Vascular Stiffening and the Brain: Direct Measures of Cerebrovascular Stiffness in Aging and Vasodilation

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Abstract

Dampening of pulsatile pressure waves within blood vessels is an essential feature of the arterial system. Vascular stiffening increases the speed and the pulsatile energy of the pressure wave, leaving low resistance organs like the brain vulnerable to microvascular mechanical damage. Due to access limitations, the effect of cerebrovascular stiffening on brain structure and neurological outcomes remains unknown. The purpose of this thesis was to assess the influence of vascular stiffening in peripheral arteries on white matter integrity (WMLv) (Chapter 2), obtain direct measures of cerebrovascular stiffness via phase contrast magnetic resonance imaging (PCMRI) (Chapter 3), and examine the impact of acute vasodilation on cerebrovascular stiffness (Chapter 4). We found that ischemic heart disease patients (IHD) had greater vascular stiffness compared with controls. However, IHD status did not influence WMLv. Regardless of vascular pathology, common carotid stiffness and ultrasound-based carotid-cerebral pulse wave transit times were associated with WMLv independently. Therefore, we applied PCMRI to the cerebral vessels to acquire direct measures of cerebrovascular stiffness in the internal carotid (ICA) and middle cerebral (MCA) arteries. Using cardiac-gated PCRMI, we collected blood flow velocity data at multiple segments of the ICA (icaPWV) and M1-M2 segment of the MCA (mcaPWV) to construct time–intensity curves and calculate PWV at temporal resolutions up to 25ms. We demonstrated that mcaPWV can detect vascular stiffening in a cross-section of young and older healthy individuals. Additionally, PWV increases from extracranial to intracranial segments, and this acceleration is amplified with age. We then measured peripheral and intracranial vascular stiffness in response to vasodilation using hypercapnia (HC; 6% CO₂, 21% O₂, balanced
N\textsubscript{2}) and nitroglycerin (NTG; 0.4mg, sublingual) in healthy young adults. Vasodilation in the MCA increased PWV and characteristic impedance. Additionally, the preferential effect of HC on conduit and downstream vascular properties of cerebral vessels versus non-specific conduit vasodilation of NTG suggests that multiple mechanisms may contribute to cerebrovascular stiffening. This thesis provides a method to obtain direct measures of intracranial PWV and demonstrates the capacity for acute modification of cerebrovasculature stiffness. This work may advance future understanding of cerebrovascular changes, damage, and therapeutics in vulnerable populations.

Keywords: Arterial stiffness, cerebrovascular, pulse wave velocity, phase contrast magnetic resonance imaging, cerebrovascular impedance, white matter.
Summary for Lay Audience

Dampening of the high-pressure flow in blood vessels is an essential feature of the arterial system. Stiffening of the vascular system, which occurs in aging and disease, increases the speed and pulsatile energy of the pressure wave. This renders low resistance organs like the brain susceptible to microvascular mechanical damage. Peripheral extracranial arterial stiffening is associated with cerebrovascular abnormalities. However, due to access challenges imposed by the skull, the influence of cerebrovascular stiffness on brain structure and neurological outcomes remains unknown. In that regard, this dissertation provides evidence that the speed of the pulse wave moving into the brain is associated with structural and functional impairments to cerebral white matter. Further, we introduce a novel application of magnetic resonance imaging that can be used to obtain direct measurements of cerebrovascular stiffness. Using this methodology, we found that the pulse wave accelerates when it moves into the brain, and this acceleration is amplified in aging arteries. Additionally, we applied laboratory and pharmacological techniques to acutely modify the cerebral vessel mechanics, which can alter the pulsatile properties of the blood pressure wave in the brain. This dissertation describes a method to obtain direct measures of intracranial pulse wave velocity while providing information on the capacity for acute modification of cerebrovascular stiffness. In the future, this work may lay the foundation for advancing our understanding of cerebrovascular damage, change, and therapeutics in vulnerable populations.
Co-Authorship Statement

Chapter 2 Author Contributions: Christopher S. Balestrini (C.S.B.), Baraa K. Al-Khazraji (B.K.A.-K.), Neville G. Suskin (N.G.S.), and J. Kevin Shoemaker (J.K.S.) conceived and designed research; C.S.B. and B.K.A.-K. performed experiments; C.S.B. analyzed data; C.S.B., B.K.A.-K., and J.K.S. interpreted results of experiments; C.S.B. prepared figures; C.S.B. drafted manuscript; C.S.B., B.K.A.-K., N.G.S., and J.K.S. edited and revised manuscript; C.S.B., B.K.A.-K., N.G.S., and J.K.S. approved final version of manuscript.

Chapter 3 Author Contributions: C.S.B., Joseph S. Gati (J.S.G.), B.K.A-K., and J.K.S. conceived and designed research; C.S.B. and Bradley J. Matushewski (B.J.M.) performed experiments; C.S.B. analyzed data; C.S.B., J.S.G, and J.K.S. interpreted results of experiments; C.S.B. prepared figures; C.S.B. drafted manuscript; C.S.B., B.J.M, J.S.G., B.K.A.-K., and J.K.S. edited and revised manuscript; C.S.B., B.J.M, J.S.G., B.K.A.-K., and J.K.S. approved final version of manuscript.

Chapter 4 Author Contributions: C.S.B., J.S.G, and J.K.S. conceived and designed research; C.S.B. performed experiments; C.S.B. analyzed data; C.S.B., J.S.G, and J.K.S. interpreted results of experiments; C.S.B. prepared figures; C.S.B. drafted manuscript; C.S.B., J.S.G., and J.K.S. edited and revised manuscript; C.S.B., J.S.G., and J.K.S. approved final version of manuscript.
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List of Abbreviations

ACA ................................................................. Anterior cerebral artery
ACh ................................................................. Acetylcholine
APOE ............................................................... Apolipoprotein E
ATP ................................................................. Adenosine triphosphate
AMP ................................................................. Adenosine monophosphate
AMPK ............................................................ Adenosine monophosphate-activated protein kinase
AU ................................................................. Arbitrary units
BP ................................................................. Blood pressure
BBB ............................................................... Blood brain barrier
BSL ............................................................... Baseline
B-Stiff .............................................................. Beta stiffness index
BW ............................................................... Bandwidth
CAD .............................................................. Coronary artery disease
CBF ............................................................... Cerebral blood flow
CBFV ............................................................. Cerebral blood flow velocity
CCA ............................................................. Common carotid artery
cPWTT .......................................................... Carotid – cerebral pulse wave transit time
CFPWV .......................................................... Carotid-femoral pulse wave velocity
cGMP ............................................................ Cyclic guanosine monophosphate
CO ............................................................... Cardiac output
CO₂ ............................................................... Carbon dioxide
CSA .............................................................. Cross-sectional area
DBP ............................................................. Diastolic blood pressure
ECG .............................................................. Electrocardiogram
eNOS .......................................................... Endothelial nitric oxide synthase
etCO₂ .......................................................... End tidal carbon dioxide
FA ............................................................. Femoral artery
FLAIR ........................................................ Fluid-attenuated inversion recovery imaging
FOV ............................................................ Field of view
HbA1c .......................................................... Hemoglobin A1c
HC ............................................................. Hypercapnia
HDL ............................................................. High density lipoprotein
ICA ............................................................ Internal carotid artery
icaPWV ....................................................... Internal carotid artery pulse wave velocity
IHD ............................................................. Ischemic heart disease
K⁺ ................................................................. Potassium
LDL ............................................................. Low density lipoprotein
L-NMMA ....................................................... L-NG-monomethyl arginine
MAP ........................................................... Mean arterial pressure
MCA ............................................................ Middle cerebral artery
mcaPWV ...................................................... Middle cerebral artery pulse wave velocity
MRI ............................................................. Magnetic resonance imaging
MMP ........................................................... Matrix metalloproteinase
MoCA ........................................................ Montreal cognitive assessment
mTOR ........................................................ Mammalian target of rapamycin
NAD .......................................................... Nicotinamide adenine dinucleotide
NADPH ...................................................... Reduced nicotinamide adenine dinucleotide phosphate
NO ............................................................ Nitric oxide
NOS .......................................................... Nitric oxide synthase
NTG ................................................................. Nitroglycerin
O₂ ................................................................. Oxygen
PCMRI .................................................. Phase contrast magnetic resonance imaging
PI .......................................................... Pulsatility index
PP .......................................................... Pulse pressure
PVS ........................................................ Perivascular space
PWTT ........................................... Pulse wave transit time
PWV ........................................................ Pulse wave velocity
RI ........................................................ Resistive index
ROS ........................................................ Reactive oxygen species
SBP ........................................................ Systolic blood pressure
SIRT ........................................................ Sirtuin
SV ........................................................ Stroke volume
TA ........................................................ Acquisition time
TCD ........................................................ Transcranial Doppler ultrasound
TE ........................................................ Echo time
TIMP ................................................... Tissue inhibitor of matrix metalloproteinase
TOF ........................................................ Time of flight angiogram
TR ........................................................ Repetition time
V_enc ...................................................... Velocity encoding factor
WMH ....................................................... White matter hyperintensity
WMLv ...................................................... White matter lesion volume
Zc ........................................................ Characteristic impedance
Chapter 1

Arterial Stiffening in the Peripheral and Cerebral Circulation

In this chapter, an introduction to arterial system mechanics is presented, focusing on arterial physiology, arterial stiffness measurements, and pathophysiological mechanisms. The purpose of this chapter is to provide a background of the current literature as well as present a rationale and studies within the thesis.

1.1 The Arterial System

1.1.1 Anatomy and Physiology of the Peripheral Arterial System

The main function of the cardiovascular system is to distribute oxygen and nutrient-rich blood to tissues. To do this in an effective manner, oxygenated blood is pumped from the left ventricle at a high pressure. The arterial system is then tasked with taking these high amplitude pressure waves and translating them to near steady-state flow in the capillaries to enable the extraction of nutrients from the blood. This elegant pump, conduit, and distribution system is complicated by branching of the arteries into smaller segments, which creates wave reflections that propagate back toward the heart (Weber et al., 2004).

The structure of the vascular wall provides the fundamental basis for their pressure-damping function. Arterial structure consists of three layers i) tunica intima, ii) tunica media, and iii) tunica adventitia (Figure 1.1; (Gasser, Ogden, & Holzapfel, 2006). The tunica intima is the innermost layer and is formed by an endothelial cell layer, subendothelial connective tissue, basement membrane made primarily of type IV collagen, and internal elastic lamina. The tunica media consists mainly of lamellar units,
which include elastin and smooth muscle fibrils within a collagen fiber network (O’Connell et al., 2008). The tunica adventitia is comprised mainly of fibroblasts and the collagen-derived (types I and III) extracellular matrix. These three layers can vary in regional density and composition, which have implications for the resistance properties of the cardiovascular system (Sawabe, 2010).

Figure 1.1. Structure of arterial layers.

Arteries are composed of three layers: intima (I), media (M), adventitia (A). I is the innermost layer consisting of a single layer of endothelial cells, a thin basal membrane and a subendothelial layer. M is composed of smooth muscle cells, a network of elastic and collagen fibrils and elastic laminae. A is the outermost layer surrounded by loose connective tissue. Reproduced with permission from Gasser et al., 2006.

The local mechanical properties of the arteries are influenced by all constituent layers. Vascular tone refers to the degree of constriction in a blood vessel relative to the maximally dilated state. The endothelium can release vasodilation (e.g. nitric oxide, prostaglandins, endothelium-derived hyperpolarizing factor) and vasoconstriction (e.g. thromboxane, endothelin) factors which can acutely influence vasomotor tone of the
vessel (Sandoo, Veldhuijzen van Zanten, Metsios, Carroll, & Kitas, 2015). The tunica media is the major load-bearing tissue at physiological pressures due to local elastin deformation to absorb the pressure pulse while smooth muscle contractile state maintains vascular tone (Michael F. O’Rourke & Hashimoto, 2007). Finally, the structural nature of the collagen in adventitia allows it to bear over half of the load at abnormal (high and low) pressures (Beenakker, Ashcroft, Lindeman, & Oosterkamp, 2012).

After ejection from the heart, the large elastic arteries distend to accommodate the dramatic increase in pressure, thereby acting as a cushioning reservoir, to store blood in systole and eject it to the tissue in diastole (Nichols, O’Rourke, Vlachopoulos, Hoeks, & Reneman, 2011). As the system moves away from the heart, the large muscular arteries act primarily as a conduit with some control via smooth muscle contraction and relaxation. Regionally, the distal tunica media contains more smooth muscle and less elastin than the central arteries. The arterioles are chiefly responsible for a large increase in resistance, which enables steady blood flow into the capillary network where nutrient exchange can occur (Nichols et al., 2011).

1.1.2 Anatomy and Physiology of Cerebral Arteries

The brain accounts for about 20% of oxygen consumption. Additionally, transient restriction of blood and oxygen supply to the brain results in unconsciousness, suggesting blood flow regulation is an important part of human survival (Clarke & Sokoloff, 1999). The brain receives blood predominantly via two pairs of arteries, anteriorly from bilateral internal carotid arteries, and posteriorly from bilateral vertebral arteries. These distributions anastomose at the Circle of Willis, where they divide into three predominant arteries, the anterior, middle, and posterior cerebral arteries (Figure 1.2).
Figure 1.2. Arterial supply of the brain.


The anterior, middle, and posterior cerebral arteries branch into the pial arteries that continue to run along the surface of the brain until they penetrate the brain tissue and to become penetrating arterioles in the Virchow-Robin space (Cipolla, 2016). The penetrating arterioles become parenchymal arterioles when they are surrounded by glial cells. At the parenchymal level, the brain tissue is supplied with oxygen and nutrients.

Unlike the pial arteries, which have collateral circulation, the long branched nature of the penetrating and parenchymal arterioles makes them susceptible to ischemic damage when obstructed (N. Nishimura, Schaffer, Friedman, Lyden, & Kleinfeld, 2007).

There are several distinct features of the cerebrovascular system. Unlike the peripheral vasculature, where the large arteries are primarily a conduit without resistive control, the
cerebrovascular large arteries have distinct resistive properties (F. M. Faraci & Heistad, 1990). Dilation of large cerebral arteries changes the microvascular gradient between the arterial and venous capillaries, suggesting that large arteries in the brain play a role in oxygen and nutrient extraction in the cerebrum (Cipolla, 2016). Additionally, autoregulation of the cerebral vessels can compensate for vasoactive stimuli, thereby maintaining blood flow to the brain over a large range of pressures (Lassen, 1959). The blood-brain-barrier (BBB) is another feature unique to the cerebral vessels. The BBB is characterized by tight and adherens junctions between cerebrovascular endothelial cells which allow closely-controlled regulation of substances into the brain (Zlokovic, 2008). This unique cerebrovascular endothelial layer can also alter the effect of humoral, neural, and metabolic stimuli (Frank M. Faraci & Heistad, 2017).

Aside from the internal characteristics that are unique to the cerebral vessels, the pressurized environment of the skull can also influence behaviour of the cerebrovasculature. In comparative modeling of the cerebral and peripheral vascular beds, the extravascular cerebral environment is suggested to minimize the ability of the cerebral vessels to express their elasticity (Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018). Additionally, increases in intracranial pressure (ICP) can cause brain tissue to stiffen when measured with MR elastography (Arani et al., 2018), which are known to change cerebrovascular pulsatile properties (Kim et al., 2015). Clinically, the stiff intracranial environment can result in faster pulse wave transmission with greater pulsatile energy moving into the cerebral microvessels, increasing the cerebral vulnerability to structural impairment (Michael F. O’Rourke & Safar, 2005).
1.1.3 Mechanical Properties of Arterial Dilation

Under conditions of maximal smooth muscle relaxation, the arterial wall becomes less compliant as it is dilated (Nichols et al., 2011). As dilation occurs in this state, the arterial wall tension increases and the load bearing tissue changes from elastin to collagen, which increases the stiffness of the artery. However, the physiological balance of vasoconstrictor and vasodilator influences creates a complex interaction that can alter the effective elastic properties of the artery. Therefore, the mechanisms by which vascular tone is altered can directly affect the elastic properties of the vessel. This thesis will discuss two common techniques to promote vasodilatory responses in humans, nitric oxide-mediated, and hypercapnia-mediated dilation.

1.1.4 Effect of Nitroglycerin on Arterial Dilation

Nitroglycerin (NTG) and other nitro-vasodilators produce nitric oxide (NO) after bioactivation in the body. The NO molecule acts on smooth muscle cells by activating soluble gyanylyl cyclase which induces the formation of cyclic guanosine monophosphate (cGMP) (Arnold, Mittal, Katsuki, & Murad, 1977). The cGMP binds to cGMP-gated ion channels and protein kinase G receptors, which promotes the reuptake of calcium from the cytosol to the sarcoplasmic reticulum, thereby relaxing the smooth muscle (R. C. Webb, 2003). Although dilation is the primary acute response of NO, it also plays a role in angiogenesis (Fukumura et al., 2001) and has anti-platelet properties (Pigazzi et al., 1999).

Exogenous forms of NTG can be administered in tablet, infusion, subdermal or sublingual form. In sublingual form, sodium NTG has an onset of 1-3 minutes and a half-life of about 2.5 minutes (Kirsten, Nelson, Kirsten, & Heintz, 1998). Additionally, NTG
can pass through the BBB, making it an ideal candidate for studying smooth muscle
vasodilation responses in both the periphery and the cerebral circulation. Vasodilation
associated with NTG in the brachial artery is shown to increase arterial elasticity and
decrease brachial pulse wave velocity (PWV) (Bank & Kaiser, 1998). This contrasts the
mechanistic thought that smooth muscle dilation reduces arterial elasticity (Peterson,
Jensen, & Parnell, 1960). Arteries exhibit inherent basal dilatory states. Therefore,
regional variations in basal vasomotor state could influence the elasticity responses to
external stimuli like NTG (Nichols et al., 2011). The thought that NTG elicits dilation of
the cerebral large arteries (Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1989) was
supported recently in studies of vascular diameter using 7-Tesla MRI (Schulz, Al-
Khazraji, & Shoemaker, 2018). However, the effect of smooth muscle vasodilation on the
dynamic pulsatile properties of the cerebral vessels have yet to be explored.

1.1.5 Effect of CO$_2$ on Arterial Dilation

The mechanisms of CO$_2$-mediated vasodilation are complex, involving extravascular pH
(Kontos, Raper, & Patterson, 1977), neuronal (Jordan et al., 2000), and endothelial
(Frank M. Faraci & Heistad, 2017) contributions. What adds to their complexity is the
longstanding notion that CO$_2$ preferentially effects the cerebral vessels (Lennox & Gibbs,
1932). To highlight this, when comparing arterial response over various respiratory end-
tidal CO$_2$ levels, vascular reactivity was 8-fold greater in the cerebral circulation than the

The mechanistic role of CO$_2$ on the cerebral vessels is multifaceted. In the pH-model,
ATP-sensitive K$^+$ and voltage-gated K$^+$ channels on vascular smooth muscle become
activated with when pH is reduced (Berger, Vandier, Bonnet, Jackson, & Rusch, 1998; H.
Xu et al., 2001). An endothelial cell-mediated opening of K\(^+\) channel in smooth muscle causes hyperpolarization and subsequent relaxation (Brayden, 1990; Nelson & Quayle, 1995). Evidence of neuronal contribution was suggested when sympathetic activation was shown to attenuate the cerebral blood flow increase during hypercapnia, suggesting a neural protective effect via innervation of the arterioles (Jordan et al., 2000). Neural regulation is also known to affect cerebral autoregulation (Zhang et al., 2002), suggesting CO\(_2\) changes may elicit both large artery and downstream effects on cerebral blood flow. Attenuation of the vasodilatory response to hypercapnia in the presence of L-NNA also suggests NO-mediated endothelial contributions in CO\(_2\)-mediated dilation (Frank M. Faraci, Breese, & Heistad, 1994). Overall, the effect of inhaled CO\(_2\) is complex in nature, likely containing multiple redundant mechanisms to maintain cerebral homeostasis.

### 1.2 Arteries in Aging and Disease

In the young, healthy cardiovascular system, the arterial system operates seamlessly to minimize pulsations from left ventricular ejections, enabling the distribution of blood at a steady rate to bodily tissues. However, the inevitability of aging results in substantial pulsations over years of life, which can cause mechanical degradation of the arterial walls. These structural changes affect wave travel and reflection beginning as early as the age of 30 and are progressive until death (Nichols et al., 2011). The main areas of arterial aging occur in the tunica intima and media, where cellular processes lead to structural remodeling over the course of life.

With age, progressive thickening occurs in the tunica intima, (Movat, More, & Haust, 1958), which is associated with development of atherosclerotic plaque. The thickening of atherosclerotic plaque is multifaceted in nature and derives from LDL cholesterol
oxidation (Navab et al., 1996), adhesion molecule signaling for monocyte recruitment (Collins et al., 2000), cholesterol-filled macrophage accumulation (Yamada, Doi, Hamakubo, & Kodama, 1998), and infiltration of medial smooth muscle cells to the intima (Ross, 1999). As this chronic inflammatory process continues, the vessel gradually dilates to compensate for the reductions in flow. However, this has consequences for the proteinaceous structure of the arterial wall.

The main proteins that are associated with vascular wall mechanics are collagen and elastin. The majority of collagen proteins in arteries are type I and III, and contribute to the tensile strength of the vessel (Mayne, 1986). In aging, the absolute number of elastin is maintained, but thinning of the laminae and reduction in relative elastin content has been observed (Spina, Garbisa, Hinnie, Hunter, & Serafini-Fracassini, 1983). While fragmentation continues, the collagen to elastin ratio increases, correlating with the progressive strain of the aorta (Newman & Lallemand, 1978). Additionally, recent evidence has shown a role for tunica media vascular smooth muscle as a contributor to age-related vascular stiffening (Mozafari, Zhou, & Gu, 2019).

Matrix metalloproteinases (MMPs) are collagenolytic and elastinolytic proteins that play a large role in the remodeling of the extracellular matrix, which occurs in arterial aging. Collagenolytic MMPs in the vessel are MMP-1 (endothelium), MMP-2 (vascular cells), and MMP-9 (inducible in vascular cells). Elastinolytic MMPs are MMP-7, MMP-12, stromelysins, and membrane type metalloproteinases (MT-MMPs) (Jacob, 2003). The regulation of MMPs is by i) production; gene expression and protein secretion, ii) activation; zymogen precursor to active form, and iii) inhibition; tissue inhibitors of MMPs (TIMPs). In a rat model, active MMP-2 and MT1-MMP increase with age, while
the associated TIMPs either remain the same or decrease, elevating the total ratio of the active proteinase (M. Wang & Lakatta, 2002). This change could be due to factors like cytokine signaling (Li, Cheng, Lederer, Froehlich, & Lakatta, 1997) or oxidative stress (Okamoto et al., 1997).

The importance of the endothelium in the dilatory response was first discovered using acetylcholine (ACh) in 1980 (Furchgott & Zawadzki, 1980). Later, nitric oxide (NO) was found to be responsible for initiating the endothelial dilatory response (Ignarro, Buga, Wood, Byrns, & Chaudhuri, 1987). Additionally, the idea that NO is an important determinant of basal vascular contractile state was confirmed when inhibition of NO resulted in constriction and consequent reduction in basal blood flow (Vallance, Collier, & Moncada, 1989). In the aging artery, impairment of NO-mediated vasodilation is seen as the endothelium is damaged. Using L-NG-monomethyl arginine (L-NMMA), a non-selective NO synthase inhibitor, reductions in forearm blood flow were diminished in older participants, suggesting the bioavailability of NO decreases with age (Lyons, Roy, Patel, Benjamin, & Swift, 1997). Also, age-associated increases in reactive oxygen species (ROS) can deactivate NO, which may contribute to the impaired vascular response (Donato et al., 2007). The oxidative stress in the endothelium is in part due to a NADPH oxidase upregulation that may occur via inflammatory (Csiszar et al., 2007), mitochondrial (Gioscia-Ryan et al., 2014), or renin-angiotensin (Flavahan, Chang, & Flavahan, 2016) mechanisms.

Energy and nutrient sensing pathways also may play a role in age-related endothelial dysfunction. These processes include the mechanistic target of rapamycin signaling (mTOR), sirtuins, and AMP-activated protein kinase (AMPK) pathways. The mTOR
pathway plays a large role in anabolic and aging processes throughout the body (Sabatini, 2017). In the arteries, over activation of the mTOR pathway causes endothelial cell senescence and endothelial NO synthase (eNOS) uncoupling in cell culture (Rajapakse et al., 2011). Inhibition of mTOR via rapamycin has also shown promise by restoring endothelial function to areas with vascular damage (Cheng et al., 2008; Lesniewski et al., 2017). Functionally, the use of mTOR inhibition on cerebrovascular outcomes like Alzheimer’s disease has suggested utility in improving blood flow and cognitive outcomes in rodent models (Lin et al., 2013). Sirtuins (SIRT) and AMPK are metabolic energy sensors and are regulated by nicotinamide adenine dinucleotide (NAD+) and the adenosine monophosphate to triphosphate ratio (AMP:ATP), respectively. In humans, the endothelial-dependent dilatory response to ACh correlates with SIRT-1 protein expression levels, which were reduced in older adults (Donato et al., 2011). Further, reductions of SIRT-1 in mice caused acetylation and deactivation of eNOS, leading to endothelial dysfunction (Donato et al., 2011). The AMPK pathway may also influence endothelial function in aging. In a rat model, AMPK was reduced with age, however, the endothelial-dependent vasodilation was restored when infused with AMPK activator aminoimidazole carboxamide ribonucleotide without increasing the bioavailability of NO, suggesting age-related decreases in endothelial-dependent vasodilation are at least in part due to the downregulation of AMPK with age (Lesniewski, Zigler, Durrant, Donato, & Seals, 2012).

Overall, these age-related factors lead to the degradation of arterial integrity, endothelial dysfunction, and concomitant stiffening. However, they can be accelerated by the presence of cardiovascular factors. While there is support for independent contributions
of metabolic syndrome (Scuteri et al., 2004), arterial degradation is largely a mechanical
issue. This notion is supported by studies that show age and blood pressure are the
primary determinants of arterial stiffness (Cecelja & Chowienczyk, 2009; McEniery et
al., 2010). Nonetheless, hypertension is one of the main risk factors for cardiovascular
disease. Arterial stiffness and hypertension are subject to a feedback loop combining both
local mechanics and global hemodynamics (Humphrey, Harrison, Figueroa, Lacolley, &
Laurent, 2016). Longitudinal evidence suggests arterial stiffness is an independent risk
factor for hypertension (Kaess et al., 2012). However, hypertension and elevated mean
arterial blood pressure elicit structural changes in the vascular smooth muscle and
extracellular matrix that cause arterial stiffening (Lacolley, Regnault, Segers, & Laurent,
2017). Regardless, in arterial stiffening and hypertension, there is preferential regional
stiffening in the central elastic arteries that elevates PWV and vascular impedance.
Impedance, the resistance to flow through a pulsatile system, is greater in the periphery in
young healthy individuals, this mismatch in impedance between the peripheral and
central arteries elicits a protective effect on the microvasculature (London & Pannier,
2010). The greater the mismatch in impedance along successive segments of the arterial
tree, the greater the reflection of a partial wave toward the heart. Thus, the forward wave
carries reduced pulsatile energy, which minimizes the stress on the microvascular
network. The consequential increase in central artery impedance with age reduces the
mismatch to the muscular arteries and may lead to propagation of damaging high
amplitude pulse waves deeper into the vascular tree (Figure 1.8; Safar et al., 2018). This
places low resistance organs like the brain at risk and gives support to the notion that
arterial stiffness is a shared risk factor for cardiovascular and cerebrovascular damage (Mattace-Raso et al., 2006).

Figure 1.3. Effect of arterial stiffening on pulse wave in microvasculature.

Effect of arterial stiffness and hypertension on impedance and pressure wave through the arterial tree. Reductions in the impedance mismatch between the central and muscular arteries translates to greater pulsatile energy moving into the microvasculature. Reproduced with permission from Safar et al., 2018.
1.3 Cerebrovascular Outcomes of Arterial Stiffening

1.3.1 White Matter in Health and Disease

White matter plays an integral role in the transmission of electrical information through various tracts in the cerebrum. The white matter makes up 40-50% of the total brain, and is comprised of axons wrapped concentrically with myelin (Filley, 2005). Myelin wraps the axons in such a way that small unwrapped sections, Nodes of Ranvier, can permit saltatory conduction, enabling high-speed propagation of the electric signal. These connections have critical roles in cognitive development in terms of attention, executive function, and general processing speed (Filley, 1998). The high fat content of myelin enables white matter images to be readily accessed via T2-weighted and FLAIR images. Another method of white matter imaging, diffusion tensor imaging (Le Bihan et al., 1986) also provides measures of organizational alignment and axonal integrity (Beaulieu, 2002). The white matter is highly vascularized, as such, vascular abnormalities can influence the structural integrity of white matter. For the purpose of this thesis, white matter integrity will be discussed using volumetric imaging correlates.

The first pathological correlates of white matter disease were discovered on computed tomography scans (Hachinski, Potter, & Merskey, 1987). More recently, researchers are using T2-weighted and FLAIR images as abnormal white matter displays with a hyperintense signal, giving rise to the term white matter hyperintensities (WMH) (Wardlaw, Valdés Hernández, & Muñoz-Maniega, 2015). The WMHs appear on T2-weighted and FLAIR images due to abnormal increases in water content and represents mixed pathology. In aging, WMHs increase progressively (De Leeuw et al., 2001) and by the 9th decade of life, over 90% of the population has a detectable amount of WMHs on
MRI scans (Zhuang, Chen, He, & Cai, 2018). Thus, in the past WMHs were typically dismissed as a part of normal aging. However, researchers discovered that WMHs are highly variable and are known to be modulated based on a variety of vascular parameters independent of age (Wardlaw et al., 2015). Vascular risk factors like hypertension (Dufouil et al., 2001; Verhaaren et al., 2013), carotid lumen diameter (Brisset et al., 2013), pulse pressure (Aribisala et al., 2014), smoking (Gons et al., 2011), and diabetes (Ferguson et al., 2003) are all associated with greater amounts of WMHs. Additionally, genetic factors may play a role in the development of WMHs (Atwood et al., 2004).

1.3.2 Pathology of White Matter Hyperintensities

The study of WMH pathological characteristics has shown large heterogeneity amongst cerebral small vessel diseases (Gouw et al., 2011). Findings include demyelination, astrogliosis, dilatation of perivascular spaces, fibrosis of vessels, and macrophage activation (Gouw et al., 2011). There are several possible mechanisms that may play a role in these outcomes. The white matter is intrinsically susceptible to insufficient blood flow due to deep anatomical positioning in watershed areas of several major cerebral arteries (Y. Wang et al., 2016). Specifically, paraventricular portions of white matter are supplied by the distal arterioles from the MCA and anterior cerebral artery. Due to the length and diameter of the penetrating arterioles, perfusion pressure is reduced, making these areas vulnerable to ischemia (Momjian-Mayor & Baron, 2005). Moreover, hypoxia may contribute to WMH pathology as hyperintense areas have reduced vessel density and greater levels of endothelial-derived hypoxic factors (Fernando et al., 2006; Moody et al., 2004). Other pathological studies have implicated BBB dysfunction, as extravasation of serum proteins like fibrinogen have been reported in glial cells tracking with WMH
severity (Tomimoto et al., 1996). More recently, greater BBB permeability was found in patients with subcortical ischemic vascular disease, as assessed by albumin index and enhanced contrast MRI (Taheri et al., 2011). Another mechanism for development of WMHs is inflammation. Microglial cells are activated in areas of white matter lesions (Simpson et al., 2007). Additionally, inhibition of microglial activation in a mouse model has shown improvement of white matter integrity (Fowler et al., 2018), suggesting glial cells as a possible therapeutic target (Ahmed, Gull, Khuroo, Aqil, & Sultana, 2017).

While vascular white matter disease is at least in-part mediated by the arterial system, a venous hypothesis has also emerged. The deposition of collagen in the paraventricular venules correlates with white matter disease, the collagen accumulates in the ventricular horns, where paraventricular WMH are typically found (Moody, Brown, Challa, & Anderson, 1995). As collagen forms and the venules dilate, vascular resistance and vasogenic edema increases, which may dilate the perivascular space and be visible as paraventricular WMHs (Black, Gao, & Bilbao, 2009).

### 1.3.3 Arterial Stiffness and Cerebral Small Vessel Disease

Clinically, there is a strong association between aortic PWV with WMH severity (Coutinho, Turner, & Kullo, 2011; Henskens et al., 2008; King et al., 2013; Mitchell et al., 2011). The vascular resistance of the brain, the ratio of pressure to flow of an artery, is similar to peripheral arteries when they are vasodilated (O’Rourke, 1982). This allows cerebral tissue to be continuously perfused through the cardiac cycle (O’Rourke & Safar, 2005). However, this also increases the susceptibility to damage of the cerebral microvessels when pulse waves are propagated faster (Poels et al., 2012) and/or with greater amplitudes (Kidwell et al., 2001; A. J. S. Webb et al., 2012). When the pulse in a
conduit artery reaches a point of greater impedance (i.e. bifurcation), a reflected wave is transmitted toward the heart. Stiffer arteries will return the reflected wave faster, leading to greater amplitude of the pulse wave, and an increase in blood flow pulsatility (T. Y. Xu et al., 2012). This increase in pulse amplitude is then propagated into the cerebrum, where it may be linked to microvascular angiopathy including dilation of the perivascular space and reductions in cerebrovascular compliance (Thomas, Cain, Nasralla, & Jackson, 2019).

1.3.4 Arterial Stiffness and Cognitive Function

When white matter is damaged, the associative network of fibers can no longer transmit electric impulses at full capacity. The resultant WMHs are associated with cognitive deficiencies in multiple domains (Debette & Markus, 2010; Filley & Fields, 2016; Prins & Scheltens, 2015). Specifically, the progression of WMHs is associated with reductions in general intelligence, attention, and executive functioning (Kloppenborg, Nederkoorn, Geerlings, & Van Den Berg, 2014). Executive functioning is an umbrella term for particularly useful high-order cognitive abilities such as working memory, cognitive flexibility, planning, reasoning, and problem solving (Cristofori, Cohen-Zimerman, & Grafman, 2019). Clinically, executive function can be assessed quickly using the Montreal cognitive assessment (Nasreddine et al., 2005) and trail making tasks (Army Individual Test Battery, 1944). However, evidence for the independent connection between arterial stiffness and cognition remains largely equivocal. Some studies suggest a strong independent relationship (Hanon et al., 2005; Scuteri, Brancati, Gianni, Assisi, & Volpe, 2005; Van Sloten et al., 2015; Waldstein et al., 2008), while others assert the
connection is confounded by other cardiovascular risk factors (Iulita, Noriega de la Colina, & Girouard, 2018; Singer et al., 2013).

1.4 Quantifying Arterial Diameter & Stiffness

There are numerous ways to collect information on the arterial diameter and stiffness. Diameter measurements can be accessed via ultrasound and imaging techniques, where vascular walls can be visualized and tracked over the course of the cardiac cycle. With adequate resolution, these images can be used to measure the luminal diameter and intima-media thickness in systole and diastole (Wikstrand, 2007). Arterial stiffness can be measured through local and regional applications (Laurent et al., 2006).

The local deformation of a vessel is related to the force that the blood exerts on the wall. Circumferential strain is the measure of the deformation compared with the original vessel length. Arterial compliance is the absolute change in diameter for a given change in pressure, and arterial distensibility is the relative diameter change for an incremental change in pressure (Michael F. O’Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002). The Peterson elastic modulus is the inverse of distensibility and represents the pressure for a given change in diameter. The Young’s modulus takes into account wall thickness and is the pressure step per centimeter for theoretical maximal stretch from a resting length (Michael F. O’Rourke et al., 2002). The $\beta$-Stiffness index is the natural logarithm of the ratio of pressure change over arterial strain (Laurent et al., 2006). These metrics are various measures of arterial stiffness at a specific point along an artery. In contrast, the stiffness along a segment of vessel may provide information of the pulsatile properties of flow, timing of wave reflection, and translation of pressure into the end
orans (Mitchell et al., 2011; Van Bortel et al., 2012). Indices of local and regional arterial stiffness are outlined in Table 1.

Table 1.1. Indices of local and regional arterial stiffness.

<table>
<thead>
<tr>
<th>Index of Vascular Stiffness</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial Strain</td>
<td>[ \text{Strain} = \frac{D_{\text{systole}} - D_{\text{diastole}}}{D_{\text{diastole}}} ]</td>
</tr>
<tr>
<td>Compliance (mm/mmHg)</td>
<td>[ \text{Compliance} = \frac{D_{\text{systole}} - D_{\text{diastole}}}{P_{\text{systole}} - P_{\text{diastole}}} ]</td>
</tr>
<tr>
<td>Distensibility (mmHg(^{-1}))</td>
<td>[ \text{Distensibility} = \frac{D_{\text{systole}} - D_{\text{diastole}}}{(P_{\text{systole}} - P_{\text{diastole}}) \times D_{\text{diastole}}} ]</td>
</tr>
<tr>
<td>Peterson’s Elastic Modulus (mmHg)</td>
<td>[ E_P = \frac{(P_{\text{systole}} - P_{\text{diastole}}) \times D_{\text{diastole}}}{D_{\text{systole}} - D_{\text{diastole}}} ]</td>
</tr>
<tr>
<td>Young’s Modulus (mmHg/cm)</td>
<td>[ E = \frac{(P_{\text{systole}} - P_{\text{diastole}}) \times D_{\text{diastole}}}{(D_{\text{systole}} - D_{\text{diastole}}) \times h} ]</td>
</tr>
<tr>
<td>( \beta )eta Stiffness</td>
<td>[ \beta eta \text{ Stiffness} = \ln \left( \frac{P_{\text{systole}}}{P_{\text{diastole}}} \right) \left( \frac{D_{\text{systole}} - D_{\text{diastole}}}{D_{\text{diastole}}} \right) ]</td>
</tr>
<tr>
<td>Pulse Wave Velocity (m/s)</td>
<td>[ PWV = \frac{\text{Distance}}{\Delta \text{Time}} ]</td>
</tr>
</tbody>
</table>

Note: \( D \), diameter; \( P \), pressure; \( h \), wall thickness; \( \ln \), natural logarithm
The most common metric of regional arterial stiffness is the measurement of segmental PWV. The PWV relates to arterial stiffness by the propagation of the arterial pressure wave through the vascular tree. After left ventricular contraction, the central elastic arteries locally distend to cushion the pressure pulse. The velocity that the pressure pulse propagates down the artery is directly related to the stiffness of that vascular segment. To calculate PWV, the distance between measurement sites is divided by the change in time it takes for the pressure pulse to move from the proximal to the distal point (Laurent et al., 2006). The PWV can be calculated via tonometry, ultrasound, and magnetic resonance imaging (MRI).

1.4.1 Tonometry Methods

Since the 1960s, applanation tonometry has been used as a quantitative measure of arterial pulsatile pressure (Pressman & Newgard, 1963). Arterial tonometry is a non-invasive measure that involves situating an artery against a bone and measuring the pressure of the passing pulse wave through a transducer. By placing two tonometry devices on different locations along the same superficial arteries, the distance between the two electrodes as well as the time delay of the pulse wave between the locations of interest can be measured and the pulse wave velocity calculated. The most common sites of measurement are the common carotid (CCA) and femoral (FA) arteries, as this measures central aortic stiffness (Van Bortel et al., 2012). This method first proved fruitful in assessing differences in both aerobic capacity and age in the Baltimore Aging Cohort (Vaitkevicius et al., 1993). Since then, carotid-femoral PWV (cfPWV) has become the “Gold Standard” for measuring central arterial stiffness and has predictive utility in many cardiovascular outcomes (Blacher et al., 2003; Mattace-Raso et al., 2010;
There are several challenges associated with using tonometry methods to measure PWV. The first is that both arteries at the ends of the segments of interest must be accessible in a manner that recordings are possible. This limits measurement to superficial vessels like the carotid, femoral, brachial, radial, and tibial arteries. Second, the vascular path distance must be estimated. This is usually based on the surface distance between measurement points and can lead to variability in calculations. Specifically, the current consensus suggests using 80% of the surface distance between the measurements of carotid and femoral pulse waves to calculate vascular distance (Van Bortel et al., 2012). This introduces variability with body weight and shape (Canepa et al., 2014), as well as the consideration that the pulse wave has already travelled past the aortic arch by the time it is detected at the CCA, which is inherently less elastic than the aorta (Van Bortel, 2006). Third, the lengths of the ascending aorta (Sugawara, Hayashi, Yokoi, & Tanaka, 2008) and aortic arch (Redheuil et al., 2011) increase with age. Overall, the estimation of path length in carotid-femoral measurements results in overestimation of cfPWV when compared with standard imaging methods (Weir-McCall et al., 2017).

1.4.2 Ultrasound Methods

Ultrasound is used primarily as a method for measurement of local arterial stiffness. Simply, tissues are comprised of various materials, and therefore have slightly different densities. The density is proportional to the tissue impedance, which have a spectrum of reflective acoustic qualities. Ultrasound waves utilize these acoustic differences to create an image (Figure 1.3; Aldrich, 2007). The high temporal resolution of ultrasound enables
continuous measures of vascular wall motion across the cardiac cycle. Therefore, arterial
diameter can then be measured throughout periods of systole and diastole to calculate the
deformation due to the force the blood exerts on the arterial wall. Combining diameter
changes with known pressure inputs enables the calculation of local arterial stiffness
(Gamble, Zorn, Sanders, MacMahon, & Sharpe, 1994).

Figure 1.4. Range of reflected ultrasound intensities.

Different tissues have inherent acoustic frequencies. The differences in acoustic
frequencies result in scaled intensity on an ultrasound image. Reproduced with
permission from Aldrich et al., 2007.

Doppler ultrasound is a technique that is used to measure blood flow velocity at a
location of interest. Echoes of stationary tissues do not change, whereas echoes from
blood in motion emit at a slightly altered frequency (Doppler frequency shift). This
change in frequency is proportional to the velocity that the blood is travelling. Doppler
ultrasound has a long history of clinical use in the periphery (Strandness, McCutcheon, &
Rushmer, 1966). The first non-invasive measurements of brain blood flow velocity were
completed in 1982 (Aaslid, Markwalder, & Nornes, 1982). This was achieved by
lowering the transducer frequency, which ultimately reduced the ultrasound wave attenuation of bone to a level that velocities could be recorded through a thin region of temporal bone (Figure 1.4). The middle cerebral artery is the most common site of transcranial Doppler (TCD), although most arteries that form the Circle of Willis can be insonated (Willie et al., 2011). Pulsatile and resistive indices can be calculated from blood flow velocities, which represent complex integration of downstream vascular resistance profiles (De Riva et al., 2012; Gosling & King, 1974; Pourcelot, 1974).

Figure 1.5. Transcranial Doppler insonation of the middle cerebral artery.

Frontal view of the ultrasound probe directed toward the middle cerebral artery (MCA). The cylinder around the MCA indicates the observation region (sampling volume) for the Doppler recording. The distance from the middle of the cylinder to the probe corresponds to the depth setting. Reproduced with permission from Aaslid et al., 1982.

Recordings from two ultrasound probes, one on the CCA, the other on the MCA (TCD), have been used to measure the pulse wave transit time (PWTT) (Giller & Aaslid, 1994) and estimate cca-mcaPWV (Fu, Huang, Wong, Chen, & Gao, 2016). This method correlates well with measures of aortic stiffness, and has demonstrated recent utility in
There are several challenges associated with using CCA-MCA measurements as markers of cerebrovascular stiffness. Due to ultrasound limitations, the vascular path between the points of interest are not clear. In the aorta, this is a minor issue as the course of the vessel is relatively linear. However, the internal carotid artery (ICA) is inherently tortuous, and the tortuosity of the vessel increases with age (Kamenskiy, Pipinos, Carson, Mactaggart, & Baxter, 2015; Vijaywargiya, Deopujari, & Athavale, 2017). This estimation can skew PWV calculations, especially over the short distances of the cerebrovascular segment. Additionally, the cca-mcaPWV measurement only contains a small vascular portion within the true intracranial cavity, where ICP is known to influence TCD recordings. Specifically, greater ICP is associated with greater pulsatility (Bellner et al., 2004; Homburg, Jakobsen, & Enevoldsen, 1993), decreases in cerebral flow velocity, and an increased resistivity (Klingelhöfer, Conrad, Benecke, Sander, & Markakis, 1988).

### 1.4.3 Magnetic Resonance Imaging Methods

In simple terms, MR imaging uses strong magnetic forces to align protons in the body to a magnetic field. A radiofrequency pulse is then emitted, causing protons to tip away from their magnetized orientation. When the radiofrequency pulse is turned off, the protons give off energy and realign to the magnetic field. The longitudinal recovery of the proton orientation is described by the time constant T1. In the transverse plane, proton spins lose coherence with one another and magnetization decays, this is known as the time constant T2. MRI takes advantage of differences in tissue T1 and T2 to produce an
image (Brown, Cheng, Haacke, Thompson, & Venkatesan, 2014). On T1 scans, tissues with short T1s appear bright. On T2 scans, tissues with long T2s appear bright. Pure liquids like water and cerebrospinal fluid, have long T1 and T2 times. Denser solids with low water content have short T2 times. This typically makes T1 scans useful for anatomical images, and T2 scans useful for pathological images, as increased water-content of lesions / tumors appear bright. A third type of scan, fluid-attenuated inversion recovery (FLAIR) uses extremely long repetition times, which enables the visualization of lesions in areas that would be masked by cerebrospinal fluid in a T2-weighted scan (Hajnal et al., 1992; Preston, 2006). This particular feature is useful in the context of imaging cerebrovascular pathologies like white matter hyperintensities (Schmidt et al., 2012).

Figure 1.6. T1-weighted, T2-weighted, and FLAIR imaging on cerebral tissue.
Angiograms are scans that produce an anatomical image of the arterial system. Time of flight angiograms (TOF) utilize short pulse repetition times to keep stationary tissues saturated, while tissue outside of the imaging slab remains magnetized. The moving blood then enters the imaging slab and exhibits high signal intensity compared with the stationary tissue (D. G. Nishimura, 1990). The TOF images can also be used as an anatomical map for phase planes in phase contrast MRI (PCMRI). In PCMRI, bipolar gradients are used to detect differences between stationary and moving spins (Moran, 1982). When subjected to a bipolar gradient, stationary spins have zero net phase change. In contrast, moving spins will have a net phase change that is proportional to their velocity. The sensitivity of the recordings is based on spacing, duration, and amplitude of the bipolar gradient, which is controlled by the velocity encoding (V_{enc}) setting. The user-collected V_{enc} is crucial for obtaining images that are sensitive enough to detect change without signal aliasing (Gatehouse et al., 2005). Two scans, stationary and flow-sensitive, are acquired and the stationary scan is subtracted from the flow-sensitive scan to produce an image that suppresses stationary tissue and highlights the moving particles (Figure 1.6; Wheaton & Miyazaki, 2012).
Figure 1.7. Concept of imaging blood flow velocity in PCMRI.

Depiction of the image produced from the phase shifts of moving particles in PCMRI. The intensity of the image produced is directly proportional to the net phase shift caused by the moving particle. Therefore, faster blood velocity produces a more intense signal. Reproduced with permission from Wheaton & Miyazaki, 2012.

Pulse wave velocity can be measured from PCMRI by acquiring cardiac-gated images at two points along a vessel. The time delay along the arterial segment is measured by reconstructing time–signal intensity graphs over a cardiac cycle and measuring the time between the upstroke of the pulse waves (Wentland, Grist, & Wieben, 2014). This can be applied over multiple images or within the same image if two vascular segments can be collected within the same imaging plane. The distance between the two points is measured via TOF and the PWV for that arterial segment is calculated. In practice, the PCMR-based approach to PWV has been used extensively on the aortic arch (Gatehouse et al., 2005; King et al., 2013; Markl et al., 2010; Rogers et al., 2001; Wentland et al., ...
2014; Yu, Peng, Wang, Wen, & Tseng, 2006). Recently, measures of carotid arterial PWV have also used the PCMR approach (Macdonald & Frayne, 2015; Rivera-Rivera et al., 2020; Sarrami-Foroushani et al., 2015).

There are some limitations when measuring PWV from PCMRI scans. The spatial resolution must be sensitive enough to detect changes in velocity. As blood flows from elastic to muscular arteries, there is a reduction in arterial diameter. The variability in flow measurements with PCMRI increases with declining arterial cross-sectional area (Macdonald & Frayne, 2015). Therefore, when imaging smaller arteries, the spatial resolution must adequately resolve that artery. The need for high spatial resolution in the brain, in turn, reduces the inherent temporal resolution of the acquisition, which can affect the detectable pulse wave upstroke (Figure 1.7; Wentland et al., 2014). In the thoracic aorta, temporal resolution of 30ms has been suggested to minimize error in PWV measurements from 2-20m/s (Dorniak et al., 2016).
Figure 1.8. Arterial pulse wave at various temporal resolutions.

Measurement of arterial pulse wave through cardiac cycle at temporal resolutions of 80ms (A), 40ms (B), 20ms (C), and 10ms (D). The upstroke of the pulse wave is harder to detect with poor temporal resolution. Reproduced with permission from Wentland et al., 2014.

1.5 Summary

Dampening of the arterial pulse wave through the vascular network is essential to ensure healthy organ functionality. In aging and cardiovascular disease, structural changes to the arterial wall cause the artery to stiffen, transmitting the pulse wave faster and with greater pulsatile energy into the microvasculature. When this occurs, low resistance and high flow organs like the brain are especially susceptible to mechanical damage. However, current methodological challenges have limited the measurement and understanding of cerebral vessel stiffness characteristics. In this thesis, we utilized tonometry, ultrasound, and MRI to characterize peripheral and cerebrovascular arterial stiffness in vascular
disease, aging, and vasodilatory conditions. Our general hypothesis is that the pressurized cranium influences cerebrovascular pulse wave transmission. Additionally, we propose that cerebrovascular stiffening can be directly measured via PCMRI and predict that aging and dilation status are determinants of cerebrovascular stiffness. Arterial mechanics through the vascular system are closely related to cerebral outcomes. Therefore, insights from this thesis will advance our understanding of the changing cerebrovascular system, which may have implications for diagnostics and therapeutics in the future.

1.6 Overview of Thesis

Throughout aging and cardiovascular disease, our large elastic and muscular arteries stiffen. However, the role of the cerebrovascular environment within the pressurized cranium, and the extent to which it influences cerebrovascular outcomes remains largely unknown. Therefore, the goal of this thesis is to examine how arterial stiffness in the peripheral and the cerebral vascular regions influences structural and functional cerebral outcomes (Study 1), to establish new applications for the direct measurement of cerebrovascular stiffness (Study 2), and to assess the effect of vasodilation on peripheral and cerebrovascular arterial stiffness (Study 3).

1.6.1 Study 1

Over the last 30 years, ischemic heart disease (IHD) mortality rates have declined (Hartley et al., 2016). However, with increased survivorship, patients are at increased risk for cognitive dysfunction after a cardiac event (Deckers et al., 2017; Roberts et al., 2010). One major component of cardiovascular disease is stiffening of the elastic central arteries, which has downstream outcomes for the end organs they supply (Michael F.
The thought that stiffening of the central arteries is associated with cerebrovascular outcomes is well substantiated in the literature (Henskens et al., 2008; Stéphane Laurent et al., 2003; Mitchell et al., 2011; Michael F. O’Rourke & Safar, 2005). However, in IHD patients with normal acute blood pressures, the relationships between peripheral and cerebral arterial stiffness to cerebrovascular outcomes remains unknown. Therefore, we investigated the connection between carotid and cerebrovascular stiffness and white matter integrity in patients with IHD and age-matched controls. We tested the hypothesis that IHD was associated with greater carotid and cerebrovascular stiffness, and that this stiffening would be associated with greater white matter impairment.

### 1.6.2 Study 2

The connection between cerebrovascular stiffening and white matter structural integrity was demonstrated in Study 1 (Balestrini et al., 2020). The cerebral vessels are unique in that they must overcome intracranial pressure to distend, which may minimize the ability of the artery to express elasticity (Zamir et al., 2018). The ultrasound method for measurement of cerebrovascular stiffness is subject to inherent access challenges due to the tortuous nature of the ICA and MCA (Kamenskiy et al., 2015; Vijaywargiya et al., 2017). Additionally, only a small portion of the CCA to MCA segment is considered to be within the pressurized cranium, creating the need for a new method of directly measuring arterial stiffness in the brain.

We applied PCMR imaging to assess the transmission of the pulse wave from the peripheral to the cerebral vessels. Specifically, we measured PWV in the ICA and in the
M1 – M2 of the MCA. We used this method to test the hypothesis that PWV increases as the pulse transmits from the extracranial to the intracranial compartments. Additionally, we compared two cohorts of healthy young and older individuals to explore whether the intracranial PWV correlates with age.

1.6.3 Study 3

The arterial system is dynamic in nature. Changes in arterial diameter in both acute and chronic circumstances have varying implications for transmission of the pressure wave down the vascular tree (Fok, Jiang, Clapp, & Chowienczyk, 2012; Mitchell et al., 2011; Michael F. O’Rourke & Hashimoto, 2007). As an artery expands circumferentially, the distensibility of that artery decreases, suggesting greater arterial stiffness (Roach & Burton, 1957). Conversely, a previous study demonstrated dilation of the radial artery via NTG caused decreased segmental PWV (Fok et al., 2012). Currently, the effect of acute dilation on the cerebral vessels within the pressurized skull remains unknown.

The PCMRI-method can be used to directly measure intracranial PWV across a range of healthy participants (Study 2). In the final study of this thesis, we use this new application to test the hypothesis that acute vasodilation influences mcaPWV in young healthy adults. By examining the capacity to alter cerebrovascular dynamics, we may advance our mechanistic understanding of cerebral small vessel disease.
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Chapter 2

2 Does vascular stiffness predict white matter hyperintensity burden in ischemic heart disease with preserved ejection fraction?

In this chapter, we investigate the effect of ischemic heart disease on carotid and cerebrovascular stiffness and determine whether vascular stiffness is associated with white matter integrity. The content of this chapter is published in the *American Journal of Physiology – Heart and Circulatory Physiology*, Volume 318, Issue 6, pages H1401-H1409.

2.1 Abstract

The survival rate of ischemic heart disease (IHD) patients is increasing. However, survivors experience increased risk for neurological complications. The mechanisms for this increased risk are unknown. We tested the hypothesis that IHD patients have greater carotid and cerebrovascular stiffness and these indices predict white matter small vessel disease. Fifty participants (age 40-78); 30 with IHD with preserved ejection fraction and 20 healthy age-matched controls, were studied using ultrasound imaging of the common carotid artery (CCA) and middle cerebral artery (MCA), as well as magnetic resonance imaging (T1, T2-FLAIR) to measure white matter lesion volume (WMLv). Carotid β-stiffness provided the primary measure of peripheral vascular stiffness. Carotid-cerebral pulse wave transit time (ccPWTT) provided a marker of cerebrovascular stiffness. Pulsatility index (PI) and resistive index (RI) of the MCA were calculated as measures of
downstream cerebrovascular resistance. Compared with controls, IHD patients exhibited
greater β-stiffness (8.5 ± 3.3 vs. 6.8 ± 2.2a.u.; \( p = 0.04 \)), MCA PI (1.1 ± 0.20 vs. 0.98 ±
0.18a.u.; \( p = 0.02 \)), and MCA RI (0.66 ± 0.06 vs. 0.62 ± 0.07a.u.; \( p = 0.04 \)). There was no
difference in WMLv between IHD and control groups (0.95 ± 1.2 vs. 0.86 ± 1.4mL; \( p =
0.81 \)). In pooled patient data, WMLv correlated with both β-stiffness (R = 0.34, \( p = 0.02 \)),
and cerebrovascular ccPWTT (R = -0.43, \( p = 0.02 \)); however, β-stiffness and ccPWTT
were not associated (\( p = 0.13 \)). In multivariate analysis, WMLv remained independently
associated with ccPWTT (\( p = 0.02 \)) and carotid β-stiffness (\( p = 0.04 \)). Patients with IHD
expressed greater β-stiffness and cerebral microvascular resistance. However, IHD did
not increase risk of WMLv or cerebrovascular stiffness. Nonetheless, pooled data
indicate that both carotid and cerebrovascular stiffness are independently associated with
WMLv.

2.2 Introduction

Ischemic heart disease (IHD) mortality rates have been steadily declining over the last 30
years (Hartley et al., 2016). However, with increased survival, these patients appear to
exhibit increased risk of stroke and cognitive dysfunction (Deckers et al., 2017; Grubb,
O’Carroll, Cobbe, Sirel, & Fox, 1996; Roberts et al., 2010), indicative of progressive
cerebrovascular impairment. One common link between IHD and cerebrovascular
damage is stiffening of the peripheral conduit arteries where structural and functional
changes lead to increased velocity of pulse wave transmission (PWV) (Poels et al., 2012).
Greater stiffness reduces the artery’s ability to distend and dampen high amplitude
pressure waves from the heart (O’Rourke & Hashimoto, 2007). This makes end organs
with high flow and low vascular resistance, such as the brain, vulnerable to mechanical
microvascular damage where the pressure waves can penetrate deeper into the microvascular beds (Byrom & Dodson, 1948; O’Rourke & Safar, 2005a; Van Sloten et al., 2015).

White matter hyperintensities (WMH) are structural white matter pathological findings, displayed through magnetic resonance imaging. Clinically, WMHs have been linked to vascular dementia (Prins et al., 2004), cognitive decline (Prins & Scheltens, 2015), cardiovascular death (Ikram, Vernooij, Vrooman, Hofman, & Breteler, 2009), and all-cause mortality (Debette et al., 2010; Kerber, Whitman, Brown, & Baloh, 2006).

Stiffening of the arterial system may contribute to proposed etiologies of WMHs including ischemia (Fazekas et al., 1993), disruption of the blood-brain-barrier (BBB) via endothelial dysfunction (Hassan et al., 2003), and/or inflammation (Rosenberg, 2009). In addition to global arterial stiffness, assessed by measures of aortic PWV (Poels et al., 2012), cerebrovascular disease is also associated with regional vascular metrics such as carotid stiffness (Van Sloten et al., 2015) and measures of cerebrovascular stiffness such as carotid-cerebral PWV (Fu, Huang, Wong, Chen, & Gao, 2016). However, despite the higher risk of stroke and cognitive decline in IHD, the impact of IHD on regional and cerebrovascular stiffness and its relation to WMH burden is unknown.

Therefore, this study investigated the association between cerebrovascular and carotid arterial stiffness and white matter structural abnormalities in patients with IHD. The study tested the hypothesis that IHD patients would express greater carotid and cerebral vascular stiffness, and that these would correlate with WMH burden.
2.3 Methods

2.3.1 Participant Recruitment

Fifty participants (IHD, n = 30; control, n = 20) were enrolled into the study. The IHD participants were recruited following hospital discharge for an IHD event (see Results section). Patients with IHD are known to exhibit long-term arterial stiffening, suggesting their vasculature has undergone systemic structural alterations (Weber et al., 2004).

Patients were excluded from the study if they had an implanted electrical device, were pregnant, had any history of head or eye injury involving metal fragments, or neurodegenerative, immunological, and/or major psychiatric conditions. Control participants had not been diagnosed with cardiac, vascular, metabolic, inflammatory, or neurological disease and were not on any medications that usually would be prescribed for these conditions in the 12 months prior to the study.

Data collection occurred over two separate visits: 1) a laboratory session to collect physiological data, and 2) a magnetic resonance imaging (MRI) session to obtain brain structure and WMH data. In the laboratory session, Lead II electrocardiography, blood pressures measurements, ultrasound of the common carotid (CCA) and middle cerebral artery (MCA), venous blood samples, and cognitive testing were completed in a temperature-controlled setting. Subsequently, MRI data were obtained during a separate testing session. All participants gave informed consent, the protocol was approved by the Health Sciences Research Board at Western University and complied with the standards set in the declaration of Helsinki.
2.3.2  Laboratory Session

2.3.2.1  Peripheral Vascular Data Acquisition

Participants were supine for at least 10 minutes before CCA sonography. The CCA diameters were measured at systole and diastole via B-mode ultrasound (Vivid I, GE Healthcare, Chicago, Illinois). Corresponding measures of systemic blood pressures were collected using photoplethysmography (Finometer, Finapres Medical Systems BV, Amsterdam, the Netherlands) obtained at the distal digital artery of the middle finger and calibrated to brachial artery blood pressures. Cognitive testing included the Montreal Cognitive Assessment (Nasreddine et al., 2005) as well as Trail Making tasks (Adjutant General’s Office, 1944).

Estimates of peripheral vascular stiffness were calculated based on the β-stiffness index (Equation 1) (Kawasaki, Sasayama, Yagi, Asakawa, & Hirai, 1987). Strain values (Equation 2) and carotid compliance were also calculated (Equation 3).

Equation (1):

$$\beta_{\text{Stiffness}} = \ln \left( \frac{\text{Pressure}_{\text{systole}}}{\text{Pressure}_{\text{diastole}}} \right) \left( \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Diameter}_{\text{diastole}}} \right)$$

Equation (2):

$$\text{Strain} = \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Diameter}_{\text{diastole}}}$$

Equation (3):
Compliance = \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Pressure}_{\text{systole}} - \text{Pressure}_{\text{diastole}}}

2.3.2.2 Cerebrovascular Data Acquisition

Participants were outfitted with a headband (Neurovision 500M, Neurovision TOC2M, Multigon Industries) and the right MCA was insonated using transcranial Doppler ultrasound (TCD). The cerebral blood flow velocity (CBFV) was recorded as the instantaneous peak velocity using a 2-MHz probe.

The carotid-cerebral pulse wave transit time (ccPWTT) was recorded as the difference in time for the blood flow pulse wave to reach the CCA (via ultrasound) and the M1 segment of the MCA (via TCD). ccPWTT was analyzed in cases where simultaneous CCA and MCA measurements were recorded (n = 33). A low-pass digital filter was applied to the velocity data (20Hz) and the second derivative method (Chiu, Arand, Shroff, Feldman, & Carroll, 1991) was used to calculate the upstroke of the pulse wave. The resistivity index (Pourcelot, 1974) and pulsatility index (Gosling & King, 1974) were calculated as well.

2.3.2.3 Magnetic Resonance Imaging

Magnetic resonance images were collected on a Siemens Magnetom Prisma 3-Tesla scanner at the Centre for Functional and Metabolic Mapping at Western University in London, Canada. Structural T1 images, an MR angiogram, and T2-Fluid-Attenuated Inversion Retention Images (FLAIR) were collected on each participant.

T1-weighted 3D SPACE pulse sequences were used with a radial trajectory to acquire sagittal images of high resolution (0.72mm isotropic, whole brain; TE = 11.0ms, TR =
700ms, FOV = 230mm, BW = 625Hz/px, iPat = 3, TA = 5:28 minutes). A 3D T1 scan was performed for full brain volume analysis (TE = 2.98ms, TR = 2300ms, FOV = 256mm, BW = 240Hz/px, iPat = 2, TA = 5:21 minutes), including the detection of white matter hypointensities.

The FLAIR images (TE = 387ms, TR = 5000ms, FOV = 230mm, BW = 751Hz/px, iPat = 2, TA = 5:42 minutes) provided information regarding white matter lesion volume (WMLv) as quantified by the open-source MATLAB software in SPM12. Lesions were segmented by the lesion growth algorithm (Schmidt et al., 2012) that overlays the T1 and FLAIR images to automatically detect areas of paraventricular white matter hyperintensities (PVH). The threshold detection value ($k$) was initially set to 0.3, if needed, visual inspection was used to determine the optimal threshold for WMH detection. WMLs were quantified again using a second approach which included measuring WMLv from T1 images using FreeSurfer software, based on automated brain segmentation techniques (Fischl, 2012; Fischl et al., 2002).

### 2.3.3 Statistical Analysis

The effect of group on participant characteristics was assessed through independent t-tests. The predictive relationship between the two modes of WMLv detection (SPM12 and FreeSurfer) was assessed using Pearson correlation. Comparisons of peripheral and cerebrovascular stiffness were assessed through Pearson correlations. Univariate correlation analysis was used to determine the association of group characteristics on WMLv. Multivariate analysis was used to determine the independent contributions of segmental vascular stiffness on WMLv using two models. First, variables were added that were significant in simple linear regression analysis. Second, the physiological
determinant of pulse pressure (PP) was added to the analysis to account for the change in pressure the vasculature must accommodate. If normality of residuals was violated in the WMLv multivariate analyses, we removed statistical outliers using the “robust regression and outlier removal method” ( ROUT ) in Prism software ( Motulsky & Brown, 2006 ). Statistical probability was set at $p < 0.05$ for all analyses. All data are presented as mean ± standard deviation, unless otherwise noted. SigmaPlot 12.5 and GraphPad Prism 8 were used for statistical analyses.

2.4 Results

2.4.1 Participant characteristics

Table 1 provides data regarding participant characteristics. Thirty IHD patients all received a diagnosis of coronary artery disease ( CAD ). IHD participants were tested in the laboratory 90 ± 36 days from the date of their hospital discharge. Patients presented with ST-elevation ($n = 15$) or non-ST elevation ($n = 7$) myocardial infarction, coronary artery disease ($n = 5$), coronary artery bypass graft surgery ($n = 4$; 3.3 ± 0.4 grafts), percutaneous coronary interventions ($n = 17$; 1.4 ± 0.6 stents), unstable angina ($n = 1$), or a combination of events. Patients remained on all prescribed medications during the study. IHD patients that disclosed medications ($n = 24$) included angiotensin II receptor blockers and/or angiotensin-converting enzyme inhibitors ($n = 21$), beta-blockers ($n = 20$), statins ($n = 24$), and antiplatelet medications including aspirin ($n = 23$). Control participants ($n = 20$) medications included statins ($n = 4$), and antiplatelet medications including aspirin ($n = 1$). Both participants and controls were all considered normotensive, and were not included in analysis if their blood pressures were above 140/90 mmHg (Williams et al., 2018).
Table 2.1. Participant characteristics of control and IHD groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>IHD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>N (males)</td>
<td>20 (10)</td>
<td>30 (26)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.75</td>
<td>11.17</td>
<td>63.97</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>27.60</td>
<td>3.80</td>
<td>29.80</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>129.2</td>
<td>16.5</td>
<td>120.34</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>77.3</td>
<td>7.8</td>
<td>71.4</td>
</tr>
<tr>
<td>Pulse Pressure (mmHg)</td>
<td>52.6</td>
<td>18.6</td>
<td>49.0</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>5.38</td>
<td>0.59</td>
<td>5.72</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.98</td>
<td>1.31</td>
<td>3.34</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.19</td>
<td>0.36</td>
<td>1.31</td>
</tr>
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<td>High Density Lipoprotein (mmol/L)</td>
<td>1.52</td>
<td>0.32</td>
<td>1.09</td>
</tr>
<tr>
<td>Low Density Lipoprotein (mmol/L)</td>
<td>2.99</td>
<td>0.96</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Total Cholesterol /</strong></td>
<td><strong>3.40 0.78</strong></td>
<td><strong>3.28 0.83</strong></td>
<td><strong>0.62</strong></td>
</tr>
<tr>
<td><strong>High Density</strong></td>
<td><strong>HbA1C (%) 5.46 0.34</strong></td>
<td><strong>5.63 0.41</strong></td>
<td><strong>0.11</strong></td>
</tr>
<tr>
<td><strong>Lipoprotein Ratio</strong></td>
<td><strong>C-Reactive Protein (mg/L) 1.40 1.11</strong></td>
<td><strong>3.52 5.95</strong></td>
<td><strong>0.12</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Insulin (pmol/L) 53.84 35.45</strong></td>
<td><strong>96.93 60.93</strong></td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td></td>
<td><strong>APOE4 Group (%) 0.20</strong></td>
<td><strong>0.31</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Montreal Cognitive Assessment 28.96 1.01</strong></td>
<td><strong>28.28 2.00</strong></td>
<td><strong>0.24</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Trail Making A (s) 29.71 11.49</strong></td>
<td><strong>35.14 15.76</strong></td>
<td><strong>0.26</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Trail Making B (s) 56.29 22.93</strong></td>
<td><strong>65.00 29.94</strong></td>
<td><strong>0.34</strong></td>
</tr>
</tbody>
</table>

### 2.4.2 Arterial stiffness

Carotid arterial stiffness ($\beta$-stiffness index) was greater in the IHD group (8.5 ± 3.3 a.u.) compared with controls (6.8 ± 2.2 a.u.; $p = 0.04$) (Figure 2.1). The corresponding CCA strain was less in the IHD group (0.067 ± 0.02 a.u.) compared with controls (0.080 ± 0.02 a.u.; $p = 0.03$). Carotid compliance in the control group (0.009 ± 0.004 mm/mmHg) was not different from in the IHD group (0.009 ± 0.004 mm/mmHg; $p = 0.95$). In the CCA, the pulsatility (2.6 ± 0.5 vs. 2.8 ± 0.7; $p = 0.17$) and resistivity (0.89 ± 0.06 vs. 0.90 ± 0.06; $p = 0.34$) indices were not different between controls and IHD participants, respectively.
Figure 2.1. Common carotid artery $\beta$-stiffness in controls and IHD patients.

IHD patients exhibit greater carotid stiffness ($p=0.04$), suggesting vascular remodeling of the common carotid artery is associated with IHD. Values are mean ± SD.

Unlike the common carotid, MCA pulsatility (1.11 ± 0.20 vs. 0.98 ± 0.18 a.u.; $p = 0.02$) and resistivity (0.66 ± 0.06 vs. 0.62 ± 0.07 a.u.; $p = 0.04$) were greater in the IHD patient group compared with the control group (Figure 2.2). Poor correlations existed between MCA PI and MoCA, ($p = 0.64$), Trail A ($p = 0.1$), Trail B ($p = 0.5$) as well as between MCA RI and MoCA ($p = 0.89$), Trail A ($p = 0.24$), Trail B ($p = 0.70$) tests of cognitive function. Measures of ccPWTT were made in 33 participants ($n = 33$). ccPWTT of the IHD group ($n = 20$; 0.013 ± 0.003 s) was not different from the control group ($n = 13$;
The carotid $\beta$-stiffness and ccPWTT measures did not correlate ($p = 0.13$).

**Figure 2.2.** Middle cerebral artery pulsatility and resistivity index in controls and IHD patients. IHD patients have greater cerebrovascular pulsatility ($p=0.02$) and resistivity ($p=0.04$), suggesting alterations in downstream vascular morphology when compared with controls. Values are mean ± SD.
2.4.3 White Matter Lesion Volume

Results of WMLv analysis by SPM12 lesion segmentation algorithm (Schmidt et al., 2012) were correlated with FreeSurfer white matter hypointensity output ($R = 0.68$, $p < 0.001$), which suggests these are areas of ischemic hypo-perfusion (Narayana et al., 2014). All WMLv data are reported using the values from SPM12. No differences were observed in the WMLv between the IHD participants (0.95 ± 1.2 mL) and the controls (0.86 ± 1.4 mL; $p = 0.81$, Figure 3).

![White Matter Lesion Volume by Group](image)

**Figure 2.3.** White matter lesion volume in control and IHD groups.

White matter lesion volume is not different between control and IHD groups. This suggests that IHD does not increase the brain’s vulnerability to white matter damage within one year of their cardiac event that required hospitalization ($p=0.81$). Values are mean ± SD.

As WMLv were statistically similar between groups, we pooled the group data to examine blood flow and velocity factors that correlate with WMLv. Before pooling, the
carotid β-stiffness ($R = 0.62, p = 0.03$) and ccPWTT ($R = -0.61, p = 0.006$) correlated with WMLv in the control group. In the IHD group, the β-Stiffness ($p = 0.40$) and ccPWTT ($p = 0.27$) correlated poorly with WMLv. In the pooled data, WMLv correlated with both carotid β-stiffness (Figure 2.4a; $R = 0.34, p = 0.02$) as well as ccPWTT (Figure 2.4b; PWTT; $R = -0.43, p = 0.02$).

Figure 2.4. Association of arterial stiffness and white matter lesion volume.

Carotid β-stiffness and carotid-cerebral transit time (ccPWTT) correlate with white matter lesion volume (WMLv) in simple linear regressions. In multivariate analysis, both carotid β-stiffness and ccPWTT remain independently associated to WMLv. In multivariate models (Table 2.2) adjusting for the significant variables from the simple linear regressions, ccPWTT ($p = 0.02$) and carotid β-stiffness ($p = 0.004$) remained independent predictors of WMLv. In model 2, PP was added to account for the pressure change that the vasculature must accommodate. In this model, both ccPWTT ($p = 0.02$) and carotid β-stiffness ($p = 0.04$) remained independently associated with WMLv. The
WMLv correlated poorly with CCA PI ($p = 0.88$), CCA RI ($p = 0.97$), MCA PI ($p = 0.84$), or MCA RI ($p = 0.89$) in the pooled data. Cognitive testing in the form of the Trail Making B test (R = 0.46, $p = 0.004$) correlated significantly with WMLv while the MoCA ($p = 0.31$) and the Trail Making A test ($p = 0.23$) did not (Figure 2.5).

Table 2.2. Regression models of physiological variables and WMLv.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>$p$</th>
<th>Model 1$^\wedge$</th>
<th>β</th>
<th>SE</th>
<th>$p$</th>
<th>Model 2$^\dagger$</th>
<th>β</th>
<th>SE</th>
<th>$p$</th>
</tr>
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<tbody>
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<td>Group</td>
<td>0.1</td>
<td>0.39</td>
<td>0.81</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>0.001</td>
<td>0.021</td>
<td>0.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SBP</td>
<td>0.017</td>
<td>0.013</td>
<td>0.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.01</td>
<td>0.02</td>
<td>0.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PP</td>
<td>0.022</td>
<td>0.013</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.004</td>
<td>0.009</td>
<td>0.70</td>
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<tr>
<td>β-Stiff</td>
<td>0.81</td>
<td>0.34</td>
<td>0.02*</td>
<td>0.11</td>
<td>0.04</td>
<td>0.004*</td>
<td>0.10</td>
<td>0.05</td>
<td>0.04*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWTT</td>
<td>-141.4</td>
<td>59</td>
<td>0.02*</td>
<td>76.5</td>
<td>29.6</td>
<td>0.02*</td>
<td>-80.9</td>
<td>32.2</td>
<td>0.02*</td>
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<td></td>
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</tbody>
</table>

Note: $^\wedge$Adjusted for significant ($p < 0.05$) variables: β-Stiffness, PWTT. $^\dagger$Adjusted for significant variables and pulse pressure.
Figure 2.5. Regression comparing trail making test B and white matter lesion volume.

Trail making B task for evaluating cognitive function was significantly correlated with white matter lesion volume. Other cognitive tests were not associated with WMLv (trail making A, $p=0.23$; Montreal cognitive assessment, $p=0.31$).

2.5 Discussion

The major findings of this study were: 1) Carotid arterial stiffness was greater in the IHD group compared to the control group. 2) MCA flow pulsatility and resistive indices were greater in the IHD group compared with the control group. 3) WMLv was not different between IHD and control groups. 4) Common carotid ($\beta$-stiffness) and cerebrovascular (ccPWTT) assessments of vascular stiffness correlated with WMLv in the pooled data but there were no differences between groups. In multivariate regression analysis, ccPWTT and carotid $\beta$-stiffness remained an independent association to WMLv in the pooled data.
This observation suggests that regardless of heart disease history, cerebrovascular PWTT and carotid β-stiffness are associated with structural white matter damage.

2.5.1 Effect of Ischemic Heart Disease

The association between arterial stiffness and cardiovascular disease is documented (Mattace-Raso et al., 2006; Mitchell et al., 2010; Weber et al., 2004). The strong association between vascular stiffening and end organ damage, particularly in renal, cardiac and cerebral vascular beds, has led to the emerging idea that the pressure waves generated by the left ventricle are damped to a lesser degree by the large arteries in these vascular beds (O’Rourke & Safar, 2005a). The transfer of high speed pulse pressure waves that penetrate deeper into the arteriolar beds of vulnerable organs resulting in microvascular damage (O’Rourke & Safar, 2005a). In humans, arterial stiffness is linked to hypertension (Mitchell, 2014; Safar et al., 2018) and increasing age (Al Ghatrif et al., 2013) through mechanisms such as increased sympathetic activity (Yamada, Miyajima, Tochikubo, Matsukawa, & Ishii, 1989), elastin degradation (Smith et al., 2012), and systemic inflammation (Sesso et al., 2003). Using a model of sinoaortic denervation and pharmacological sympathectomy in rodents, Lacolley and colleagues (Lacolley et al., 1995) demonstrated that arterial stiffness can occur in the absence of hypertension. In their model, arterial wall stiffness increased and distensibility decreased due to a combination of increased collagen:elastin ratio (Lacolley et al., 1995) and increasing α5 and αν integrin concentration in the vessel walls (Bouissou et al., 2014) with no change in mean blood pressure (Lacolley et al., 1995). In our study, the IHD patients (8.5 ± 3.3 a.u.) had greater carotid arterial stiffness than the controls (6.6 ± 2.2 a.u.; p = 0.04) with normal blood pressures in both groups. The groups were of similar age as well. The
mechanisms mediating vascular stiffness were not addressed in the current study and the
impact of historical blood pressure is not known. Nonetheless, their respective blood
pressure contributions to the association between vascular stiffness indices and WMLv
were statistically small.

The current data are complicated by a large degree of variability. One cause of this
variability likely lies in the heterogeneity of conditions underlying the ischemic heart
disease. First, anthropometric factors such as excess subcutaneous lipid deposition occurs
in approximately 50% of IHD populations (Nabipour et al., 2007). Additionally, in our
cohort, 57% of IHD participants (17/30) and 20% of our control population (4/20) were
considered prediabetic with HBA1c levels of 5.7% or more. This may explain why the
IHD cohort had greater fasting insulin levels compared with the control population ($p = 0.007$). However, insulin levels were not significantly related to ccPWTT ($p = 0.69$),
carotid $\beta$-stiffness ($p = 0.62$), or WMLv ($p = 0.42$) outcome variables in pooled analysis.

2.5.2 Pulsatility and Resistivity in Ischemic Heart Disease

Cerebral blood flow pulsatility and resistivity are indices of vascular resistance, reflecting
the overall downstream vascular bed caliber. Therefore, under steady-state conditions,
these indices may provide some measure of morphological changes in the
cerebrovascular bed downstream from the site of measurement in the MCA. Distinctly
different from vascular stiffness (or compliance), MCA pulsatility correlates modestly
with pulse pressure ($R = 0.34$-$0.48$) and weakly with peripheral arterial stiffness
measured via carotid-femoral ($R = 0.12$) and brachial-ankle ($R = 0.23$) PWV in patients
of varying blood pressures (Xu et al., 2012). Also, a link between hypertension and
greater MCA PI is consistent with pressure-induced remodeling of microvascular vessel
walls (Cho, Sohn, Kim, & Kim, 1997). Acutely, elevated baseline blood pressures are expected to induce a passive distension of a vessel segment, effectively reducing that vessel’s distensibility by mechanical means. In our study, the IHD group had greater MCA PI and RI compared with the control group, but similar blood pressures, suggesting that IHD patients exhibit differences in the morphology of the cerebral microvessels.

2.5.3 Pulsatility, Resistivity, and White Matter Lesion Volume

Low resistance to flow in the CCA is proposed as a means of permitting highly pulsatile flow and pressure waves to reach further into the microvasculature of the cerebrum, causing damage to the endothelium (Byrom & Dodson, 1948; O’Rourke & Safar, 2005a). Webb et al. found an association between pulsatility of the MCA and various degrees of WMLv (Webb et al., 2012). Our results stand in contrast to this earlier conclusion. Specifically, neither in the between-group contrasts, nor in the regression analysis of the pooled data did we observe an association between WMLv and the pulsatility or resistivity of the MCA blood velocity patterns. Furthermore, the current study does not support any relationship between pulsatile flow resistance indices in the CCA and WMHs, supporting observations from the earlier large scale (n = 668) Reykjavik study (Mitchell et al., 2011a).

2.5.4 Vascular Stiffness and White Matter Lesion Volume

Earlier studies indicate that greater stiffness of the peripheral vasculature, assessed by brachial-ankle pulse wave velocity, associates with greater WML burden in otherwise healthy adults (Hatanaka et al., 2011; Kuo et al., 2010). Two large-scale studies, the Rotterdam Study (Poels et al., 2012) and the Framingham Offspring Study (Tsao et al., 2013) established a strong correlation between WMHs and aortic arterial stiffness.
Although WMHs are commonly reported with greater arterial stiffness, no methodology directly measures cerebrovascular stiffness. Giller and Aaslid first estimated carotid-cerebral PWV in healthy individuals under normo- and hypocapnic conditions (Giller & Aaslid, 1994). They found the PWV to be higher in the cerebrovasculature compared to the aorta, which did not change when they constricted the cerebrovascular bed using hypocapnia. Fu et al. later found that ccPWV independently associated with peripheral vascular stiffness (brachial-ankle PWV), age, and diastolic blood pressure using a similar method (Fu et al., 2016). In their study, they examined healthy participants and excluded people with hypertension, coronary artery disease, and metabolic factors. In the current study, there is support for the cerebrovasculature playing a determining role in white matter structural integrity. The proposition that greater cerebral stiffness causes cerebrovascular damage aligns with recent findings that greater levels of ccPWV were predictive of carotid-MCA segment atherosclerosis (Fu et al., 2018) as well as stroke severity (Fu et al., 2019).

In order to gain greater insight into structural effects of cerebrovascular stiffness, Webb and colleagues (Webb et al., 2012) quantified the ECG-MCA transit time, and reported an association between this value of PWV and the severity of WMLv after adjusting for physiological factors (Webb et al., 2012). The ECG-MCA transit time is convenient, but carries the possible challenge of inter-individual variability in pre-ejection periods that can induce non-negligible variations in vascular transit time (Harris, Schoenfeld, & Weissler, 1967; Newlin & Levenson, 1979). The current study avoided this limitation by insonating the CCA and MCA concurrently.
To our knowledge, the current study represents the first attempt to correlate cerebrovascular stiffness, as measured by ccPWTT, with WMLv in patients known to exhibit vascular disease (IHD). The results indicate that the burden of IHD confers no greater risk to the individuals studied. Thus, while these data support the general hypothesis that greater cerebrovascular stiffness is associated with cerebral damage, they challenge the specific hypothesis that IHD exacerbates cerebrovascular stiffness and WMLv. The IHD patients in this study were examined within one year following their cardiac event (mean = 90 ± 26 days) that required hospitalization. Perhaps a longer-term follow up of these patients will expose accelerated decline in cerebrovascular health.

The proposal that vascular stiffness contributes to WMLv has received observational support, but the mechanisms of action remain unclear. Nonetheless, a vascular component is proposed. Specifically, WMLs depict structural brain regions with altered fluid mobility and water content and are often linked to vascular impairment (Joanna M. Wardlaw, Valdés Hernández, & Muñoz-Maniega, 2015). Further, perhaps endothelial dysfunction from central vascular parameters like PP and PWV may lead to BBB breakdown and subsequent extravasation of damaging substances or direct neuronal damage (J. M. Wardlaw, Sandercock, Dennis, & Starr, 2003). Inflammation may also contribute to the vascular abnormalities via dilation of perivascular spaces (Gutierrez et al., 2017; Wuerfel et al., 2008) which is associated with white matter damage (Zhu et al., 2010) through altering the drainage potential of cerebral interstitial fluid (Weller, Subash, Preston, Mazanti, & Carare, 2008).

The association between WMLv and vascular or neurological impairment may also be affected by the actual volume of white matter lesions. WMLv varies widely from ~0 mL
in young healthy adults to an average of over 30 mL in multiple sclerosis patients (Alfano et al., 2000). Functionally, when WMLv increases to 3-4mL, amnesic mild cognitive impairment is reported (Ye et al., 2017). However, neither the IHD or control participants in the current study presented with abnormal values of WMLv (pooled; 0.91 ± 1.3 mL), and the pooled values agreed with other reported values from older healthy cohorts (Mortamais et al., 2014).

2.5.5 Limitations

There are several limitations to this study. First, the heterogeneity of the IHD participants, in both etiology and prescribed medications discussed above, may have affected outcomes. Whether or not the types of IHD affect vascular stiffness differently was beyond the scope of this study although the question warrants additional research. However, a recent study determined that arterial stiffness was not different between various etiologies of coronary artery diseases such as ST elevated myocardial infarction and non-ST elevated myocardial infarction (Akkus et al., 2013). Many of the IHD participants were on vasoactive medications throughout the course of the study (IHD: n = 24; control: n = 5). While these may modify vascular properties they also enabled the examination of ccPWTT and carotid β-stiffness under normotensive conditions, avoiding the acute impact of hypertension on mechanical vascular distention (Safar et al., 2018). Nonetheless, even within drug classifications (i.e. beta-blockers) variability exists in the arterial stiffness response (McEniery et al., 2004). The direct effect of these pharmaceuticals was not studied in the current report. Heterogeneity of these factors may contribute to non-significant differences in hemodynamic variables, such as pulse.
pressure, which can affect arterial stiffness measures (Safar, Levy, & Struijker-Boudier, 2003).

Sex differences may have impacted the current results. In the current study, 87% of the IHD group were males, whereas 50% were male in the control group. Males exhibit greater arterial stiffness compared to females (Coutinho, Borlaug, Pellikka, Turner, & Kullo, 2013). In a post-hoc sub-analysis of our pooled data, no difference was observed between males and females in either carotid ($p = 0.60$) or cerebrovascular ($p = 0.25$) stiffness indices.

Another limitation is the method used to measure cerebrovascular stiffness. The carotid-MCA method has been established as a repeatable measure of vascular stiffness (Fu et al., 2016). However, this surrogate for intracranial stiffness includes extracranial components and a portion within the carotid canal before entering the true intracranial environment with elevated extravascular pressure (Cipolla, 2016; Faraci & Heistad, 2017; Vijaywargiya, Deopujari, & Athavale, 2017; Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018). We expect that each of these anatomical features exhibit varying effects on the measured ccPWTT across the entire segment. Thus, our value of ccPWTT must be considered a composite value. To our knowledge, no data exist on the distinct contributions of each segment to this composite value reported here. However, our data that carotid and cerebrovascular stiffness indices did not correlate with each other, suggest heterogeneous contributions from extra-, versus intra-cranial vascular segments. Theoretically, the association between intravascular and intracranial pressures will affect the transmission of the pulse wave within the intracranial segment (Doyle & Mark, 1992;
Further research is needed to articulate the effect that the cranium exerts on the pulse wave transmission into the microcirculation of the brain.

### 2.5.6 Conclusions

Ischemic heart disease patients with medically controlled blood pressures exhibit increased carotid arterial stiffness when compared with controls. MCA pulsatility and resistivity were higher in IHD patients; however, these measures did not correlate with WML. Regardless of vascular pathology, carotid β-stiffness and ccPWTT stiffness measures were correlated with the degree of white matter tissue abnormality (WMLv). In the pooled data, both carotid and cerebrovascular stiffness correlated independently with WMLv. The currently available measures of carotid and cerebrovascular stiffness did not correlate with one another suggesting heterogeneous contributions from extra- versus intra-cranial vascular segments. We found that increased stiffness in the cerebral vascular segment, not the pulsatility, increases the likelihood of white matter lesions in the brain, regardless of vascular pathology.

### 2.6 Acknowledgements

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2.7 References


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2016
In investigating the arterial stiffness of patients with cardiovascular disease, we discovered that the velocity of the pulse wave moving into the cerebrum is associated with severity of white matter damage. However, there are several inaccuracies involved with current methods to measure cerebrovascular stiffness including the estimation of vessel path length and the inclusion of extracranial arterial segments. In this chapter, we apply phase contrast magnetic resonance imaging to young and older healthy adults to take direct measurements of intracranial pulse wave velocity.

3.1 Abstract

Elevations in central arterial stiffness correlate with cerebrovascular outcomes like stroke, cognitive decline, and small vessel disease. These findings infer abnormal cerebrovascular function as well. However, limited by access challenges imposed by the skull, direct measures of cerebral arterial stiffness remain unreported. We present an approach to measure cerebrovascular pulse wave velocity (PWV) using phase contrast MRI (PCMRI). Eight healthy adults (4 young, age 19-25 yrs; 4 older, age 51-66 yrs) with no history of cardiovascular or cerebrovascular disease were studied. Arterial stiffness measurements were collected via tonometry (common carotid (CCA) and femoral arteries), and ultrasound (CCA and middle cerebral artery (MCA)). PCMRI was used to assess PWV and calculate characteristic impedance of CCA and MCA segments using gradient-recalled echo imaging with prospective cardiac-gating. Internal carotid (icaPWV) and middle cerebral artery PWV (mcaPWV) were assessed in separate PCMR scans using time-to-upstroke characteristics of segmental normalized waveforms. PCMRI of the
intracranial vessels was completed with 0.7mm in-plane spatial and up to 25ms temporal resolution. The mean mcaPWV was $3.4 \pm 1.5$ m/s (young, $2.1 \pm 0.5$ m/s; older, $4.7 \pm 0.8$ m/s; $p = 0.001$). PWV increased on moving from the carotid ($1.8 \pm 0.5$ m/s) to the MCA ($3.4 \pm 1.5$ m/s; $p = 0.004$). The MCA to ICA PWV ratio increased to a greater extent in the older than the younger cohort ($1.39 \pm 0.27$ vs. $2.22 \pm 0.50$, $p = 0.03$). Using a novel application of PCMR imaging, we obtained direct measures of cerebrovascular PWV in the MCA. Arterial PWV increased from the ICA to the M1-M2 segment of the MCA. Age appears to amplify this regional difference. This application may provide an improved understanding of cerebrovascular stiffness with aging and disease.

### 3.2 Introduction

Vascular arterial stiffening is a major cardiovascular health determinant, producing detrimental outcomes in many organs including the brain. Approximately 30% of global mortality is attributed to cardiovascular disease (Roth et al., 2015), including 14% of deaths attributed to cerebrovascular disease (Lozano et al., 2012). The conduit arteries offer a direct link between the heart and the brain, contributing to delivery of oxygen and nutrient-containing blood while dampening pressure pulsations via local distension to provide consistent flow to the tissue. However, the ability to damp pressure oscillations diminishes with age, an outcome that can be affected by lifestyle, nutritional, and genetic factors (Lee & Oh, 2010). This stiffening of peripheral arteries, particularly the aorta (Van Sloten et al., 2015), is associated with structural and functional deficits such as increased risk for stroke (Mattace-Raso et al., 2006) and cognitive decline (Waldstein et al., 2008). Links between peripheral stiffness and cerebrovascular outcomes are well documented (Henskens et al., 2008; Laurent et al., 2003; O’Rourke & Safar, 2005; Rundek et al., 2017). However, vascular stiffness and propagation characteristics of the pulse wave within the cranium remains unknown.
Various approaches have been advanced to quantify cerebrovascular stiffness. For example, Giller and Aaslid first proposed using simultaneous ultrasound of the common carotid artery (CCA) and the middle cerebral artery (MCA) to estimate the carotid-cerebral PWV (ccPWV) (Giller & Aaslid, 1994). Ultrasound measures of carotid-cerebral transit times correlate well with white matter lesion volume in adults with varying vascular pathologies (Balestrini, Al-Khazraji, Suskin, & Shoemaker, 2020), suggesting cerebral pulse wave propagation may relate to cerebrovascular integrity. However, previous estimates of ccPWV cannot be considered direct measurements of cerebrovascular stiffness due to the inclusion of extracranial and petrous carotid segments together with intracranial MCA segments (Vijaywargiya, Deopujari, & Athavale, 2017). Unlike extracranial arteries of the neck, cerebral vessels are subject to extravascular intracranial pressure that will produce a unique impact on the effective pulsatile properties (Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018) such as vascular compliance. Thus, approaches are needed to provide values of vascular stiffness within the pressurized cranium.

Phase contrast magnetic resonance imaging (PCMRI) was adapted to measure aortic PWV noninvasively in the 1990s (Mohiaddin, Firmin, & Longmore, 1993) and the outcomes predict cardiovascular (Mitchell et al., 2010; Vlachopoulos, Aznaouridis, & Stefanadis, 2010) and cerebrovascular disease (Laurent et al., 2003). This method provides a direct imaging perspective in arteries with acoustic window limitations, thereby making it ideal for cerebrovascular imaging.

In the current study, we present a PCMRI-based approach to assess the transmission of the pulse wave from the peripheral vasculature to the cerebral vessels. Specifically, we applied PCMRI to an extracranial-dominant segment from the internal carotid artery (ICA) to the initiating point of the MCA (icaPWV), and to a segment of intracranial artery along the M1-M2 portion of the
MCA (mcaPWV) to provide direct measures of intracranial arterial stiffness. This approach was used to test the overall hypothesis that vascular stiffness increases in intracranial vessels compared to extracranial vessels.

Additionally, we compared small cohorts of young and older healthy individuals to investigate whether the measured mcaPWV will correlate with age. This approach may have diagnostic and mechanistic implications for cerebrovascular disease.

3.3 Methods
3.3.1 Participants
Eight participants volunteered for the study (young healthy, n = 4, age 19 – 26 years; old healthy, n = 4, age 50 – 66 years). Participants were healthy, non-smokers, and did not have diagnosed cardiovascular, inflammatory, or psychological impairments. Three participants were taking medication at the time of testing, which included levothyroxine (n = 2), fluticasone/salmeterol (n = 1), and/or antihistamines (cetirizine, n = 1; bilastine, n = 1). All participants were normotensive and were not on blood pressure medications. Baseline participant characteristics, cognitive testing, and blood profiles are described in Table 3.1. All participants provided informed consent and the experimental protocol was in accordance with the standards set in the declaration of Helsinki and approved by the Health Sciences Research Board at Western University.

The overall study incorporated data from two sessions: 1) A laboratory session, and 2) an MRI session. When combined, and as detailed below, these two sessions provided indices of i) carotid-femoral PWV (cfPWV) using tonometric methods, ii) common carotid artery mechanical metrics, iii) PWV along the internal carotid (ICA)-to-MCA origin (icaPWV) to provide a largely extracranial segment, and iv) along the M1-M2 portion of right MCA (mcaPWV) to provide an intracranial segment.
Table 3.1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
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<th>Older</th>
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<td></td>
<td>Mean</td>
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<td>Trail Making A (s)</td>
<td>22</td>
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<td>26</td>
<td>8</td>
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<td>Trail Making B (s)</td>
<td>33</td>
<td>5</td>
<td>58</td>
<td>24</td>
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</tbody>
</table>

3.3.2 Laboratory Session

Participants were asked to refrain from alcohol, caffeine, and strenuous exercise for the 12 hours prior to testing. A fasting blood sample was taken from the medial antecubital vein. Participants
were equipped with lead II electrocardiography and laid supine for 10 minutes to measure resting heart rate. Measures of systemic blood pressure were obtained at the distal digital artery using photoplethysmography (Finometer, Finapres Medical Systems BV, Amsterdam, Netherlands) and calibrated to brachial blood pressures. Using B-mode ultrasound (Vivid I, GE Healthcare, Chicago, Illinois) right CCA diameters were measured at systole and diastole. Carotid arterial strain (equation 1) and compliance (equation 2) were calculated. β-stiffness was calculated as an index of carotid artery stiffness (equation 3) (Kawasaki, Sasayama, Yagi, Asakawa, & Hirai, 1987). Cerebral blood velocities from the M1 segment of the right MCA were collected via transcranial Doppler ultrasound (TCD; 2 MHz transducer, Neurovision 500M, Neurovision TOC2M, Multigon Industries).

Equation (1):

\[
\text{Strain} = \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Diameter}_{\text{diastole}}}
\]

Equation (2):

\[
\text{Compliance} = \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Pressure}_{\text{systole}} - \text{Pressure}_{\text{diastole}}}
\]

Equation (3):

\[
\beta \text{eta Stiffness} = \ln \left( \frac{\text{Pressure}_{\text{systole}}}{\text{Pressure}_{\text{diastole}}} \right) \frac{\text{Strain}}{}
\]

Additionally, cfPWV was quantified using two tonometric probes (10,000Hz) placed on the CCA and superficial femoral artery. Carotid-femoral lengths were recorded as 80% of the direct path between the transducers (Van Bortel et al., 2012). Participant completion of the Montreal
Cognitive Assessment (Nasreddine et al., 2005) and Trail Making Tasks (Army Individual Test Battery, 1944) provided indices of cognitive function.

### 3.3.3 MRI Session

All MRI data were acquired using a Siemens Prisma Fit 3-Tesla scanner at the Centre for Functional and Metabolic Mapping at Western University in London, Ontario, Canada. A 64-channel head/neck radiofrequency receive array was used to provide optimal signal properties in both the CCA and MCA vessels. Volumetric structural T1-weighted (MPRAGE; TE = 2.98ms, TR = 2300ms, TI = 900ms, FOV = 256mm, BW = 240Hz/px, TA = 5:21 minutes) and fluid-attenuated inversion recovery (FLAIR; TE = 387ms, TR = 5000ms, TI = 2400ms, FOV = 230mm, BW = 750Hz/px, TA = 5:30 minutes) were acquired with 1mm isotropic resolution.

Images from MPRAGE and FLAIR scans were overlaid to automatically detect paraventricular WMHs using the lesion growth algorithm (Schmidt et al., 2012). The threshold value ($\kappa$) was set to 0.3, if needed, visual inspection was used to determine the optimal value for detection of WMHs.

A 3D time of flight (TOF) angiogram (Figure 3.1ab; TE = 3.42ms, TR = 21ms, FOV = 174mm, BW = 185Hz/px, TA = 6:25 minutes) was used as a localizer to prescribe imaging planes for subsequent scans as well as measure the distance between regions of interest on the PCMR images. PCMR images were acquired using a gradient-recalled echo sequence (TE = 3.66ms, TR = 25.4 ms, FOV = 188mm, BW = 445Hz/px, in-plane resolution = 0.73mm, slice thickness = 2mm, $V_{enc} = 100$cm/s, TA = ~5 minutes) to measure the onset of the pulse wave. Blood flow velocity profiles at various locations along the ICA and MCA were created with the use of prospective ECG-gating.
3.3.4 MRI Analysis

Imaging planes were selected manually to ensure they intersected at angles close to perpendicular with each vascular segment being studied (Figure 3.1ab, panel 1). Based on anatomical positioning, two phase contrast images were acquired. The first image captured two points along the ICA-MCA segment to analyze icaPWV (Figure 3.1a, panel 1-2). The second image captured two points along the M1-M2 portion of the intracranial MCA and was used to analyze mcaPWV (Figure 3.1b, panel 1-2). For each PCMR series, individual images were used to plot a time-intensity curve over the course of the cardiac cycle (Figure 3.1a-b, panel 3) (Rebergen, van der Wall, Doornbos, & de Roos, 1993). Each vessel segment on the PCMR image was normalized to the respective region of interest maximum and minimum phase intensity, and curve fitting was applied to the waveforms. The normalized foot of the pulse was set as 30% of the increase in pixel intensity to denote the upstroke of the waveform (Figure 3.2).

The temporal delay of the normalized PCMRI velocity waveforms between the proximal and distal measurement sites for each of the icaPWV and mcaPWV segments was calculated. This allowed the time of the pulse upstroke to be calculated from multiple reconstructed frames over the cardiac cycle to reduce the measurement variability (Wentland, Grist, & Wieben, 2014). The distance between the points of interest on vascular segments was measured using Osirix software (Pixmeo, Geneva, Switzerland) (Rosset, Spadola, & Ratib, 2004). Pulse wave velocity was reported as the distance/time of the pulse wave between the points of interest.
Figure 3.1. Phase plane selection, measurement, and time-intensity curves of ICA and MCA

Panel A, ICA; Panel B, MCA. 1) An angiogram is taken and the plane for the phase contrast slice is manually selected (yellow dashed line) and measured (red solid lines). 2) Phase contrast images of the cerebral vessels. 3) Normalized phase-change plotted over the upstroke of the pulse wave.

Figure 3.2. Comparison of ICA and MCA pulse wave upstroke.

Internal carotid artery (left) and middle cerebral artery (right) time-intensity curves. The 30% of the normalized phase-change was used as the marker of pulse wave upstroke. Arrows represent the time between proximal and distal artery segments.
Regional differences in vascular impedance can be used to assess reflection characteristics and downstream pressure wave amplitude propagation (Mitchell et al., 2004). Characteristic impedance is related to the PWV of the pressure wave through a vessel (equation 4) (Nichols, O’Rourke, Vlachopoulos, Hoeks, & Reneman, 2011). The ICA and MCA characteristic impedance (equation 5) was reported using the PWV calculated from the PCMR images (described above) and the diameters of the CCA and MCA (TOF angiogram).

\[
PWV = \frac{Z_c \cdot A}{\rho}
\]

\[
Z_c = \frac{4\rho \cdot PWV}{\pi \cdot D^2}
\]

Where \(Z_c\) is vascular impedance, \(\rho\) is blood density, \(PWV\) is pulse wave velocity, \(A\) is cross-sectional area, and \(D\) is arterial diameter.

### 3.3.5 Statistical Analysis

The effect of location (e.g. difference between icaPWV and mcaPWV) as measured by PCMRI was assessed using a paired t-test. A secondary analysis comparing young and older age groups was performed using independent t-tests and Pearson regression analysis. Statistical probability was set at \(p < 0.05\) for all analyses. Data are presented as mean ± standard deviation unless otherwise noted. Statistical analyses were conducted using SigmaPlot 12.5 (Systat software Inc., San Jose, CA, USA) and GraphPad Prism 8 (GraphPad software Inc., La Jolla, CA, USA).
3.4 Results

3.4.1 Arterial Segment Characteristics

The estimated carotid-femoral distance (60 ± 4cm) was used for the central tonometry PWV calculations. The average diameter of the phase contrast ICA segment was 4.4 ± 0.5mm and did not correlate well with age (p = 0.86). The average diameter of the M1 segment was 2.8 ± 0.2mm and did not correlate with age (p = 0.14). The phase contrast segment distance for the icaPWV calculation was 7.9 ± 2.8cm. For the phase contrast mcaPWV calculation, the distance between proximal and distal locations was 5.6 ± 1.2cm. The time between the onset of the corresponding flow velocity waves measured at the proximal and distal ends of the ICA segment was 49 ± 24ms and 19 ± 8ms for the MCA segment.

3.4.2 Laboratory Measures of Arterial Stiffness

Common carotid arterial strain was 0.107 ± 0.029 AU (young, 0.131 ± 0.016 AU; older, 0.083 ± 0.014 AU; p = 0.004) and correlated with age (R = -0.86, p = 0.006). Common carotid compliance was 0.016 ± 0.006 AU (young, 0.021 ± 0.003 AU; older, 0.012 ± 0.005 AU; p = 0.02) and correlated with age (R = -0.78, p = 0.02). Common carotid β-stiffness was 4.8 ± 1.6 AU (young, 3.8 ± 0.4 AU; older, 5.8 ± 1.7 AU; p = 0.06) and did not correlate with age (R = 0.62, p = 0.1). Tonometry-based PWV measures from the carotid-femoral segment were 5.9 ± 1.3m/s (young, 5.0 ± 0.7m/s; older, 6.7 ± 1.2 m/s; p = 0.06) and correlated with age (R = 0.73, p = 0.04).

3.4.3 Cerebrovascular Phase Contrast Pulse Wave Velocity and Impedance

Velocity-based waveforms of ICA and MCA blood flow are shown in Figure 3.2. Phase contrast PWV measured in the internal carotid segment (icaPWV) was 1.8 ± 0.5m/s (young, 1.4 ± 0.3m/s; older, 2.2 ± 0.5 m/s; p = 0.03) and was correlated with age (R = 0.77, p = 0.03). The mean
mcaPWV was $3.5 \pm 1.5 \text{m/s}$ (young, $2.1 \pm 0.5 \text{m/s}$; older, $4.7 \pm 0.8 \text{m/s}$; $p = 0.001$) and was correlated with age ($R = 0.92$, $p = 0.001$, Figure 3.3a). In a multiple regression model, the effect of age on mcaPWV was independent of corresponding values of MAP and HR ($p = 0.02$). The ratio of PCMRI-based measures of PWV increased from the ICA to the MCA (mcaPWV : icaPWV, $1.85 \pm 0.45$, $p = 0.004$), which was displayed across all participants. The increase in PWV from ICA to the MCA was amplified in the older cohort (mcaPWV : icaPWV; $1.39 \pm 0.27$ vs. $2.22 \pm 0.50$, $p = 0.03$, Figure 3.4a).

Figure 3.3. Effect of age on MCA PWV and Impedance

Panel A) The effect of age on measured MCA PWV. The mcaPWV in the older cohort was greater than in young individuals. Panel B) The effect of age on calculated MCA impedance. The calculated MCA impedance was greater in the older group compared with young individuals.

Characteristic impedance ($Z_c$) was calculated using propagation velocity (PCMRI; icaPWV and mcaPWV) and arterial diameter (TOF angiogram). Characteristic impedance in the ICA was 131
± 52 dynes*s/cm³ (young, 99 ± 13 dynes*s/cm³; older, 162 ± 57 dynes*s/cm³; p = 0.11) and did not correlate significantly with age (p = 0.07). Characteristic impedance in the MCA was 627 ± 316 dynes*s/cm³ (young, 345 ± 96 dynes*s/cm³; older, 910 ± 175 dynes*s/cm³; p = 0.003) and was strongly associated with age (R = 0.89, p = 0.003, Figure 3.3b). The Zc increased from the ICA to the MCA segment (p = 0.002). The increase in Zc from the ICA to the MCA was amplified in the older cohort (mcaZc : icaZc; 3.5 ± 1.0 vs. 6.0 ± 1.4, p = 0.05, Figure 3.4b).

![Figure 3.4](image)

**Figure 3.4.** Effect of pulse moving into the intracranial compartment on MCA PWV and Impedance

Panel A) Comparison of age group on ratio of mcaPWV to icaPWV. The increase from the extracranial to the intracranial PWV is accelerated in older individuals. Panel B) Effect of age group on ratio of MCA impedance to ICA impedance. The older cohort experiences a greater increase in vascular impedance when the pulse wave enters the cranial cavity.
3.5 Discussion

The major findings of the study were: 1) PCMRI can be used to measure intracranial PWV at the ICA and MCA, 2) PWV increases going from extracranial to intracranial segments, and 3) vascular impedance increases going from the ICA to the MCA. Additionally, sub analyses suggested that the increase in PWV and vascular impedance on going from extracranial to intracranial vascular segments was amplified in older adults. Therefore, the current results indicate that PCMRI can be used to study the variations in PWV between extracranial and intracranial sites in intact and conscious humans. Further, although the sample size was small, the current results indicate that age amplifies the stiffening of vascular beds in the brain.

3.5.1 Measures of Cerebrovascular Stiffness

To our knowledge, this study marks the first time that PCMRI has been applied to the cerebrovasculature to obtain direct measurements of vascular stiffness within the rigid confines of the skull. This technique provides advantages over earlier approaches (Fu, Huang, Wong, Chen, & Gao, 2016; Giller & Aaslid, 1994) that rely on ultrasound-based measures of PWV from the CCA to the M1 MCA segment because distances can be quantified accurately, taking into account the inherent tortuosity of the cerebral vessels, which increases with age (Kamenskiy, Pipinos, Carson, Mactaggart, & Baxter, 2015; J. B. Thomas et al., 2005). Furthermore, the PCMRI approach provides measures of intracranial vascular segments that are not confounded by inclusion of extracranial vascular segments that apparently express greater elasticity.

Many studies have used PCMRI to collect cerebral artery flow measurements as it allows for the simultaneous quantification of blood flow velocity and arterial diameter (Spilt et al., 2002). Recently, 2D PCMRI measures of PWV in the carotid artery in younger and older participants (Kröner et al., 2014) provided evidence that both aortic and carotid measures of vascular stiffness were directly associated with age. Importantly, measures of carotid stiffness in the
previous study were assessed with separate acquisitions at proximal and distal points. In contrast, our approach relied on the tortuosity of the ICA and MCA to obtain proximal and distal arterial segment measurements with a single-phase acquisition. This advancement allowed us to remove the influence of inter-scan variability as the velocities were calculated from the same image. Also, the prospective gating and image reconstruction into 34 frames allowed an effective temporal resolution of up to 25ms and spatial resolution of 0.7*0.7mm, which was adequate to resolve the MCA outcomes in our participants.

3.5.2 Vascular Impedance

Based on the Water-Hammer formula, characteristic vascular impedance (McDonald, 1974) can be calculated if PWV and arterial diameter are known (Mitchell et al., 2003). Impedance is the resistance to flow through a pulsatile system. In young, healthy individuals, there is a significant difference between central aortic PWV (~4-6 m/s) and peripheral muscular artery PWV (~8-10 m/s) (Mitchell et al., 2004) creating a mismatch in impedance between the two sites. This mismatch allows the minimization of forward amplitude propagation, which functions to protect the microvasculature (London & Pannier, 2010). With aging, artery diameter remains rather stable (as shown in the current study) but central stiffness increases at a rate greater than peripheral vascular stiffness, minimizing the impedance mismatch and shifting wave reflections distally in the arterial system (Sugawara, Hayashi, & Tanaka, 2010). This distal-shift may enhance the risk for low-resistance organs like the brain, as damaging high amplitude pulse waves may reach deeper into the microvasculature. Greater aortic stiffness is hypothesized to propagate greater amplitude pressure waves into the cerebral vessels, leading to dilation of the perivascular space, a biomarker for small vessel disease (O. Thomas, Cain, Nasralla, & Jackson, 2019). The current study did not aim to explore clinical outcomes and no participants in our study had underlying white matter damage. Nonetheless, compared to the younger group, the
older group had greater impedance in the MCA (345 ± 96 vs. 910 ± 175 dynes*s/cm³). Thus, our
data suggest that with aging, PWV and characteristic impedance increase in the cerebral vessels
more than in the extracranial arteries. This presents a complex interaction that may be
responsible for the greater stress on the vascular system in aging. While the forward propagation
of the pulse wave reaches the branching points faster, the reflected waves caused by the
mismatch of impedance between vessel beds is also greater, adding stress to both the end organ
as well as the heart. Previous work demonstrated that reduced pulse wave transit time (inverse of
PWV) in the CCA to MCA M1 segment correlated with structural white matter damage in
patients with ischemic heart disease and healthy controls (Balestrini et al., 2020). Future studies
are needed to provide insight into the temporal relationship between direct markers of
cerebrovascular stiffness and corresponding indices of structural changes in the microvascular
beds supplied by these larger arteries.

3.5.3 Cerebrovascular Considerations
While vascular segments are anatomically continuous, they express regional variations in their
mechanical properties. Additionally, advancing age exerts heterogeneous effects on regional
arterial stiffness (Rogers et al., 2001). While contributing to impedance, the PWV is greater in
muscular arteries than elastic arteries in young participants (Latham et al., 1985). However, a
PCMRI study found that aging increases the vascular stiffness preferentially in central elastic
arteries versus distal muscular segments (Rogers et al., 2001). While connecting the periphery to
the cerebrum by measuring stiffness from the aorta (cpPWV; tonometry), internal carotid
(icaPWV, PCMRI), and cerebral (mcaPWV, PCMRI) vessels, we found a strong association
between age and regional measures of stiffness (aorta, ICA, MCA) in healthy individuals. For a
method of direct comparison, PCMRI was used both peripherally in the ICA and intracranially in
the MCA. While the absolute measurements of PWV were low relative to other studies in the
abdominal aorta (Rogers et al., 2001; Yu, Peng, Wang, Wen, & Tseng, 2006), the PWV increased when moving from the periphery to the cranium by a factor of 1.85 (95% CI: 1.45 – 2.26). This suggests differential patterns of regional stiffening occur in the cerebrovasculature than in the extracranial muscular peripheral arteries.

3.5.4 Perspectives

While the connection between elevated aortic stiffness and cerebral vessel disease and cognitive functioning has been established (Henskens et al., 2008; Mitchell et al., 2011; Poels et al., 2012; Singer, Trollor, Baune, Sachdev, & Smith, 2014; Waldstein et al., 2008), there remains a need for a method to quantify the vascular stiffness in the confines of the skull. The need to overcome intracranial pressure to distend is unique to the cerebral vessels. Mechanically, this may play a critical role in transferring elevated pulsatile pressure in the cerebral microvessels. This conjecture is supported with our data that mcaPWV is greater than icaPWV in a manner that is amplified with age (Figure 3.4). In this regard, our current application of PCMRI analysis for direct measures of intracranial vascular stiffness provides a basis for enhanced and large-scale studies to test this hypothesis.

3.5.5 Limitations

The heterogeneity of metabolic factors between study participants may have influenced arterial stiffness measures. All participants were healthy controls, with no underlying cardiac, metabolic, neurological, or inflammatory impairment. However, age correlated well with cholesterol (total and LDL) and HBA1c levels. Metabolic factors like hypercholesterolemia are associated with regional changes in arterial stiffness parameters (Ershova et al., 2016). However, none of the current participants were high-risk for metabolic syndrome or had cholesterol levels above 6.3 mmol/L.
The purpose of this study was to apply PCMRI to measure arterial stiffness in the brain. Therefore, the participant cohort was selected based on the expected difference in PWV seen in aging (McEniery et al., 2005). Sample size was calculated based on Mohiaddin et al. (Mohiaddin et al., 1993), where a strong association was reported for age-related changes in PCMRI-based aortic PWV recordings ($r^2 = 0.76$). In post-hoc analysis, our mcaPWV measure had an effect size of 0.92.

To analyze mcaPWV from PCMR imaging, an image plane that intersects the MCA vessel at near perpendicular angles in multiple locations is required. Additionally, the distance between intersecting locations of the MCA must be adequate to allow the discrimination of the pulse wave, which is subject to the temporal resolution of the scan. While novel to the cerebral vessels, a temporal resolution of 30ms has been suggested to minimize error when using PCMRI for PWV calculations in the thoracic aorta at velocities from 2 to 20m/s (Dorniak et al., 2016). The current study achieved temporal resolutions up to 25ms and used MCA segmental distances of $5.6 \pm 1.2$cm which was adequate to detect mcaPWV in the 1 to 6m/s range.

### 3.5.6 Conclusions

To our knowledge this is the first study to obtain direct measurements of PWV within the cranium, using the M1-M2 segment of the MCA. This was possible by applying cardiac-gated PCMRI with a gradient-recalled echo sequence to the cerebral vessels. Arterial stiffness was greater in older participants, measured by conventional methods (cfPWV) and our novel PCMR imaging approach (icaPWV and mcaPWV). Additionally, the characteristic impedance of the MCA was greater with age in our cross-sectional cohort with limited sample size. Using PCMRI of both the ICA and MCA, the PWV increased within the cranium, and this increase was amplified with age. This novel application of PCMRI in the cerebral vessels allows for further insight into the connection between pulse wave transmission and cerebral abnormalities.
3.6 Acknowledgements

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Chapter 4

4 Effect of hypercapnia and sodium nitroglycerin on multimodal measurements of peripheral and cerebral arterial stiffness

By applying PCMRI imaging to the cerebral vessels in a cross-sectional population of young and older healthy adults, it appears PCMRI-based pulse wave velocity measurements can be used to detect differences in arterial stiffness (Chapter 3). In this chapter, we applied the PCMRI intracranial PWV approach during hypercapnia and nitroglycerin conditions in healthy young adults to assess the effect of vascular dilation on cerebrovascular stiffness.

4.1 Abstract

Stiffening of the central and peripheral arteries is commonly associated with negative cerebrovascular outcomes like small vessel disease. The non-linear arterial distension – tension relationship suggests arterial stiffness may vary when arterial diameter is altered. However, the affect of vasodilation on acute changes in peripheral and cerebral vessel stiffness remains unknown. We investigated effect of carbon dioxide (HC; 6% CO₂) and sodium nitroglycerin (NTG; 0.4mg sublingual) on the peripheral and cerebral vascular responses in six young healthy adults (age 23-25). Local arterial distension and pulse wave velocity (PWV) measurements were collected via tonometry (common carotid (CCA) to femoral artery (FA; cfPWV)), ultrasound (local CCA stiffness) and PCMRI (M1-M2 MCA; mcaPWV). During HC, local distension (CCA diameter; Δ4.7 ± 2.6%, p = 0.01; MCA cross-sectional area (CSA); Δ6.6 ± 2.2%, p < 0.001) and regional PWV (cfPWV 7.3 ± 1.4m/s vs. 6.1 ± 1.5m/s, p = 0.001; mcaPWV 4.4 ± 1.3m/s vs. 2.4 ± 0.9m/s; p = 0.003) increased over baseline levels, while FA diameter (p = 0.37) was not affected. During NTG, local distension (FA diameter; Δ7.3 ± 4.1%; p = 0.008; CCA diameter; Δ6.3 ± 2.5%, p = 0.002; MCA CSA; Δ11.3 ± 5.2%, p = 0.003) and regional PWV (cfPWV; 6.7 ± 1.5m/s vs. 6.1 ± 1.5m/s; p = 0.003; mcaPWV; 7.3 ± 4.4m/s vs. 2.4 ± 0.9m/s; p = 0.04) increased
over baseline levels. Using a multimodal approach to measure regional arterial parameters during HC and NTG conditions, we found that the acute dilatory response increased peripheral and cerebral PWV. Understanding how dilation affects pulse wave propagation into the cerebrum may have direct implications for interpreting mechanisms of cerebrovascular disease.

4.2 Introduction

Stiffening of the arterial system is a major element of cardiovascular health with direct implications for end organs like the brain. Vascular stiffening reduces circumferential dampening of pulse pressure fluctuations resulting in higher amplitude and increased rate of travel along the vascular system. In this manner, aortic arterial stiffness is associated with adverse cerebral outcomes such as increased risk of small vessel disease (Brisset et al., 2013; Poels et al., 2012) and neurological impairment such as cognitive decline (Singer, Trollor, Baune, Sachdev, & Smith, 2014; Waldstein et al., 2008). An emerging hypothesis to explain this relationship outlines the potential for high amplitude and faster pressure waves to travel deeper into the microvasculature causing damage (O'Rourke & Safar, 2005; Safar et al., 2018), particularly in high-conductance organs such as the brain. However, measuring vascular stiffness in the cerebrum is difficult due to limited access for traditional tonometry or ultrasound-based approaches.

Recently, we applied phase contrast MRI (PCMRI) to measure intracranial pulse wave velocity (PWV) across the M1 – M2 segment of the middle cerebral artery (MCA) (Balestrini, Matuschewski, Gati, Al-Khazraj, & Shoemaker, 2020). This direct measurement of cerebrovascular PWV has distinct advantages over traditional extracranial measures for evaluating cerebrovascular outcomes. Specifically, imaging removes path length estimations by allowing explicit measurement of the vascular distance. Additionally, the M1 – M2 segment is entirely within the cranial cavity, providing an accurate illustration of how arteries are affected
within the pressurized cerebral environment. The previous study found that PWV increased from the extracranial ICA to the intracranial M1 – M2 segment of the MCA. The acceleration of the pulse wave was amplified in older adults (Balestrini et al., 2020). However, the previous study focused solely on a descriptive characterization of baseline vascular characteristics, leaving open the question of whether the differences were related to vascular contractile state or other features related to the MCA residing within the pressurized cranium. In the present study we aimed to use PCMRI to measure cerebrovascular pulse wave characteristics following acute changes in dilation status.

Hypercapnia (HC) (Al-Khazraji, Shoemaker, Gati, Szekeres, & Shoemaker, 2018; Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014) and sodium nitroglycerin (NTG) (Schulz, Al-Khazraji, & Shoemaker, 2018) promote dilation of the cerebral conduit arteries. Hypercapnia provokes a complex cerebral dilatory response that includes endothelial (Faraci & Heistad, 2017), extravascular pH (Kontos, Raper, & Patterson, 1977), and neuronal (Jordan et al., 2000) contributions. Additionally, HC is accompanied by downstream pial artery dilation and subsequent increase in total cerebral blood flow (Al-Khazraji et al., 2018; Coverdale et al., 2014) whereas the effect of NTG appears to be localized to the conduit arteries alone. Specifically, NTG administered sublingually is absorbed through mucous membranes (Zhang, Zhang, & Streisand, 2002) and acts on smooth muscle cells via a cyclic guanosine monophosphate (cGMP) mechanism to induce passive dilation of the muscular arteries (Moncada, Palmer, & Higgs, 1991) without an ensuing change in total cerebral blood flow (Schulz et al., 2018).

The elasticity of a vascular segment can be affected by its diameter. When a vessel is stretched circumferentially, the relative load-bearing contributions of collagen and elastin are altered such that the vessel wall experiences greater tension with incremental increases in distension (Roach & Burton, 1957). However, whether active vasodilation produces the same effect as passive
distension remains unclear because the involvement of the contractile element in concert with the stiff collagen and elastic elastin elements will vary. Thus, Bank et al. (1995) reported increased compliance of the human brachial artery following administration of intra-arterial NTG under conditions of controlled transmural pressure (Bank et al., 1995). Additionally, Harvey and colleagues (2017) reported reduced peripheral artery PWV following ganglionic blockade of sympathetic vasoconstriction, but this effect was due to the concurrent reduction in blood pressure (Harvey et al., 2017).

Unlike peripheral arteries, vascular compliance within the skull is affected by both intravascular and intracranial elements (Mair Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018). Specifically, higher extravascular pressures in the cranium may minimize the ability of these vessels to express their latent elasticity. This expectation is supported by modeling data (M. Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018), as well as PWV values that increased markedly on going from the internal carotid artery to the M1 – M2 segment of the MCA (Balestrini et al., 2020). Nevertheless, how the pressurized cranium affects the relationship between dilation and PWV in the cerebral circulation is unknown.

In the present study, we used PCMRI in young healthy adults to assess PWV in the M1 – M2 segment of the MCA (mcaPWV) during baseline, HC, and NTG conditions. This methodology was used to test the hypothesis that vascular dilation influences cerebral arterial stiffness. Additionally, we examined the effect of HC and NTG on the femoral (FA) and common carotid (CCA) arteries to evaluate the impact of the vascular environment and region on arterial stiffness parameters. If cerebral arterial stiffness can be altered acutely, there may be mechanistic implications for understanding the etiology of small vessel disease in the brain.
4.3 Methods

4.3.1 Participants
Six young participants (age = 23 – 25 years) volunteered for the study. Participants were healthy non-smokers, and did not have any diagnosed metabolic, cardiovascular, or psychological condition at the time of the study. All participants were normotensive and were not on any medications. Three participants were taking vitamin supplementation at the time of testing including iron (n = 3), vitamin C (n = 3), and vitamin D (n = 2). Participants refrained from exercise, alcohol, and caffeine for 12 hours prior to testing. A fasted venous blood sample was taken from the median antecubital vein. Participants completed cognitive testing including the Montreal Cognitive Assessment (Nasreddine et al., 2005) and trail making tests (Army Individual Test Battery, 1944). Participant characteristics, blood profiles, and cognitive testing are described in Table 4.1. All participants gave informed consent and the protocol was in accordance with the standards set in the declaration of Helsinki and approved by the Health Sciences Research Board at Western University.

4.3.2 Laboratory Testing
Participants were laying in a supine position throughout testing. Heart rate was measured via lead II electrocardiography. Measures of systemic blood pressure were collected from the distal digital artery using photoplethesmography (Finometer, Finapres Medical Systems BV, Amsterdam, Netherlands) and were calibrated to manual blood pressures. Ultrasound (Vivid I, GE Healthcare, Chicago, Illinois, 2D 7-12 MHz transducer) of the common carotid (CCA) and femoral (FA) arteries was used to measure arterial diameter during systole and diastole. Vessel diameter is reported during diastole. Calculated $\beta$-Stiffness provided a measure of local vascular stiffness (equation 1) (Kawasaki, Sasayama, Yagi, Asakawa, & Hirai, 1987). Arterial strain, compliance, distensibility, and
Peterson elastic modulus were calculated as well (Laurent et al., 2006). Cerebral blood flow velocities were recorded via transcranial Doppler ultrasound (TCD; 2 MHz transducer, Neurovision 500M, Neurovision TOC2M, Multigon Industries) of the M1 segment of the MCA. For each artery, the pulsatility (PI) (Gosling & King, 1974) and resistive (RI) (Pourcelot, 1974) indices were calculated as measures of downstream vascular resistance.

The cfPWV was calculated using the time delay between two tonometric probes (10,000Hz) on the CCA and superficial FA. Carotid-femoral lengths were taken as 80% of the surface length between the two measurement locations (Van Bortel et al., 2012).

Characteristic impedance ($Z_c$) was calculated as a measure of regional changes in wave reflection characteristics and downstream propagation of the pulse wave amplitude (Mitchell et al., 2004). The $Z_c$ was calculated based on arterial diameters (equation 2.1; FA and CCA) and CSA (equation 2.2; MCA) as well as cfPWV (FA) and mcaPWV (MCA) (Mitchell et al., 2003).

Where $\rho$ is the density of the blood (1.06 g/mL), D is diameter, and CSA is the cross-sectional area of the artery.

Equation (1):

$$\beta \text{eta Stiffness} = \ln \left( \frac{\text{Pressure}_{\text{systole}}}{\text{Pressure}_{\text{diastole}}} \right) \left( \frac{\text{Diameter}_{\text{systole}} - \text{Diameter}_{\text{diastole}}}{\text{Diameter}_{\text{diastole}}} \right)$$

Equation (2.1):

$$Z_c = \frac{4\rho \times PWV}{\pi \times D^2}$$

Equation (2.2):
4.3.3 Magnetic Resonance Imaging

Magnetic resonance images were acquired via a Siemens Prisma Fit 3-Tesla scanner at the Centre for Functional and Metabolic Mapping at Western University in London, Ontario, Canada. Volumetric structural 3D T1 images (MPRAGE; TE = 2.98ms, TR = 2300ms, TI = 900ms, FOV = 256mm, BW = 240Hz/px, TA = 4:51 minutes) and fluid attenuated inversion recovery (FLAIR; TE = 387ms, TR = 5000ms, TI = 2400ms, FOV = 230mm, BW = 750Hz/px, TA = 5:30 minutes) images were acquired. Using the lesion growth algorithm (Schmidt et al., 2012), T1 and FLAIR images were overlaid to quantify paraventricular white matter hyperintensities (WMH) to confirm no small vessel disease.

A time of flight angiogram (TOF; TE = 3.63ms, TR = 22ms, FOV = 181*199mm, BW = 165Hz/px, TA = 6:25 minutes) was used to map the MCA and as a localizer for phase contrast plane selection in order to image perpendicular to the M1 and the M2 segments. Whole-brain T2 images (TE = 96ms, TR = 3000ms, FOV = 160mm, BW = 225Hz/px, TA = 1:15 minutes) were used to measure the CSA of the M1 segment of the MCA during each condition. Phase contrast MR images (TE = 3.66ms, TR = 25.4ms, FOV = 188mm, BW = 445Hz/px, in-plane resolution = 0.73mm, slice thickness = 2mm, velocity encoding (V_{enc}) = 100-120cm/s, TA = ~5 minutes) were acquired to collect flow data at two points along the M1 – M2 segment of the MCA. Velocity encoding parameters for PCMRI were adjusted based on laboratory MCA velocities (100-120cm/s). Time – intensity curves were plotted using prospective ECG-gating to reproduce 34 frames through the cardiac cycle. Protocols from our previous work were used to normalize and determine the upstroke of the waveform (Balestrini et al., 2020). Vascular distance between...
M1 and M2 segments of interest was measured using Osirix software (Prixmeo, Geneva, Switzerland) (Rosset, Spadola, & Ratib, 2004).

### 4.3.4 Vascular Dilation States

In both the laboratory and the MRI sessions we assessed the above metrics in three conditions. First, a baseline (BSL) condition while breathing room air in the supine position. Second, participants breathed a gas mixture with elevated CO₂ levels (HC; 6% CO₂, 21% O₂, balanced N₂) to achieve a hypercapnic state using a 5L Douglas bag and a ventilation tube fastened to a face mask. Hypercapnia was confirmed by measurement of end tidal CO₂ (etCO₂) levels. After the hypercapnic challenge, participants rested for 10 minutes to allow for etCO₂ measures to return to baseline levels. Finally, participants were administered a sublingual dose of nitroglycerin spray (NTG; 0.4mg Nitrolingual Pumpspray; Sanofi, Laval, Qc, Canada) to initiate passive dilation of the large arteries via smooth muscle relaxation. Treatments were non-randomized and remained in order (BSL, HC, NTG) due to long washout periods after NTG administration.

### 4.3.5 Statistical Analysis

Descriptive values and standard deviations were calculated as a group. The effect of condition was tested using a one-way repeated measures ANOVA with Bonferronni correction for significant differences. When an effect of condition was established, conditions were compared to BSL using paired t-tests. Linear regression analysis was used to assess the relationship between changes in vascular dilation and PWV. Statistical significance was set at $p < 0.05$ for all analyses. Data are presented as mean ± standard deviation. All statistical analyses were conducted using SigmaPlot 12.5 (Systat software Inc., San Jose, CA, USA) and GraphPad Prism software (GraphPad software Inc., La Jolla, CA, USA).
4.4 Results

4.4.1 Participant characteristics

Participants’ physical, hematological, and cognitive measures are outlined in Table 4.1. Normal blood values ruled out cases of acute inflammation or metabolic conditions. Cognitive testing confirmed that the population was cognitively normal.

4.4.2 Laboratory Vascular Parameters

Central hemodynamic factors across the treatment conditions are displayed in Table 4.2. The HC condition increased diastolic blood pressure (DBP; \( p = 0.02 \)) and mean arterial blood pressure (MAP; \( p = 0.03 \)). Similarly, NTG increased systolic blood pressure (SBP; \( p = 0.003 \)), DBP (\( p = 0.006 \)) and MAP (\( p = 0.005 \)) over BSL measures.

Table 4.3 illustrates the PI and RI for each artery and condition. Femoral artery diameter (Figure 4.1a) was unchanged in response to HC (\( \Delta -0.5 \pm 1.0\%; \ p = 0.37 \)) and was increased in response to NTG (\( \Delta 7.3 \pm 4.1\%; \ p = 0.008, \ d = 0.73 \)). Carotid artery diameter (Figure 4.1b) increased in both the HC (\( \Delta 4.7 \pm 2.6\%, \ p = 0.01, \ d = 0.45 \)), and NTG (\( \Delta 6.3 \pm 2.5\%, \ p = 0.002, \ d = 0.61 \)) compared with BSL diameters.

The cfPWV was 6.1 ± 1.5m/s in the BSL condition and increased to 7.3 ± 1.4m/s (\( p = 0.001, \ d = 0.83 \)) during HC and 6.7 ± 1.5m/s (\( p = 0.003, \ d = 0.40 \)) during NTG. The FA Zc was 280 ± 120 dynes*s/cm\(^3\) in the BSL condition. The FA Zc was unchanged in the HC condition (304 ± 108 dynes*s/cm\(^3\); \( p = 0.07 \)) and the NTG condition (269 ± 99 dynes*s/cm\(^3\); \( p = 0.39 \)) when compared with BSL.
Table 4.1. Participant baseline, cognitive, and venous blood characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171</td>
<td>6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66</td>
<td>11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>60</td>
<td>7</td>
</tr>
<tr>
<td><strong>Cognitive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trail A (s)</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Trail B (s)</td>
<td>36</td>
<td>6</td>
</tr>
<tr>
<td>MoCA</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td><strong>Bloods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.0</td>
<td>0.3</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>2.7</td>
<td>0.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.1</td>
<td>0.2</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>39.7</td>
<td>19.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Note: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PP, pulse pressure; HR, heart rate; MoCA, Montreal cognitive assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, Hemoglobin A1c; CRP, C-reactive protein; HOMA-IR, hemostatic model assessment of insulin resistance.

Table 4.2. Central, femoral, and carotid characteristics across conditions

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean±SD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Central</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>60±7</td>
<td>59±7</td>
<td>60±8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>94±8</td>
<td>97±8</td>
<td>98±9*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>62±10</td>
<td>63±9*</td>
<td>65±8*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73±5</td>
<td>75±5*</td>
<td>76±4*</td>
</tr>
<tr>
<td>TPR</td>
<td>16±6</td>
<td>17±7</td>
<td>17±5</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>85±24</td>
<td>88±24</td>
<td>85±22</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>5.1±1.8</td>
<td>5.1±1.7</td>
<td>5.0±1.6</td>
</tr>
<tr>
<td>EtCO2 (mmHg)</td>
<td>43±2</td>
<td>47±2*</td>
<td>43±2</td>
</tr>
<tr>
<td><strong>FA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>0.03±0.01*</td>
</tr>
<tr>
<td>Compliance</td>
<td>0.010±0.007</td>
<td>0.008±0.005</td>
<td>0.008±0.006</td>
</tr>
<tr>
<td>Distensibility</td>
<td>0.0016±0.0012</td>
<td>0.0013±0.0008</td>
<td>0.0012±0.0009*</td>
</tr>
<tr>
<td>Ep (mmHg)</td>
<td>799±339</td>
<td>968±464</td>
<td>1111±532</td>
</tr>
<tr>
<td>B-Stiff</td>
<td>10.6±4.8</td>
<td>12.4±6.1</td>
<td>13.8±6.4</td>
</tr>
<tr>
<td><strong>CCA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>0.13±0.05</td>
<td>0.11±0.05*</td>
<td>0.11±0.04*</td>
</tr>
</tbody>
</table>
### Compliance (mm/mmHg)

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>0.028±0.020</td>
<td>0.025±0.017</td>
<td>0.024±0.013</td>
</tr>
</tbody>
</table>

### Distensibility (mmHg⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>0.0051±0.0034</td>
<td>0.0043±0.0028*</td>
<td>0.0040±0.0021</td>
</tr>
</tbody>
</table>

### Ep (mmHg)

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>252±108</td>
<td>308±158</td>
<td>295±115*</td>
</tr>
</tbody>
</table>

### B-Stiff

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>3.4±1.6</td>
<td>4.0±2.2</td>
<td>3.7±1.6*</td>
</tr>
</tbody>
</table>

**Note:** FA, femoral artery; CCA, common carotid artery; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; TPR, total peripheral resistance; SV, stroke volume; CO, cardiac output; etCO₂, end tidal CO₂; Ep, Peterson elastic modulus; B-Stiff, β-stiffness. * p < 0.05.

### Table 4.3. Arterial pulsatility and resistive indices across conditions

<table>
<thead>
<tr>
<th></th>
<th>BSL</th>
<th>HC</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### FA

- **Pulsatility Index**: Mean±SD 13.0±5.1, 14.1±3.5, 19.9±5.2*
- **Resistive Index**: Mean±SD 1.26±0.06, 1.28±0.03, 1.39±0.06*

### CCA

- **Pulsatility Index**: Mean±SD 3.03±0.42, 2.82±0.41, 3.14±0.48
- **Resistive Index**: Mean±SD 0.89±0.04, 0.88±0.02, 0.90±0.04

### MCA

- **Pulsatility Index**: Mean±SD 0.87±0.08, 0.74±0.11*, 0.82±0.11
- **Resistive Index**: Mean±SD 0.56±0.03, 0.50±0.05*, 0.53±0.04

**Note:** FA, femoral artery; CCA, common carotid artery; MCA, middle cerebral artery; BSL, baseline; HC, hypercapnia; NTG, nitroglycerin. * p < 0.05.
4.4.3 MRI Vascular Parameters

The etCO$_2$ (Figure 4.2) was 44.1 ± 1.8mmHg in the BSL condition. Compared to BSL, etCO$_2$ increased with HC (Δ 4.1 ± 1.6mmHg, $p = 0.002$, $d = 2.12$) but did not change during NTG (Δ 0.1 ± 1.0mmHg, $p = 0.88$). Middle cerebral artery CSA (Figure 4.1c) was 7.1 ± 0.9mm$^2$ in the BSL condition. Compared to baseline, MCA CSA increased in both the HC (Δ 6.6 ± 2.2%, $p < 0.001$, $d = 0.53$) and NTG conditions (Δ 11.3 ± 5.2%, $p = 0.003$, $d = 0.85$).

Figure 4.1. Arterial diameter across conditions

This figure shows the effect of hypercapnia (HC) and nitroglycerin (NTG) on the femoral (A), common carotid (B), and middle cerebral (C) arteries are shown. Continuous lines represent individual data. Bars are mean ± SD. * $p < 0.05$. 
**Figure 4.2. Change in end tidal CO2 across conditions**

This figure shows the effect of hypercapnia (HC) and nitroglycerin (NTG) on end tidal CO2. The contributions of CO2 as a stimulus was only present in the HC condition. Continuous lines represent individual data. Bars are mean ± SD. * p < 0.05.

Cardiac-gated time-intensity curves were generated for the flow velocities in the M1 and M2 segment of the MCA during BSL, HC, and NTG conditions (Figure 4.3). The mcaPWV in the BSL condition was 2.4 ± 0.9m/s. The mcaPWV increased in both the HC (4.4 ± 1.3m/s, p = 0.003, d = 1.84) and the NTG treatments (7.3 ± 4.4m/s, p = 0.04, d = 1.53), respectively (Figure 4.4). In a linear regression using both HC and NTG conditions compared with BSL, the change in CSA of the MCA associated with the change in mcaPWV (R = 0.61, p = 0.04; Figure 4.5).
This figure shows an example of the waveforms of the proximal (●) and distal (○) segments of the M1-M2 region of the MCA. The time delay of the waveforms between the segments is reduced with hypercapnia (HC) and nitroglycerin (NTG).

The MCA Zc was 370 ± 136 dynes*s/cm³ in the BSL condition. The MCA Zc increased in the HC condition (634 ± 212 dynes*s/cm³; \( p = 0.005 \)) and the NTG condition (1002 ± 598 dynes*s/cm³; \( p = 0.04 \)) when compared with BSL.

### 4.5 Discussion

The key findings in this study were: 1) Intrapersonal changes in cerebrovascular arterial stiffness in response to changes in diameter can be detected using PCMR-based measurement of mcaPWV. 2) Hypercapnia dilated the CCA and MCA but not the FA. 3) Nitroglycerin dilated the FA, CCA, and MCA. 4) Tonometric cifPWV and PCMRI mcaPWV increased with both the HC and NTG interventions. 5) In the MCA, there was a direct association between the increase in CSA and the increase in mcaPWV. 6) Calculated characteristic impedance was higher in the MCA compared with the CCA-FA segment, and impedance increased when the MCA stiffened. Therefore, in the cranium, the dilation of the MCA in the supine position translated to an increase in arterial stiffness. Additionally, HC preferentially affects the cerebral vessels, both at
the conduit artery dilation and downstream resistive components. The NTG appears to act non-
specifically on conduit vessels to change dilation. Both aortic and cerebrovascular PWV
increased with dilation, which may have been influenced by changes in central arterial pressure.

Figure 4.4. mcaPWV across conditions

This figure shows the effect of hypercapnia (HC) and nitroglycerin (NTG) on middle
cerebral artery pulse wave velocity (mcaPWV). The PWV increased in the HC and the
NTG conditions. Continuous lines represent individual data. Bars are mean ± SD. * p <
0.05.

4.5.1 Cerebrovascular Pulse Wave Velocity

Stiffening of the aorta has direct implications on cerebrovascular outcomes such as cognitive
impairment (Scuteri, Brancati, Gianni, Assisi, & Volpe, 2005), stroke (Mattace-Raso et al.,
2006), and white matter hyperintensity burden (King et al., 2013; Mitchell et al., 2011; Van
Sloten et al., 2015). These relationships raise the question of whether the cerebral arteries
participate in conveying pressure waves from the aorta to the small vessels of the brain. Until
recently, challenges in acquiring direct measurements of cerebrovascular stiffness limited the
interpretation of arterial stiffening to central and extracranial peripheral arteries. In our previous
work we presented a phase contrast application to obtain intracranial measurements of ICA and MCA PWV (Balestrini et al., 2020). This method provided the cross-sectional observation that mcaPWV was different in young and older healthy individuals and correlated with cfPWV measured via tonometry. Our current study demonstrates that the PCMRI method can also be used to detect intrapersonal differences in mcaPWV in various vascular dilation states (Figure 4.4). The temporal resolution up to 25ms achieved with our PCMR prospective ECG-gated approach was able to detect mcaPWVs of 2.4 ± 0.9 m/s at baseline, which was similar to the young healthy cohort in our earlier study (2.1 ± 0.5 m/s). However, with greater velocities, there appears to be greater in the variability of the PWV recordings in the cerebrum. Although no computational data exists for the MCA, temporal resolution of 30ms has been suggested to be adequate to detect the initiation of the pulse wave upstroke in the thoracic aorta at velocities of 2-20 m/s (Dorniak et al., 2016).
This figure shows the relationship between the change in middle cerebral artery (MCA) cross-sectional area (CSA) and the change in pulse wave velocity (PWV) with conditions versus the baseline parameters.

### 4.5.2 Vascular Response to Hypercapnia

A long history of measurements indicate that hypercapnia preferentially affects the cerebral circulation over the peripheral circulation (Lennox & Gibbs, 1932). In the HC condition in the periphery, FA diameter was unaffected \((p = 0.37)\), while there was an increase in CCA diameter \((p = 0.01)\), supporting earlier observations (Ainslie, Ashmead, Ide, Morgan, & Poulin, 2005).

However, the cfPWV \((p = 0.001)\) increased in response to HC. Moreover, HC did not affect the FA using either the local (Zc, distensibility) or downstream (PI, RI) markers. Therefore, the effect of HC on cfPWV likely represents changes in aortic compliance or central parameters like the modest \((+2.2 \text{ mmHg})\) increase in MAP (Kim et al., 2007). In contrast, CCA strain and distensibility decreased with HC. The mechanisms mediating this local effect are not clear.

However, this could suggest that in the CCA, the tension load bearing may shift from the vascular smooth muscle contractile element to the stiffer collagenous element (Bank et al., 1995) as the vessel dilates actively, or is distended passively by the increase in MAP.

In the supine state with normal intracranial pressure, the cerebral conduit arteries are in a relatively dilated state, which is needed to ensure the blood flow meets metabolic demand in the brain. Hypercapnia elicits both local and downstream effects at the MCA. An increase in MCA CSA by \(~1.5\%/\text{mmHg}\) of change in etCO\(_2\) when breathing 5% CO\(_2\)/balanced O\(_2\) gas mixture was shown to occur locally at the M1 segment in young healthy individuals (Al-Khazraji et al., 2018). Also, we saw a downstream response to HC seen via decreased MCA PI and RI, which has been reported previously (Czosnyka, Richards, Whitehouse, & Pickard, 1996), supporting the overall concept that HC elicits changes throughout the cerebrovascular network. Our HC challenge prompted a modest change in etCO\(_2\) \((4.1 \pm 1.6 \text{ mmHg})\) and an increase in MCA CSA.
of 1.6%/mmHg of etCO₂, which aligns with the earlier study. In contrast, Kellawan et al. (Mikhail Kellawan et al., 2016) reported no increase in MCA CSA ($p = 0.06 - 0.07$) with a small increase in etCO₂ (~3.7 mmHg). Differences in etCO₂ change may be due to paced versus spontaneous breathing and/or the composition of the inhaled gas mixtures. Spontaneous breathing was utilized in our study over the course of the hypercapnia stimulus (Figure 4.6). This combined with a potential threshold effect may contribute to an undetectable dilatory response in studies with small changes in etCO₂.

In our study, the HC condition prompted MCA dilation ($\Delta 6.6 \pm 2.2\%, p < 0.001, d = 0.53$), and increased the mcaPWV ($p = 0.003$, Figure 4.4). In the first measurements of cerebrovascular pulse wave transit time, Giller and Aaslid used ultrasound at the ICA and MCA during periods of hyperventilation, inducing relative hypocapnia and cerebral vasoconstriction. In their work, there was no observable difference in cca-mcaPWV when etCO₂ was reduced (Giller & Aaslid, 1994). The difference in constriction and dilation may reflect distinct threshold points on the arterial distensibility curve (Roach & Burton, 1957). It is important to note that our vascular segment is entirely within the cranium and that, in contrast to the surface distance measures used earlier, we incorporated accurate measures of vascular length along the tortuous vessel pathway (Vijaywargiya, Deopujari, & Athavale, 2017).

### 4.5.3 Vascular Response to Nitroglycerin

Nitroglycerin initiates activation of soluble guanylyl cyclase to induce cGMP to passively dilate large arteries via smooth muscle relaxation (Arnold, Mittal, Katsuki, & Murad, 1977). The expected vasodilation of the large muscular arteries (Ignarro, Ross, & Tillisch, 1991) occurred in the FA and CCA. The dilation response in the FA and CCA was accompanied by an increase in strain as well as cfPWV. This contrasts an early study that reported decreased brachial-radial PWV when the radial artery was dilated with NTG infusion (Fok, Jiang, Clapp, & Chowienczyk,
Regional differences in arterial composition can alter the effective stiffness curve depending on pressure and vessel diameter (Cox, 1975; Gavish & Izzo, 2016), which may have influenced PWV. The NTG only altered PI ($p = 0.002$) and RI ($p = 0.02$) in the FA, not the CCA, suggesting an effect on peripheral muscular arteries downstream in the lower limb, but not in the cerebral microcirculation.

**Figure 4.6. Changes in etCO$_2$ over time.**

This figure shows the onset and time course of etCO$_2$ in a representative subject over the course of hypercapnia. The thick black line represents the start of the hypercapnic challenge.

Nitroglycerin was first suggested to increase the diameter of the large cerebral vessels in the 1980s, with reports of decreased cerebral blood velocity and maintained blood flow (Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1989). Using 7T MRI and sublingual NTG, Schulz et al. (Schulz et al., 2018) demonstrated a 24% dilation of the MCA in healthy subjects compared with a placebo. In this previous study, MCA CSA increased while blood velocity was reduced, resulting in no change of total blood flow to the brain. Our data support the earlier findings, as all
participants had an increase in MCA CSA without change to PI \((p = 0.15)\) and RI \((p = 0.08)\) suggesting that exogenous NTG primarily influences the vascular smooth muscle of the conduit arteries without affecting downstream vascular resistance. While the mechanisms for unaffected downstream metrics are still to be determined, baroreflex sensitivity and heart rate variability analysis suggest an increase in sympathetic balance following nitroglycerin treatment (Gori, Floras, & Parker, 2002), which presents competing mechanisms that may modulate regional blood flow despite conduit artery dilation.

4.5.4 Dilation – Stiffness Considerations

Vessel wall elasticity is related to pulse wave propagation speed by the Moens-Korteweg equation (equation 3).

Equation (3):

\[
Pulse\ Wave\ Velocity = \sqrt{\frac{E_{inc}h}{2r\rho}}
\]

Where \(E_{inc}\) is the incremental elastic modulus, \(h\) is the wall thickness, \(r\) is the vessel radius, and \(\rho\) is the density of the blood. \(E_{inc}\) is commonly used as a metric of elasticity and is not dependent on arterial size (Nichols, O’Rourke, Vlachopoulos, Hoeks, & Reneman, 2011). Additionally, large regional differences in \(E_{inc}\) have been reported between the CCA and other central and peripheral arteries (Bia et al., 2005), suggesting increased diameter will disproportionally affect certain arteries. We did not measure incremental elastic modulus, and our Peterson elastic modulus results may have not achieved adequate power to detect a change across conditions. However, our PWV results indicate that \(E_{inc}\) may increase when the conduit arteries are dilated during HC and NTG conditions. It appears that dilation via HC and NTG cause conduit cerebral
vessels to shift to a low gain threshold of the curvilinear wall tension and pressure curve, which may have implications for pulse wave transmission into the microvascular beds.

4.5.5 Limitations

There are several limitations to this study. The need for refined spatial resolution can limit the temporal resolution in PCMR imaging. We achieved spatial resolution of 0.73mm with temporal resolution of up to 25ms. This exceeds the 30ms resolution suggested as the minimum for thoracic aorta PWV measurements (Dorniak et al., 2016). However, there is greater flow variability in PCMR recordings with smaller CSA (Macdonald & Frayne, 2015). As a result, when the time delay between flow waveforms is reduced, such as during the conditions of this study, there may be the need to improve temporal resolution in order to detect differences at higher values of PWV. Therefore, future studies may want to consider interweaving multiple phase contrast scans with offset time delays to reconstruct time – intensity plots with improved effective temporal resolution (Yu, Peng, Wang, Wen, & Tseng, 2006). Another consideration is the small sample size. Nonetheless, the changes in mcaPWV with hypercapnia and/or NTG achieved a large effect size of $d = 1.84$, and the response was consistent across individuals, producing confidence in the outcomes.

4.5.6 Conclusions

To our knowledge, this is the first study to use PCMRI to detect intrapersonal changes in cerebrovascular PWV. The HC and NTG conditions elicited dilation of the MCA as well as an increase in mcaPWV and characteristic impedance. The increased dilation and subsequent increase in arterial stiffness was also exhibited in the FA (cfPWV; tonometry). Hypercapnia and NTG altered downstream vascular resistance in a vessel-dependent manner such that HC decreased MCA PI and RI, while NTG increased FA PI and RI. Our results support the use of PCMRI to measure intracranial PWV, providing a method to examine intracerebral vascular
stiffness and advance our understanding of cerebrovascular changes, damage and therapeutics in vulnerable populations.
4.6 References


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https://doi.org/10.1148/radiol.13121598


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https://doi.org/10.1016/j.arr.2014.02.002


https://doi.org/10.1097/HJH.0b013e32834fa8b0


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https://doi.org/10.1161/HYPERTENSIONAHA.107.093674


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Chapter 5

5 General Discussion & Summary

Using tonometry, ultrasound, and MRI techniques, this thesis describes the independent effect of the cranium on vascular considerations in white matter disease (Chapter 2), while providing the first direct measurements of intracranial pulse wave velocity (Chapter 3) and how it is influenced by acute vasodilation in young, healthy individuals (Chapter 4). The current chapter will discuss several key findings as they relate to mechanisms of cerebrovascular outcomes and examine future avenues of related research in the field.

5.1 General Discussion

5.1.1 Vascular Considerations of Brain Health

Cardiovascular disease, including associated cerebrovascular disease, is the leading cause of global mortality (Roth et al., 2015). The physiological underpinnings of systemic oxygen and nutrient transport to tissues translates to vulnerability when the conduit mechanics of the cardiovascular system are altered. In the diseased and aging artery, the ability to dampen pressure waves and distribute steady blood flow to peripheral capillaries is diminished due to degradation of structural integrity (O’Rourke & Hashimoto, 2007). End organs like the brain and kidney have low vascular resistance, which leaves them especially susceptible to damaging flow and pulsatile pressure waves travelling into their microvascular structure (Mitchell, 2018; Mitchell et al., 2011; O’Rourke & Safar, 2005). As such, stiffening of the arterial system is a major determinant of cardiovascular health that is directly related to cerebrovascular outcomes like small vessel disease (Ding et al., 2015; Poels et al., 2012; Thomas, Cain, Nasralla, & Jackson, 2019) and cognitive decline (Angelo Scuteri, Brancati, Gianni, Assisi, & Volpe, 2005; Singer, Trollor, Baune, Sachdev, & Smith, 2014; Waldstein et al., 2008). In Chapter 2, IHD patients with preserved ejection fraction but normal blood pressures were investigated to
determine whether arterial stiffness influences cerebral white matter structure in the absence of acute hypertension. We found that while IHD patients exhibited greater carotid arterial stiffness, their vascular pathology alone did not reflect changes in white matter integrity. Instead, both the local CCA stiffness and the cca-mcaPWTT influenced the severity of WMHs independently regardless of IHD status, after adjustment for one another and pulse pressure. This finding complimented an earlier cross-sectional study that showed an association of central arterial stiffness and WMHs, which was not influenced by previous diagnosis of IHD (Saji et al., 2011).

In contrast to earlier studies that focused on the pulsatility of the cerebral blood vessels (Kidwell et al., 2001; Lee et al., 2017), we did not see any relation of PI or RI to WMLv. In the cerebrum, these indices represent complex interactions of intracranial factors and downstream resistance profiles of pial arteries and the parenchymal microcirculation, and are not easily interpreted (Bellner et al., 2004; De Riva et al., 2012). In our study, the greater velocity of the pulse wave, not the pulsatility, was associated with white matter irregularities in the cerebrum.

5.1.2 Measurement of Cerebrovascular Stiffness

The idea of measuring the time delay in pulse pressure transmission between the CCA and MCA has emerged as a diagnostic tool and a predictor of large-vessel atherosclerosis, and is currently undergoing clinical trials for stroke outcomes (Fu et al., 2018; Zeng et al., 2019). In Chapter 2, we found direct associations between cca-mcaPWTT and both WMLv and the cognitive Trail Making task-B, supporting the notion that the transmission of the pulse wave into the cerebral vessels has implications for structural and function cerebral outcomes. However, the CCA-MCA measurement is complicated by access limitations that the skull imposes on ultrasound. Additionally, the majority of the vascular path from the CCA to the MCA contains extracranial segments or is encased within the bony carotid canal before entering the skull (Vijaywargiya, Deopujari, & Athavale, 2017). Once inside the cranium, unique characteristics of intracranial
pressure (ICP) and brain tissue influence extravascular pressure on the vasculature (Arani et al., 2018; M. O. Kim et al., 2015), which can diminish the ability of the artery to express elasticity (Zamir, Moir, Klassen, Balestrini, & Shoemaker, 2018). Therefore, in Chapter 3 we used PCMR imaging to acquire direct measurements of time-resolved flow through the cerebral vessels. The PCMRI approach is used to measure PWV in the abdominal aorta (Mohiaddin et al., 1993; Rogers et al., 2001; Yu, Peng, Wang, Wen, & Tseng, 2006), with emerging applications in the carotid arteries (Rivera-Rivera et al., 2020). We applied cardiac-gated PCMRI to the ICA and MCA to measure PWV in young and older healthy adults, as aging is known to increase arterial wall stiffness. Further, we eliminated the estimation of arterial distance via surface length between ultrasound probes as imaging enables direct measurement of vascular segments. In Chapter 3, we found that age correlates with greater velocity of the pulse wave in both the ICA and MCA. Additionally, PWV increases upon moving from the extracranial to the intracranial compartment, and this acceleration is amplified in older adults. The conjecture that PWV increases in the peripheral vasculature has been met with mixed evidence (Latham et al., 1985; Roccabianca, Figueroa, Tellides, & Humphrey, 2014; Taviani et al., 2011). Nonetheless, aging is known to alter the central elastic arteries preferentially compared with peripheral arteries (Rogers et al., 2001), likely due to ongoing elastin fragmentation (Schlatmann & Becker, 1977). This increase in central PWV with age reduces the mismatch in vascular impedance between central and peripheral extracranial arteries and may diminish wave reflections, allowing high energy pulsations to travel deeper into the vascular bed (Mitchell et al., 2004). In our cohort of healthy older participants, we did not detect any form of white matter impairment. One possible explanation for this is that the increased vascular impedance in the MCA may have maintained the mismatch between the central and intracranial arteries, conveying a protective effect on the cerebral microvasculature.
5.1.3 Cerebrovascular Stiffness and Vasodilation

The temporal and spatial resolution of ICA and MCA PCMRI was adequate to differentiate cerebrovascular stiffness in young and older healthy individuals (Chapter 3). This study substantiated the utility of this technique to determine whether acute changes in vascular state influence intrapersonal arterial stiffness within the cranium (Chapter 4). To address this question, we implemented two conditions known to dilate the MCA, hypercapnia (HC) (Al-Khazraji, Shoemaker, Gati, Szekeres, & Shoemaker, 2018) and sublingual sodium nitroglycerin (NTG) (Schulz, Al-Khazraji, & Shoemaker, 2018), to measure peripheral and cerebral arterial stiffness in young healthy adults. We found that HC and NTG initiated vasodilation in both the CCA and MCA. The vasodilatory response to hypercapnia in the cerebral arteries is likely due to complex interactions of endothelial (Faraci & Heistad, 2017), extravascular pH (Kontos, Raper, & Patterson, 1977), and neuronal contributions (Jordan et al., 2000). Sublingual NTG is an NO donor, and initiates dilation of the MCA via a cGMP mechanism to cause a relaxation of the smooth muscle of large muscular arteries (Arnold, Mittal, Katsuki, & Murad, 1977; Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1989; Schulz et al., 2018). In our investigation, regardless of the mechanism, MCA vasodilation was associated with an increase in intracranial PWV. This finding suggests that when cerebral arteries are dilated in the supine position, they operate at a low gain area of the diameter – pressure curve such that greater pressures are needed to induce local distension (Roach & Burton, 1957), resulting in greater PWV of the vessel. There appear to be regional differences in the dilation – PWV relationship, as a previous study found that dilation via NTG infusion of the peripheral radial artery elicits a reduction in PWV (Fok, Jiang, Clapp, & Chowienczyk, 2012). This contrast may be interpreted as a difference in 1) basal vasomotor tone and 2) extravascular factors affecting the effective pulsatile dynamics of each artery. Compared with the radial artery, the cerebral vessels are subject to reduced vasomotor tone to ensure that blood flow can meet the high metabolic demands of the brain. Additionally, modelling of
cerebral vessels has demonstrated the ability of the artery to express elastic features is reduced in the pressurized skull (Zamir et al., 2018). Our results were complicated by the finding that blood pressure increased with both treatments, which may have affected mcaPWV from a central hemodynamic perspective (E. J. Kim et al., 2007). However, the increase in MAP was small (~3mmHg) and we are not sure if this would affect PWV. Nonetheless, cerebral vessels in the resting state appear to be dilated with low compliance. In this dilated state, the contractile force of the vascular smooth muscle is negligible (Barra et al., 1993) and the load shifts to the passive elastic components of the arterial wall. The elastic modulus of the passive component is non-linear, such that as pressure increases, there is a shift in load-bearing from elastin to the less-compliant collagen protein. Our interpretation is that vascular smooth muscle and elastin are stretched in the resting cerebrovasculature, and collagen bears the additional mechanical load of dilation, resulting in increased intracranial PWV.

5.2 Future Directions

The findings of this thesis demonstrate that cerebrovascular arterial stiffness is associated with integrity of white matter, establish a PCMRI application to directly measure intracranial stiffness, and reveal that intracranial stiffness can be acutely modified by changes in vascular dilation. The connection between aortic arterial stiffness and structural and functional cerebral impairment has been well established (Henskens et al., 2008; Mitchell et al., 2011; Poels et al., 2012; Singer et al., 2014; Waldstein et al., 2008). The cerebral vessels are unique in that they must overcome ICP to dilate. Mechanically, greater stiffness of the cerebral vessels may translate the pressure wave into the microvascular beds, which may not be able to handle higher pulsations structurally (O’Rourke & Hashimoto, 2007). While we can now directly measure cerebrovascular stiffness, the PCMRI-based method remains to be done in populations with known white matter impairment. Higher values of mcaPWV may require improved temporal
resolution to produce accurate results. We found that in the HC and NTG conditions, the values beyond 10m/s were disproportionately altered, suggesting that these values approached the limit of our resolution capabilities. In these populations, multiple scans with staggered ECG-trigger delays could be interweaved to improve the effective temporal resolution of the flow curve through the cardiac cycle (Peng, Chung, Yu, & Tseng, 2006; Yu et al., 2006). Improved temporal resolution while evaluating patient populations with HC and NTG could also provide information on cerebral endothelial function. While aging and disease is known to compromise endothelial function in the peripheral vasculature (Seals, Jablonski, & Donato, 2011), the endothelial response may vary regionally in the cerebral vessels (Pretnar-Oblak, Sabovic, & Zaletel, 2007).

Improved acquisition and gating techniques have emerged with the use of 4D PCMRI in the cerebral vessels (Holmgren, Wåhlin, Dunås, Malm, & Eklund, 2020; Meckel et al., 2013). Recently, the 4D PCMRI technique was applied to measure PWV in the thoracic aorta, where they achieved temporal resolution of ~34ms and validated PWV values to standard tonometry cfPWV (Soulat et al., 2020). Attempts to improve the temporal resolution to measure smaller segments of the ICA have been attempted using local low-rank (LLR) reconstruction to achieve 100 frames per cardiac cycle (Rivera-Rivera et al., 2020). However, their superior temporal resolution came at a tradeoff, shown by reduced spatial resolution of 1.7mm. There is an increase in variability of PCMRI flow measurements when cross-section area decreases, which would be exacerbated with inadequate spatial resolution of smaller cerebral vessels (Macdonald & Frayne, 2015). In determining cerebral PWV, only the timing of the blood velocity upstroke is important. Therefore, some spatial resolution can be sacrificed in favour of improved temporal resolution. However, the greater number of reconstructed frames increases scan times, limiting the clinical utility at higher effective temporal resolutions. Nevertheless, if LLR reconstruction can be
completed in the range of 30 – 40 frames per cardiac cycle, this may provide adequate temporal
and spatial resolution to accurately measure changes along the vessel using 4D PCMRI. This
possibility would expand the horizons of cerebrovascular stiffness measurements, as 4D PCMRI
would navigate the geometrical limitations of 2D PCMRI and allow regional-specific data for
precise arteries supplying cerebral tissue.

Aside from the technological aspects of using phase contrast to measure cerebrovascular
stiffness, there are several avenues that this research could be applied to answer new questions.
The influence of sex on the cerebrovascular stiffness was not assessed in this dissertation.
Peripheral arterial stiffness is suggested to be decreased in premenopausal females (McEniery et
al., 2005) and in post-menopausal women taking estrogen replacement therapy, suggesting
estrogen confers cardiovascular protection (DuPont, Kenney, Patel, & Jaffe, 2019; A. Scuteri,
Lakatta, Bos, & Fleg, 2001). While estrogen levels were not collected in our studies, future work
could determine if the protective effect is consistent in the cerebral vessels.

All data in this dissertation was collected in the supine position, where ICP is elevated (Ng et al.,
2004). Additionally, all participants in these studies had normal blood pressures. In the
periphery, reducing transmural pressure results in decreased PWV (Zheng & Murray, 2011). The
effect of hypertension on cerebrovascular stiffness is an important question. Specifically, our
data indicate the window for elastic-oriented dilation in the cerebral vessels is narrow, suggesting
that alterations in central hemodynamics and / or arterial structure may influence the dilation –
stiffness relationship to a greater extent in the brain. Future investigations should consider the
impact of altered transmural pressure on intracranial cerebrovascular stiffness through alterations
in venous or arterial pressures.
5.3 Conclusions

Cerebrovascular stiffness is an important factor for transmission of the pulse wave into the brain. Regardless of vascular pathology, the local and regional stiffening of conduit arteries supplying the brain influence the severity of white matter structural and functional impairment in older adults. While ultrasound provided estimates of cerebrovascular stiffness, we demonstrated that by applying PCMR imaging to the cerebral vessels, direct measurements of cerebrovascular stiffness can be elucidated. It is apparent that the cerebral vessels are susceptible to stiffening with age, and that pulse wave velocity accelerates upon entering the cranial cavity. The cerebral conduit arteries can be dilated acutely via HC and NTG, which reduces their elasticity. Moreover, modification of vasodilatory status may influence regional flow velocity and pulsatile properties of cerebral arteries. The findings in this thesis suggest that cerebrovascular stiffness is a determinant of cerebral structural and functional outcomes. Additionally, new applications of PCMRI were used to directly measure and detect changes in cerebrovascular stiffness, presenting a technique that will enhance our understanding of cerebrovascular damage and therapeutics in vulnerable populations.
5.4 References


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https://doi.org/10.5115/acb.2017.50.3.163


Appendices

5.5 Ethics

Research Ethics

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kevin Skeen
Department & Institution: Health Sciences, Western University

Review Type: Full Board
ISREB File Number: 13720
Study Title: Confirmatory outcomes in ischemic heart disease patients undergoing cardiac rehabilitation
Sponsor: Canadian Institutes of Health Research

HSREB Initial Approval Date: April 29, 2016
HSREB Expiry Date: April 29, 2019

Document Approved and/or Received for Information:

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The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tricyclic Practices (ICH E6(R1)), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part C of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00009660.

Ethics Officer, (on behalf of Dr. Robert berry): HSREB Vice Chair

Western University, Research Support Services, Room 5500
London, ON, Canada N6G 3G3 1-519-661-3035 1-519-573-2466 www.uwo.ca/research/ethics
Western University Health Science Research Ethics Board

**HSREB Amendment Approval Notice**

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences|Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107620
Study Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation
Sponsor: Canadian Institutes of Health Research

**HSREB Amendment Approval Date:** October 31, 2016
**HSREB Expiry Date:** April 29, 2017

**Documents Approved and/or Received for Information:**

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The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer: Erkka Basit, Katelyn Harris, Nicole Kamini, Grace Kelly, Vicki Trax, Karen Gopaul

Western University, Research, Support Services Bldg., Rm. 5150
London, ON, Canada N6G 1L9 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/ethics
Western University Health Science Research Ethics Board
HSREB Annual Continuing Ethics Approval Notice

Date: March 15, 2017
Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences/Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107620
Study Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation
Sponsor: Canadian Institutes of Health Research

HSREB Renewal Due Date & HSREB Expiry Date:
Renewal Due - 2018/03/31
Expiry Date - 2018/04/29

The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 0000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair
EO: Erica Bashe Nicole Kaniki Grace Kelly Katelyn Harris Nicola Morphet Karen Gopaul
Dear Kevin Shoemaker,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonization Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 0000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).
Dear Kevin Shoemaker,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).
Dear Kevin Shoemaker,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

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Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonization Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wypyski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).
5.6 Letter of Information

TITLE: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: Control Group

Principal Investigator: Dr. Kevin Shoemaker

Research Staff: Jen Vording, Mark Badrov, Arlene Fleischhauer, Jeff Risdon, Baraa Al-Khazraji, Peter Prior, Neville Suskin.

Sponsor: Canadian Institutes of Health Research

INTRODUCTION AND PURPOSE

You are being invited to participate in a research study that will examine the role of vascular disease on the size and function of the brain and the health of blood vessels in the brain. We are particularly interested in how vascular disease affects brain blood flow as well as whether or not exercise training improves brain blood flow in individuals with, or at risk for, cardiovascular disease. The study will consist of three visits to our lab, which may be repeated before and following a period of exercise rehabilitation or training. The experiments on each visit day will last anywhere from two to three hours depending on the tasks being performed. A total of 270 participants will be recruited in this study.

Before agreeing to participate, please read this LETTER OF INFORMATION and ask any questions you wish.

Participant Inclusion/Exclusion Criteria

Overall, this investigation will study three groups of individuals: 1) healthy participants (Control Group), 2) participants with risk for cardiovascular disease, and 3) those with Coronary Artery Disease (CAD) who have recently had a cardiac event. You are invited to participate in the Control group.

Inclusion Criteria:

You may be included in this Control group if you are between the ages of 18 and 80 years, and if you are normally physically active and have not been diagnosed with any medical concern. Your inclusion in the Control group will be confirmed following measures of levels of glucose and triglycerides in your blood, as well as blood pressure, body size, and waist circumference. These measures will be made during your first visit (see Visit 1 below).

Exclusion Criteria:

You will not be included in the study if you smoke or have any of the following: Raynaud’s disease, respiratory illnesses, diabetes, claustrophobia, history of psychosis, eating disorders, manic or bipolar disorder, major psychiatric conditions, dependence on alcohol or drugs within the past year. In addition, you will not be included in the study if you are, or think you might be, pregnant. A routine pregnancy test may be performed on women of child-bearing potential. If you are a woman of child-bearing potential you must be using an effective method of contraception.
Magnetic resonance imaging (MRI) will be used to examine the brain’s vascular system in this experiment. You will not be included in this study if you have any history of head or eye injury involving metal fragments, if you have some type of implanted electrical device (such as a cardiac pacemaker). If you have severe heart disease (including susceptibility to heart rhythm abnormalities) you should not have an MRI scan unless supervised by a physician. Additionally, you should not have an MRI scan if you have conductive implants or devices such as skin patches, body piercing or tattoos containing metallic inks because there is a risk of heating or induction of electrical currents within the metal elemental causing burns to adjacent tissue.

Finally, participants will be excluded if they are unable to provide written informed consent, or to complete questionnaires or health history forms due to language or cognitive difficulties.

**STUDY DESIGN and PROCEDURE**

If you agree to participate you will be assigned in a random manner (by the tossing of a coin) to one of two groups. Each group will be tested at three stages corresponding to 0, 6 and 12 months. Group 1 will begin the exercise training immediately whereas Group 2 will wait for six months before beginning the training. At each test period (i.e., 0, 6 and 12 months) you will be asked to come to the laboratory for a series of visits (see below).

Training will occur at the Laboratory for Brain and Heart Health, Room 402, Labatt Health Sciences Building. We ask you to commit to exercising at the designated site three times each week as per a program provided to you by the staff. The exercise staff include a Nurse and a Certified Exercise Physiologist. The exercise program will include approximately 30 minutes of aerobic exercise (on a bicycle ergometer or treadmill for example) and 30 minutes of strength training. The levels of exercise will be determined by a pre-study examination of your fitness level. The exercise will be progressive in the sense that as you improve, the exercise loads will increase accordingly. Your blood pressure and heart rate will be measured at each visit. Emergency equipment includes a defibrillator.

Tests: All testing and training will occur in the Laboratory for Brain and Heart Health at Western University (Room 402 Labatt Health Sciences Building). We try to schedule your testing to fit into three visits. Here is a sample of what those visits include (the order of testing may vary depending on schedules):

**Visit 1 - Laboratory Testing 1 (Consent, Neurovascular Health) (3-4 hours)**

<table>
<thead>
<tr>
<th>Read LOI Consent Enrolment</th>
<th>30min supine rest</th>
<th>Blood Draw</th>
<th>Snack</th>
<th>Instrumentation</th>
<th>Cerebrovascular Reactivity (supine 5% CO2 inhalation; Sit-to-Stand protocol)</th>
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</thead>
<tbody>
<tr>
<td>(15min)</td>
<td>(30m)</td>
<td>(10m)</td>
<td>(15m)</td>
<td></td>
<td>(1 hour)</td>
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</tbody>
</table>

**Visit 2 - Laboratory Testing 2 (Vascular Imaging, Exercise Capacity, Brain blood flow) (1-2 hours)**

<table>
<thead>
<tr>
<th>Psychology Tests</th>
<th>Vascular Imaging</th>
<th>6-min walking test</th>
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</thead>
<tbody>
<tr>
<td>(45 min)</td>
<td>(30 min)</td>
<td>(10 min)</td>
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Initials: _____________
Visit 3 - MRI Testing (1-2 hours)

<table>
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<tr>
<th>MR Safety Screen</th>
<th>Instrumentation: Finger pulse oximeter Respiration, blood pressure, MoCA test</th>
<th>MRI brain imaging of brain blood vessels and blood flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>(15min)</td>
<td>(15min)</td>
<td>(1 hour)</td>
</tr>
</tbody>
</table>

**Pre-Visit Preparation:** We will ask that you abstain from exercise for 24 hours, and fast for 12 hours before Visit 1. Also, we ask that you abstain from exercise, and that you do not consume alcohol, nicorette gum (or any source of nicotine), coffee, tea, caffeinated soft drinks and chocolate for at least 12 hours before each Visit. At each visit, the testing will require approximately 2-4 hours of your time depending on the test sequence.

**Visit 1 - Laboratory Testing 1 - Laboratory for Brain and Heart Health, HSB 402**

On arrival for your first visit, you will be given opportunity to read this information letter as you decide whether or not to participate in the study. You may wish to take more time to ponder a decision about whether or not to participate in this study. If so, please feel free to take this form with you and take your time in deciding. After signing the form (and returning to the lab at the scheduled appointment), you will rest quietly for 30 minutes after which we will take a resting, fasted blood sample. A venous catheter will be inserted into a large vein near your elbow through which the research nurse (Arlene Fleischhauer) will take a blood sample. This is similar to the blood sample you would give for your annual physical, but we analyse your blood for a number of additional markers of health. We will not take more than 12 tablespoons of blood at each visit. Your blood will be analyzed for general health markers (glucose, cholesterol) as well as markers of inflammation, hormones and markers that reflect blood vessel health. One of the markers we analyze your blood for is a genetic marker called apolipoprotein (APOE). The APOE is present in 15-20% of Caucasians and has been associated with the risk of changes to brain blood vessels and cortical thickness in the brain of aging individuals.

After the blood draw is finished, we will provide you with a light snack and something to drink. You will be asked to fill in some questionnaires about your medical history while you eat and rest. You may then wish to go to the bathroom, before we measure your height and weight, and abdominal girth.

During the subsequent tests we will measure your heart rate using an electrocardiogram (ECG) and a pulse monitor attached to one of your toes. We will measure your blood pressure with a cuff around your finger, and also with a larger cuff placed around the upper part of your arm, just like it is done in a doctor’s office. The arm cuff will be inflated to a high pressure for about 30 seconds to measure your blood pressure. Your rate and depth of breathing will be measured by placing a respiratory belt around your ribcage.

**Brain Blood Flow Stimulation:** For this task, we will have you breathe through a mask. For the first few minutes, you will breathe normal room air. You will then breathe a gas mixture that contains a higher (5%) level of carbon dioxide but the normal level of oxygen (21%) and nitrogen (74%). Carbon dioxide is a gas that your body normally produces and it increases brain blood flow. We will examine the reaction of your blood vessels and nerves to the increased level of carbon dioxide. Breathing the carbon dioxide will last up to five minutes, and will be followed by a five minute recovery period. You may be asked to breathe more frequently (in time with a rhythmic tone) for up to five minutes in order to reduce levels of carbon dioxide.
While measuring your brain blood flow, and breathing room air through this mask (or mouth piece), you will be asked to perform a series of “sit-to-stand” tasks where you will sit quietly in a chair for up to 3 minutes, stand for two minutes, and then sit again. This would be repeated up to 5 times. This task may be repeated while you are asked to either breathe a little faster or while you are breathing the 5% carbon dioxide gas mixture outlined above.

**Visit 2 - Laboratory Testing 2 - Laboratory for Brain and Heart Health, HSB 402**

Your second visit to the lab will involve completion of some psychological tests, characteristics of your blood vessel health, and a 6-minute walking test of your overall physical fitness.

**Psychological measurements:** You will undergo brief standardized testing to measure cognition, (certain features of your brain’s information processing) and emotional state (mood and anxiety). This will take about 45 minutes and will consist of a series of paper-and-pencil measures and a test on the computer. In addition, the Stroop Test is a psychological test of your mental vitality and flexibility. The task takes advantage of our ability to read words more quickly and automatically than we can name colors. If a word is printed or displayed in a color different from the color it actually names; for example, if the word "green" is written in blue ink we will say the word "green" more readily than we can name the color in which it is displayed, which in this case is "blue."

**Vascular Properties:** You will be asked to rest quietly on the bed as we collect 10 minutes of baseline data. The ECG and blood pressure systems outlined above for Visit 1 will be used again. Then, images of your blood vessels and blood flow will be measured using ultrasound probes placed on the skin over the arteries in your neck, arm and/or leg and brain. We will also measure the blood ejected from your heart using ultrasound. Then, we will measure the change in vessel diameter at your elbow before and for 3 minutes following, a brief (e.g., 5 minute) period where blood flow to your arm will be stopped by a cuff placed around your forearm. These ultrasound measures will be repeated before and for 4 minutes following a small dose of sodium nitroglycerine sprayed just under your tongue.

**Six-minute walking test:** The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in a hallway. Six minutes is a long time to walk, so you will be exerting yourself. You may become out of breath or tired. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.

**Visit 3 - MRI**

The MRI visit will occur at Robarts Research Institute, 100 Perth Drive, Western University Campus.

At the beginning of your visit to the MRI we will confirm whether or not it is safe for you to enter the MRI suite by completing an MRI Safety Screen. We will put a small cuff around your finger to monitor your heart rate, and a respiratory belt around your chest to measure the depth and rate of your breathing. You will lie on a bed for about two hours while the MRI machine gathers data. MRI makes images of the interior of your body using strong magnetic and radio waves. You will not feel either. You will, however, hear loud, repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones to wear which will minimize the sound and protect your hearing.

You will rest lying down on a padded bed quietly for 10 minutes for measurement of resting heart rate. During the MRI visit we will measure the structural properties of your brain and its blood vessels while you breathe room air. Your brain’s blood vessels will be measured again when you breathe the 5% carbon dioxide gas mixture outlined above for up to 5 minutes.
STUDY BENEFITS

There is the possibility that you will receive no personal benefit from this study. However, it is likely that you may be able to lower your risk for heart disease from participating. Your participation may also increase your awareness of new health habits. In reports about the study, your contributions will be grouped with those of other participants to develop conclusions that could be used to improve the education and support available for people with heart disease or who are at high risk of heart disease or stroke.

STUDY RISKS

Laboratory Test Procedures Risk

There is a small risk of bruising or infection when collecting blood from your vein. Some people may experience mild pain and discomfort and some may feel nauseous or dizzy when blood is taken. To avoid this, we will be collecting blood from you while you are lying down.

The adhesive on the electrodes used to measure your heart rate may lead to temporary redness of the skin on your chest.

There are no known harmful effects with the measures of blood vessels or blood flow using ultrasound imaging, or blood pressure, as used in this study.

The sodium nitroglycerine is a common self-administered treatment for angina pain. It may give you a mild headache for a few minutes.

Breathing a slightly higher level of carbon dioxide may give you a small headache and it may make you feel breathless. These feelings vanish quickly when you start breathing room air again.

Exercise Tests: Walking or running can be a physically challenging activity. You will breathe harder and may begin to sweat. This is part of your body’s normal response to exercise. There is minimal risk associated with the self-paced six-minute walking test.

Psychology Tests

There are no risks with the psychology tests.

MRI Risk

This MRI machine uses a strong magnet and radio waves to make images of the body interior. You will be asked to lie on a long narrow couch for about 1.5 hours in each MRI session while the machine gathers data. During this time you will be exposed to magnetic fields and radio waves. You will not feel either. You will, however, hear repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones that you will be required to wear to minimize the sound and protect your hearing. The space within the large magnet in which you lie is somewhat confined, although we have taken many steps to relieve the “claustrophobic” feeling. There are no known significant risks with this procedure at this time because the radio waves and magnetic fields at the strengths used, are thought to be without harm. The exception is if you have a cardiac pacemaker, or a metallic clip in your body (e.g., an aneurysm clip in your brain), have severe heart disease, body piercings, tattoos containing metallic ink or slow release pharmaceutical skin patches.
There is the possibility that you will experience a localized twitching sensation or perhaps a little dizzy due to the magnetic field changes during the scan. These responses are not unexpected and should not be painful. However, you can stop the exam at any time. The magnetism and radio waves do not cause harmful effects at the levels used in the MRI machine. However, because the MR scanner uses a very strong magnet that will attract metal, all metallic objects must be removed from your person before you approach the scanner. In addition, watches and credit cards should be removed as these could be damaged (these items will be watched for you).

As with any technology there is a risk of death or injury. For MRI the risk of death is less than 1 in 10 million and the risk of injury is less than 1 in 100,000. These risks do not arise from the MRI process itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the participant or the scanner room. It is therefore very important that you answer all the questions honestly and fully on the MRI screening questionnaire. Almost all the deaths and injuries related to MRI scans have occurred because the MRI operator did not know that surgically implanted metal hardware (such as a cardiac pacemaker) was present inside the participant during the MRI scan. For comparison, the risk of death in an MRI is similar to travelling 10 miles by car, while the risk of injury during an MRI is much less than the risks associated with normal daily activities for 1 hour. Any unusual findings from the MRI images will be provided to you so that you can seek further medical attention.

Exercise training Risk

All participants will obtain their family physician’s signed permission to participate in exercise before they may participate in the exercise training segment.

YOUR PARTICIPATION

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care, academic status, or employment. If you withdraw from the study before its completion then you may decide whether to also withdraw your data. Participation in this study will be brought to the attention of your family doctor.

The blood specimens will be discarded or destroyed once they have been used for the purposes described in the protocol. All other study data (e.g., paper files, digital files) will be kept for a minimum of 20 years.

If you are participating in another study at this time, please inform the study coordinator right away to determine if it is appropriate for you to participate in this study.

Whether you agree to participate in this study or not, you will be asked if you consent to having your name and contact information added to a master database of individuals who would be willing to be contacted in the future regarding your interest in other research studies.

Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. Representatives of Lawson Quality Assurance (QA) Education Program may look at study data for quality assurance purposes.

Confidentiality

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Initials: ______________
Your research records will be stored in a secure office at Western University. To further protect your confidentiality, your name will be replaced with a participant ID number on all documents. The master list linking your identity and participant ID number and your contact information will be stored separately in a secure office at Western University. Your contact information will be securely maintained at Western University to allow for setting up follow up visits. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published. No information that could reveal your identity will be released to anyone with the exception of your Family Doctor if you give permission for this.

If we find information we are required by law to disclose, we cannot guarantee confidentiality.

**ALTERNATIVES TO STUDY PARTICIPATION**

You may choose not to participate in this study.

**Reimbursement**

You will be reimbursed for travel costs up to $60 for your participation in each of the pre and post series of tests outlined above. For coronary artery disease participants, the costs associated with your participation in the cardiac rehabilitation program are provided for you. However, we cannot provide support for the travel associated with your participation in the cardiac rehabilitation program. For all participants, your parking and exercise costs will be covered for six months if you perform the exercise training in the Laboratory for Brain and Heart Health.

**CONTACT PERSONS**

If you have any questions about the study please contact:

**IHD Research Staff:** Jen Vording / Mark Badrov

**Research Nurse:** Arlene Fleischhauer

**Principal Investigator:** Dr. Kevin Shoemaker

Or send an email to

Please note that email is not considered a secure method of communication and you should not send any personal health information via email.

If you have any questions about your rights as a research participant or the conduct of the study you may contact: Dr. David Hill, Scientific Director, Lawson Health Research Institute at

You will receive a copy of the fully signed informed consent document for your records. You do not waive any legal rights by signing the consent.

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Initials: ____________
Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: CONTROL GROUP

Principal Investigator: Dr. Kevin Shoemaker

Research Staff: Jen Vording, Mark Badrov & Arlene Fleischhauer

CONSENT

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

☐ I consent to be contacted for future research

SIGNATURES

_________________________________________  ____________________________
Signature of Participant                      Date

_________________________________________
Print

_________________________________________  ____________________________
Signature of Person Obtaining Informed Consent Date

_________________________________________
Print
5.7 Permissions

THE AMERICAN PHYSIOLOGICAL SOCIETY ORDER DETAILS

Jul 29, 2020

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Title: Vascular stiffening and the brain: Direct measures of cerebrovascular stiffness in aging and vasodilation

Institution name: University of Western Ontario

Expected presentation date: Sep 2020

Mr. Christopher Balestrini

Requestor Location: Canada

Attn: Mr. Christopher Balestrini

Total: Not Available
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| Title | Vascular stiffening and the brain: Direct measures of cerebrovascular stiffness in aging and vasodilation |
| Institution name | University of Western Ontario |
| Expected presentation date | Sep 2020 |
| Portions | Figure 1 |
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Thank you!

For the permission and the timely response, I appreciate it!

Cheers,

Chris

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Neurovascular Research Laboratory  
Western University

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State: Ontario
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Country: CA
Phone:
Fax:
Website:
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5.8 Curriculum Vitae

CHRISTOPHER S. BALESTRINI

Education

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<thead>
<tr>
<th>Degree</th>
<th>Institution</th>
<th>Specialization</th>
<th>Supervisor</th>
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<tbody>
<tr>
<td>MD / PhD</td>
<td>Western University, Anatomy &amp; Cell Biology</td>
<td>Neurobiology</td>
<td>Dr. Kevin Shoemaker</td>
<td>2017-2024</td>
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<tr>
<td>MSc.</td>
<td>Western University, Anatomy &amp; Cell Biology</td>
<td>Clinical Anatomy</td>
<td>Dr. Marjorie Johnson</td>
<td>2015-2017</td>
</tr>
<tr>
<td>BSc.</td>
<td>Western University, Kinesiology</td>
<td>Honours Specialization</td>
<td></td>
<td>2011-2015</td>
</tr>
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</table>

Teaching Experience

Western University, London, Ontario September 2015 to Present

Teaching Assistant, Schulich School of Medicine & Dentistry
- ANATCELL 2221 – Functional Gross Anatomy
- BIOLOGY 2290 – Scientific Methods of Biology
- ANATCELL 3309 – Mammalian Histology
- ANATCELL 3319 – Human Systemic Anatomy
- ANATCELL 9501 – Anatomy for Physical Therapy Students
- DENTIST 5160 – Systemic Anatomy and Histology
- DENTIST 5186 – Head and Neck Anatomy for Dentistry Students
- ANATCELL 9560 – Gross Anatomy for Clinical Anatomy

Publications

Journal Papers Accepted


Presentations and Invited Lectures

Oral Presentations


Invited Lectures
