the equation (11 s). Participants in our study were cycling at 0 Watts and were already engaged in the condition (Control, Hypercapnia, or Hypocapnia) when the exercise load was applied without warning. Therefore, the baseline contained all aspects of the exercise load condition, prior to the exercise load. It was our intention that this would reduce the anticipatory neurovascular response and cardiovascular adjustment to load and provide a more appropriate baseline from which the effect of exercise alone could be observed in each condition.

In every participant we observed a steep decline in MCAV followed by an increase at the onset of exercise load and lasting up to almost a minute. This initial response can also be observed in the data shown by Billinger et al. (2017) once the final load was achieved. The initial disturbance in MCAV may reflect the activation of mechanisms known to regulate exercise response such as central command, neurovascular coupling, type III afferents, carbon dioxide, blood pressure, and neurohumoral responses to exercise (for reviews see (Querido & Sheel, 2007; Smith & Ainslie, 2017). Central command is a feed-forward mechanism with signals arising from the forebrain or midbrain that activate descending motor neurons and concomitantly, stimulate the cardiovascular control centre (CCC) in a dose dependent fashion (Asahara et al., 2018). Deformation of contracting muscles stimulates mechanoreceptors (Afferent III) which have been shown to increase CBF (Braz et al., 2014; Jorgensen et al., 1993). Activation of CCC elicits rapid withdrawal of parasympathetic nervous system activity (PNA) and a slightly slower increase in sympathetic nervous system activity (SNA) increasing HR, CO, and BP. With exercise onset, CCC also resets the

baroreceptors set point to a new blood pressure consistent with exercise intensity (reviewed by (Murphy et al., 2011)) allowing for increases in sympathetic nervous system activity. In healthy individuals, cerebral autoregulation (CA) has a lag over a few cardiac cycles. With increasing metabolism, alveolar ventilation is increased to tightly regulate $PaCO_2$. All of these responses would need to meet the principles of a first order system and hold the same first order properties in order for MCAV kinetics to be appropriately fitted by a single exponential equation. It is therefore, reasonable to conclude that the aforementioned mechanisms collectively would confound the MCAV kinetic response to exercise, prohibiting a first order response, and creating interindividual variability. In this study we examined how two of these mechanisms, $PaCO_2$ and MAP, influence MCAV kinetic response to exercise.

Prediction of MCAV by changes in $PaCO_2$ and MAP

Models

Three models, $MCAV_{CO2}$, $MCAV_{MAP}$, and $MCAV_{CO2MAP}$, were developed to predict MCAV response to exercise that were associated specifically to changes in $PaCO_2$ or MAP, or both $PaCO_2$ and MAP, respectively and their corresponding gains determined by slopes of linear regressions (see equations 4.2, 4.3, and 4.4). In this way, the models represent the MCAV response predicted by the change and gain of the corresponding variable only. The gain for $MCAV_{CO2}$ and $MCAV_{MAP}$ is represented by the slope of the single linear regressions while the gain for $MCAV_{CO2MAP}$ is represented by the slopes for CO_2 and MAP of the multiple

linear regression. The models allow a statistical comparison of MCAV predicted by each or both variables with the measured MCAV. The limitation of the models may be in the use of regression slopes to represent gain for three reasons. First, linear regression is primarily influenced by the last 2.5 to 3 min of the response which is relatively stable. Conversely, MCAV kinetic response is greatest in the first 1 to 2 min as indicated by a tau of less than 45 sec in 7 of the 10 participants and mean tau of 52 sec in Control. Second, some regression equations failed to meet some or all of the assumption testing. Third, regressions did not include baseline but started at the onset of exercise, including some of the "adjustment" phase of MCAV. Therefore, to assess the validity of slope to represent gain, slopes were compared to apposite delta ratios as follows. DeltaMCAV/deltaPaCO_2 was not statistically different from MCAV vs PaCO_2 regression slope in any of the Conditions. Likewise, deltaMCAV/deltaMAP was also similar to MCAV vs MAP regression slopes in both Control and Hypocapnia but not Hypercapnia. In Hypercapnia, MCAV continued to rise throughout the last min of exercise without a concomitant and equivalent increase in MAP which impacted deltaMCAV/deltaMAP to a greater extent than regression slope. Finally, power for all regressions was greater than 0.80. It is our conclusion that the models adequately represent the change in MCAV that would occur due only to the changes and gain in PaCO_2 ($MCAV_{CO2}$), MAP ($MCAV_{MAP}$), or both PaCO_2 and MAP ($MCAV_{CO2MAP}$).

Changes in both $PaCO_2$ and MAP influence MCAV kinetics

Consistent with our hypothesis, changes in $PaCO_2$ and MAP, and their respective gains effectively predict the kinetic response of MCAV to exercise. Visual inspection of Figures 4.3, 4.4, and 4.5 shows that for most participants, $MCAV_{CO2MAP}$ closely aligned with MCAV changes over time, especially under Control conditions but also during Hypocapnia and Hypercapnia. Considerable support for the influence of both $PaCO_2$ and MAP responses in MCAV dynamics is provided in Figure 4.6. The difference between MCAV and $MCAV_{CO2MAP}$ was more precisely and consistently spread around zero compared to models with $PaCO_2$ or MAP alone, at least in Control and Hypercapnia. Furthermore, in all three conditions there were fewer time points when the difference between MCAV and $MCAV_{CO2MAP}$ was significant. This was especially true in Control where $MCAV_{CO2MAP}$ differed from MCAV in only 7 sec of the 5 min exercise. In Control regression analysis, more variation in MCAV was explained by $PaCO_2$ and MAP together ($R^2 = 0.58$) than by MAP or $PaCO_2$ ($R^2 = 0.47$ and 0.40, respectively) alone. Furthermore, a statistical main effect showed variability in linear regression (SEE) was lower when both MAP and $PaCO_2$ were included in the model.

Further support for the influence of $PaCO_2$ and MAP on MCAV kinetics can be seen in Figure 4.2 where changes in each variable are aligned with the MCAV response.

$PaCO_2$ as the primary influence in MCAV kinetics

In agreement with our hypothesis, change in arterial CO_2 and its respective gain was shown to play the primary role in the kinetic response of MCAV to exercise. In Control and Hypercapnia conditions, significant differences between MCAV and $MCAV_{CO2}$ (see Figure 4.6A) are also observed in $MCAV_{CO2MAP}$ (Figure 4.6C) whereas differences between MCAV and $MCAV_{MAP}$ (Figure 4.6B) are not always reflected in $MCAV_{CO2MAP}$. More specifically, of the 297s of exercise, MCAV was significantly different from $MCAV_{CO2}$ during 26 s which overlapped with the same 7 s in which $MCAV_{CO2MAP}$ differed from MCAV. However, $MCAV_{MAP}$ was different from MCAV for 91 s, not all of which overlapped with either or both of $MCAV_{CO2MAP}$ and $MCAV_{CO2.}$ Further evidence of the primary influence of $PaCO_2$ compared to MAP in MCAV kinetics is seen from manipulation of CO_2. Hypercapnia resulted in a greater MCAV at last minute and a greater rise from baseline to last minute (deltaMCAV, Table 4.1; amplitude, Table 4.2) compared to Control and Hypocapnia. Conversely, there is evidence that Hypocapnia dampened the MCAV response to exercise. When CO_2 was suppressed, MCAV response was slower and inhibited in most participants (compare Figures 4.3 and 4.4). BLMCAV and SSMCAV were lower in Hypocapnia compared to Control. Furthermore, the linear regression slope of MCAV versus $PaCO_2$ was negative in three cases in Hypocapnia, while all relationships in Hypercapnia and Control were positive. While BL and SSMAP were greater in Hypercapnia than Control and Hypocapnia, deltaMAP was not different between conditions. The dominant influence of $PaCO_2$ demonstrated in this study is in contrast to conclusions by Billinger et al. (2017) and

Ward et al. (2018) who reported no significant correlation between changes in MCAV and changes in $P_{ET}CO_2$ or MAP.

Effect of Hypercapnia on prediction of MCAV by $PaCO_2$ and MAP

Hypercapnic condition shows some interesting patterns in the ability of $PaCO_2$ and MAP to predict MCAV during the transition from baseline to about 150s and then during a more consistent period in the last 2-3 min of exercise (see Figure 4.6). In this section, the patterns will be described with possible mechanisms proposed first for the initial transition period and then for the final minutes of exercise.

Transition:

$MCAV_{CO2MAP}$ overestimated MCAV during Hypercapnia for about the first 150 s (see Figure 4.6C, Hypercapnia), approximately the time to a more stable MCAV response estimated from Figure 4.5C. During this transition period, MCAV predicted by changes in CO_2 also overpredicted MCAV. MAP, on the other hand, underestimated MCAV in a progressive and somewhat linear fashion. An explanation may be described by Edwards et al. (2004) who administered two breaths of elevated CO_2 (step increase) in YA at rest under three conditions: hypocapnia, normocapnia, and hypercapnia and measured MCAV, MAP at the level of MCA ($MAP_{MCA)}$, and cerebrovascular resistance index (CVRi: $MAP_{MCA} \div$ MCAV). Results showed an elevated steady state MAP and MCAV, and lower CVRi during

hypercapnia. Using autoregressive moving average analysis (ARMA) the authors determined that the dynamic cerebral autoregulation response to the step increase in CO_2 was impaired during hypercapnia. In addition, the step change in CO_2 during hypercapnia resulted in a smaller and slower CO_2 induced cerebrovascular response compared to control and hypocapnia (Edwards et al., 2004). Applied to our data, these findings suggest that the higher $MCAV_{CO2}$ compared to MCAV during transition may be due to a smaller and slower cerebral vasodilation in response to changes in $PaCO_2$ that was not reflected in the average slope of the MCAV versus $PaCO_2$ regression over 297 s used to calculate $MCAV_{CO2}$. The higher curve fitting coefficient, tau, in Hypercapnia compared to Control also supports a slower cerebrovascular response to CO_2. Similar discrepancies between $MCAV_{CO2MAP}$ and MCAV can be seen during transition in individual responses in Figure 4.5, most notably in participants D8, D9 and D4 and to a lesser extent, D1 and D10, where predicted MCAV lies at the upper end of MCAV. The rise in MAP with exercise in concert with impaired CA may have contributed to increasing MCAV during transition compensating somewhat for the dampened effect of CO_2. However, the lower relative contribution of MAP to MCAV compared to CO_2 is seen in the transfer of the initial transient overestimation of MCAV to $MCAV_{CO2MAP}$ (see Figure 4.6 Hypercapnia).

Last 2 to 3 min of exercise

First, it is clear from observation of Figure 4.5 that some participants showed continuous increase in MCAV during Hypercapnia, most obviously D3, D6 and D7, but also any

individual with a tau greater than 60 s which includes all but D2 and D4. Therefore, rather than SS, we refer to the last 2-3 min of exercise, where MCAV response tends toward flattening. $MCAV_{CO2}$ sufficiently predicted MCAV during this timeframe but there seems to be a steady increase in under-prediction by $MCAV_{MAP}$. This may be the results of increasing cerebrovascular sensitivity to $PaCO_2$ and some CA impairment discussed above. An increased cerebrovascular reactivity in hypercapnia has been reported during steady state exercise. In 13YA who breathed 5%CO_2 (in room air) during cycling, hypercapnic cerebrovascular reactivity almost doubled at workloads of 30 and 70%HRR (2.7 ± 0.1 and 2.6 ± 0.6 cm/s/mmHg, respectively) compared to rest (1.46 ± 0.6 cm/s/mmHg) (Murrell et al., 2013). $MCAV_{MAP}$ showed a slow but progressive underprediction of MCAV which became significant in the last 2-3 min of exercise.

Effect of Hypocapnia on prediction of MCAV by $PaCO_2$ and MAP

Prediction of MCAV by the models employed in this study was less effective in Hypocapnia. Indeed, there was a significant difference between measured and predicted MCAV by all models consistently after about 90 s. $MCAV_{CO2MAP}$ apparently underestimated measured MCAV in four participants (see Figure 4.4). Both $MCAV_{CO2}$ and $MCAV_{MAP}$ also underestimated MCAV (see Figure 4.6A and 4.6B, Hypocapnia). Regression analyses showed that $PaCO_2$ and MAP explained less of the variation in MCAV during Hypocapnia compared to Control and Hypercapnia (R^2, Table 4.3). $PaCO_2$ was successfully suppressed by hyperventilation as evidenced by lower $SSPaCO_2$ and $deltaPaCO_2$ during Hypocapnia

compared to Control. Accordingly, both BLMCAV and SSMCAV were lower in Hypocapnia compared to Control. Also, visual inspection of the individual MCAV responses to exercise during Hypocapnia (Figure 4.4) shows a dampened MCAV kinetic response to exercise compared to Control (Figure 4.3). Furthermore, slope of linear regressions applied to MCAV versus $PaCO_2$ was lower in Hypocapnia than Control. Surprisingly however, there was no significant difference in deltaMCAV between Hypocapnia and Control which was confirmed by the curve fitting coefficient amplitude, that also showed no difference between Hypocapnia and Control. In summary, while Hypocapnia suppressed the kinetic response to exercise, MCAV did eventually rise by a similar magnitude as that in Control, however, $PaCO_2$ and MAP did not effectively predict the change in MCAV during exercise. These findings suggest that MCAV was influenced by factors other than $PaCO_2$. To understand this further, we asked one participant to rest while sitting on a chair for 5-10 min, then inhale 5%CO_2 for 5 min, and then hyperventilate hard enough to decrease $P_{ET}CO_2$ to resting levels while still inhaling 5%CO_2. The participant watched the $P_{ET}CO_2$ values on the capnograph display and changed their frequency and/or tidal volume at will. Values of $P_{ET}CO_2$ and MCAV were 50.7 mmHg and 54 cm/s respectively at rest and 58.3 mmHg and 64.2 cm/s, respectively at 5% CO_2 inhalation. During hyperventilation, the participant was successful at reducing $P_{ET}CO_2$ to 50.4 mmHg but MCAV remained high at 65.1 cm/s. Therefore, in this one participant, MCAV was elevated by about 11 cm/s by factors other than $P_{ET}CO_2$. It should be noted that the cost of hyperventilation in this participant would be greater than during our study because they were hyperventilating against a high inhaled concentration of CO_2. There is some evidence in the literature that hyperventilation that successfully reduces

$PaCO_2$ still produces similar MCAV or CO_2 reactivity to control. When $P_{ET}CO_2$ was elevated at rest using supplemental CO_2 (5%) or reduced to the same extent by hyperventilation alone, cerebrovascular reactivity was not different between hypercapnia and hypocapnia at rest (Murrell et al., 2013). There is a paucity of research employing hypocapnia during exercise. However, in a similar protocol to our study participants watched a monitor to match tidal volume to a preset level and listened to a metronome to match breathing frequency (Ogoh et al., 2008). The workload of 32% VO_2max was too low to significantly increase MCAV however, the authors found that cerebrovascular reactivity during steady state exercise was greater during hypercapnia than hypocapnia, with no difference between hypocapnia and normocapnia. Edwards et al. (2004) controlled for the effect of controlled breathing by applying similar breathing protocols to all conditions. Participants were asked to maintain breathing rate and tidal volume while $P_{ET}CO_2$ was maintained at baseline (normocapnia), 8 mmHg above (hypercapnia) or 8 mmHg below (hypocapnia) normocapnia using a computerized dynamic end-tidal forcing system. The authors found that steady state MCAV was lower during hypocapnia compared to normocapnia. Our data and the results of these studies suggest that one or more mechanisms other than $PaCO_2$ are responsible for the elevated MCAV during hyperventilation-induced hypocapnia. There is some evidence that one of these factors is MAP. It may be that an increased MAP compensated to some extent for a reduced $PaCO_2$. Significant differences between MCAV and $MCAV_{MAP}$ (Figure 5.4A, Hypocapnia) are also observed in $MCAV_{CO2MAP}$ (Figure 5.4C) whereas most of the significant differences between MCAV and $MCAV_{CO2}$ (Figure 5.4B) not seen in $MCAV_{MAP}$ were not apparent in the combined Model $MCAV_{CO2MAP}$. Alternatively, increases in MCAV

during Hypocapnia may be at least partially explained by the increased demand for cerebral oxygen required to adjust breathing pattern to $P_{ET}CO_2$. Hyperventilation to induce hypocapnia was controlled by setting a metronome for breathing frequency and by verbal feedback to "breathe more or less" in order to alter tidal volume enough to keep $P_{ET}CO_2$ about 20% lower than that during normal cycling at 0 Watts. This requirement for working memory/executive function may have increased neurovascular coupling reflected in elevated MCAV.

Individual variability

The individual variability in MCAV kinetics can be appreciated in the exponential responses to exercise as well as the regression analyses. Figures 4.3, 4.4, and 4.5 illustrate various amplitudes, rate of responses, and fluctuations. Control curve-fitting parameters showed large standard deviations (SD) in amplitude where SD was more than half of the mean (11 ± 6.1), tau where SD was 67% of the mean (53 ± 35), and time delay where SD was 138% of mean (16 ± 22). In regression analyses, $PaCO_2$ and MAP together explained a range of between 34 and 84% of the MCAV kinetic response to exercise under control conditions in young adult participants in this study (refer to R^2 values in Appendix Table 4.9). Furthermore, the relative contributions to MCAV in Control for $PaCO_2$ ranged between 0.13 and 0.82 and for MAP ranged between 0.31 and 0.88 (refer to standard coefficients in Appendix Tables 4.7 and 4.8, respectively). Together these observations exemplify the

complexity of the MCAV kinetic response to moderate exercise and the interindividual variability.

In conclusion, this study demonstrates that changes in both $PaCO_2$ and MAP and their respective gains predict the kinetic response of MCAV to exercise onset. Furthermore, the dynamic response of MCAV does not reliably or adequately meet the requirements of a first order system and is therefore not well represented by a single exponential equation. Finally, there is a great deal of between-individual variability in MCAV dynamics.

4.6 Limitations

1. A limitation of this study is that hyperventilation was controlled by setting a metronome for breathing frequency and by verbal feedback to "breathe more or less" which was achieved by increasing or decreasing tidal volume while maintaining breathing frequency. The ability to control $P_{ET}CO_2$ by breathing was performed well by participants as apparent in Figures 4.1 and 4.3B as well as final minute data and delta values, but some variability was inevitable. Furthermore, this technique introduced a cognitive task that may have elevated MCAV. The cognitive task was minimized by verbal reassurance and minimal verbal instruction.
2. $PaCO_2$ was predicted from $P_{ET}CO_2$ and tidal volume. Although the validation study was based on results of only 5 young, male participants, measured and predicted $PaCO_2$ were highly correlated ($r = 0.98$) over the range of 25 to 58 mmHg (Jones et al., 1979).

3. Models to predict MCAV from changes in $PaCO_2$ and/or MAP employed slopes from linear regressions which were influenced primarily by the last 1-2 min of exercise and did not all pass assumptions testing. Arguments for use of the models are discussed under the subheading “Models” in the Discussion section of this chapter. Briefly, the deltaMCAV/deltaPaCO$_2$ and deltaMCAV/deltaMAP were not statistically different from their respective regression slopes in any of the Conditions with one exception (MAP in Hypercapnia as discussed above). The power for the linear regressions were almost always 1.0 but at least greater than 0.8.

Table 4-1. Effect of condition on variables during baseline, last minute exercise and the difference between them (Delta).

Variable	Control	Hypocapnia	Hypercapnia
BL-$P_{ET}CO_2$ (mmHg)	41.2 ± 4.3	33.0 ± 2.7*	53.4 ± 1.2*!
BL-$PaCO_2$ (mmHg)	42.5 ± 3.7	35.2 ± 2.5*	53.6 ± 1.0*!
BL-MCAV(cm/s)	56.4 ± 9.2	47.5 ± 7.9*	78.2 ± 10.6*!
BL-MAP (mmHg)	94.4 ± 11.3	93.0 ± 10.2	104.9 ± 13.2*!
SS-$P_{ET}CO2$ (mmHg)	46.7 ± 3.6	34.1 ± 2.5*	63.5 ± 2.3*!
SS-$PaCO_2$ (mmHg)	47.5 ± 3.2	36.1 ± 2.2*	62.8 ± 2.1*!
SS-MCAv (cm/s)	65.4 ± 10.5	54.3 ± 7.9*	102.5 ± 15.8*!
SS-MAP (mmHg)	104.4 ± 8.9	103.7 ± 11.3	119.6 ± 13.5*!
$\Delta P_{ET}CO_2$ (mmHg)	5.5 ± 3.3	1.0 ± 1.0*	10.1 ± 2.5*!
$\Delta PaCO_2$ (mmHg)	5.0 ± 3.0	0.9 ± 0.9*	9.2 ± 2.3*!
ΔMCAV (cm/s)	9.1 ± 5.7	6.8 ± 4.8	24.3 ± 9.3*!
ΔMAP (mmHg)	9.1 ± 11.6	10.7 ± 7.2	14.7 ± 6.3

Abbreviations: BL: baseline, SS: last minute of exercise, Δ: delta. Delta indicates change from the last minute of baseline to the last minute of exercise. SS indicates average over the last min of exercise. * difference from Control. ! difference between Hypocapnia and Hypercapnia

Table 4-2. Group-averaged results of single exponential fit of MCAV data from exercise onset to 5 min.

Condition		Baseline (cm/s)	Amplitude (cm/s)	Tau (s)	Time Delay (s)	SEE
Control	Mean	56.4	11.1	52.5	16.0	3.5
	SD	9.2	6.1	35.3	22.1	0.9
Hypocapnia	Mean	47.5*	18.2	520.0	-0.7	2.4*
	SD	7.9	22.9	925.7	12.9	0.4
Hypercapnia	Mean	78.2*!#	27.3#	97.8#	11.0	2.9*
	SD	10.0	8.8	54.0	11.9	0.5

Abbreviations are standard error of the estimate (SEE). MCAV response for participant 8 in Control condition was identified as an outlier (see D8, Figure 4.3 and Appendix 4.6) and was omitted from Control averages. There is no Hypocapnia condition for participant 7. MCAV response was suppressed in Hypocapnia and in several subjects did not reflect an exponential curve. See individual responses in Figure 4.4 and Appendix Table 4.6. * different from Control. ! difference between Hypocapnia and Hypercapnia. When Hypocapnia was removed from the analyses, # indicates difference between C and 5%, $p<0.05$.

Table 4-3. Group-averaged results of linear regressions with MCAV (dependent variable), $PaCO_2$, and MAP and multiple linear regression during three conditions; Control, Hypocapnia, and Hypercapnia.

Regression	Regression coefficients	Control	Hypocapnia	Hypercapnia
A. MCAV vs. CO_2	Std. Coeff.	0.58 (0.26)	0.12 (0.27)	0.77 (0.84)
	R^2	0.40 (0.25)	0.08 (0.08)	0.72 (0.12)#
	SEE	3.66 (0.80)	3.11 (0.97)	4.15 (1.09)
B. MCAV vs. MAP	Std. Coeff.	0.66 (0.17)	0.40 (0.22)	0.76 (0.13)
	R^2	0.47 (0.20)	0.20 (0.16)	0.59 (0.18)$
	SEE	3.42 (0.90)	3.07 (0.88)	5.03 (1.42)
C. MCAV vs. CO_2 and MAP	$PaCO_2$	0.36 (0.22)	0.07 (0.29)	0.61 (0.19)
	MAP	0.46 (0.18)	0.37 (0.21)	0.32 (0.23)
	R^2	0.58 (0.19)**	0.24 (0.11)$	0.79 (0.09)#
	SEE*	3.00 (0.74)	2.81 (0.79)	3.59 (0.99)

Values are mean and (SD). Acronyms are standard coefficient (Std. Coeff) and standard error of the estimate (SEE). Statistical analyses conducted on R^2 and SEE only. * Main effect for SEE showing significant difference of Regression C from A and B. Regression by Condition interaction showing within Condition**difference from Regressions A and B, $ difference from Regression A, # difference from Regression B. $p < 0.05$. Participant 7 did not complete Hypercapnia condition and Participant 8 is missing MAP data. Individual regression equations are found in Appendix tables 4.7, 4.8, and 4.9.

Table 4-4. Comparison of slopes from single linear regressions with related delta values to compare methods of determining gain in MCAV as a function of $PaCO_2$ or MAP.

Variable	Control	Hypocapnia	Hypercapnia
Slope MCAV vs. $PaCO_2$	1.4 (0.8)	0.6 (0.4)#	2.6 (0.9)#
Slope MCAV vs. MAP	0.5 (0.2)	0.2 (0.1)#	0.9 (0.3)#
DeltaMCAV/Delta$PaCO_2$	1.3 (1.1)	7.0 (18.5)	2.7 (0.9)
DeltaMCAV/DeltaMAP	0.7 (0.5)	-1.9 (7.8)	1.7 (0.3)*

*different from slope of respective MCAV vs. MAP. # different from Control

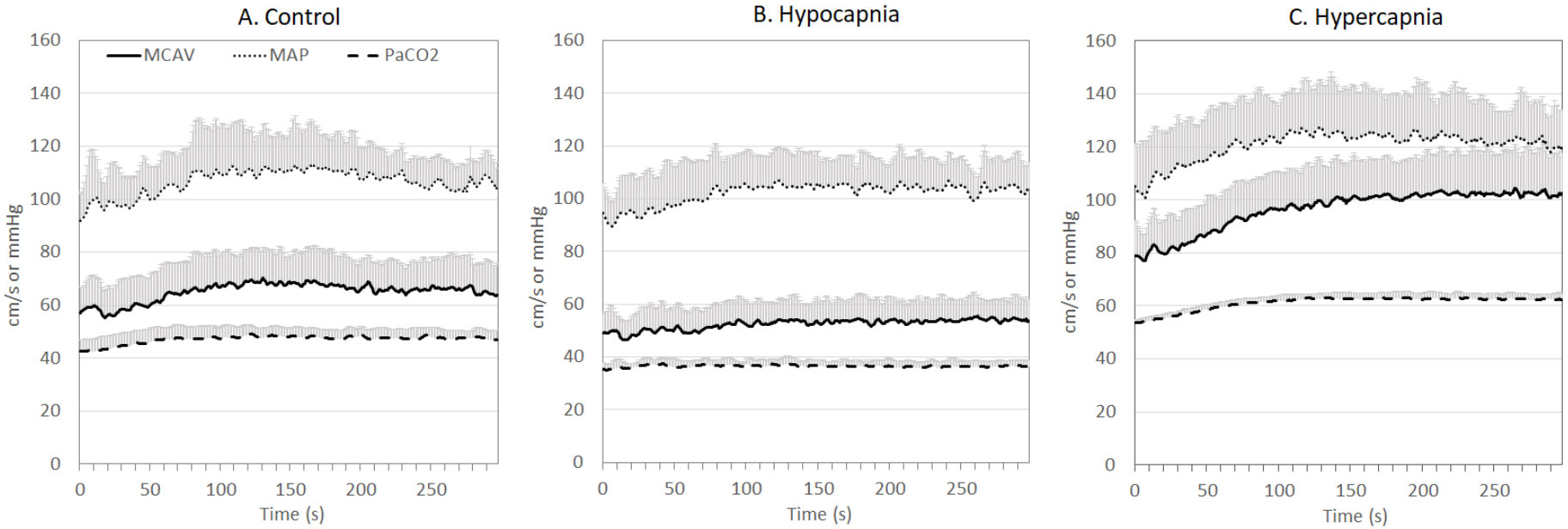

Figure 4-1. Effect of moderate cycling at constant load for 5 min on MCAV, calculated $PaCO_2$, and MAP during three conditions, A. Control, B. Hypercapnia, and C. Hypercapnia.

Dotted, solid, and dashed lines represent MAP, MCAV, and calculated $PaCO_2$, respectively. Values are mean and SD for every second of exercise. Onset of exercise load from a baseline of 0 W (loadless pedalling) is at time = 0.

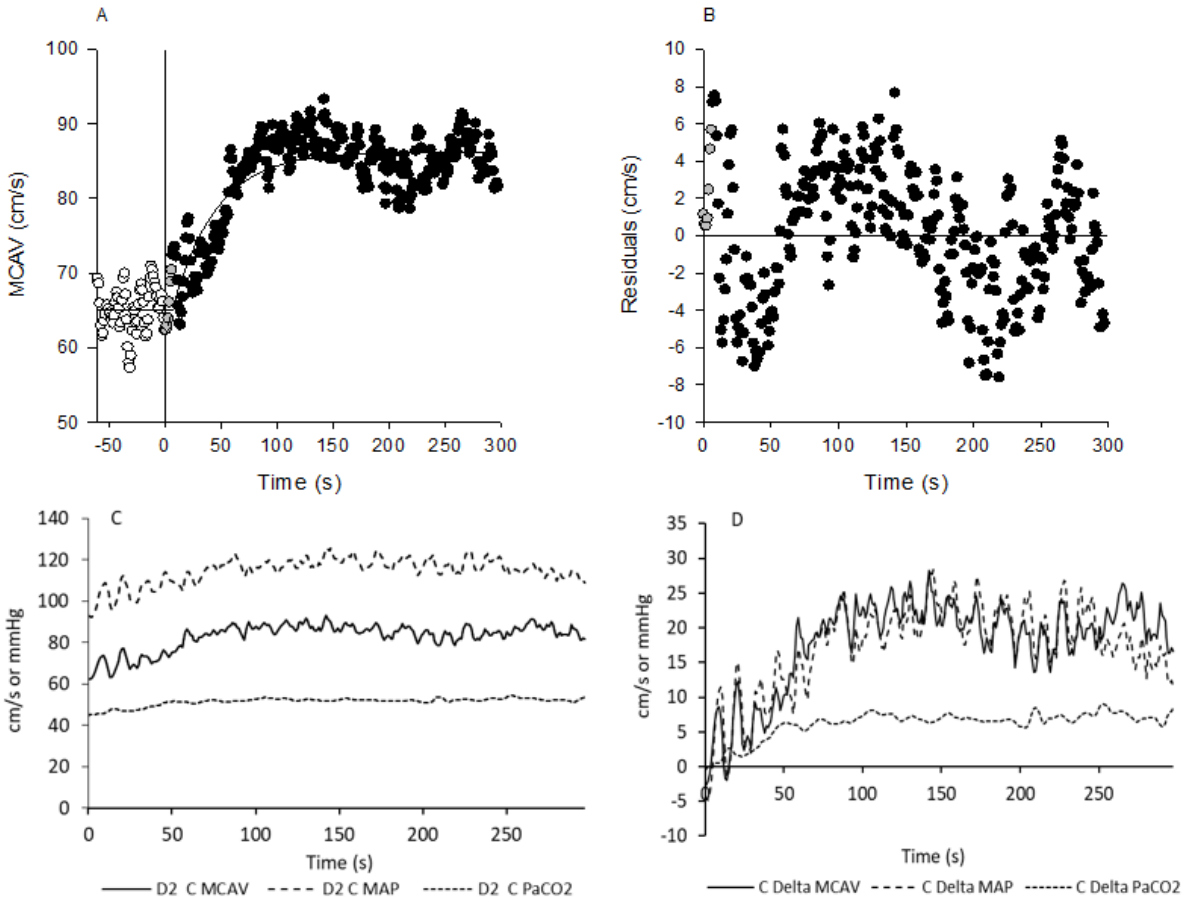

Figure 4-2. Example of beat-by-beat data set for one participant (D2) during exercise in Control with a single exponential fit to MCAV response to exercise from onset to 5 min with residuals of the fit shown to the right.

Time -60 to 0 s represents baseline data. Exercise onset is at time 0 s marked by vertical line. See Figure 5.3 that illustrates individual responses of measured MCAV without curve fit demonstrating the variability in response and presence or absence of TD. In addition, Appendix Table 4.6 shows individual curve fitting coefficients for each condition. The temporal response of MCAV in relation to $PaCO_2$ and MAP (C) and the change in these variables from baseline (D) support a causal role for MAP and $PaCO_2$ in driving MCAV kinetics.

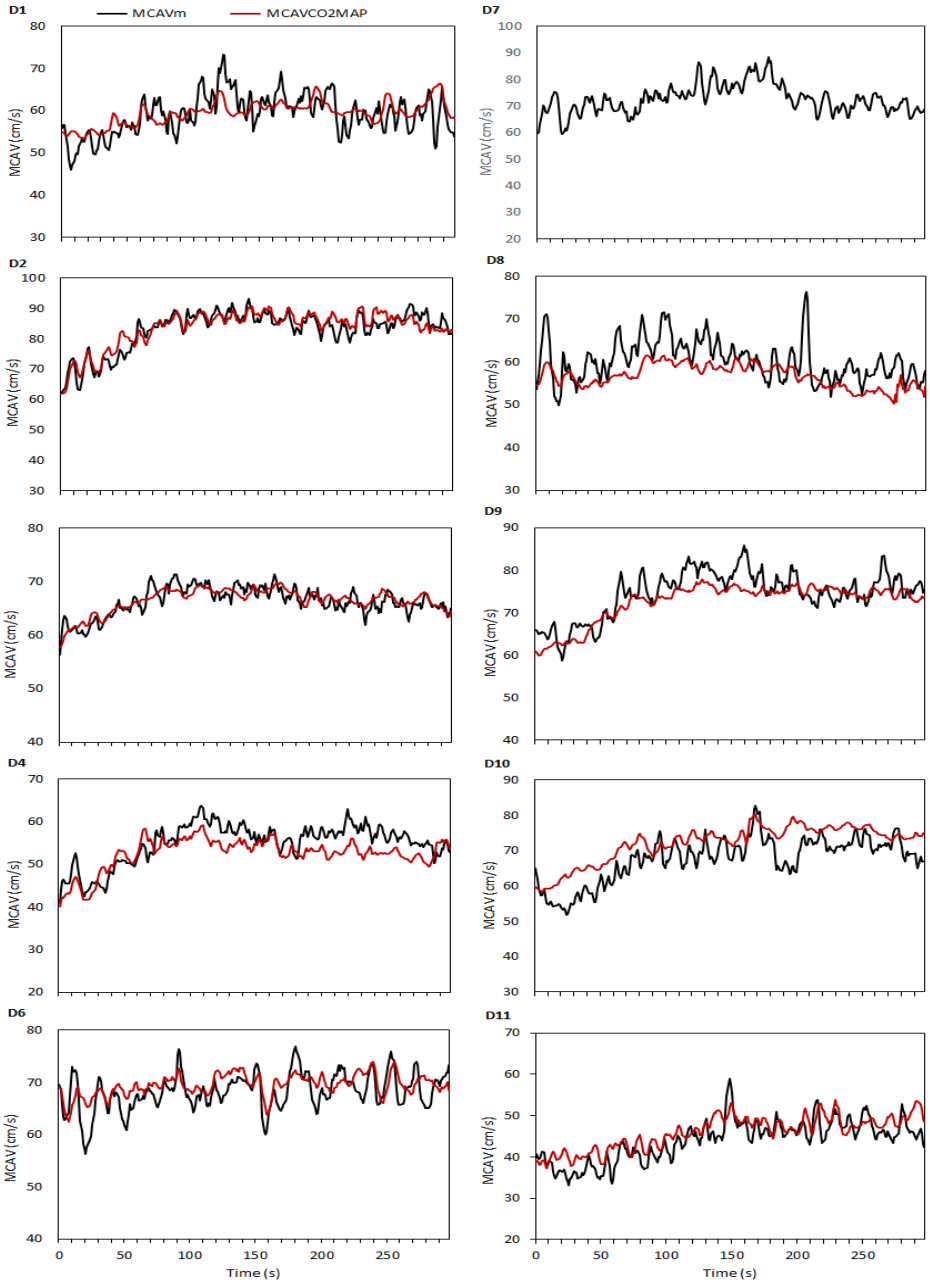

Figure 4-3. Measured MCAV (black line) and MCAV predicted from changes in $PaCO_2$ and MAP ($MCAV_{CO2MAP}$), red line) following the step increase in work rate in the Control condition. Each plot represents second-by-second interpolated data from one participant, without curve fitting. Participant 7 does not show predicted MCAV because MAP measurements were unreliable.

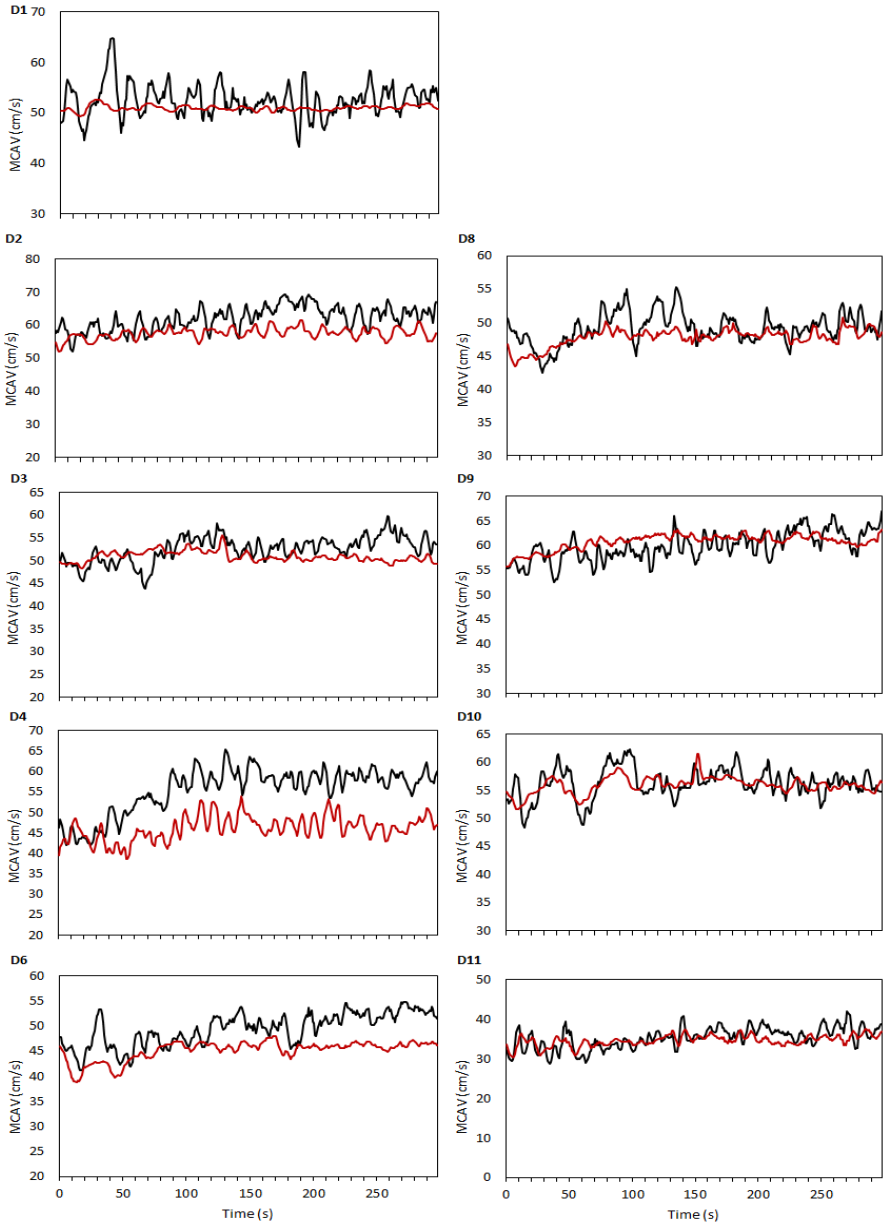

Figure 4-4. Measured MCAV (black line) and MCAV predicted from changes in $PaCO_2$ and MAP ($MCAV_{CO2MAP}$), red line) following the step increase in work rate in the Hypocapnic condition.

Each plot represents second-by-second interpolated data from one participant. Participant 7 was unable to perform adequate hyperventilation.

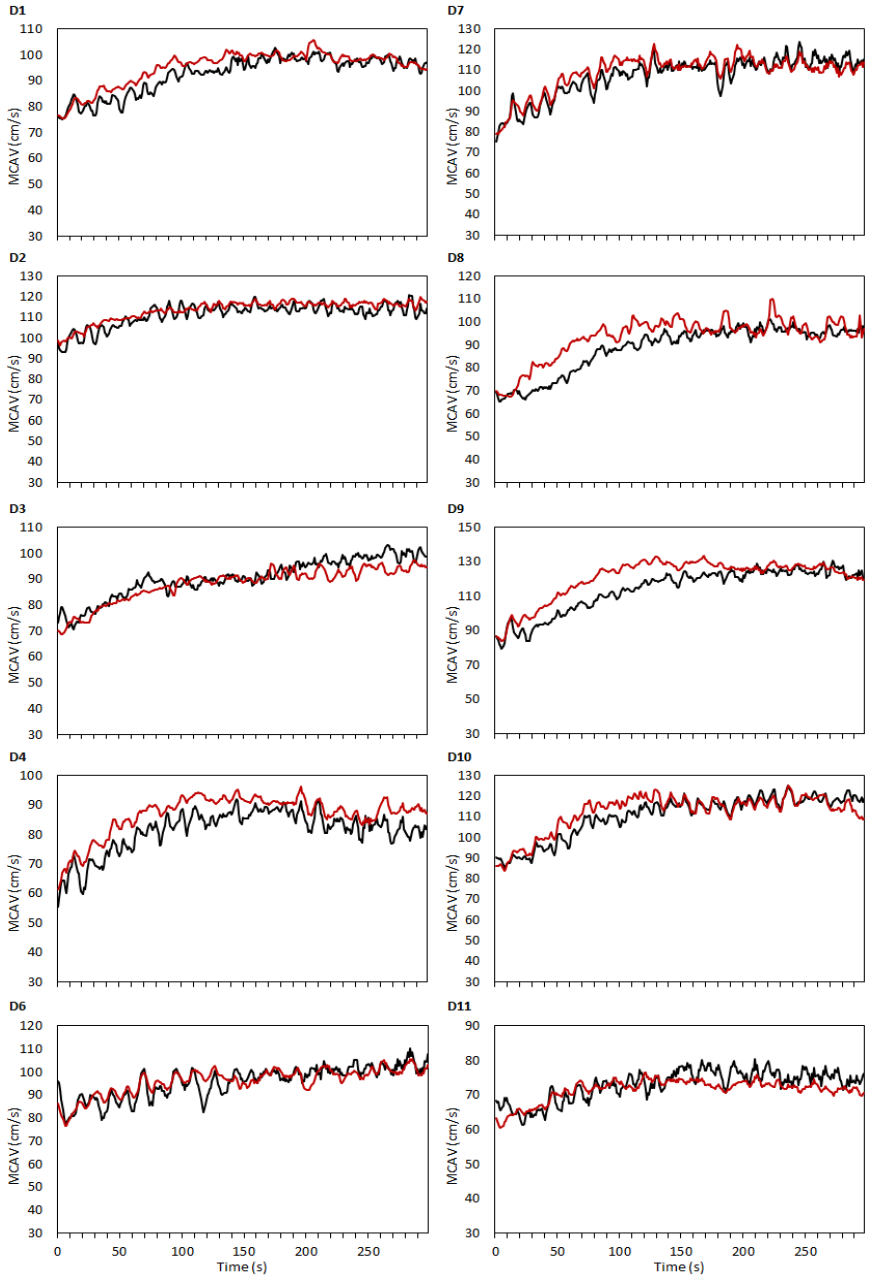

Figure 4-5. Measured MCAV (black line) and MCAV predicted from changes in $PaCO_2$ and MAP ($MCAV_{CO2MAP}$), red line) following the step increase in work rate in the Hypercapnic condition.

Each plot represents second-by-second interpolated data from one participant.

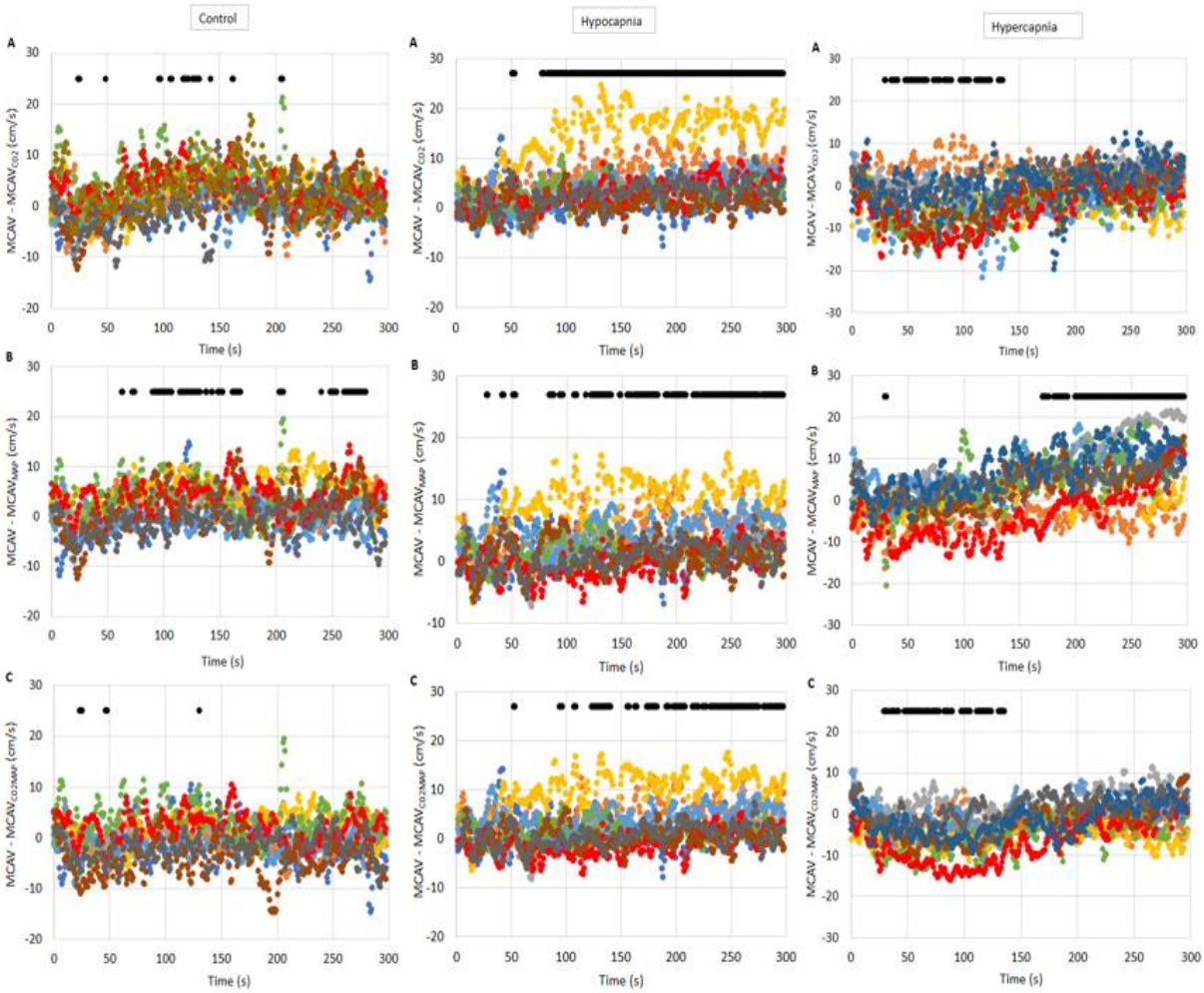

Figure 4-6. Individual values of the second-by-second difference between measured MCAV (MCAV) and MCAV predicted by $PaCO_2$ ($MCAV_{CO2}$) (A), MAP ($MCAV_{MAP}$) (B), and both PaCO2 and MAP ($MCAV_{CO2MAP}$) (C) during Control (Left), Hypocapnia (Centre), and Hypercapnia (Left).

Each colour plot indicates second-by-second interpolated data from one participant. The black solid symbols above each plot indicate significant difference between measured and calculated MCAV at the given time ($p < 0.05$). In Control n=9, Hypocapnia n=9, and Hypercapnia n=10.

4.7 Appendix

Table 4-5. Participant characteristics and graded exercise test results.

Participant	Age (y)	Height (cm)	Weight (Kg)	VO_2peak (ml/kg/min)	Wmax (Watts)	Exercise Load (Watts)	Exercise Load (%Wmax)	Peak HR (bpm)
D1	22	181.0	82.0	39.7	280	130	46.4	194
D2	21	173.7	73.7	37.7	250	130	52.0	188
D3	22	181.3	70.0	39.6	250	130	52.0	182
D4	21	185.7	75.0	59.7	300	150	50.0	206
D6	21	183.6	82.8	39.9	250	130	52.0	167
D7	23	181.3	69.4	49.6	250	120	48.0	181
D8	26	187.0	97.9	38.7	250	130	52.0	173
D9	24	187.8	73.8	48.1	250	120	48.0	179
D10	26	181.4	76.2	44.1	250	100	40.0	190
D11	24	170.8	77.1	32.1	225	110	48.9	194
Mean	**23.0**	**181.4**	**77.8**	**42.9**	**255.5**	**125.0**	**48.9**	**185.4**
SD	**1.94**	**5.5**	**8.3**	**7.8**	**20.3**	**13.5**	**66.6**	**11.4**

Table 4-6. Results of single exponential fit of MCAV data from exercise onset to 5 min during each Condition.

Time series data for MCAV are shown for each participant, without curve fits, in Figures 4.3, 4.4, and 4.5 for Control, Hypocapnia, and Hypercapnia, respectfully.

Participant	Condition	Baseline cm/s	Amplitude cm/s	Tau s	Time Delay s	SEE
D1	Control	56.8	3.9	38.6	44.9	3.9
D2	Control	65.0	21.3	38.8	6.5	3.4
D3	Control	59.8	7.4	27.8	6.0	2.0
D4	Control	40.6	16.7	43.6	-2.2	2.9
D6	Control	66.0	4.0	98.9	31.9	3.2
D7	Control	60.2	13.6	38.7	-13.5	5.0
D8	Control	55.7	4.6	0.8	0.3	4.7
D9	Control	60.2	17.1	46.2	1.8	3.5
D10	Control	60.1	12.3	70.0	34.6	3.7
D11	Control	39.5	10.2	121.7	49.3	3.1
D1	Hypocapnia	50.2	2.4	1.3	1.0	3.2
D2	Hypocapnia	55.3	9.7	106.0	-10.7	2.8
D3	Hypocapnia	48.6	6.5	114.6	12.5	2.2
D4	Hypocapnia	40.2	19.1	57.9	1.5	2.7
D6	Hypocapnia	44.6	42.7	1312.2	-10.7	2.1
D7	Hypocapnia					
D8	Hypocapnia	46.2	3.5	47.5	2.9	2.2
D9	Hypocapnia	55.7	68.8	2730.8	-21.9	2.1
D10	Hypocapnia	54.7	1.9	27.0	21.5	2.6
D11	Hypocapnia	31.8	9.0	283.2	-2.2	2.1
D1	Hypercapnia	77.9	21.8	85.5	14.4	2.6
D2	Hypercapnia	94.5	20.2	46.2	2.2	2.6
D3	Hypercapnia	73.1	33.3	187.9	-8.7	2.4
D4	Hypercapnia	63.2	21.9	40.1	9.4	3.5
D6	Hypercapnia	87.8	22.3	212.7	38.0	3.7
D7	Hypercapnia	80.4	34.5	67.2	0.9	3.8
D8	Hypercapnia	67.8	31.3	83.8	17.0	2.5
D9	Hypercapnia	85.9	41.9	89.3	12.5	2.9
D10	Hypercapnia	85.8	35.0	84.4	6.5	2.9
D11	Hypercapnia	65.6	11.0	80.7	18.3	2.4

Table 4-7. Individual linear regression analysis results with dependent variable MCAV and independent variable $PaCO_2$.

A. Control						
Participant	Equation	Std. Coeff.	$F_{(1,297)}$	P (α=.05)	R^2	SEE
D1	MCAV = 27.09 + (0.72 * $PaCO_2$)	0.56	133.54	<0.001	0.31	3.96
D2	MCAV = -58.62 + (2.75 * $PaCO_2$)	0.82	606.43	<0.001	0.67	3.76
D3	MCAV = 2.69 + (1.36 * $PaCO_2$)	0.75	384.95	<0.001	0.57	1.77
D4	MCAV = -29.35 + (1.61 * $PaCO_2$)	0.68	250.59	<0.001	0.46	3.44
D6	MCAV = 46.75 + (0.44 * $PaCO_2$)	0.13	4.99	0.026	0.02	3.51
D7	MCAV = -25.04 + (1.97 * $PaCO_2$)	0.54	118.72	<0.001	0.29	4.56
D8	MCAV = 48.57 + (0.25 * $PaCO_2$)	0.12	4.38	0.037	0.01	4.67
D9	MCAV = 7.59 + (1.58 * $PaCO_2$)	0.79	488.98	<0.001	0.62	3.33
D10	MCAV = -23.46 + (2.05 * PaO_2)	0.81	580.53	<0.001	0.66	3.67
D11	MCAV = -1.61 + (1.04 * $PaCO_2$)	0.61	170.95	<0.001	0.37	3.93
B. Hypocapnia						
D1	MCAV = 38.67 + (0.40 * $PaCO_2$)	0.16	7.62	0.006	0.03	3.18
D2	MCAV = 75.86 - (0.39 * $PaCO_2$)	-0.11	3.56	0.06	0.01	3.55
D3	MCAV = 69.14 - (0.48 * $PaCO_2$)	-0.26	21.58	<0.001	0.07	2.77
D4	MCAV = 82.70 - (0.71 * $PaCO_2$)	-0.19	11.52	<0.001	0.04	5.43
D6	MCAV = 23.31 + (0.73 * $PaCO_2$)	0.26	21.35	<0.001	0.07	3.14
D7						
D8	MCAV = 21.85 + (0.67 * $PaCO_2$)	0.33	36.14	<0.001	0.11	2.20
D9	MCAV = 49.02 + (0.29 * $PaCO_2$)	0.08	2.08	0.15	0.01	2.90
D10	MCAV = 2.36 + (1.50 * $PaCO_2$)	0.53	117.30	<0.001	0.28	2.30
D11	MCAV = 16.82 + (0.51 * $PaCO_2$)	0.29	27.09	<0.001	0.08	2.56
C. Hypercapnia						
D1	MCAV = -26.01 + (1.93 * $PaCO_2$)	0.91	1370.59	<0.001	0.82	3.03
D2	MCAV = 20.18 + (1.45 * $PaCO_2$)	0.86	836.50	<0.001	0.74	2.86
D3	MCAV = -28.71 + (1.93 * $PaCO_2$)	0.86	865.53	<0.001	0.75	3.82
D4	MCAV = -88.21 + (2.66 * $PaCO_2$)	0.90	1300.88	<0.001	0.82	3.08
D6	MCAV = -45.68 + (2.41 * $PaCO_2$)	0.72	312.35	<0.001	0.51	4.76
D7	MCAV = -66.33 + (2.82 * $PaCO_2$)	0.84	734.86	<0.001	0.71	5.17
D8	MCAV = -104.53 + (3.15 * $PaCO_2$)	0.87	922.80	<0.001	0.76	5.05
D9	MCAV = -142.96 + (4.25 * $PaCO_2$)	0.89	1168.33	<0.001	0.80	5.78
D10	MCAV = -131.49 + (3.91 * $PaCO_2$)	0.88	1000.13	<0.001	0.77	4.88
D11	MCAV = -36.83 + (1.89 * $PaCO_2$)	0.70	281.16	<0.001	0.49	3.09

Table 4-8. Individual linear regression analysis results with dependent variable MCAV and independent variable MAP.

A. Control						
Participant	Equation	Std. Coeff.	$F_{(1,297)}$	P (α=.05)	R^2	SEE
D1	MCAV = 35.97 + (0.23 * MAP)	0.31	31.91	<0.001	0.10	4.53
D2	MCAV = -20.29 + (0.90 * MAP)	0.88	990.52	<0.001	0.77	3.15
D3	MCAV = 19.46 + (0.39 * MAP)	0.69	275.60	<0.001	0.48	1.93
D4	MCAV = -4.80 + (0.58 * MAP)	0.74	347.99	<0.001	0.54	3.17
D6	MCAV = 23.27 + (0.46 * MAP)	0.58	149.29	<0.001	0.34	2.89
D7						
D8	MCAV = 40.25 + (0.16 * MAP)	0.57	141.40	<0.001	0.32	3.87
D9	MCAV = 18.77 + (0.52 * MAP)	0.78	471.65	<0.001	0.61	3.37
D10	MCAV = -6.33 + (0.80 * MAP)	0.63	195.41	<0.001	0.40	4.90
D11	MCAV = -3.19 + (0.48 * MAP)	0.80	510.34	<0.001	0.63	2.99
B. Hypocapnia						
D1	MCAV = 54.75 - (0.02 * MAP)	-0.03	0.31	0.578	0.00	3.22
D2	MCAV = 46.62 + (0.15 * MAP)	0.27	22.99	<0.001	0.07	3.44
D3	MCAV = 31.70 + (0.17 * MAP)	0.31	30.50	<0.001	0.09	2.74
D4	MCAV = 4.77 + (0.52 * MAP)	0.57	139.10	<0.001	0.32	4.57
D6	MCAV = 28.86 + (0.47 * MAP)	0.70	291.67	<0.001	0.50	4.26
D7						
D8	MCAV = 30.57 + (0.16 * MAP)	0.52	112.10	<0.001	0.28	1.99
D9	MCAV = 38.46 + (0.21 * MAP)	0.50	99.55	<0.001	0.25	2.51
D10	MCAV = 40.73 + (0.16 * MAP)	0.28	24.29	<0.001	0.08	2.61
D11	MCAV = 9.66 + (0.26 * MAP)	0.49	95.70	<0.001	0.24	2.33
C. Hypercapnia						
D1	MCAV = -18.35 + (0.96 * MAP)	0.69	268.15	<0.001	0.48	5.20
D2	MCAV = 3.68 + (0.83 * MAP)	0.83	642.30	<0.001	0.69	3.15
D3	MCAV = 20.89 + (0.49 * MAP)	0.46	80.27	<0.001	0.21	6.71
D4	MCAV = -23.00 + (0.89 * MAP)	0.85	798.77	<0.001	0.73	3.72
D6	MCAV = 49.56 + (0.52 * MAP)	0.78	463.73	<0.001	0.61	4.26
D7	MCAV = -29.96 + (1.13 * MAP)	0.81	551.13	<0.001	0.65	5.70
D8	MCAV = -33.37 + (0.84 * MAP)	0.74	350.52	<0.001	0.54	6.93
D9	MCAV = -37.39 + (1.30 * MAP)	0.86	865.00	<0.001	0.75	6.49
D10	MCAV = -45.82 + (1.39 * MAP)	0.88	1026.87	<0.001	0.78	4.83
D11	MCAV = 5.08 + (0.57 * MAP)	0.65	218.52	<0.001	0.43	3.27

Table 4-9. Individual multiple linear regression analysis results with dependent variable MCAV and independent variables $PaCO_2$ and MAP.

A. Control							
Participant	Equation	Std. Coef. $PaCO_2$	Std. Coef. MAP	$F_{(2,297)}$	P (α=.05)	R^2	SEE
D1	MCAV = 17.70 + (0.66 * $PaCO_2$) + (0.12 * MAP)	0.51	0.16	74.40	<0.001	0.34	3.90
D2	MCAV = -52.98 + (1.27 * $PaCO_2$) + (0.61 * MAP)	0.38	0.60	751.64	<0.001	0.84	2.66
D3	MCAV = -8.08 + (0.98 * $PaCO_2$) + (0.24 * MAP)	0.54	0.43	346.25	<0.001	0.70	1.47
D4	MCAV = -32.92 + (0.88 * $PaCO_2$) + (0.40 * MAP)	0.37	0.51	249.64	<0.001	0.63	2.86
D6	MCAV = 8.45 + (0.31 * $PaCO_2$) + (0.46 * MAP)	0.09	0.57	77.22	<0.001	0.34	2.87
D7							
D8	MCAV = 39.59 + (0.02 * $PaCO_2$) + (0.16 * MAP)	0.01	0.57	70.48	<0.001	0.32	3.87
D9	MCAV = 4.03 + (0.92 * $PaCO_2$) + (0.29 * MAP)	0.46	0.44	360.56	<0.001	0.71	2.93
D10	MCAV = -32.67 + (1.74 * $PaCO_2$) + (0.25 * MAP)	0.69	0.20	321.67	<0.001	0.69	3.54
D11	MCAV = -11.11 + (0.34 * $PaCO_2$) + (0.41 * MAP)	0.20	0.68	284.14	<0.001	0.66	2.89
B. Hypocapnia							
D1	MCAV = 41.87 + (0.42 * $PaCO_2$) - (0.04 * MAP)	0.17	-0.06	4.31	=0.014	0.03	3.18
D2	MCAV = 70.88 - (0.80 * $PaCO_2$) + (0.20 * MAP)	-0.23	0.35	19.60	<0.001	0.12	3.36
D3	MCAV = 48.33 - (0.48 * $PaCO_2$) + (0.17 * MAP)	-0.26	0.31	28.78	<0.001	0.16	2.63
D4	MCAV = 15.03 - (0.23 * $PaCO_2$) + (0.51 * MAP)	-0.06	0.55	70.53	<0.001	0.32	4.56
D6	MCAV = 13.48 + (0.49 * $PaCO_2$) + (0.22 * MAP)	0.18	0.55	85.58	<0.001	0.37	2.59
D7							
D8	MCAV = 12.75 + (0.48 * $PaCO_2$) + (0.15 * MAP)	0.24	0.48	71.97	<0.001	0.33	1.92
D9	MCAV = 43.21 - (0.14 * $PaCO_2$) + (0.21 * MAP)	-0.04	0.51	50.00	<0.001	0.25	2.52
D10	MCAV = -1.94 + (1.39 * $PaCO_2$) + (0.09 * MAP)	0.50	0.15	64.24	<0.001	0.30	2.27
D11	MCAV = 1.41 + (0.29 * $PaCO_2$) + (0.24 * MAP)	0.17	0.45	54.54	<0.001	0.27	2.29
C Hypercapnia							
D1	MCAV = -46.55 + (1.63 * $PaCO_2$) + (0.34 * MAP)	0.77	0.24	916.26	<0.001	0.86	2.68
D2	MCAV = 0.52 + (0.92 * $PaCO_2$) + (0.41 * MAP)	0.55	0.41	611.56	<0.001	0.81	2.47
D3	MCAV = -14.29 + (2.33 * $PaCO_2$) - (0.28 * MAP)	1.05	-0.26	525.84	<0.001	0.78	3.55
D4	MCAV = -80.06 + (1.77 * $PaCO_2$) + (0.42 * MAP)	0.60	0.40	1103.51	<0.001	0.88	2.46
D6	MCAV = -12.32 + (1.29 * $PaCO_2$) + (0.37 * MAP)	0.38	0.55	349.19	<0.001	0.70	3.72
D7	MCAV = -86.35 + (1.87 * $PaCO_2$) + (0.65 * MAP)	0.56	0.46	805.085	<0.001	0.85	3.80
D8	MCAV = -106.84 + (2.53 * $PaCO_2$) + (0.28 * MAP)	0.70	0.25	546.91	<0.001	0.79	4.72
D9	MCAV = -116.37 + (2.80 * $PaCO_2$) + (0.53 * MAP)	0.59	0.35	701.73	<0.001	0.83	5.37
D10	MCAV = -100.68 + (2.04 * $PaCO_2$) + (0.76 * MAP)	0.46	0.48	692.96	<0.001	0.83	4.29
D11	MCAV = -37.79 + (1.28 * $PaCO_2$) + (0.30 * MAP)	0.48	0.35	185.31	<0.001	0.56	2.87

Chapter 5: General Discussion

5.1 Purpose and Findings

The summarized purpose of the three studies in this thesis, was to investigate the MCAV response to ADL by assessing both the magnitude of change in MCAV to ADL and the kinetic response of MCAV to activity of similar intensity to ADL. In addition, the mechanisms responsible for the dynamic MCAV response were investigated. A treadmill pilot study (Supplement) showed that, MCAV was elevated in a common ADL, walking at usual pace, and to the same extent as walking at an intensity of 60%HRR. In Chapter 2, MCAV was shown to increase during ambulatory usual pace and fast walking in both YA and OA and during slow walking in OA. Chapter 3 continued this investigation in OA with the addition of three new ambulatory ADL; stair climbing and descension, vacuuming, and carrying and shelving groceries, in addition to the walking speeds in Chapter 2. Results showed elevated MCAV above seated rest during all ADL. In addition to MCAV, ADL showed cardiorespiratory (HR and CO) and metabolic ($P_{ET}CO_2$ and VO_2) increases. ADL, including usual pace and fast but not slow walking, were performed at moderate to low vigorous intensities determined by MET. Chapter 4 builds on Chapters 2 and 3 by investigating the second-by-second kinetic response of MCAV to moderate and low vigorous cycling, a similar intensity to ADL in Chapters 2 and 3. It was determined that MCAV kinetics could not be adequately described by a single exponential equation based on visual assessment of residual plots. The conclusion in Chapter 4 that $PaCO_2$ is a primary regulator of MCAV kinetic response to exercise is in contrast to findings in Billinger et al. (2017) and

Ward et al. (2018), who found no relationship, but in agreement with studies that show a relationship between $PaCO_2$ and MCAV with increasing intensities of steady state exercise (Hellstrom et al., 1996) as well as dynamic response to exercise (Steventon et al., 2018). In Chapter 4, the three models that predicted MCAV due to changes in $PaCO_2$, MAP, or both during Control (normocapnia), hypercapnia, and hypocapnia conditions showed that $PaCO_2$ and MAP were regulators of the MCAV dynamic response.

5.2 Significance of Findings

The integration of results from these three studies provides evidence for the effectiveness of ADL in raising MCAV. This is the first study to measure MCAV during ambulatory ADL. These studies show that moderate to low vigorous ADL in YA and OA, and even light intensity ADL in OA raise MCAV. The kinetic response of MCAV to exercise and the role of $PaCO_2$ in that response are prominent in understanding the parameters of ADL that raise MCAV.

Kinetic Response of MCAV to Exercise

Recent literature reports that the MCAV response to exercise is described by a single exponential equation (Billinger et al., 2017; Ward et al., 2018; Witte et al., 2019). Data from Chapter 4 clearly show that MCAV dynamics with exercise cannot be adequately described by a first order system as observed in residuals of the fit. Both $PaCO_2$ and MAP regulate

MCAV kinetics and both would need to respond with the same first order properties and meet the principles of a first order system to validate a single exponential description of the data. Contrary to the pattern of residuals observed with the single exponential model, there was no discernable pattern in residuals comparing measured MCAV with the data modeled from changes in $PaCO_2$ and MAP after the onset of exercise. Compared to our data, Billinger et al. (2017) and Ward et al. (2018) showed a much longer TD in YA but both authors used a protocol that likely confounded TD with a graded workload onset over 30 s. In contrast, our study applied workload in a square wave onset while participants were already cycling under no load. There is a close temporal relationship between MCAV, $PaCO_2$ and MAP (see Figure 4.2C and D). Furthermore, at least in Control, $PaCO_2$ and MAP adequately modelled the change in MCAV from the onset of exercise for 5 minutes and therefore, account for the dynamic responses of MCAV (see Figure 4.6, Control). Several mechanisms such as central command (Asahara et al., 2018) and Afferent III mechanoreceptors (Braz et al., 2014), are proposed to contribute to MCAV regulation at the onset of exercise (reviewed by Querido & Sheel, 2007; Smith & Ainslie, 2017)). However, according to our data, these were not required to account for the MCAV response to exercise. Together, the results of this thesis and previous work suggest that sudden onset type ADL will have a shorter TD and therefore, earlier rise in MCAV than ADL that slowly and progressively increase in intensity.

$PaCO_2$ as a primary regulator of the MCAV kinetic response to exercise

The results of Chapters 3 and 4 show the importance of $PaCO_2$ in the MCAV response to exercise and are used here to discuss the value of intensity and duration in ADL. First, in Chapter 4, there was a tight temporal relationship between increases in $PaCO_2$ and MCAV (Figure 4-2). $PaCO_2$ increased by 5 mmHg and MCAV increased by 9 cm/s during moderate to low vigorous cycling (Table 4-1). Second, ADL that are of moderate intensity but not vigorous may be more effective in raising MCAV. ADL below VT elevated $PaCO_2$ and MCAV. ADL that elicited the greatest increase in $PaCO_2$ were ascending and descending stairs, usual pace and fast walking; the same ADL that produced the highest MCAV (Tables 3-2 and 3-4). Conversely, carrying and shelving groceries elicited the smallest change in $PaCO_2$ and MCAV. Literature shows a decline in MCAV at exercise intensities greater than about 60% VO_2max or VT when ventilation increases and CO_2 is reduced (Hellstrom et al., 1996). Together, these observations suggest that ADL should be performed at moderate to low vigorous intensities below or only slightly above VT where $PaCO_2$ is elevated optimally, rather than higher intensities where $PaCO_2$ declines with increasing ventilation. Therefore, ADL performed at a higher level of $PaCO_2$, closer to 60%VO_2max, are more likely to experience the greatest rise in MCAV. According to results in Chapters 2 and 3, all walking speeds except slow walking, vacuuming and stairs were, on average, performed at moderate to low vigorous intensities where both MCAV and $PaCO_2$ were elevated above rest. However, there was also a large inter-individual variability in the MCAV response which was at least partially due to a variability in walking speed and %HRR. Variability in relative

workload impacts $PaCO_2$ thereby influencing the effectiveness of these ADL in raising MCAV. Those who perform a given ADL with more vigor may elicit a greater $PaCO_2$ and therefore, MCAV.

Short duration ADL may elevate MCAV. During Control, the kinetic response of MCAV to cycling at similar intensities to ADL had an average TD of 16 s and tau of 53 s suggesting that 63% of the MCAV achieved during ADL would occur within an average of about 70 s of the onset of activity. The range in TD was 0 to 50 s indicating that MCAV could begin to rise immediately upon onset of activity. One may speculate that several ADL such as meal preparation, cleaning, walking to and from the car or elsewhere, gardening and lawn work may take several minutes or more while climbing one or two flights of stairs may not be long enough to elicit an increase in $PaCO_2$ and MCAV.

Possible benefits of ADL to Brain Health

There is some evidence to suggest that elevation in shear stress improves endothelial function in cerebral conduit arteries (Hoiland et al., 2016; Smith et al., 2017, Iwamoto et al., 2018). Shear-mediated vasodilation was found in VA in response to exercise (Smith et al., 2017). Exercise programs with walking at moderate to vigorous intensity have been shown to reduce CA stiffening (Moreau et al., 2003; Tanaka et al., 2000) and increase CR_{CO2} (Murrell et al., 2013). With a paucity of research on cerebral endothelial function, we look to research in peripheral arteries with the expectation that they may reflect to some extent, cerebral

vasculature. Research with the brachial artery suggests that even short exposure to rises in shear stress (10s every 15s for 30 min), three times per week can elicit improvements in endothelial function in as little as three weeks as shown by rises in brachial artery FMD (Hodges et al., 2018). Elevated FMD has been associated with lower cardiovascular risks including cerebrovascular incidents such as stroke (Shechter et al., 2009; Yeboah et al., 2007). Together these studies suggest that repeated habitual ADL may improve endothelial function in cerebral arteries and lower the risk of cerebrovascular accidents due to episodic exposure to shear stress. There are several other benefits of moderate activity to brain health including elevations in BDNF, IGF-1, VEGF, that promote neurogenesis, synaptic plasticity and angiogenesis (Lucan et al, 2015; Voss et al., 2010) that may apply to ADL performed at moderate intensity (Boyne et al., 2019). The findings in this thesis in conjunction with previous literature are particularly important to those who perform fewer ADL as they age for any number of reasons such as hired or volunteer support or by moving to retirement homes where less time is spent in ADL (Regan et al. 2016) or by personal choice to drive more rather than walk, take elevators rather than climb several flights of stairs etc. MCAV was elevated during usual pace walking which may be a mechanism for the reduced risk of cognitive decline in YA who are active in teenage (Middleton et al., 2010) suggesting YA should choose walking more often for transportation. Furthermore, research that considers habitual exercise should also assess parameters of ADL in prospective participants by questionnaire or accelerometry since these activities may influence brain health.

Some pathologies show a reduced CR_{CO2}. This is true of patients with heart failure, even in those with severity level II in the New York Heart Association categories, characterized with mild symptoms and slight limitation during ordinary activity (Georgiadis et al., 2000). The primary role of CO_2 in regulating MCAV kinetics observed in this thesis suggests that those with lower CR_{CO2} would benefit from high moderate intensity ADL (closer to 60%VO_2 max) that would raise CO_2 to, or close to optimal levels.

5.3 Limitations

1. Although MCAV has been shown to be a reliable surrogate for middle cerebral artery blood flow (Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1992; Bishop, Powell, Rutt, & Browse, 1986), the relationship is dependent on a constant arterial diameter. The effect of various perturbations on cerebral arterial diameter is under debate (Brothers & Zhang, 2016; Hoiland & Ainslie, 2016). MCA diameter was not measured. During exercise, where MCA flow and velocity are elevated, vessel diameter changes if any, would increase from rest due to increasing CO_2 production. Our data show that in Control, MCAV stayed elevated or decreased slightly from the peak but still remained elevated above baseline during the last 2-3 min of exercise (Figure 4-3). If MCAV was dilated due to $PaCO_2$, CBF could be underestimated. Therefore, diameter changes if applicable, would underestimate the reported magnitude of change with exercise rather than overstate our findings.

2. Several studies have shown no significant difference between right and left MCAV (Nedeltchev et al., 2002; Billinger et al., 2017; Robertson, 2013). Accordingly, the right MCA was typically insonated unless the insonation attempt was unsuccessful and then the insonation of the left artery was attempted.
3. Workload for the MCAV kinetics study was set at 50%Wmax which elicited an oxygen uptake that placed the exercise in the high end of moderate in three participants and the low end of vigorous in the others. Ideally, a lower workload would have related better to ADL intensity shown in Chapters 2 and 3, however we were concerned that lower work intensities may not elevate MCAV high enough to evaluate kinetics well. Exclusion criteria could have included a lower limit of MCAV response e.g., failure to show an increase in MCAV of 4 cm/s, which was the minimum amplitude during Control in this study. Furthermore, participants in Chapter 4 were YA and results may not apply to OA. Previous literature indicates that tau is almost double but TD is similar or lower in OA compared to YA (Billinger et al., 2017; Ward et al., 2018).
4. A metronome to regulate walking pace or breathing frequency introduced a cognitive task that may have elevated MCAV (Gatouillat et al., 2015). Furthermore, to regulate breathing in the MCAV kinetics study, participants also adjusted tidal volume to verbal instructions from the experimenter. Verbal assurance and minimal instruction were techniques used to minimize effects of cognitive tasks. Furthermore, exercise load was applied in a square wave, without warning, to minimize any mental preparation for an exercise load onset that may have influenced MCAV.

5.4 Future Direction

This research shows that MCAV is elevated during ADL. However, we do not currently know if the increase in shear stress related to this elevated MCAV is sufficient to improve endothelial function. Since the role of endothelial function is critical in the aging process and development of vascular disease, future work should be conducted with the end goal of setting a criterion in common units such as relative HR or workload that would stimulate improvements in endothelial function.

The ADL performed in this study do not necessarily reflect the performance of ADL in community living adults, especially in the fluctuation of activity intensity and duration. The workload used to assess MCAV dynamic response to exercise was on the high end of representation of ADL. Future work might focus on in-home/community analysis of kinetics and continuous cardiovascular, cerebrovascular, and hemodynamic measures to provide a greater understanding of how MCAV responds to ambulatory ADL performed at realistic intensities and durations in a community setting.

The major mechanisms responsible for the MCAV dynamic response to exercise were identified in this thesis. However, we did not measure neurovascular coupling, a mechanism shown to significantly influence brain blood flow. Further research should assess the relative role of neurovascular coupling during exercise.

Understanding that the MCAV kinetic response to exercise is not a first order system will allow further research to more accurately describe the response. The primary mechanisms responsible for MCAV changes were changes in MAP and $PaCO_2$ and the data

show that measured MCAV was adequately predicted by both MAP and $PaCO_2$ suggesting that the MCAV kinetic response may be adequately predicted by a higher order system. Understanding that there are numerous factors that influence MCAV, even if only to a small extent compared to MAP and $PaCO_2$, may explain variability in the model. In Chapter 4 we found that MCAV and $PaCO_2$ were lower with hyperventilation during baseline zero watt cycling than in Control. With exercise, $PaCO_2$ stayed low (within <1 mmHg), while MCAV increased from baseline (albeit very slowly (tau equals 520 s), so that deltaMCAV was similar to the Control condition. Baseline and deltaMAP were similar in hypocapnia and Control conditions. An interesting follow-up study to determine the role of changes in MAP to changes in MCAV with exercise would be to clamp $PaCO_2$ more consistently and precisely with a dynamic end-tidal forcing system and concurrently manipulate MAP using a technique to lower MAP (e.g., lower body negative pressure) during exercise.

Chapter 6: Supplement: Walking at usual pace increases brain blood velocity

6.1 Introduction

Aging is associated with declining endothelial function (Seals et al., 2008) resulting in stiffening of conduit arteries (Santhanam, Christianson, Nyhan, & Berkowitz, 2008; Seals et al., 2008; Modrick et al., 2009), a process associated with white matter hyperintensities, cognitive decline, and Alzheimer's disease (Austin et al., 2013; Cooper & Mitchell, 2016; Purkayastha et al., 2014). Regular exercise has been shown to slow or even repair vascular stiffening, partially by improved endothelial function in central and peripheral arteries (Bolduc et al., 2013; Di Francescomarino S. et al., 2009; Hoiland et al., 2017; Niebauer & Cooke, 1996; Smith et al., 2017). Exercise-induced shear stress elicits vasodilation via increased nitric oxide synthesis and bioavailability (Di Francescomarino S. et al., 2009; Smith et al., 2017), known to reduce arterial stiffness and reduce the risk of atherosclerosis and arteriosclerosis (Leung et al., 2008; Tsao et al., 1996). It is known that exercise increases global CBF (Sato et al., 2011) and MCAV (Hellstrom et al., 1996). It has been argued that only regular moderate or harder exercise maintains endothelial function (Di Francescomarino S. et al., 2009). Health organizations and government agencies recommend adults accumulate at least 150 minutes of moderate-to-vigorous exercise a week; however, only about 20% of Canadian adults attain this level of activity the Canadian Health Measures Survey (CHMS) (Statistics Canada, 2015a) . Moderate exercise is accepted to be 40-60%HRR (Wallace 2006). In a recent article that assessed physical activity over the life span

in women, exercise in all decades was associated with lower probability of cognitive impairment later in life; however, greater benefits were seen with regular exercise early in life, during teenage . Although most adults do not engage in planned moderate-to-vigorous activity, adults perform activities of daily living that require a range in energy expenditure. The most common of these is walking at usual pace. This leads us to wonder if Usual would be strenuous enough to elicit cerebrovascular responses seen in higher intensity exercise in YA.

6.2 Purpose

This study was the foundation of future studies in that it was designed to determine whether usual pace walking was intense enough to stimulate significant cerebrovascular responses above resting levels that may have a role to play in healthy vascular aging.

6.3 Question

Is walking at usual pace of sufficient intensity to elicit increases in MCAV in young healthy adults or is it necessary to walk at moderate to high intensity workloads generally performed for cardiorespiratory fitness.

6.4 Hypothesis

Usual pace walking will be strenuous enough to elevate MCAV.

6.5 Methods

Participants

Six females and four males aged 24.4± 3.1 y were recruited via posters at the University of Waterloo. Exclusion criteria included any conditions that limited the ability to exercise, diabetes, stroke, medications that affect blood pressure or heart rate, uncontrolled hypertension, or heart disease. Participants were fully informed of the study and encouraged to ask questions before signing the consent form. The consent form and study procedures were approved by the Office of Research Ethics at the University of Waterloo (ORE# 17714).

Protocol

Usual pace was determined in six female and four male healthy adults (170.7 ± 10.5cm, 66.2 ± 14 kg) using the 8 m walk test from stand position. Participants were seated during instrumentation. Following instrumentation, participants remained seated for another three min (Sit), stood still for 3 min on the treadmill (Stand), then straddled the treadmill while speed was set to usual pace. Subjects walked for 5 min at usual pace (Usual). The treadmill

speed was then increased to between 3.2 and 3.7 mph and gradient increased to between 8 and 12% to elicit a heart rate of 60%HRR (percent heart rate reserve, %HRR) for 5 min (Exercise). Measurements were taken in the last min of each state (Sit, Stand, Usual and Exercise).

Instrumentation

The right middle cerebral artery was insonated using a 2-MHz Transcranial Doppler Device (TCD) (Compumedics DWL, Singen DE). Blood pressure was measured using portable continuous finger-cuff plethysmography (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands). Beat to beat cardiac output (Q) was estimated using the Modelflow algorithm (Beatscope 1.1a, AD Instruments, Colorado Springs CO USA). All data was monitored using a data acquisition system (PowerLab; AD Instruments, Colorado Springs CO USA) and compatible display and analyses software (Chart 5, AD Instruments, Colorado Springs CO USA). Oxygen uptake (VO_2) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) were measured breath by breath with a COSMED portable metabolic system (K4b2, COSMED, Italy). Heart rate was measured using a Polar chest strap coupled with the COSMED.

Calculations

%HRR was calculated using Karvonen formula (Karvonen et al., 1957). Cerebrovascular resistance index (CVRi) was estimated using the equation MAPmca/MCAVmean where MAP is corrected to the level of the TCD probe over the middle cerebral artery using the equation MAPmca = MAP – (distance above heart in cm*0.78). Total peripheral resistance (TPR) was calculated as MAP/Q.

6.6 Statistical Analyses

Differences between four conditions (Sit, Stand, Usual, and Exercise) were analyzed using a linear model one factor analyses of variance repeated measure design. Significance was inferred at $p<0.05$. Significant F-tests were further analyzed using Tukey post-hoc.

6.7 Results

All variables were similar during Sit and Stand therefore, Stand measures were used for comparison since the exercise was performed in a standing position. Average Usual speed measured during the 8 m standing start test was 1.37 ± 0.17 mps. HR was elevated from Stand during Usual and Exercise ($p<0.0001$) (Figure 1A). The relative intensities were 22.5 ± 9 %HHR during Usual and 59.24 ± 5.8 %HRR during Exercise. VO_2 increased from Stand to Usual and again from Usual to Exercise ($p<0.0001$) (Figure 1B). MAP was elevated by 19.8% during Usual and a further 8.7% during Exercise ($p<0.0001$) (Figure 1C). SBP

increased from Stand to Usual and Exercise with no difference between them ($p<0.02$). DBP increased only at Exercise ($p<0.02$). MCAVmean during Usual and Exercise was higher than Stand ($p=0.007$) (Figure 4D) with no difference between Usual and Exercise. MCAVsys was also greater during both Exercise and Usual ($p<0.0001$) with no difference between them and there was no effect of activity on MCAVdia ($p=0.72$). $P_{ET}CO_2$ increased 18.6% ($p<0.0001$) from rest to Usual with no further change during Exercise (Figure 1E). CVRi was unchanged from resting measures (Figure 1F). Q increased by 74% during Usual and by another 11.9% during Exercise compared to Stand. TPR decreased from Stand to Usual by 38% ($p<0.0001$) from 17 ± 4 to 10 ± 2 mmHg/cm/s with a small insignificant decrease to 8.3 ± 2 mmHg/cm/s during Exercise.

6.8 Discussion

Clearly, walking at usual pace is strenuous enough to elicit significant cardiovascular and cerebrovascular responses compared to rest. Most importantly, and in support of the hypothesis, MCAV was significantly elevated by walking at usual pace.

Interestingly, MCAV, $P_{ET}CO_2$, and CVRi leveled off between Usual and Exercise. This is similar to earlier findings, where MCAV and $P_{ET}CO_2$ were not different during cycling between intensities of 30 and 70%HRR (Murrell et al., 2013). Therefore, exercise at about 23-30 % HRR elicited the same MCAV as exercise at 60-70%HRR. The typical cerebrovascular response to increasing exercise intensity is an increase in MCAV and $PaCO_2$

until about 60%VO_2max and then a decrease in both (Hellstrom et al., 1996) in spite of continued increases in metabolic rate and MAP (discussed in detail earlier in Chapter 1). It is possible that our participants were experiencing some level of hyperventilation which led to comparable $P_{ET}CO_2$. To be certain that a significant change in MCAV from Stand is indeed due to the stimulus of activity, it is important that participants were, in fact, in a resting state during Stand. To assess their state of rest, $P_{ET}CO_2$ and respiratory exchange ratio (RER) were evaluated. Participants began the experiment at Sit and Stand with relatively low $P_{ET}CO_2$ which might suggest higher ventilation indicative of stress or a non-resting state. However, measurements of RER were below 0.85 in all but two participants suggesting most were at a resting state during Sit (0.82 ± 0.09) and Stand (0.81 ± 0.09). These two participants with higher RER were also the only participants to show a greater than 30% increase in $P_{ET}CO_2$ during walking, which may be indicative of higher ventilation at rest.

Although there was an increase in $P_{ET}CO_2$ and MCAV during Usual Pace and Exercise reflective of increased cerebral and systemic metabolism, CVRi was stable suggesting similarity in the relative change in CBF and MAP. Usual pace walking induced a typical exercise response with increased metabolic rate, heart rate and MAP, leading to vasodilation evident in the lower TPR. The slightly higher MAP reflects both the greater cardiac output and the lower TPR. These typical cardiovascular responses to exercise were also evident when walking intensity was increased to 60%HRR and not surprisingly, VO_2, HR, MAP, and Q, were significantly greater than walking at usual pace.

6.9 Conclusion

The important message in this study is that walking at usual pace, the most common activity of daily living, was strenuous enough to elicit cardiovascular and cerebrovascular responses in YA. Walking is highly recommended as an exercise due to its low impact and feasibility, especially in OA. This study leads to questions about whether or not over-ground walking and other activities of daily living increase brain blood flow and elicit similar responses in OA and how OA and YA compare. Furthermore, if usual pace walking and activities of daily living are an effective stimulus for CBF in seniors, how much of their daily activity is spent at intensities high enough to stimulate increased blood flow.

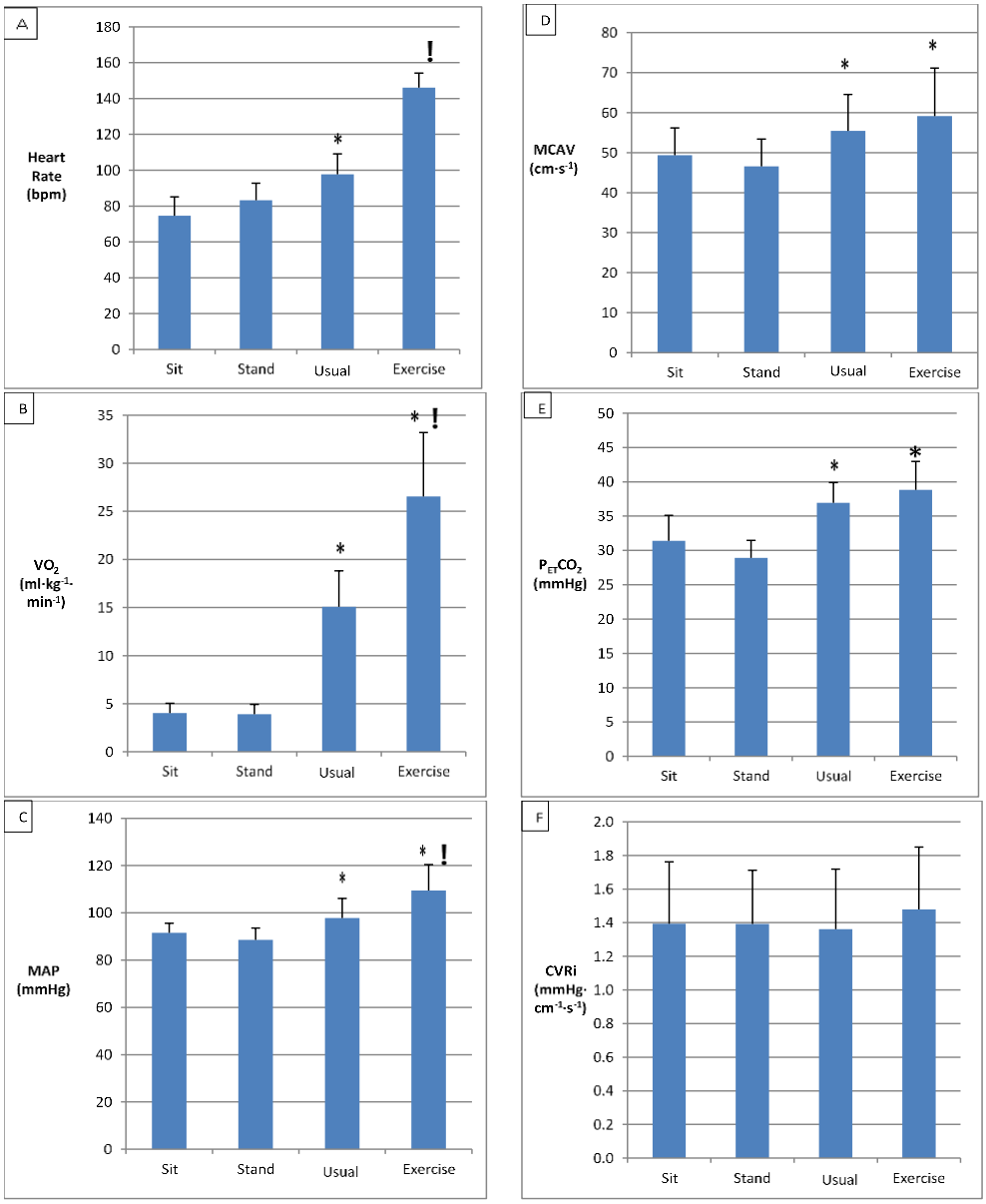

Figure 6-1. Cardiovascular and cerebrovascular responses to walking at usual pace (Usual) and at 60%HRR (Exercise). Heart rate (A), oxygen uptake (VO_2) (B), mean arterial pressure (MAP) (C), mean middle cerebral artery velocity (MCAV) (D), partial pressure of CO_2 ($P_{ET}CO_2$) (E) and cerebrovascular resistance index (CVRi) (F) are presented. *different from Stand, $p<0.05$. ! different from Usual, $p<0.05$.

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