# VASCULAR HEALTH IN ADULTS WITH AND WITHOUT STROKE

# NOVEL VASCULAR RISK MARKERS IN COMMUNITY-DWELLING ADULTS WITH AND WITHOUT STROKE

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## LAY ABSTRACT

Novel measures of arterial health, such as arterial stiffness and endothelial function, may provide early stroke risk detection. This study aimed to assess the day-to-day reliability and between-side differences of these new arterial measures in people with stroke, and to also investigate relationships between these measures and cognitive function in older adults with and without stroke. Arterial stiffness was assessed using pressure sensors that measure the speed of the pulse waves, and endothelial function was assessed using ultrasound to measure the capacity of the arteries to expand following an increase in blood flow. Results found that the most well-established measures of arterial health were reliable and there were no between-side differences in any measures. No relationships were observed between arterial measures and cognitive function. Overall, this study increases knowledge surrounding novel measures of arterial health in older adults with and without stroke.

## ABSTRACT

Stroke is the leading cause of adult neurological disability. Novel markers of vascular disease progression, such as arterial stiffness and endothelial function, may provide early and sensitive stroke risk detection. However, measurement properties of these novel vascular risk markers have not yet been established in the post-stroke population, nor has the association between these risk markers and measures of physical function. Moreover, given that subclinical vascular disease may initially manifest as a decline in cognitive function, understanding vascular disease progression in older adults with and without cognitive impairment may provide information regarding early stroke risk. This study aimed to investigate 1) test-retest reliability and between-side differences in novel vascular risk markers in individuals with stroke, 2) differences in arterial stiffness and endothelial function between older adults without cognitive impairment, with cognitive impairment, and with stroke, and 3) relationships between novel vascular risk markers and physical function in individuals with and without stroke. Participants were 50-80 years of age, able to ambulate ≥10 meters, and for those with stroke, ≥1 year post-stroke. Carotid-femoral, carotid-radial, and femoral-foot pulse wave velocity (cfPWV, crPWV, and ffPWV, respectively) were used to quantify systemic arterial stiffness, and compliance and distensibility were used to quantify local carotid arterial stiffness. Flow-mediated dilation (FMD) was used to quantify endothelial function. Findings revealed almost perfect test-retest

reliability of cfPWV (ICC>0.91, *P*<0.0001) and substantial test-retest reliability of FMD in individuals with stroke (ICC>0.70, *P*<0.01). There were no between-side differences in novel vascular measures, no differences in these measures across subgroups of increasing vascular risk, and a positive correlation between cfPWV and walking speed. Findings suggest that in the post-stroke population, cfPWV and FMD may be the most appropriate measures of vascular risk progression, that it may be more clinically feasibly to assess these measures on the unaffected side.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| BBS | Berg Balance Scale |
| BMI | Body mass index |
| BP | Blood pressure |
| CAD | Coronary artery disease |
| CHD | Coronary heart disease |
| CI | Confidence interval |
| cfPWV | Carotid-femoral pulse wave velocity |
| CMSA | Chedoke McMaster Stroke Assessment |
| crPWV | Carotid-radial pulse wave velocity |
| CV | Cardiovascular |
| CVD | Cardiovascular disease |
| DBP | Diastolic blood pressure |
| ECG | Electrocardiogram |
| eNOS | Endothelial nitric oxide synthase |
| ffPWV | Femoral-foot pulse wave velocity |
| HDL-c | High-density lipoprotein cholesterol |
| HR | Hazard ratio |
| ICC | Intraclass correlation coefficient |
| LDL-c | Low-density lipoprotein cholesterol |
| MoCA | Montreal Cognitive Assessment |
| MRI | Magnetic resonance imaging |
| NIHSS | National Institutes of Health Stroke Scale |
| NO | Nitric oxide |
| NTG | Nitroglycerin |
| OR | Odds ratio |
| PAD | Peripheral artery disease |
| PP | Pulse pressure |

| | |
|---|---|
| PWV | Pulse wave velocity |
| RHR | Resting heart rate |
| RR | Relative risk |
| SBP | Systolic blood pressure |
| SD | Standard deviation |
| SEM | Standard error of measurement |
| SVD | Small vessel disease |
| TC | Total cholesterol |
| TG | Triglyceride |
| WC | Waist circumference |
| WHR | Waist-hip ratio |
| 5MWT | 5 Meter Walk Test |
| 6MWT | 6 Minute Walk Test |

## DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis was completed by the candidate and recognizes the contribution of Dr. Ada Tang, Dr. Maureen MacDonald and Dr. Julie Richardson in providing helpful insight into its preparation and in the research process. Daria Shkredova and Derek Stouth contributed to data collection and analysis.

# Chapter 1

# BACKGROUND

## 1.1 Stroke and Vascular Disease Progression

### 1.1.1 Stroke

Stroke is the fourth leading cause of death in Canada (Heart and Stroke Foundation, 2016) and the leading cause of disability in adults worldwide (World Heart Federation, 2016), leaving the majority of its survivors with impairments such as hemiparesis (Hankey, Jamrozik, Broadhurst, Forbes, & Anderson, 2002), spasticity (Thibaut et al., 2013), and cognitive decline (Levine et al., 2015; Rajan, Aggarwal, Wilson, Everson-Rose, & Evans, 2014). Individuals with history of stroke are also at higher risk of recurrent cardiovascular (CV) events, with over 25% risk of stroke recurrence at 5 years following stroke (Mohan et al., 2011). Recent statistics estimate that 405,000 Canadians are currently living with the effects of stroke (Krueger et al., 2015), nearly 30% higher than previous estimates (Public Health Agency of Canada, 2011), underscoring the impact of stroke in our society. Moreover, the incidence, prevalence and burden of stroke is likely to continue to increase because of the growing population of elderly Canadians, and it is expected that 726,000 people will be living with stroke by the year 2038 (Krueger et al., 2015).

Strokes may be either ischemic or hemorrhagic in origin. Ischemic strokes, caused by an interruption of blood flow to the brain, account for approximately 87% of all strokes (American Stroke Association, 2015). The ischemic stroke type may be divided into two subtypes: embolic, caused by the travel of a clot to the brain that has originated in a part of circulatory system outside of the brain, or

thrombotic, caused by the formation of a blood clot in an artery directly supplying blood to the brain (American Stroke Association, 2015). The thrombotic subtype may be further divided into large-vessel thrombosis or small-vessel thrombosis (lacunar infarcts) (American Stroke Association, 2015). In contrast, hemorrhagic strokes are caused by a rupture of blood vessels in the brain, and while they are less common, they often result in more devastating outcomes, such as increased risk of death or dependency (Bhalla, Wang, Rudd, & Wolfe, 2013).

### 1.1.2 Small Vessel Disease

Covert strokes are characterized by cerebral infarcts that fail to cause symptoms that are clinically recognized as stroke (Price et al., 1997). A contributing factor in the development of covert stroke is cerebral small vessel disease (SVD), whereby pathological processes affect the small arteries, arterioles, venules, and capillaries of the brain and result in vascular narrowing, endothelial damage, white matter lesions, and cerebral microbleeds (Pantoni, 2010). Indeed, cerebral SVD is associated with lacunar infarcts (Mantyla et al., 1999; Wardlaw, Lewis, Keir, Dennis, & Shenkin, 2006), which account for the majority of covert strokes (Longstreth et al., 1998; Vermeer, Koudstaal, Oudkerk, Hofman, & Breteler, 2002).

The prevalence of covert stroke is fivefold that of overt stroke (Vermeer et al., 2002), and while covert strokes are not associated with clinical symptoms, they should not be considered silent or benign. They share similar CV risk factors with

overt stroke, such as hypertension (Vermeer et al., 2002), and their presence more than doubles the risk of future stroke (Vermeer, Longstreth, & Koudstaal, 2007). Covert strokes may initially manifest as declines in cognition (Longstreth et al., 1998; Price et al., 1997; Vermeer et al., 2003), and therefore cognitive function may be an important clinical marker of vascular disease progression. Indeed, previous research suggests that individuals with cognitive impairment may be at increased risk of incident overt stroke (Rajan et al., 2014), and that individuals with overt stroke, with or without baseline cognitive impairment, may experience greater cognitive decline compared to those without stroke (Levine et al, 2015; Rajan et al., 2014).

### 1.1.3 Proposed Continuum of Increasing Vascular Risk

In order to assist in our understanding of vascular disease progression, a continuum of increasing vascular risk may be created that considers the presence of cerebrovascular disease and cognitive impairment. Figure 1 outlines a proposed framework that reflects a spectrum spanning low to high vascular disease risk that includes older adults 1) without known cerebrovascular disease and without cognitive impairment, 2) without known cerebrovascular disease and with cognitive impairment, and 3) with overt stroke. Understanding CV risk in individuals across these subgroups may increase information concerning vascular disease progression, which may ultimately allow for early and effective interventions to reduce risk of future CV events.

## 1.2 Traditional Cardiovascular Risk Factors

There are many well-established risk factors associated with stroke, some of which are non-modifiable, such as age and family history (World Heart Federation, 2016). Other risk factors are modifiable, the most well established being hypertension, dyslipidemia, diabetes, obesity and physical inactivity (O'Donnell et al., 2010). These traditional risk factors explain approximately 90% of the risk of stroke and other cardiovascular disease (CVD) in a population (O'Donnell et al., 2010; Yusuf et al., 2004) (Figure 2).

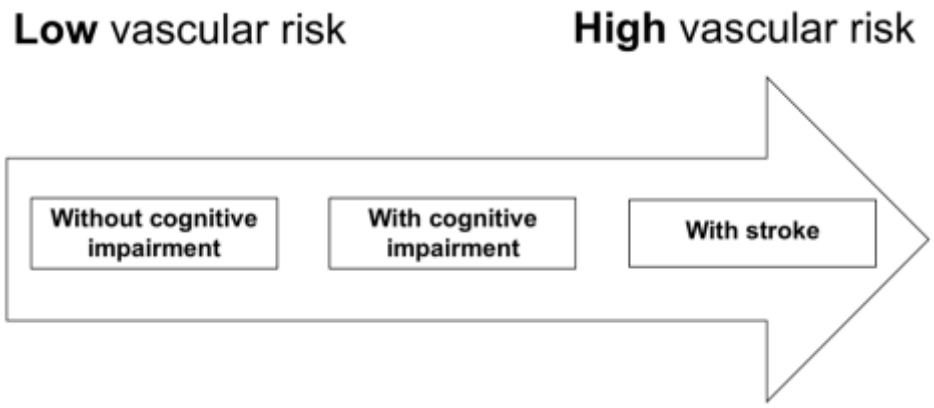


**Figure 1.** Proposed continuum of increasing cardiovascular risk

Given that cognitive decline may be the first manifestation of covert stroke (Longstreth et al., 1998; Price et al., 1997; Vermeer et al., 2003), cognitive function may be an important clinical marker of vascular disease progression. A continuum of increasing vascular risk may serve as a model of vascular disease progression, in which the vascular risk is low for individuals without cognitive impairment, moderate for individuals with cognitive impairment, and high for individuals with overt stroke.

Traditional CV risk factors are not adequately managed in individuals with stroke. In a study of 364 participants, nearly all (99%) had at least one sub-optimally controlled risk factor, and two or more inadequately controlled risk factors were present in 91% of participants (Kopunek et al., 2007). Reasons for high rates of sub-optimal risk factor management are multifactorial and complex (Kopunek et al., 2007). There may exist a gap between evidence and clinical practice, but physical impairments, lack of motivation, and environmental factors may also contribute to sedentary lifestyle and poor management of CV risk factors (Damush, Plue, Bakas, Schmid, & Williams, 2007).

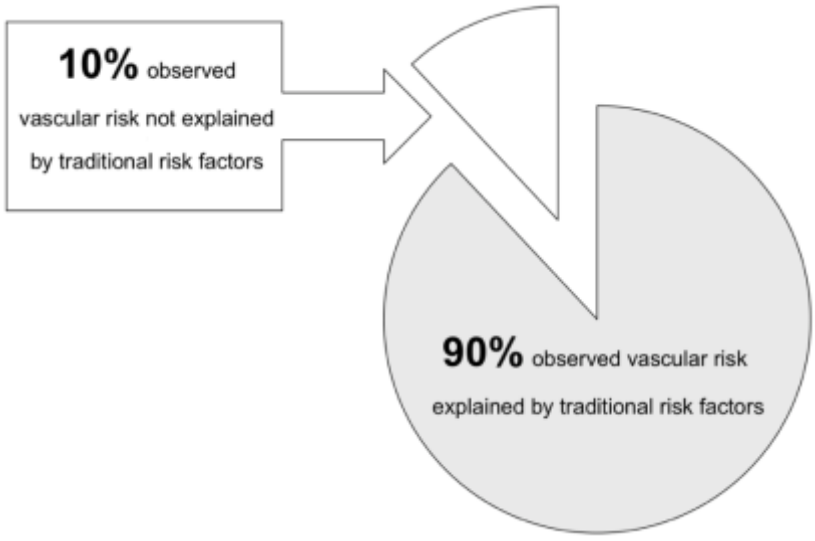


**Figure 2.** Explanation of observed stroke and other cardiovascular disease risk

Approximately 90% of the risk of stroke and other cardiovascular disease (CVD) in a population is explained by traditional cardiovascular risk factors (O'Donnell et al., 2010; Yusuf et al., 2004). However, a portion of observed CVD risk (approximately 10%) may not be explained by these traditional risk factors alone.

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### 1.2.1 Blood Pressure

Elevated blood pressure (BP), or hypertension, is a common CV risk factor and the leading modifiable risk factor for stroke (O'Donnell et al., 2010). BP may be classified as optimal, prehypertension, stage one hypertension, and stage two hypertension, and the cut-points for these categories are displayed in Appendix 1, Table A1.

Hypertension can cause both ischemic and hemorrhagic stroke through many mechanisms. Elevated BP leads to complex structural alterations in the cerebral arteries, which have important hemodynamic consequences. Chronic hypertension stimulates arterial smooth muscle hypertrophy, thereby increasing vascular resistance (Johansson, 1999). While increased vascular resistance enables arteries to tolerate increased pressure acutely, over the long-term it

reduces the collateral circulation, a process in which a system of arteries connect to deliver blood to ischemic tissue, thereby compromising this important compensatory mechanism (Johansson, 1999). Chronic hypertension leads to adaptations that reflect pathological processes, such as impaired vasodilator responsiveness and the development of atherosclerotic plaques and thrombosis (Gkaliagkouski et al., 2009), which in combination with impaired collateral circulation may predispose risk of cerebral ischemia (Heistad, Mayhan, Coyle, & Baumbach, 1990). In addition, hypertension induces degenerative changes in the cerebral arteries and arterioles, which predisposes intracerebral hemmorhage (Johansson; 1999; Lammie, 2002).

The association between hypertension and increased risk of CVD and stroke is well established. In the Cardiovascular Health Study, a large-scale, prospective, cohort study of older adults (n=4,902, mean age 73 years) (Psaty et al., 2001), elevated systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) were associated with greater risk of coronary and cerebrovascular events. Elevated SBP had the strongest association, with a 1.24 hazard ratio (HR) for risk of first myocardial infarction associated with one standard deviation (SD) increase in SBP (95% confidence interval [CI] 1.15–1.35, $P$<0.001), and HR 1.34 for risk of first stroke associated with one SD increase in SBP (95% CI 1.21–1.47, $P$<0.001) [HRs adjusted for age, sex, smoking, diabetes mellitus, clinical CVD, and carotid artery intimal medial wall thickness] (Psaty et al., 2001). Similarly, another large-scale, prospective, population-based, cohort

study found that in older adults (n=6,287, mean age 70 years), first stroke was associated with hypertension, relative to optimal BP (relative risk [RR] 1.6, 95% CI 1.2–2.0, *P*<0.05), and with isolated systolic hypertension, relative to SBP <160 millimeters of mercury (mmHg) and DBP <90 mmHg (RR 1.7, 95% CI 1.1–2.6, *P*<0.05 (RRs adjusted for age, sex, smoking, and diabetes mellitus) (Voko et al., 1999). Moreover, a recent systematic review and meta-analysis (n=762,393, mean age 53 years) found that prehypertension was associated with increased risk of stroke, relative to optimal BP (RR 1.66, 95% CI 1.51–1.81, *P*<0.00001) (Huang et al., 2014).

In addition to increased risk of first stroke, previous reports have observed associations between hypertension and increased risk of recurrent stroke, however this relationship is less clear. An earlier study (n=17,398, age range 60–89 years) found that risk of stroke recurrence within 14 days of ischemic stroke increased by 4.2% for every 10 mmHg increase in SBP (*P*=0.023) (Leonardi-Bee, Bath, Phillips, Sandercock, & IST Collaborative Group, 2002). Findings from another report (n=11,560, mean age 66 years) suggested that hypertension may be related to recurrent stroke in individuals with small-vessel occlusion (odds ratio (OR) 1.52 for stroke recurrence at 12 months, 95% CI 1.03-2.30, *P*<0.05; OR adjusted for age, sex, history of stroke, and other CV risk factors) but not with other subtypes of ischemic stroke (Wang et al., 2013).

It is well-established that hypertension increases the risk of first and recurrent stroke and other CVD in adults, independent of sex, age, and other CV risk

factors, such as diabetes mellitus. Given that hypertension is the leading modifiable risk factor for stroke (O'Donnell et al., 2010), it is important to understand the association between hypertension and CV risk in order to in order to target rehabilitation interventions to effectively reduce BP, and in turn, reduce the risk of adverse CV events.

### 1.2.2 Blood Lipids

Dyslipidemia, characterized by elevated triglyceride (TG), total cholesterol (TC), or low density lipoprotein cholesterol (LDL-c) levels, or reduced high density lipoprotein cholesterol (HDL-c) levels, is a known CV risk factor (World Heart Federation, 2015) and has also been established as a risk factor of ischemic stroke (Putaala et al., 2009). Cut-off TG, TC, LDL-c, and HDL-c values indicative of optimal levels, borderline CV risk, and high CV risk are displayed in Appendix 1, Table A2.

There are multiple mechanisms by which dyslipidemia may increase stroke risk. LDL-c is important in the development and progression of atherosclerosis (Gorelick, 2002), a disease process that commonly precedes ischemic stroke (Arenillas, 2011). Oxidized LDL-c accelerates endothelial damage, macrophage recruitment, uptake of LDL-c by foam cells, and abnormalities in vascular tone (Gorelick, 2002). In contrast, HDL-c prevents lipid oxidation and is a carrier of enzymes involved in reversing oxidative odamage (Sanossian, Saver, Navab, & Ovbiagele, 2007) and thus higher HDL-c levels reduces atherosclerotic

development and arterial damage. In addition, TGs represent an important biomarker of CV risk, as they are associated with atherogenic remnant particles and proatherogenic proteins (Talayero & Sacks, 2011).

Previous research has established associations between dyslipidemia and stroke occurrence. The Copenhagen City Heart Study, a large-scale, prospective study (n=19,698, aged ≥20 years), found a positive association between TG levels and the risk of ischemic stroke (RR 1.12 for every 1 millimole/liter [mmol/L] increase, 95% CI 1.07–1.16, $P$<0.05) as well as a negative association between HDL-c levels and risk of ischemic stroke (RR 0.53 for every increase of 1 mmol/L, 95% CI 0.34–0.83, $P$<0.05) (Lindenstrom, Boysen, & Nyboe, 1994). Another prospective study found that in middle-aged adults (n=2,351, mean age 57 years), risk of first atherothrombotic stroke increased from the first to fourth quartiles of LDL-c levels (≤2.7 and ≥3.9 mmol/L, respectively), with a HR of 2.84 (95% CI 1.17–6.93, $P$=0.02, adjusted for age, sex, and other CV risk factors) (Imamura et al., 2006). Moreover, results from another prospective study in older adults (n=9,940, mean age 65 years) suggest an association between dyslipidemia and ischemic stroke recurrence at 5 years (OR 1.2, 95% CI 1.02-1.42, $P$<0.05) (Kumral, Evyapan, Gokcay, Karaman, & Orman, 2014). It is important to understand the association between dyslipidemia and stroke in order to develop targeted interventions that effectively decrease TG and LDL-c levels and increase HDL-c levels, and in turn reduce the risk of stroke.

### 1.2.3 Blood Glucose

Diabetes mellitus, a disease characterized by elevated blood glucose levels, resulting from defects in insulin secretion, insulin action, or both, as well as hyperglycemia, a condition of elevated blood glucose that typically precedes diabetes (American Diabetes Association, 2014) have been linked with increased risk of stroke and CVD (Poppe et al., 2009; Shou, Zhou, Zhu, & Zhang, 2015; Sui et al., 2011). Blood glucose levels indicative of hyperglycemia and diabetes are displayed in Appendix 1, Table A3.

Diabetes and hyperglycemia, through elevated blood glucose levels and insulin resistance, may increase the risk of stroke through several mechanisms. Insulin deficiency liberates circulating free fatty acids, which in combination with elevated glucose levels, reduces vascular reactivity and accelerates development of microvascular disease and atherosclerosis in the cerebral vasculature, predisposing stroke (Garg, Chaudhuri, Munschauer, & Dandona, 2006). Hyperglycemia may cause anaerobic metabolism, lactic acidosis and free radical production, which results in membrane lipid peroxidation and cell lysis in metabolically-altered ischemic tissue, furthering ischemic tissue damage (Garg et al., 2006).

Earlier findings have established associations between diabetes and stroke incidence and recurrence. Findings from a large-scale, prospective study of middle-aged men (n=43,933, mean age 44 years) suggest an association between diabetes and incident stroke (HR 1.57, 95% CI 1.06–2.32, *P*=0.01,

adjusted for age and other CV risk factors) (Sui et al., 2011), and findings from a meta-analysis found that stroke recurrence risk was greater in individuals with diabetes compared to those without (n=43,899, age not reported, HR 1.45, 95% CI 1.32–1.59, *P*=0.0001) (Shou et al., 2015). Elevated blood glucose is also associated with worse outcomes following stroke. A multicentred, prospective trial found that in older adults (n=1,098, mean age 71 years), post–stroke hospital admission hyperglycemia was independently associated with increased risk of death (RR 1.5, 95% CI 1.2–1.9, *P*<0.001) and poor functional status at 90 days following stroke (favourable function status outcome RR 0.7, 95% CI 0.5–0.9, *P*<0.001) (RRs adjusted for (Poppe et al., 1999). These previously established associations between elevated blood glucose levels, indecent and recurrent stroke, and worse outcomes following stroke suggest that interventions geared towards decrease blood glucose levels may be effective in reducing the risk and severity of stroke.

### 1.2.4 Obesity

Body mass index (BMI) (weight/height$^2$ [kilogram/meter$^2$ or kg/m$^2$]) (Hu et al., 2007), is a traditional anthropometric marker of obesity status (Bodenant et al., 2011) associated with risk of CVD and stroke (Chen et al., 2013; Kurth et al., 2002). In addition, measures of visceral adiposity, namely waist circumference (WC), a measure of the circumference around the waist, and waist-hip ratio (WHR), the ratio of the circumference of the waist to that of the hips, are also

associated with increased risk of CVD and stroke (Bodenant et al., 2011; de Koning, Merchant, Pogue, & Anand, 2007). While BMI takes into account excess adiposity that accumulates in both the subcutaneous and visceral compartments, WHR and WC are stronger indicators of visceral fat (Despres, 2012) and may be more closely related to CVD risk (Coutinho et al., 2011). Indeed, previous studies have found that relative to subcutaneous fat, visceral fat has stronger endocrine activity and inflammatory characteristics and is more closely associated with insulin resistance (Despres & Lemieux, 2006). Established BMI, WHR, and WC cut-off values indicative of anthropometric category are displayed in Appendix 1, Table A4.

Associations between measures of obesity and stroke incidence have been previously established. A large scale, prospective study found that relative to normal BMI (<23 kg/m$^2$), elevated BMI (>30 kg/m$^2$) was associated with increased risk of stroke in middle-aged, healthy males (n=21,414, mean age 53 years; RR 2.0, 95% CI 1.48–2.71, *P*<0.001; RR adjusted for age and other CV risk factors) (Kurth et al., 2002). Similarly, another large-scale, prospective trial found one SD increases in WHR and WC were associated with increased risk of stroke in middle-aged men (n=31,201, median age 52 years; RR 1.22, 95% CI 1.10–1.34, *P*<0.001 and RR 1.26, 95% CI 1.09–1.47, *P*=0.002, respectively) and women (n=23,516, median age 48 years; RR 1.14, 95% CI 1.03–1.26, *P*=0.01, and RR 1.19, 95% CI 1.02–1.34, *P*=0.03, respectively) (RRs adjusted for age and other CV risk factors) (Bodenant et al., 2011). Moreover, a systematic review and

meta-analysis (n=258,114, mean age 57 years) found that the RR of coronary heart disease (CHD) and stroke increased by 2% (95% CI 1–3%, *P*<0.05) for a 1 centimeter (cm) increase in WC, and by 5% (95% CI 4–7%, *P*<0.05) for a 0.01 unit increase in WHR, after adjusting for age, cohort year, and medication (de Koning et al., 2006). Together, these findings suggest an association between obesity and increased risk of stroke and other CVD, independent of age and other CV risk factors. It is important to understand this association to develop targeted interventions to reduce obesity, and in turn decrease risk of CV events.

### 1.2.5 Physical Inactivity

Although commonly used interchangeably, physical activity and exercise are terms that explain different concepts. Physical activity refers to "any bodily movement produced by the skeletal muscles that results in energy expenditure", while exercise refers to "a subset of physical activity that is planned, structured and repetitive, that has a final or intermediate objective the improvement or maintenance of physical fitness" (Caspersen, Powell, & Christenson, 1985).

It is well established that physical inactivity increases the risk of stroke and other CVD (Sesso, Paffenbarger, & Lee, 2000; Shah et al., 2013), but engagement in physical activity is typically reduced following stroke (Butler & Evenson, 2014; Field, Gebruers, Sundaram, Nicholson, & Mead, 2013; Gebruers, Vanroy, Truijen, Engelborghs, & De Deyn, 2010). A recent study found that, compared to age-matched individuals without history of stroke, fewer adults with

stroke (n=262, aged 20–80 years) met 2008 Physical Activity Guidelines for Americans (18% versus 28%, $P<0.05$) and reported less vigorous leisure activity (9% versus 19%, $P<0.05$) (Butler & Evenson, 2014). In addition, previous systematic reviews and meta-analyses have observed reduced walking endurance and upper extremity activity in adults with stroke relative to healthy, age-matched controls (Gebruers et al., 2010), as well as lower levels of physical activity duration and intensity compared to previously established levels in healthy populations (Field et al., 2013).

Recent guidelines developed by the American Heart Association (Billinger et al., 2014) recommend that individuals with stroke engage in regular exercise, consisting of low- to moderate-intensity aerobic and muscle-strengthening activity, to reduce sedentary behaviour and improve secondary prevention. The effectiveness of exercise interventions in improving physical function and CV fitness following stroke is well established and has been the focus of earlier meta-analyses (Pang, Eng, Dawson, & Gylfadottir, 2006; Saunders et al., 2016). Fewer studies have examined effects of exercise training on reducing CV risk factors in this population, however research suggests that exercise following stroke may decrease traditional CV risk factors such as BP and fasting glucose levels (Kono et al., 2013; Tang et al., 2014b).

### 1.3 Novel Vascular Risk Markers

Although traditional risk factors are well established and explain approximately 90% of observed stroke risk (O'Donnell et al., 2010) (Figure 2), there remains approximately 10% of stroke risk that is not explained by these risk factors alone. Novel vascular risk markers, such as arterial stiffness and endothelial function, may provide insight into the variance that is not explained by traditional risk factors (Figure 3) and may also provide earlier and more sensitive risk detection. Arterial stiffness and endothelial dysfunction are important factors in the development of atherosclerosis, a disease process characterized by the deposition of plaque on the arterial walls that typically precedes CVD (Duprez & Cohn, 2007). Increased arterial stiffness reduces shock-absorbing capacity of the arteries during pulsatile flow, increasing stress on the arterial wall, and impaired endothelial function reduces the ability of the arteries to expand following an increase in shear stress (Verma & Anderson, 2002). Thus, increased arterial stiffness and impaired endothelial function contribute to endothelial damage and the development of plaque and calcification, factors that may predispose development of atherosclerosis, and in turn, CVD and stroke (Mackey, Venkitachalam & Sutton-Tyrrell, 2007; Verma & Anderson, 2002).

### 1.3.1 Arterial Stiffness

*Mechanisms of link with CVD*

Arterial stiffness is an important determinant of PP, left ventricular load and coronary perfusion pressure (Mac-Way, Leboeuf, & Agharazii, 2011; Safar, Levy,

& Struijker-Boudier, 2003). Ejection of blood from the heart into the aorta generates an incident pressure wave that is propagated to arteries throughout the body and reflected back to the aorta (reflected wave). Thus, incident and reflected pressure waves are in constant interaction and result in summated PP waves (Safar et al., 2003). In healthy arteries, reflected waves return to the aorta during diastole (Mac-Way et al., 2011) but as the stiffness of the arteries increases, so does the transmission velocity of both forward and reflected traveling waves. This causes the reflected waves to arrive earlier in the central aorta, during late systole rather than diastole, resulting in increased aortic pressures during systole and reduced aortic pressure during diastole (Safar et al., 2003). Greater SBP causes an increase in left ventricular afterload, which compromises normal ventricular relaxation and increases myocardial oxygen consumption, augmenting the workload of the heart and increasing risk for coronary artery disease (CAD) (Mac-Way et al., 2011; Safar et al., 2003). Cardiac perfusion pressure, a determinant of the blood flow to cardiac muscle controlled by the pressure gradient between aortic diastolic and right atrial diastolic pressure, is greatest during diastole (Cruickshank, 1992). Decreased aortic diastolic blood pressure (DBP) reduces coronary perfusion pressure and predisposes the heart to ischemia (Namasivayam, Adji, & O'Rourke, 2011).

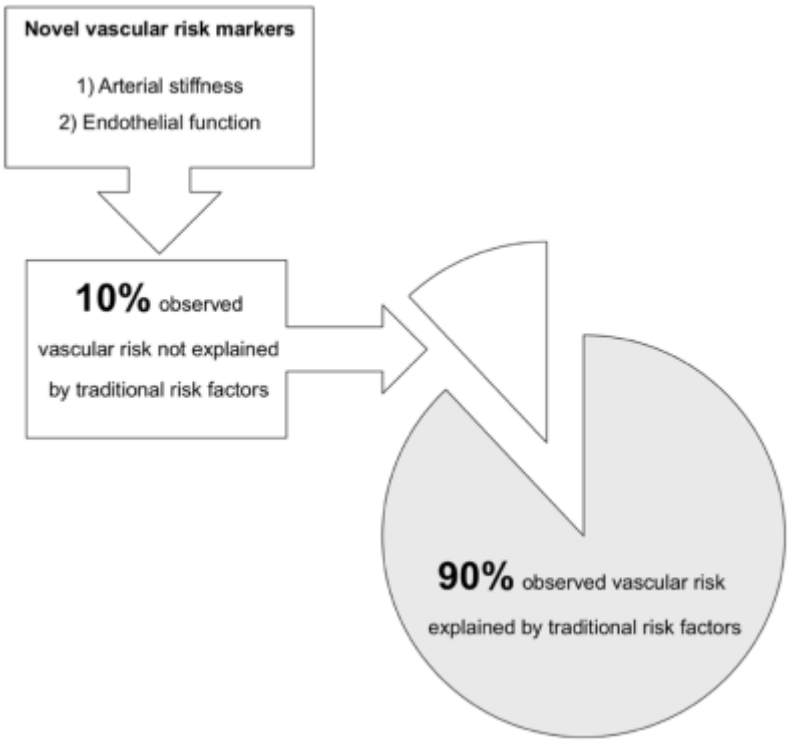



**Figure 3.** Proposed model to explain cardiovascular disease risk

Approximately 10% of observed cardiovascular disease (CVD) risk is not explained by traditional risk factors alone (O'Donnell et al., 2010; Yusuf et al., 2004), but may be explained by novel CVD risk factors, such as arterial stiffness and endothelial dysfunction.

Arterial stiffening also increases risk of cerebrovascular disease through multiple mechanisms. Increased central aortic or local carotid arterial stiffness leads to increased pulsatile pressure and flow in the brain, and due to the low impedance of the cerebral microcirculation, this increased pressure and flow penetrates deep into the microvascular bed and may cause cerebral ischemia or hemmorhage (Mitchell, 2008; O'Rourke & Safar, 2005; Tzourio, Laurent, &

Debette, 2014). Though increased pulsatile load may induce a protective effect through hypertrophic remodelling and increased vascular resistance, this may also lead to detrimental effects, such as impaired vasoreactivity, hypoperfuson, and chronic ischemia (Tzourio et al., 2014). Increased arterial stiffening may also contribute to arterial damage and development and rupture of atherosclerotic plaques (Cheng, Loree, Kamm, Fishbein, & Lee, 1993; Witteman et al., 1994), further increasing the risk of adverse atherothrombotic CV and cerebrovascular events (Cohn, 2006).

*Measuring arterial stiffness*

Arterial stiffness may be assessed non-invasively. Applanation tonometry can be used to measure pulse waveforms at two separate points along the arterial tree in order to determine the **pulse wave velocity (PWV)**, a systemic measure of arterial stiffness (Laurent et al., 2006). Central, or aortic, PWV, measured between the carotid and femoral arteries (carotid-femoral PWV, cfPWV), is the criterion standard of arterial stiffness measurement and is an independent predictor of vascular risk (Van Bortel et al., 2012). Central PWV may also be assessed using heart-femoral PWV (measured from the heart to the femoral artery), however this measure is less established than cfPWV. Common peripheral PWV assessments include upper and lower extremity arterial stiffness, measured between the carotid and radial arteries (crPWV), and the femoral and dorsalis pedis arteries (ffPWV), respectively. Upper extremity PWV may also be

assessed using carotid-brachial PWV (measured between the carotid and brachial arteries) or brachial-radial PWV (measured between the brachial and radial arteries), though these measures are less commonly used. In addition, whole body PWV, a composite measure of central and peripheral PWV, may be assessed using measures such as brachial-ankle PWV (measured between the brachial artery and tibialis posterior artery) or heart-foot PWV (measured from the heart to the dorsalis pedis artery).

PWV is calculated as:

$$\frac{d}{\Delta t}$$

where d is the distance between the two points along the arterial tree (meters [m]) and $\Delta t$ is the transit time of the pulse wave between these two points (seconds [s]). Greater PWV values indicate increased arterial stiffness, and in turn, poorer vascular health. (Laurent et al., 2006).

Historically, the distance between the carotid and femoral arteries was estimated by calculating a subtracted distance (sternal notch to femoral artery minus carotid artery to sternal notch) (Vermeersch et al., 2009). Current guidelines (Van Bortel et al., 2012) have revised this estimation after it was established that the real traveled distance between the carotid and femoral arteries (defined as the distance from the ascending aorta (aortic valve) to the right common femoral artery distance, minus the distance from the ascending aorta to right common carotid artery) measured by magnetic resonance imaging

(MRI) was overestimated by the direct anthropometric distance between the carotid and femoral arteries, but underestimated by previously used subtracted distances (Huybrechts et al., 2011). Thus, current guidelines suggest that 80% of the measured distance between the carotid and femoral arteries appears to be the most accurate in determining cfPWV, only slightly overestimating the real traveled distance (Huybrechts et al., 2011; Van Bortel et al., 2012).

In addition to PWV, **compliance** and **distensibility** are non-invasive, local measures of arterial elasticity that may be used to assess arterial stiffness of the carotid artery. In order to assess compliance and distensibility, carotid artery images and PPs are collected simultaneously using B-mode ultrasound and applanation tonometry (Currie, Proudfoot, Timmons, & MacDonald, 2010).

Compliance is calculated as:

$$\frac{d_{max} - d_{min}}{PP}$$

Disensibility is calculated as:

$$\frac{d_{max} - d_{min}}{d_{min} \times PP}$$

where $d_{max}$ is the maximum diameter of the artery (millimeters [mm]), $d_{min}$ is the minimum diameter of the artery (mm) and PP is the pulse pressure (mmHg).

While both compliance and distensibility refer to the change in arterial diameter relative to unit of pressure, distensibility accounts for baseline arterial diameter, such that the change in arterial diameter is in proportion to baseline arterial diameter and PP. Lower values of compliance and distensibility indicate greater arterial stiffness, and in turn, poorer vascular health.

Local arterial stiffness may also be assessed by calculating the Elastic Modulus, which assesses the pressure change required for a theoretical 100% stretch from resting diameter, as well as Young's Modulus, which accounts for wall thickness in the Elastic Modulus calculation. In addition, Stiffness Index, the ratio of the SBP to DBP natural logarithm to the relative change in arterial diameter, may also be used to calculate local arterial stiffness (Oliver & Webb, 2003).

*Test-retest reliability of arterial stiffness measurement*

Almost perfect test-retest reliability of cfPWV (intraclass correlation coefficient [ICC] >0.94, *P*-value not reported) has been observed in healthy adults (n=60, mean age 44 years) when assessed using an automatic device Compilor, a validated non-invasive device (Asmar et al., 2001), with one minute between measurements (Papaioannou et al., 2012). Another report observed substantial to almost perfect test-retest reliability of applanation tonometry assessed cfPWV, ffPWV, and brachial-ankle PWV in older adults (n=79; mean age 76 years; ICC 0.7, 0.69, and 0.84, respectively, *P*-values not reported; four to eight weeks

between measurements) (Meyer et al., 2015). Additionally, test-retest reliability of heart-foot PWV (measured using electrocardiogram [ECG] and a photoplet-hsmograph sensor) was found to be substantial in children (n=20; mean age 4 years; ICC 0.76, $P \leq 0.001$; two to 31 days between measurements) (Currie et al., 2010).

In adults with spinal cord injury (n=20, mean age 43 years), the test-retest reliability of cfPWV and ffPWV, measured within two weeks using Doppler flowmeter to record pulse waves, was almost perfect (ICC 0.92 and ICC 0.91, respectively, $P<0.001$) but that of brachial-radial PWV was moderate (ICC 0.60, $P=0.03$) (Miyatani et al., 2012). Although reasons for lower test-retest reliability of upper extremity PWV in this study were not clear, the authors speculated this to be in part due to measurement error, given that results showed no variations in BP or RHR measures between the two days that would affect variations in the measure (Miyatani et al., 2012). Similarly, another study reported almost perfect test-retest reliability of cfPWV in individuals with spinal cord injury (n=18, mean age 46 years), when assessed using applanation tonometry one to seven days apart (ICC 0.98, $P<0.0001$) (Currie, Hubli, & Krassioukov, 2014).

Although some preliminary studies have examined the reliability of carotid artery compliance and distensibility, it is not well-established. In healthy adults (n=169, mean age 62 years), test-retest reliability of carotid artery compliance and distensibility was moderate when measured using B-mode and M-mode ultrasound and a standard oscillometric device, with one day to three months

between measurements (ICC 0.75 and 0.77, *P*-values not reported) (Caviezel et al., 2013). Substantial test-retest reliability of carotid artery compliance and distensibility has been observed in children (n=20; mean age 4 years; ICC 0.61 and 0.63, respectfully, *P*≤0.01), when assessed using B-mode ultrasound and applanation tonometry with two to 31 days between measurements (Currie et al., 2010).

These previous findings suggest substantial to almost perfect test-retest reliability of arterial stiffness measures in healthy adults, adults with spinal cord injury, and pre-school age children. The test-retest reliability of arterial stiffness measures has not yet been established in individuals with stroke, but it is important to investigate in order to determine the clinical applicability of this novel vascular risk markers in this population.

*Associations between arterial stiffness and CV risk*

Previous studies have established associations between arterial stiffness, age, and CV risk in various populations (Mattace-Raso et al., 2006; Sutton-Tyrell et al., 2005). In healthy adults (n=2,835, mean age 71 years), elevated cfPWV was found to be an independent predictor of CVD, with HRs (second tertile of cfPWV relative to reference category, adjusted for age, gender, mean arterial pressure and HR) of 2.45 for CHD (95% CI 1.29-4.66, *P*=0.02) and 2.28 for stroke (95% CI 1.05-4.96, *P*=0.03) (Mattace-Raso et al., 2006). Similarly, another study found associations between cfPWV and adverse CV outcomes in healthy

adults (n=2,388, mean age 74 years), with RRs (fourth quartile of cfPWV relative to first quartile, adjusted for age, gender, race, SBP, and site) of 1.53 for CHD (95% CI 1.09-2.13, *P*<0.05), 3.21 for stroke (95% CI 1.56-6.63, *P*<0.01), 1.98 for CV mortality (95% CI 1.03-3.81, *P*<0.05), and 1.60 for total mortality (95% CI 1.10-2.32, *P*<0.05) (Sutton-Tyrell et al., 2005).

There may be differences in associations with CV risk between arterial regions due to varying molecular, histological and cellular structure along the arterial tree. Typically, central arteries exhibit more elastic characteristics compared to peripheral arteries, which tend to have stiffer properties (Laurent et al., 2006). However, the opposite effect is observed with aging and presence of CV risk factors, as earlier findings suggest greater arterial stiffening in central arteries with increasing age and presence of CVD, relative to peripheral arteries, which may stiffen less with these same factors (Kimoto et al., 2003; Tsuchikura et al., 2010).

In contrast to central PWV, associations between upper and lower extremity PWV and increased CV risk are not as well-established. A previous report suggests that in older adults with and without atherosclerotic disease (n=2,798, mean age 64 years), heart-femoral PWV, may be more strongly associated with risk of CVD, CAD, and peripheral artery disease (PAD) (HR 1.28, 95% CI 1.06-1.55, *P*<0.05; HR 1.26, 95% CI 1.01-1.58, *P*<0.05; and HR 1.77, 95% CI 1.38-2.28, *P*<0.001, respectfully, for one SD increase in PWV) than upper or lower extremity PWV (*P*>0.05) (Tsuchikura et al., 2010). However, this study also found

that compared to subjects without atherosclerotic disease, those with CAD, CVD and PAD had higher levels of both central and peripheral PWV ($P$<0.05) (Tsuchikura et al., 2010). Similarly, results from another study suggest that relative to healthy adults, those with type 2 diabetes mellitus (n= 290, mean age 60 years) had greater PWV, and the effect of diabetes on central PWV (heart-carotid PWV β 0.113, $P$<0.05 and heart-femoral PWV β 0.236, $P$<0.001) was greater than on peripheral PWV (heart-brachial or femoral-ankle PWV, β 0.09–0.096, $P$>0.05) (Kimoto et al., 2003). Although these observed associations are consistent and suggest greater stiffening of the central arteries relative to the peripheral arteries with increasing CV risk, additional research in this area is warranted to better establish these associations.

Greater CV risk has also been associated with measures of local carotid arterial stiffness in numerous studies (Van Stolen et al., 2014; Yuan, Wang, & Ying, 2016). Findings from an earlier population-based cohort study (n=579, mean age 67 years) suggest associations between carotid artery distensibility and CV events (HR 1.19 for one SD decrease, 95% CI 1.00–1.41, $P$<0.05), as well as between carotid artery compliance and distensibility and all-cause mortality (HR 1.43 for one SD decrease, 95% CI 1.10–1.86, $P$<0.05 and HR 1.51 for one SD decrease, 95% CI 1.11–2.06, $P$<0.05, respectively) (Van Stolen et al., 2014). In addition, a recent systematic review and meta-analysis (n=20,361, mean age 68 years) found that, in a wide range of populations, including adults with CV risk factors, decreased carotid artery distensibility (lowest quartile

compared to all higher quartiles) was associated with CV events, such as stroke and CHD (RR 1.19, 95% CI 1.06–1.35, *P*<0.05), CV mortality (RR 1.09, 95% CI 1.01–1.18, *P*<0.05), and all-cause mortality (RR 1.65, 95% CI 1.15– 2.37, *P*<0.05) (Yuan et al., 2016). Moreover, this report suggests that a one SD decrease in distensibility may increase the risk of CV events, CV mortality, and all-cause mortality by 13%, 6%, and 41%, respectively (Yuan et al., 2016).

It is important to note that while associations between arterial stiffness and CV risk have been determined in various populations, only few studies have investigated arterial stiffness in individuals with stroke, and thus these associations are not well-established in the post-stroke population.

### 1.3.2 Endothelial Function

*Mechanisms of link with CVD*

The vascular endothelium senses and responds to various physical and chemical stimuli, which leads to the synthesis and release of factors necessary for self-regulation (Corretti et al., 2002). Vascular tone is regulated through the release of factors in response to stimuli that determine smooth muscle contractile activity (Paniagua, Bryant, & Panza, 2001). When the stimulus is an increase in blood flow, and thus an increase in shear stress against the vascular endothelium, a cascade of events occurs that ultimately results in vasodilation. Over short time periods (seconds) endothelial-dependent vasodilation occurs through the opening of the potassium channels, which hyperpolarize the

endothelial cells and increase the driving force for calcium entry into the cell. Calcium activates endothelial nitric oxide synthase (eNOS), which in turn generates nitric oxide (NO) to relax the arterial smooth muscle, resulting in vasodilation. Over longer time periods (minutes), activation of eNOS occurs through a serine/threonine protein kinase (Corretti et al., 2002).

Endothelial dysfunction is characterized by a reduction of the bioavailability of vasodilators, predominantly NO, or an increase in endothelium contracting factors. This imbalance leads to an impairment of endothelial-dependent vasodilation (Hadi, Carr, & Al Suwaidi, 2005). Endothelial dysfunction promotes the early and late mechanisms of atherosclerosis, including the upregulation of adhesion molecules, increased cell permeability, enhanced low density lipoprotein oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration. Endothelial dysfunction is therefore an important factor in the development of atherosclerosis and hypertension and is associated with future risk of adverse CV events (Verma & Anderson, 2002).

*Measuring endothelial function*

**Flow-mediated dilation (FMD)** is used to assess endothelial-dependent vasodilation, or reactive hyperemia, in response to an increase in shear stress against the arterial wall. This assessment involves occlusion of blood flow to the arm with a pneumatic cuff and B-mode ultrasound to image the brachial artery

pre- and post-occlusion to obtain baseline diameter and maximum dilation, respectively (Corretti et al., 2002). This test assesses the ability of the artery to expand following increases in shear stress against the arterial wall.

FMD is calculated as:

$$\frac{d_{max} - d_{min}}{d_{min}} \times 100$$

where $d_{max}$ is the maximum diameter of the artery post-occlusion (mm) and $d_{min}$ is baseline diameter (mm). Lower FMD values indicate greater endothelial dysfunction, and in turn, poorer vascular health (Corretti et al., 2002; Gori et al., 2008).

The placement of the pneumatic cuff on the arm varies throughout different FMD methodologies. Results from an earlier study (Doshi et al., 2001) suggest that FMD following lower arm occlusion may be a more valid marker of endothelial function when compared to upper arm occlusion, given that dilation following lower arm occlusion was exclusively mediated by NO, whereas dilation following upper arm occlusion may have been due to tissue ischemia around the brachial artery. FMD tests may also be performed in the leg to evaluate the function of the superficial femoral artery, particularly in disease states that specifically affect the lower extremities or to examine the effects of exercise training on the lower extremity (Kooijman et al., 2008). Some FMD methodologies have used handgrip exercise, rather than blood flow occlusion, to stimulate

increased shear stress against the arterial wall (Szijgyarto, Poitras, Gurd, & Pyke, 2014), however this method may not be practical for individuals with impaired arm function.

In addition to FMD, endothelial-independent vasodilation, the dilation of the arteries produced by vasodilating drugs, may be quantified in order to assess maximum arterial dilation. This test involves administration of nitroglycerin (NTG), a vasodilator that acts directly on smooth muscle cells to release NO (Corretti et al., 2002). In order to assess changes in diameter caused by endothelial-independent vasodilation, ultrasound images are taken each minute for 10 minutes following NTG administration. NTG (%) is calculated using the same equation as FMD (%), where $d_{max}$ is the maximum diameter of the artery achieved during the 10 minutes following NTG administration (mm) and $d_{min}$ is the baseline diameter of the artery prior to NTG administration (mm).

*Test-retest reliability of endothelial function measurement*

In young, healthy adults (n=26, mean age 37 years), test-retest reliability of brachial artery FMD (forearm occlusion) was high (ICC 0.92, $P$<0.0001), with seven days between measurements (Welsch, Allen, & Geaghan, 2002). Similarly, another report of healthy, young adults (n=23, mean age 33 years) suggests moderate to high test-retest reliability of brachial artery FMD (upper arm occlusion) on the same day (ICC 0.70, $P$<0.05), and different days (ICC 0.84, $P$<0.05), with a minimum of 1.5 hours and three days between measurements,

respectively (Meirelles, Leite, Montenegro, & Gomes, 2007). The reliability of brachial artery FMD has not yet been established in the post-stroke population, however a previous study found that in individuals with chronic stroke (n=18, mean age 68 years, mean time since stroke 5.7 years), test-retest reliability of femoral artery blood flow, a surrogate marker of endothelial function, was high in both the affected and non-affected limbs (ICC 0.96 and ICC 0.98, respectively, $P<0.0001$) (Billinger & Kluding, 2009b). Given that individuals with stroke are at increased risk of recurrent CV events (Mohan et al., 2011), it is important to establish the test-retest reliability of novel vascular risk markers in order to determine their clinical utility in this population.

*Associations between endothelial function and CV risk*

Previous research suggests an association between FMD and increased CV risk. A previous systematic review and meta-analysis of prospective studies (n=14,753, mean age 60 years) in various groups (healthy adults, post-menopausal women, adults with increased CV risk, and population-based cohorts) found an inverse association between brachial artery FMD and CVD risk (RR 0.92 per 1% increase in FMD, 95% CI 0.88–0.95, $P<0.01$), and this association was stronger in clinical populations compared to asymptomatic populations (Ras, Streppel, Draijer, & Zock, 2013). Similarly, a multi-ethnic, prospective cohort study found that in healthy individuals (n=842, mean age 67 years), decreased FMD values were predictive of CV events (HR 1.12 per 1%

decrease in FMD, 95% CI 1.01–1.25, *P*=0.03) (Shimbo et al., 2007). Although previous studies have reported associations between endothelial function and CV risk in various populations, only few studies have investigated endothelial function in individuals with stroke, and consequently these associations are not well-established.

## 1.4 Novel Vascular Risk Markers: Associations with Stroke, Subclinical Vascular Disease, and Physical Function

### 1.4.1 Novel Vascular Risk Markers and Stroke

*Arterial Stiffness*

Few studies have examined arterial stiffness in individuals with stroke. An earlier study found that cfPWV was elevated in individuals with acute ischemic stroke (1 week post-stroke) compared to controls with CV risk factors or history of CV or cerebrovascular morbidity (n=209; mean age 70 years; cfPWV 11.8 ± 3.3 m/s versus 10.0 ± 2.3 m/s, *P*<0.001) (Tuttolomondo et al., 2009). Findings from this study also suggest an association between cfPWV and other traditional CV risk factors, such as older age (r=0.40, *P*<0.001), hypertension (r=0.40, *P*<0.001), and diabetes (0.40, *P*<0.05). Similarly, in another study of individuals with acute ischemic stroke (n=198, median age 62 years), older age (*P*<0.001), hypertension (*P*=0.015), and diabetes (*P*<0.001) were independently associated with elevated cfPWV (De Silva et al., 2008). Elevated cfPWV has also been observed in individuals with chronic stroke. A previous study conducted by Tang et al. (2014a) found that cfPWV was elevated in community-dwelling, ambulatory

individuals with chronic stroke (n=35, mean age 67 years, median time since stroke 3.7 years, cfPWV 11.2 ± 2.8 m/s) relative to values previously reported for healthy, older adults (Tang et al., 2014a).

*Endothelial Function*

Endothelial function has been previously investigated in the post-stroke population. Findings from earlier reports suggest reduced FMD in individuals with acute ischemic stroke (n=43, mean age 62 years) (Blum et al., 2012) and chronic ischemic stroke (n=75, mean age 67 years) (Adachi et al., 2015) compared to healthy controls (FMD 16.6 ± 7.6% [control] versus –4.4 ± 7.4% [chronic ischemic stroke], *P*=0.001 (Blum et al., 2012) and FMD 8.2 ± 3.4% [control] versus 3.8 ± 2.0% [large artery atherosclerosis], 4.9 ± 3.0% [cardioembolism], and 5.7 ± 3.3% [small vessel occlusion], *P*=0.01–0.05 (Adachi et al., 2015)). In contrast, another study found that individuals with all subtypes of acute ischemic stroke (n=143, mean age 69 years) had impaired FMD, but only those with the lacunar stroke subtype had lower FMD compared to healthy controls (FMD 4.3 ± 6.1% versus 8.8 ± 6.0%, *P*=0.008) (Chen et al., 2006). Similarly, another study (n=62, mean age 61 years) also found reduced FMD in individuals with lacunar stroke (FMD 0.4 ± 5.0%) compared to individuals without stroke but with similar risk factors, such as hypertension (FMD 3.8 ± 4.8%), and healthy controls (FMD 7.9 ± 6.0%) (Pretnar-Oblak, Sabovic, Pogacnik, Sebestjen, & Zaletel, 2006).

*Between-limb differences in novel vascular risk markers after stroke*

Individuals with stroke may demonstrate unique unilateral adaptations in the affected limb, such as hemiparesis and changes in muscle tone, which may result in limited function and mobility on the affected side (Hankey et al., 2002; Thibaut et al., 2013). It is possible that differences in limb function between the affected and unaffected limbs may also lead to between-limb differences in vascular health and function. Indeed, earlier studies have reported between-side differences in resting blood flow, resting arterial diameter and arterial wall thickness in post-stroke populations (Billinger, Gajewski, Guo, & Kluding, 2009a; Billinger & Kluding, 2009b, Ivey, Gardner, Dobrovolny, & Macko, 2004). as well as between-side differences in arterial stiffness and endothelial function (Billinger et al., 2012; Ivey et al., 2004; Ivey, Hafer-Macko, Ryan, & Macko, 2010).

The earliest studies in this area have reported reduced reactive hyperemic blood flow, measured by strain-gauge plethysmography, in the affected compared to the unaffected leg in individuals with chronic stroke (>6 months post-stroke) (Ivey et al., 2004; Ivey et al., 2010). However, the use of reactive hyperemic blood flow as an indicator of endothelial function is limited, as this measure does not account for changes in arterial diameter. In a more recent study, endothelial function, measured using brachial artery FMD, was lower in the affected compared to the unaffected limb in individuals <6 months post-stroke (n=10, mean age 61 years, FMD 4.7 ± 2.5% affected limb versus 6.5 ± 2.2% unaffected limb) (Billinger et al., 2012). Whether these between-side differences

in FMD persist in the later post-stroke phases has not yet been examined.

It is important to establish between-side differences in novel vascular measures in individuals with stroke, as impairments of peripheral vascular function are reflective of systemic vascular health and function (Korkmaz & Onalan, 2008). In addition, given limited physical function and mobility, positioning the affected limb for assessments of arterial stiffness and endothelial function may be challenging to obtain and difficult to maintain for an extended period of time, which may potentially affect the ability to achieve accurate measurements. Investigating between-side differences in novel vascular risk markers in individuals with stroke may increase insight into the feasibility of these measures in the post stroke population, such that given no between-side differences, it may be more clinically feasible to perform assessments on the non-affected limb.

### 1.4.2 Novel Vascular Risk Markers and Subclinical Vascular Disease

Previous work has reported associations between novel vascular risk markers and subclinical vascular disease. Results from a large-scale, population-based study in older adults (n=2512, mean age 75 years) found an association between decreased carotid artery distensibility and incident deep cerebral microbleeds (RR per one SD decrease 1.14, 95% CI 1.05-1.24, $P<0.05$; RR adjusted for age, sex and follow-up interval) (Ding et al., 2015). Similarly, another study in older adults (n=208, mean age 72 years) observed a positive association

between cfPWV and white matter hyperintensities, adjusted for age and sex (OR 1.58, 95% CI 1.04–2.40, *P*<0.05), although this effect was reduced following adjustments for several CV risk factors (Gustavsson et al., 2015). In addition, findings from another study in older adults with a range of CVD, such as myocardial infarction and heart failure, (n=25, mean age 72 years) found an inverse association between brachial artery FMD and white matter hyperintensity volume (r=–0.53, *P*=0.02) (Hoth et al., 2007).

Given that cerebral SVD may initially manifest as declines in cognition (Longstreth et al., 1998; Price et al., 1997; Vermeer et al., 2003), cognitive function may serve as an important clinical marker of vascular disease progression. However, although earlier findings suggest a possible association between novel vascular risk markers and cerebral SVD, only few studies have investigated the association between these novel vascular risk markers and clinically-measured cognitive function. A previous study in individuals with chronic stroke (n=102, mean age 61 years, mean time since stroke 6 years) found a negative association between cfPWV and global cognitive function, quantified using the Mini Mental State Examination, which assesses multiple domains of cognition, such as memory, attention, and language (r=–0.45, *P*<0.01) (Lee et al., 2014). In contrast, findings from a large-scale, population-based study of older adults (n=1820, mean age 80 years) suggest a negative association between cfPWV and memory, assessed using the California Verbal Learning test immediate and delayed recall, but not with other cognitive domains, such as

executive functioning or processing speed (Cooper et al., 2015). Similarly, another study suggests that brachial artery FMD was reduced in individuals with mild cognitive impairment (MCI), assessed using an extensive neurophysiological examination evaluating criteria such as objective memory impairment and impairment of one or more non-memory domains, compared to healthy controls (MCI n=34, mean age 68 years, FMD 8.7 ± 1.3%; control n=37, mean age 66 years, FMD 11.8 ± 1.5%; between-group FMD $P$=0.001) (Vendemiale, Romano, Dagostino, de Matthaeis, & Serviddio, 2013). These preliminary findings suggest an association between novel vascular risk markers and clinical measures of cognitive function. Further research in this area may increase understanding regarding the detection of cerebrovascular disease progression without use of neuroimaging techniques.

To our knowledge, no study has investigated differences in novel vascular risk markers across a continuum of increasing vascular risk that includes presence of cerebrovascular disease and cognitive impairment. Given previously established associations between novel vascular risk markers and stroke (Adachi et al., 2015; Blum et al., 2012; Chen et al., 2006; De Silva et al., 2008; Pretnar-Oblak et al., 2006; Tang et al., 2014a; Tuttolomondo et al., 2009) and cerebral SVD (Ding et al., 2015; Gustavsson et al., 2015; Hoth et al., 2007), as well as between cerebral SVD and cognitive impairment (Longstreth et al., 1998; Price et al., 1997; Vermeer et al, 2003), it is possible that we may observe an increase in vascular risk across a continuum that includes older adults without cognitive

impairment, with cognitive impairment, and with stroke (Figure 4). A comparison of novel vascular risk markers across such a continuum may increase what is currently known regarding early vascular disease detection and may also work to establish cognitive function as a clinical marker of vascular disease progression.

### 1.4.3 Novel Vascular Risk Markers and Physical Function

Measures of physical function, such as walking ability and balance, are commonly used as indicators of physical activity in the post-stroke population (Pang et al., 2006; Saunders et al., 2016) and limited physical function following stroke is associated with low levels of cardiorespiratory fitness, muscle strength and muscle power (Flansbjer, Lexell, & Brogardh, 2012; Patterson, Ross-Edwards, & Gill, 2010; Saunders, Greig, Young, & Mead, 2008).

Previous reports have suggested that novel vascular risk markers are associated with physical fitness in individuals with stroke (Billinger 2012 et al., Tang et al., 2014a). Findings from an observational, cross-sectional study suggests that in individuals with chronic stroke (n=35, mean age 67 years, median time post-stroke 3.7 years), aerobic capacity (VO2 peak) may contribute to the variance of cfPWV after accounting for the effects of age and medication use (Tang et al., 2014a). In addition, findings from a prospective study (n=10, mean age 61 years) suggest that aerobic exercise may be effective in improving brachial artery FMD in both the affected (pre-intervention 4.7 ± 2.5%, post-intervention 5.4 ± 2.4%, $P$=0.001) and unaffected limbs (pre-intervention 6.5 ±

2.2%, post-intervention 7.7 ± 2.3%, *P*=0.001) in individuals with stroke (Billinger et al., 2012).

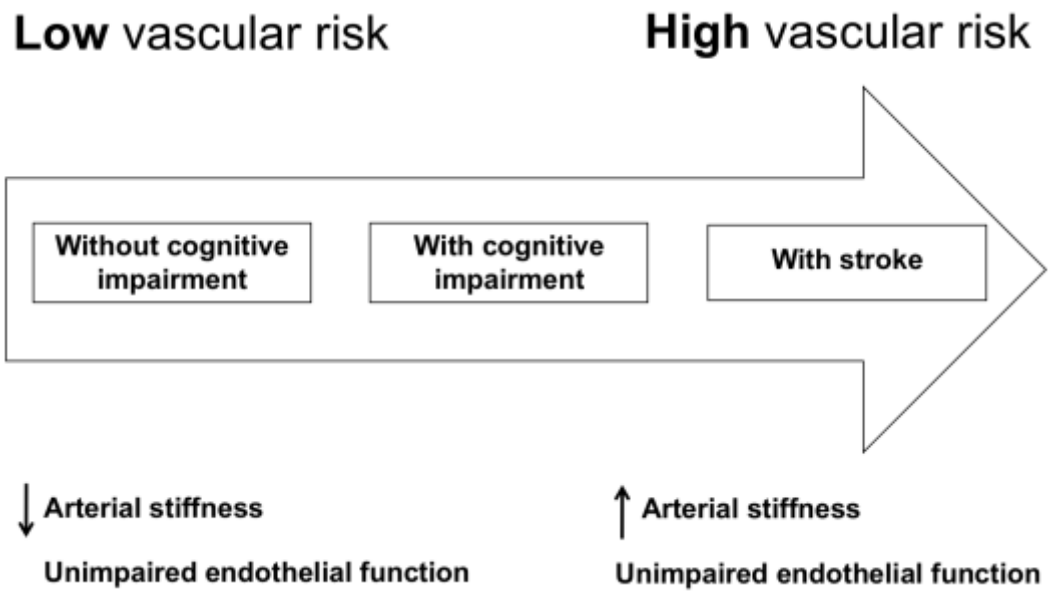



**Figure 4.** Integration of novel vascular risk markers into proposed continuum of increasing vascular risk

Given previous findings regarding the associations between novel risk factors and stroke (Adachi et al., 2015; Blum et al., 2012; Chen et al., 2006; De Silva et al., 2008; Pretnar-Oblak et al., 2006; Tang et al., 2014a; Tuttolomondo et al., 2009) and cerebral SVD (Ding et al., 2015; Gustavsson et al., 2015; Hoth et al., 2007), as well as between cerebral SVD and cognitive impairment (Longstreth et al., 1998; Price et al., 1997; Vermeer et al., 2003), it is possible that individuals at the low end of the continuum will have low arterial stiffness and impaired endothelial function, individuals in the middle of the continuum will have moderate arterial stiffness and moderately impaired endothelial function, and individuals at the high end of the continuum with have high arterial stiffness and impaired endothelial function

However, associations between novel vascular risk markers and measures of physical function, such as walking and balance ability, are not well-established in

the post-stroke population. Although an earlier study involving individuals with chronic stroke (n=102, mean age 61 years, mean time since stroke 6 years) observed negative associations between cfPWV and measures of physical function, namely flexibility, muscular strength, balance, and walking endurance (r=–0.45 – –0.55, *P*=0.001) (Lee et al., 2014), no other studies have investigated associations between physical function and other novel vascular risk markers. Given that individuals with stroke are at elevated risk for recurrent events (Mohan et al., 2011), and that post-stroke rehabilitation interventions typically focus on improving physical function, it is important to understand associations between physical function and novel vascular risk markers in order to optimize rehabilitation interventions. If findings suggest negative associations between vascular risk and physical function, rehabilitation interventions may be designed to concurrently improve physical function and reduce vascular risk.

### 1.5 Overall Thesis Objectives

The test-retest reliability of arterial stiffness and endothelial function measures has not yet been determined in individuals with stroke, however given the elevated risk of recurrent CV events (Mohan et al., 2011) it is important to establish this measurement property in order to determine the clinical applicability of these assessments in the post-stroke population. Applying arterial stiffness and endothelial function measures in this population may lead to early CV risk detection and implementation of future strategies to effectively manage this risk.

In addition, differences in novel vascular risk markers between the affected and unaffected sides have not yet been established in the post-stroke population. Given limited physical function and mobility of the affected limbs, it is important to investigate between-side differences in novel vascular measures in individuals with stroke in order to increase insight into the feasibility of these measures.

Although few studies have investigated novel vascular risk markers in individuals with stroke, cerebral SVD, and cognitive impairment, none have compared them across continuum of vascular risk in older adults that considers the presence of stroke and cognitive impairment. Such a comparison would increase knowledge regarding vascular disease progression in older adults and potentially lead to early CV risk detection. In addition, the association between physical function and novel vascular risk markers has not yet been established in individuals with stroke, but may help to improve post-stroke rehabilitation programs to effectively target both physical function and CV risk.

This thesis aims to:

1) Investigate test-retest reliability of arterial stiffness and endothelial function in individuals with stroke (Chapter 2: Measurement Properties of Novel Vascular Risk Markers).

2) Investigate between-side differences of arterial stiffness and endothelial function in individuals with and without stroke (Chapter 2: Measurement Properties of Novel Vascular Risk Markers).

3) Investigate differences in arterial stiffness and endothelial function across a

continuum of increasing vascular risk: older adults without cognitive impairment, with cognitive impairment, and with stroke (Chapter 3: Comparison of Novel Vascular Risk Markers Across a Proposed Continuum of Increasing Vascular Risk).

4) Examine relationships between novel vascular risk markers (arterial stiffness and endothelial function) and physical function in individuals with and without stroke (Chapter 3: Comparison of Novel Vascular Risk Markers Across a Proposed Continuum of Increasing Vascular Risk).

# Chapter 2

## STUDY 1 – MEASUREMENT PROPERTIES OF NOVEL VASCULAR RISK MARKERS

## 2.1 Background

Stroke is the leading cause of adult neurological disability (Heart and Stroke Foundation, 2012), with the majority of its survivors at elevated risk of recurrent stroke and other adverse cardiovascular events (Dhamoon, Sciacca, Rundek, Sacco, & Elkind, 2006; Mohan et al., 2011). Traditional cardiovascular (CV) risk factors, such as hypertension, dyslipidemia and diabetes, are highly prevalent and inadequately managed following stroke (Kopunek et al., 2007), which contributes to this risk. However, these traditional risk factors do not fully explain the observed risk of stroke in a population (Figure 2) (O'Donnell et al., 2010, Yusuf et al., 2004). Novel markers of vascular health and function, namely **arterial stiffness** and **endothelial function**, may provide earlier and more sensitive risk detection. These novel markers are important factors in the development of atherosclerosis (Widlansky, Gokce, Keaney, & Vita, 2003; van Popele et al., 2001) and are associated with elevated risk of stroke and other cardiovascular disease (CVD) (Blum et al., 2012; Mattace-Raso et al., 2006; Ras et al., 2013; Sutton-Tyrell et al., 2005).

*Arterial Stiffness.* Increased arterial stiffness reduces the elastic capacity of the arterial wall, thus increasing the transmission velocity of pulsatile flow (Safar et al., 2003). Increased central arterial stiffness causes reflected pulse waves to arrive earlier in the central aorta during systole rather than during diastole, which normally occurs with less arterial stiffening. This leads to increased aortic systolic blood pressure (SBP) and reduced aortic diastolic blood pressure (DBP), both of

which have negative consequences (Safar et al., 2003). Chronically high SBP increases left ventricular afterload, augmenting the workload of the heart and increasing risk for coronary artery disease (CAD) (Mac-Way et al., 2011; Safar et al., 2003). Reduced DBP decreases coronary perfusion pressure, predisposing the heart to ischemia (Cecelja & Chowienczyk, 2012).

Moreover, increased arterial stiffening of the aorta and carotid artery also exposes the arterioles in the brain to high pulsatile pressure and flow, which may contribute to the pathogenesis of cerebral small vessel disease and stroke (Cecelja & Chowienczyk, 2012). Increased arterial stiffening of the central and peripheral arteries may also contribute to arterial damage and development and rupture of atherosclerotic plaques (Cheng et al., 1993; Witteman et al., 1994), further increasing the risk of adverse CV events (Cohn, 2006).

*Endothelial Function.* The vascular endothelium regulates vascular tone through the release of factors in response to various stimuli (Paniagua et al., 2001). When the stimulus is an increase in blood flow, the vascular endothelium responds to the resultant increase in shear stress by producing and releasing factors (such as nitric oxide [NO]) to cause vasodilation in order to mitigate shear stress (Corretti et al., 2002). Endothelial dysfunction refers to an impairment of endothelial-dependent vasodilation, characterized by a reduction of the bioavailability of endothelial vasodilatory factors, predominantly NO, or an increase in endothelial constricting factors (Hadi et al., 2005). Endothelial dysfunction promotes the development of atherosclerosis through mechanisms

such as the upregulation of adhesion molecules, enhanced low density lipoprotein oxidation and platelet activation, and is thus an important factor in the development of atherosclerosis (Widlansky et al., 2003) and hypertension (Panza, Garcia, Kilcoyne, Quyyumi, & Cannon, 1995) and is associated with future risk of adverse CV events and stroke (Blum et al., 2012; Ras et al., 2013).

Measurement properties of arterial stiffness and endothelial function have been reported previously in various populations. Earlier studies have reported substantial to almost perfect test-retest reliability of systemic and local measures of arterial stiffness in pre-school aged children (age 4.0±1.0 years; intraclass correlation coefficient [ICC] 0.61-0.76) (Currie et al., 2010), healthy adults (mean age 44.4–75.7 years, ICC 0.69–0.94) (Caviezel et al., 2013; Meyer et al., 2015; Papaioannou et al., 2012), and individuals with spinal cord injury (mean age 43.0–46.0 years, ICC 0.60–0.98) (Currie et al., 2014; Miyatani et al., 2012). Previous findings also suggest high test-retest reliability of endothelial function measures in young, healthy adults (mean age 33.0–41.0 years, ICC 0.84–0.92) (Meirelles et al., 2007; Welsch et al., 2002)

However, the test-retest reliability of novel vascular risk marker measures in not well-established in the post-stroke population, despite the knowledge that these individuals are at elevated risk of recurrent events. To our knowledge, only one study has investigated the test-retest reliability of these measures in individuals with chronic stroke (age 68 ± 4 years, time since stroke 5.7 ± 1.1 years), findings of which suggest high test-retest reliability of femoral artery blood

flow, a surrogate marker of endothelial function, in both the affected and non-affected limbs (ICC 0.96 and ICC 0.98, respectively) (Billinger & Kluding, 2009b). It is important to determine the measurement properties of these early and sensitive measures of vascular risk progression to establish their clinical applicability in the post-stroke population, which may ultimately allow for early CVD risk detection and implementation of strategies to effectively manage this risk.

Moreover, a unique feature of stroke that may contribute to challenges with measuring vascular health is the presence of unilateral impairments, such as hemiparesis (muscle weakness) and changes in muscle tone, which may result in limited function and mobility on the affected side (Thibaut et al., 2013). Thus, positioning the affected limb for assessments of arterial stiffness and endothelial function may be challenging to obtain and difficult to maintain for an extended period of time, which may potentially affecting the ability to obtain accurate measurements.

Differences in limb function between the affected and unaffected sides may impact vascular function. Earlier studies in individuals with stroke have reported between-side differences in resting femoral artery blood flow, resting diameter, and arterial wall thickness (Billinger et al., 2009a; Billinger & Kluding, 2009b; Ivey et al., 2004), but few studies have reported between-side differences in arterial stiffness and endothelial function (Billinger et al., 2012; Ivey et al., 2004, Ivey et al., 2010). It is important to establish between side differences in novel vascular

measures in individuals with stroke, as impairments of peripheral vascular function are reflective of systemic vascular health and function (Korkmaz & Onalan, 2008). Additionally, given the challenges of obtaining accurate measures on the affected side in individuals with stroke, determining between-side differences may increase insight into the feasibility of novel vascular measures in this population.

The earliest work in this area reported reduced endothelial function, quantified as reactive hyperemic blood flow measured by strain-gauge plethysmography, in the affected compared to the unaffected limb (Ivey et al., 2004; Ivey et al., 2010). More recently, one study (Billinger et al., 2012) found reduced endothelial function in the affected compared to the unaffected limb using flow-mediated dilation, the most well-established and commonly-used non-invasive measure of endothelial function (Corretti et al., 2002). Of note however, this preliminary study had a small sample size (n=10) and only assessed individuals in the early stage of stroke recovery (<6 months post-stroke). No study to date has examined between-side differences in those in the later stages post-stroke (>6 months post-stroke). Given the challenges of obtaining accurate measures on the affected side in individuals with stroke, determining between-side differences may increase insight into the feasibility of novel vascular measures in this population.

## 2.2 Objectives and Hypotheses

*Objective 1. To investigate test-retest reliability of arterial stiffness and endothelial function in individuals with stroke.* It was hypothesized that there would be almost perfect test-retest reliability (ICC > 0.80) (Landis & Koch, 1977) for the measurement of arterial stiffness and endothelial function in individuals with stroke.

*Objective 2. To investigate between-side differences of arterial stiffness and endothelial function in individuals with and without stroke.* It was hypothesized that arterial stiffness and endothelial dysfunction would be greater on the affected side compared to the unaffected side in individuals with stroke, and that there would be no between-side differences in individuals without stroke.

## 2.3 Methodology

### 2.3.1 Study Design

This was a cohort study with repeated measures. This protocol was approved by the Hamilton Integrated Research Ethics Board (HIREB # 13-348). Informed written consent was obtained from all participants (participant information and consent displayed in Appendix 2).

### 2.3.2 Participants

Community-dwelling adults with or without stroke were recruited for this study and screened to fit the following criteria:

*Inclusion Criteria*

- 50-80 years of age
- ability to walk 10 meters independently with or without an assistive device
- for individuals with stroke, ≥ 12 months post stroke

*Exclusion Criteria*

- for individuals with stroke, stroke of non-cardiac origin
- significant cardiovascular, musculoskeletal or other neurological conditions
- significant cognitive or communication impairment or behavioural issues affecting ability to understand instructions for testing

### 2.3.3 Assessments

Data were collected in the temperature-controlled Vascular Dynamics Laboratory at McMaster University. Participants with stroke visited the laboratory for two visits over two days, no more than seven days apart, at the same time of day. Participants without stroke visited the laboratory for one visit. All participants were asked to abstain from food or drink for 4 hours, smoking, caffeine and alcohol for 12 hours and exercise for 24 hours before each visit.

*Participant Characteristics*

Participant characteristics were collected, including age, sex, body mass index (BMI), waist-hip ratio (WHR), resting SBP, DBP, and heart rate (RHR).

Resting supine blood pressure (BP) was measured at the brachial artery from the dominant arm in participants without stroke and both affected and unaffected arms in participants with stroke (DINAMAP, Critikon Inc). Two readings were taken and averaged, but if values differed >5 mmHg, an additional 2 readings were taken and all 4 readings were averaged (Pickering et al., 2005). RHR was measured continuously using single-lead electrocardiography.

Self- and fast-paced walking speed were assessed using the 5 Meter Walk Test (5mWT), which provides a measure of the speed at which an individual is able to walk 5 meters, with or without an assistive device (Salbach et al., 2001). Walking endurance was assessed using the 6-Minute Walk Test (6MWT), which provides a measure of the distance walked over 6 minutes, with or without an assistive device and with rest breaks permitted (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002).

Balance ability was assessed using the 14-item Berg Balance Scale (BBS) (Berg Balance Scale, 2016; Berg, Wood-Dauphinee, Williams, & Maki, 1992). Items on this scale include functional balance activities, such as standing with eyes closed, retrieving an object from the floor and standing on one foot. Each item was scored on a 5-point scale (0-4), with the highest achievable score being 56 and higher scores representing better balance ability.

Cognitive function was assessed using the 16-item Montreal Cognitive Assessment (MoCA) (McLennan, Mathias, Brennan, & Stewart, 2011), which assesses multiple cognitive domains including visualspatial and executive

functions, naming, memory, attention, language, and orientation. The highest achievable score is 30, with higher scores representing better cognitive function and a score of <26 indicating mild cognitive impairment (Nasreddine et al., 2005). The MoCA is displayed in Appendix 3.

In addition, details of the previous stroke event were collected from participants with stroke (date of stroke, stroke location, type of stroke, stroke severity using the National Institutes of Health Stroke Scale (NIHSS) (NIH and limb impairment using the Chedoke-McMaster Stroke Assessment (CMSA) impairment inventory (Miller et al., 2008). The NIHSS is a 11-item assessment used to quantify stroke-related impairments, with each item scoring between 0-4 and higher scores indicating greater impairment (NIH Stroke Scale, 2003). The CMSA impairment inventory assesses impairment of specific domains (arm, hand, leg and foot), scoring each on a seven-point scale, with lower scores indicating greater impairment (Miller et al., 2008).

*Novel Vascular Measures*

*Arterial Stiffness*

Arterial stiffness was evaluated using several of non-invasive measures. Pulse wave velocity (PWV), a systemic measure of arterial stiffness, was measured according to the most recent guidelines (Van Bortel et al., 2012). Simultaneous measurements of arterial pressure waveforms were obtained by two trained assessors at two points along the arterial tree using applanation

tonometry (Figure 5) (Model SPT-301; Millar Instruments Inc., Houston, TX, USA). A surface tape measure was used to assess distance between the two measurement sites. Central, or carotid-femoral, PWV (cfPWV), upper extremity, or carotid-radial, PWV (crPWV) and lower extremity, or femoral-foot, PWV (ffPWV) were assessed between the carotid and femoral, carotid and radial, and femoral and dorsalis pedis arteries, respectively.

**Figure 5.** Representation of carotid-femoral pulse wave velocity assessment

Applanation tonometry measuring pulse waveforms at the carotid and femoral arteries.

PWV was calculated as:

$$\frac{d}{\Delta t}$$

where d is the distance between the two points along the arterial tree (meters [m]) and $\Delta t$ is the transit time of the pulse wave between these two points (seconds [s]) (Figure 6). Greater PWV values indicated increased arterial stiffness, and in turn, poorer vascular health. In accordance with recent PWV guidelines (Van Bortel et al., 2012), 80% of the direct measured distance between the carotid and femoral arteries was used for the calculation of cfPWV. Since the direct distance between the carotid and femoral arteries was not measured for the majority of participants with stroke (13/17 [76%] participants), a validated equation was used to convert subtracted distances (sternal notch to femoral artery minus sternal notch to carotid artery) to the direct distance Vermeersch et al., 2009), and 80% of this direct distance was used to calculate cfPWV. The direct distance between the other sites was used for the calculation of crPWV and ffPWV. PWV was tested bilaterally in all participants and on two separate visits (no more than seven days apart) in participants with stroke.

In addition to PWV, common carotid artery compliance and distensibility, local measures of carotid artery elasticity, were used to assess local artery stiffness. Compliance and distensibility, which refer to the absolute and relative changes in artery diameter with pressure, respectfully, were measured using applanation tonometry and B-mode ultrasound (System FiVe; GE Medical Systems, Horten, Norway) to collect simultaneous carotid artery pulse pressures and images for 10 consecutive cardiac cycles (Currie et al., 2010).

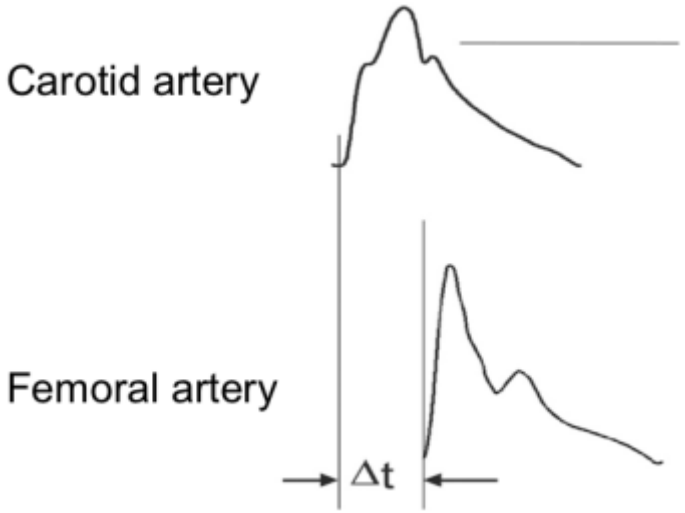



**Figure 6.** Time delay (Δt) between carotid and femoral pulse waveforms

Compliance was calculated as:

$$\frac{d_{max} - d_{min}}{PP}$$

Distensibility is calculated as:

$$\frac{d_{max} - d_{min}}{d_{min} \times PP}$$

where $d_{max}$ is the maximum lumen diameter of the artery (millimeters [mm]), $d_{min}$ is the minimum lumen diameter of the artery (mm) and PP is the pulse pressure (millimeters of mercury [mmHg]) (Currie et al., 2010).

Pulse pressure data were collected and analyzed offline using Powerlab acquisition equipment (Labchart 7; ADInstruments IC, Colorado Springs, CO, USA). Carotid artery diameter was assessed from ultrasound images using semi-automated edge-detection software (Artery Measurement System; Image and Data Analysis, Gothenburg, Sweden). Compliance and distensibility were assessed bilaterally and on two separate visits in participants with stroke (no more than seven days apart). Repeated measures were not performed with participants without stroke.

*Endothelial Function*

Endothelial function (endothelial-dependent vasodilation) was assessed using brachial artery flow-mediated dilation (FMD) (Corretti et al., 2002). Participants were in the supine position with a pneumatic cuff positioned on the forearm distal to the antecubital fossa. The pneumatic cuff was inflated to suprasystolic pressure (200 mmHg) using a rapid cuff inflator (model E20 and AG101; Hokanson, Bellvue, WA) to occlude blood flow in forearm for 5 minutes. B-mode ultrasound was used to obtain images of the brachial artery for 30 seconds pre-occlusion to assess baseline arterial diameter and for 3 minutes post-deflation to assess maximum arterial diameter (Corretti et al., 2002). Figure 7 shows a schematic representation of the FMD assessment.

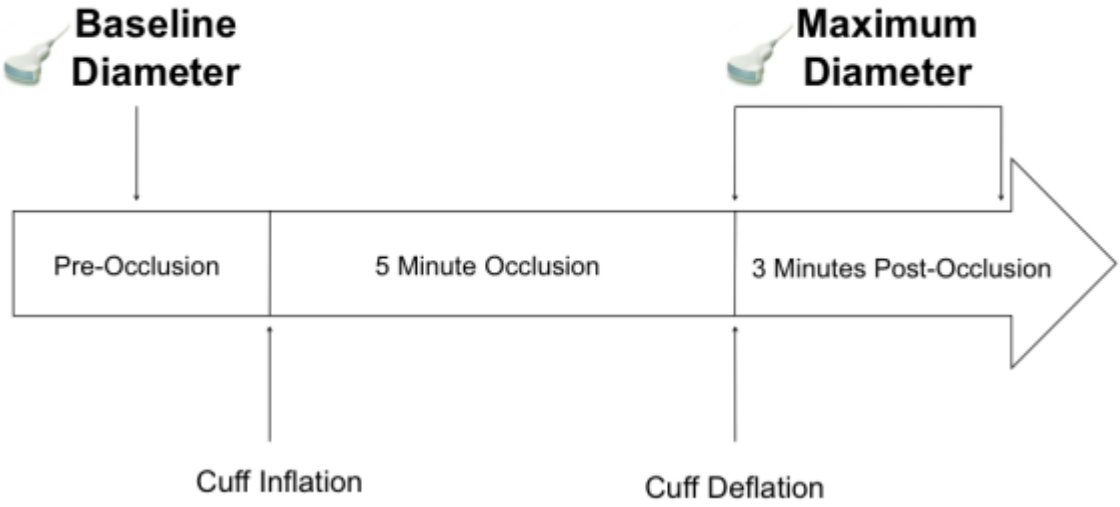



**Figure 7.** Schematic representation of flow-mediated dilation assessment

A pneumatic cuff was placed on the forearm and inflated to suprasystolic pressure (200 mmHg) for 5 minutes, occluding blood flow to the forearm. Brachial arterial diameter was assessed before cuff inflation and following cuff deflation in order to obtain baseline and maximum arterial diameter, respectfully.

FMD (%) was calculated as

$$\frac{d_{max} - d_{min}}{d_{min}} \times 100$$

where $d_{max}$ is the maximum diameter of the artery (mm) and $d_{min}$ is the minimum diameter of the artery (mm). Lower FMD values indicate greater endothelial dysfunction, and thus poorer vascular health (Corretti et al., 2002).

In addition to FMD, endothelial-independent vasodilation was quantified in order to assess maximal arterial dilation capacity in response to agonists acting

on vascular smooth muscle. At least 10 minutes following FMD assessment, a single dose of 0.4 mg nitroglycerin (NTG), a vasodilator that acts directly on smooth muscle cells to release NO (Corretti et al., 2002), was administered sublingually. B-mode ultrasound images of the brachial artery were obtained prior to NTG administration for 30 seconds in order to assess baseline arterial diameter, and for 30 seconds each minute thereafter for 10 minutes to assess maximum arterial diameter. NTG (%) was calculated using the same equation as FMD (%), where $d_{max}$ is the maximum diameter of the artery achieved during the 10 minutes following NTG administration (mm) and $d_{min}$ is the baseline diameter of the artery prior to NTG administration (mm).

For all ultrasound image analysis, the R-spike of the simultaneous ECG trace was used to determine end-diastolic image frames. These frames were extracted and stacked using commercially available software (Sante DICOM Editor, Version 3.0.12; Santesoft, Athens, Greece) to create an image file (Digital Imaging and Communications in Medicine [DICOM] file). Brachial artery diameter was then assessed from this file of end-diastolic ultrasound images using semi-automated edge-detection software (Artery Measurement System; Image and Data Analysis, Gothenburg, Sweden). FMD and NTG were assessed bilaterally and on two separate visits in participants with stroke. Repeated measures were not performed with participants without stroke.

*Measurement Summary*

Test-retest reliability and between-side differences in all vascular measures were assessed in participants with stroke and between-side differences in PWV were assessed in participants without stroke and used as a comparison arm (Figure 8)

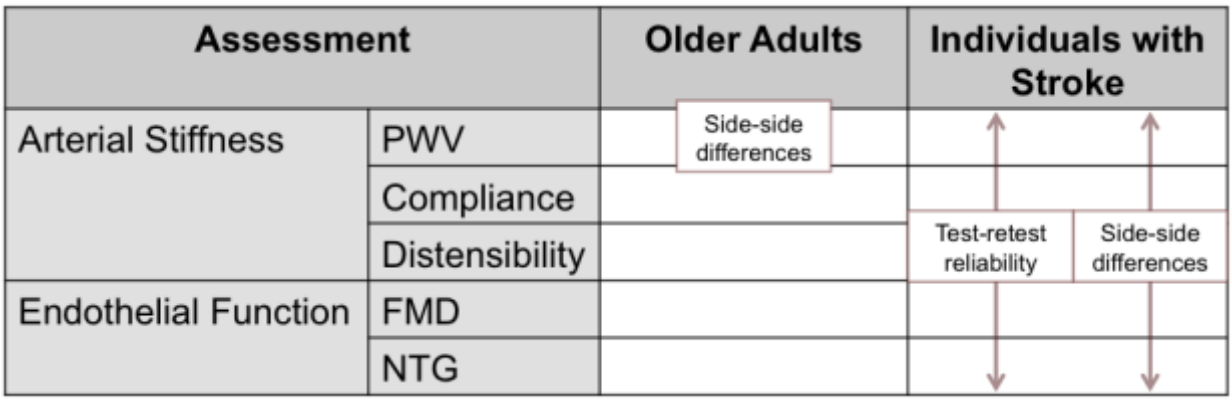



**Figure 8.** Schematic representation of vascular assessments

Test-retest reliability and between-side differences of all vascular measures (pulse wave velocity, PWV; compliance; distensibility; flow-mediated dilation, FMD; nitroglycerin, NTG) were assessed in all participants and between-side differences in PWV were also assessed in participants without stroke.

### 2.3.4 Statistical Analyses

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 20.0; IBM Corporation, Armonk, NY, USA). Descriptive statistics were performed on all measures (mean ± SD for continuous variables, n (%) for non-continuous variables). Independent t-tests were used to evaluate between-visit differences in all vascular measures in individuals with

stroke. ICCs (two-way random model) were used to determine relative test-retest reliability of all vascular measures. ICC values of 0.00 – 0.20, 0.21 – 0.40, 0.41 – 0.60, 0.61 – 0.80, and 0.81 – 1.00 represent poor, fair, moderate, substantial and almost perfect agreement, respectfully (Landis & Koch, 1977). Standard error of measurement (SEM) was calculated using Microsoft Excel, version 12.1.0. (Microsoft Corporation, Washington D.C.) to assess absolute test-retest reliability of all vascular measures. Bland-Altman plots were created to visualize the between-visit agreement of all vascular measures (Bland & Altman, 1986). Independent t-tests were used to evaluate between-side differences in all vascular measures in individuals with stroke and PWV in older adults without stroke. Little's missing completely at random (MCAR) test was performed to determine if data was missing at random. Statistical significance was set at $p<0.05$.

## 2.4 Results

*Participant characteristics*

Forty-four participants (17 with stroke and 27 without stroke) were included in this study. Participant characteristics are displayed in Table 1. Participants with stroke were younger in age, and presented with higher DBP, BMI and WHR. They also had poorer BBS and MoCA scores, slower self- and fast-paced walking speed on the 5MWT, and lower distance walked on the 6MWT (Table 1).

*Missing data*

Due to a number of different factors, 18% of the total data was missing (25% and 8% from participants with and without stroke, respectfully). Data was missing at random (Little's MCAR chi$^2$ 216.9, *P*=1.0) and casewise deletion was performed for analyses. Reasons for missing data are displayed in Appendix 4.

*Test-retest reliability*

Group means, SEMs and ICC's for all vascular measures from visit 1 and visit 2 are reported in Table 2. There were no differences in any vascular measures between visit 1 and visit 2. Test-retest reliability was almost perfect for cfPWV, substantial for FMD, moderate for crPWV, and low for ffPWV on both the affected and unaffected sides. Test-retest reliability was moderate for compliance (unaffected side) and distensibility (affected side), and low for compliance (affected side) and distensibility (unaffected side). Scatter plots for cfPWV and FMD are displayed in Figure 9.

It is worth noting that on Bland-Altman plots for FMD measurements taken on visit 1 and visit 2, we observed values that were boarding or outside the upper and lower limits of agreement (n=1 affected side, n=2 unaffected side) (Figure 10). When analyses were performed with these values removed, the ICC increased to 0.90 (95% CI 0.64–0.98, *P*<0.0001; n=9) on the affected side and 0.88 (95% CI 0.48–0.97, *P*<0.0001; n=9) on the unaffected side.

*Between-side differences*

Between-side comparisons for all vascular measures in individuals with stroke are reported in Table 3, and for PWV in individuals without stroke in Table 4. There were no between-side differences in PWV, compliance or distensibility, or FMD in individuals with stroke or in PWV in individuals without stroke (Table 4, Figures 11-14). In addition, there were no between-side differences in NTG (Table 3).

## 2.5 Discussion

Our results suggest that in individuals with previous stroke, test-retest reliability was almost perfect for cfPWV, substantial for FMD, moderate for crPWV, compliance (unaffected side only), and distensibility (affected side only), and low for ffPWV, compliance (affected side only), and distensibility (unaffected side only). In addition, results suggest no between-side differences in PWV, compliance, distensibility, FMD and NTG in individuals with stroke, and in PWV in individuals without stroke.

*Test-retest Reliability*

To our knowledge, this study is the first to report test-retest reliability of arterial stiffness and endothelial function in individuals with stroke. CfPWV is the criterion standard of arterial stiffness measurement, and is an independent predictor of vascular risk (Laurent et al., 2001, Van Bortel et al., 2012). FMD is

Table 1. Characteristics for Participants with and without Stroke

| Characteristics | Individuals with Stroke | | | Older Adults | | | *P*-Value |
|---|---|---|---|---|---|---|---|
| | n | n (%) or Mean ± SD | Range | n | n (%) or Mean ± SD | Range | |
| Age (years) | 17 | 63.9 ± 8.6 | 50.0 – 80.0 | 27 | 70.4 ± 4.3 | 62.0 – 78.0 | 0.002* |
| Sex (males/females) | 17 | 13(76) / 4(24) | | 27 | 12(44) / 15(56) | | 0.06 |
| Systolic blood pressure (mmHg) | 16 | 136.1 ± 15.6 | 112.6 – 174.5 | 27 | 127.7 ± 4.3 | 100.8 – 156.2 | 0.08 |
| Diastolic blood pressure (mmHg) | 16 | 79.5 ± 10.3 | 65.6 – 100.0 | 27 | 73.0 ± 9.7 | 55.2 – 97.2 | 0.048* |
| Resting heart rate (bpm) | 16 | 65.7 ± 13.1 | 45.0 – 96.8 | 27 | 62.2 ± 10.2 | 45.0 – 91.4 | 0.34 |
| Body mass index (kg/m$^2$) | 17 | 31.2 ± 5.5 | 23.1 – 41.6 | 27 | 25.4 ± 6.3 | 17.9 – 33.5 | 0.0001† |
| Waist-hip ratio | 17 | 1.0 ± 0.1 | 0.9 – 1.1 | 27 | 0.9 ± 0.1 | 0.8 – 1.1 | < 0.0001† |
| Time since stroke (years) | 17 | 4.8 ± 2.8 | 1.5 – 11.2 | | | | |
| Stroke type | 17 | | | | | | |
| Lacunar | | 4(24) | | | | | |
| Infarct | | 7(41) | | | | | |
| Hemorrhagic | | 2(12) | | | | | |
| Unknown | | 4(24) | | | | | |
| Stroke location | | | | | | | |
| Cortical | | 6(35) | | | | | |
| Subcortical | | 4(24) | | | | | |
| Cerebellar | | 1(6) | | | | | |
| Unknown | | 6(35) | | | | | |
| Hemisphere affected (right/left) | 17 | 7(41)/10(59) | | | | | |
| National Institutes of Health Stroke Scale | 17 | 1.8 ± 1.1 | 0 – 3 | | | | |
| Chedoke-McMaster Stroke Assessment | 17 | | | | | | |
| Arm | | 4.5 ± 1.7 | 2 – 7 | | | | |
| Hand | | 4.8 ± 1.9 | 1 – 7 | | | | |
| Leg | | 5.6 ± 1.2 | 3 – 7 | | | | |
| Foot | | 4.6 ± 1.8 | 2 – 7 | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Berg Balance Scale | 16 | 50.4 ± 4.2 | 42.0 – 56.0 | 27 | 55.2 ± 1.0 | 52.0 – 56.0 | < 0.0001† |
| 5-Meter Walk Test | 17 | | | 27 | | | |
| Self-paced (m/s) | | 1.0 ± 0.3 | 0.4 – 1.4 | | 1.2 ± 0.2 | 0.7 – 1.6 | 0.02* |
| Fast-paced (m/s) | | 1.3 ± 0.4 | 0.6 – 1.8 | | 1.6 ± 0.3 | 1.0 – 2.1 | 0.003* |
| 6-Minute Walk Test (m) | 16 | 398.4 ± 108.5 | 203.4 – 549.0 | 27 | 520.5 ± 75.7 | 367.2 – 749.5 | 0.0001† |
| Montreal Cognitive Assessment | 17 | 21.8 ± 3.4 | 15 – 28 | 27 | 25.4 ± 3.5 | 13.0 – 30.0 | 0.002* |

Abbreviations: bpm, beats per minute; kg/m$^2$, kilogram/meter$^2$; m, meter; m/s, meter/second; mmHg, millimeters of mercury; SD, standard deviation

* p<005

† p<0.001

Table 2. Test-retest Reliability of Novel Vascular Risk Markers in Participants with Stroke

| Outcome | Side | n | Visit 1 Mean ± SD | Visit 2 Mean ± SD | t-test *P*-value | SEM | ICC | 95% CI | ICC *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| crPWV (m/s) | Affected | 14 | 8.40 ± 1.18 | 7.97 ± 1.22 | 0.85 | 0.87 | 0.48 | -0.01 – 0.79 | 0.03* |
| | Unaffected | 13 | 8.11 ± 1.15 | 7.40 ± 1.43 | 0.57 | 0.95 | 0.46 | -0.03 – 0.79 | 0.03* |
| cfPWV (m/s) | Affected | 13 | 10.56 ± 2.28 | 10.30 ± 2.21 | 0.77 | 0.67 | 0.91 | 0.74 – 0.97 | <0.0001† |
| | Unaffected | 12 | 10.52 ± 2.82 | 10.37 ± 2.76 | 0.73 | 0.68 | 0.94 | 0.81 – 0.98 | <0.0001† |
| ffPWV (m/s) | Affected | 10 | 9.30 ± 2.05 | 8.00 ± 1.58 | 0.41 | 1.70 | 0.12 | -0.38 – 0.64 | 0.34 |
| | Unaffected | 11 | 9.53 ± 1.47 | 9.22 ± 1.82 | 0.48 | 1.61 | 0.04 | -0.62 – 0.62 | 0.45 |
| Compliance ($mm^2$/mmHg) | Affected | 13 | 0.11 ± 0.04 | 0.12 ± 0.03 | 0.38 | 0.03 | 0.03 | -0.57 – 0.57 | 0.46 |
| | Unaffected | 10 | 0.10 ± 0.05 | 0.12 ± 0.06 | 0.94 | 0.04 | 0.46 | -0.22 – 0.83 | 0.09 |
| Distensibility ($mmHg^{-1}$) | Affected | 13 | 0.003 ± 0.001 | 0.004 ± 0.001 | 0.09 | 0.0008 | 0.50 | -0.001 – 0.81 | 0.03* |
| | Unaffected | 10 | 0.003 ± 0.001 | 0.003 ± 0.001 | 0.79 | 0.001 | 0.10 | -0.60 – 0.67 | 0.40 |
| FMD (%) | Affected | 10 | 5.88 ± 3.58 | 4.48 ± 4.40 | 0.59 | 1.94 | 0.76 | 0.32 – 0.94 | 0.002* |
| | Unaffected | 11 | 4.14 ± 4.10 | 5.59 ± 5.46 | 0.30 | 2.61 | 0.70 | 0.25 – 0.91 | 0.004* |

Abbreviations: CI, confidence interval; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; ICC, intraclass correlation coefficient; m/s, meter/second; mm, millimeter; mmHg, millimeters of mercury; SD, standard deviation; SEM, standard error of measurement

* $p<0.05$

† $p<0.0001$

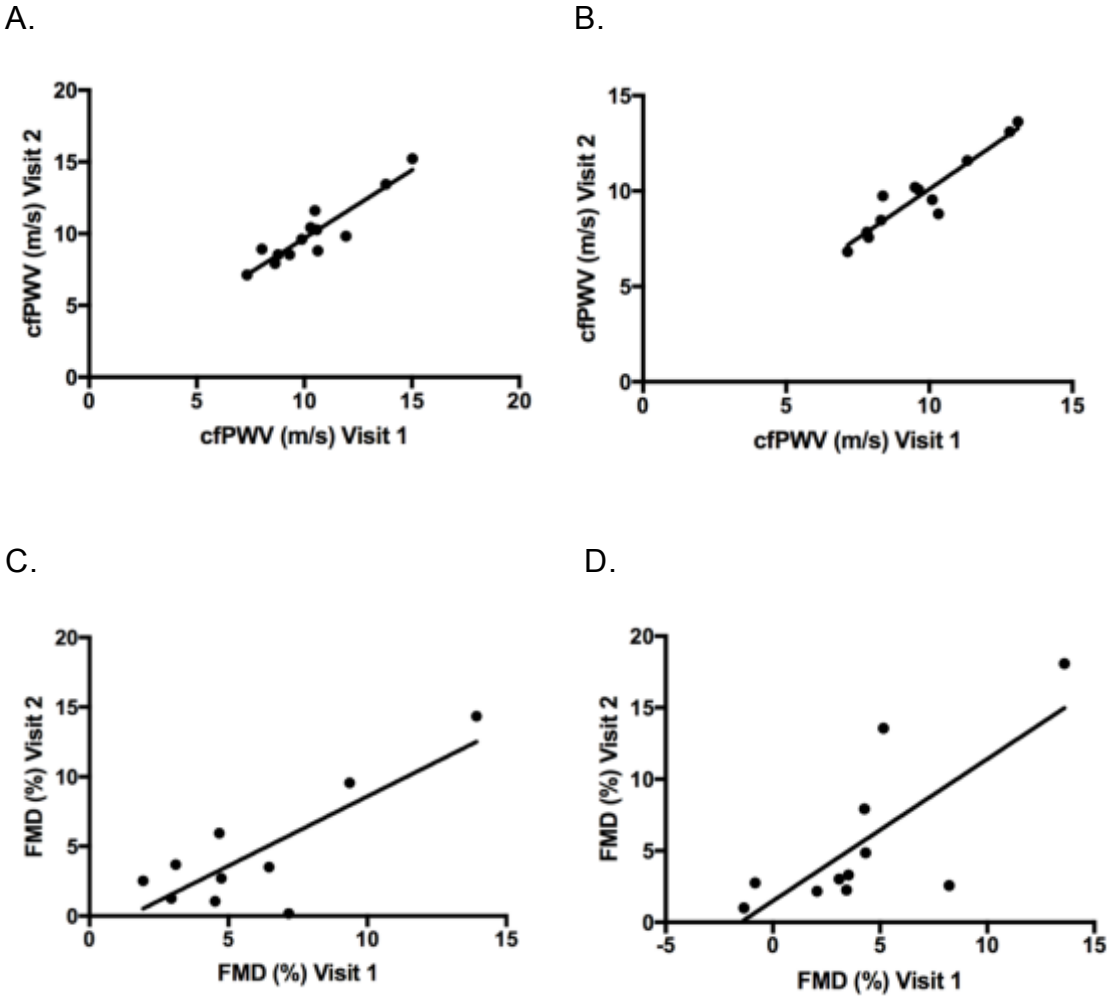
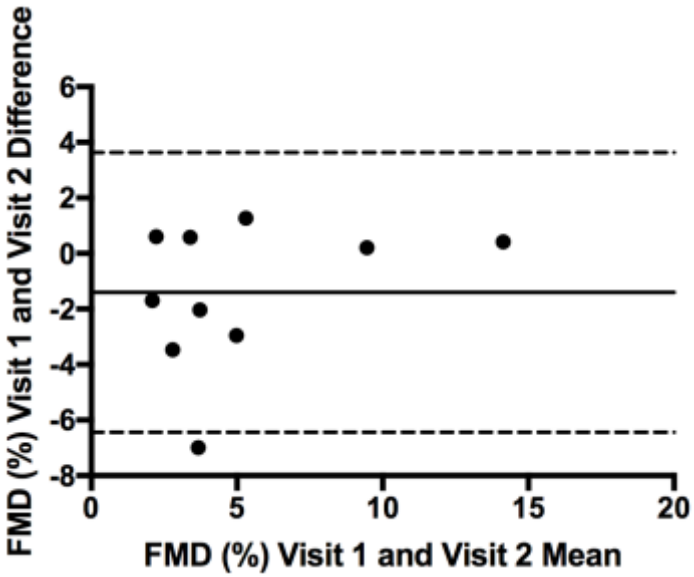



**Figure 9.** Scatter plots of visit 1 plotted against visit 2 for A. affected side carotid-femoral pulse wave velocity (cfPWV), (n=13), B. unaffected side cfPWV (n=12), C. affected side flow-mediated dilation (FMD) (n=10), and D. unaffected side FMD (n=11)

A.




B.

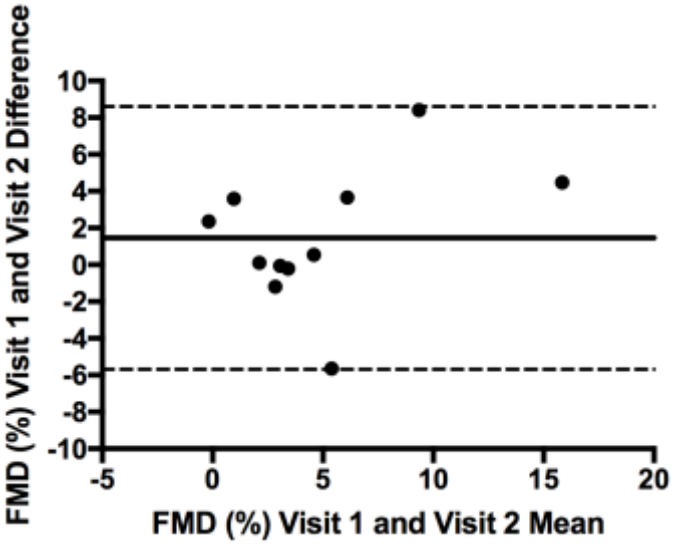



**Figure 10.** Bland-Altman plots of measurements taken on visit 1 and visit 2 for A. flow-mediated dilation (FMD) affected side (n=10) and B. FMD unaffected side (n=11). The solid horizontal line represents the mean of the differences between visit 1 and visit 2, and the dashed horizontal lines represent the 95% confidence intervals.

Table 3. Between-side Differences in Novel Vascular Risk Markers in Participants with Stroke

| Outcome | Visit | n | Affected Side Mean ± SD | Unaffected Side Mean ± SD | Between-group *P*-value |
|---|---|---|---|---|---|
| crPWV (m/s) | 1 | 12 | 8.32 ± 1.26 | 8.24 ± 1.10 | 0.54 |
| | 2 | 15 | 7.78 ± 1.39 | 7.32 ± 1.35 | 0.82 |
| cfPWV (m/s) | 1 | 12 | 10.34 ± 2.29 | 9.70 ± 1.94 | 0.77 |
| | 2 | 13 | 10.02 ± 2.27 | 9.47 ± 2.32 | 0.85 |
| ffPWV (m/s) | 1 | 11 | 9.32 ± 1.94 | 10.00 ± 1.61 | 0.72 |
| | 2 | 11 | 8.21 ± 1.60 | 9.11 ± 1.72 | 0.83 |
| Compliance ($mm^2$/mmHg) | 1 | 9 | 0.12 ± 0.04 | 0.09 ± 0.04 | 0.47 |
| | 2 | 12 | 0.12 ± 0.03 | 0.12 ± 0.06 | 0.12 |
| Distensibility ($mmHg^{-1}$) | 1 | 9 | 0.003 ± 0.0006 | 0.003 ± 0.0008 | 0.85 |
| | 2 | 12 | 0.004 ± 0.001 | 0.003 ± 0.001 | 0.28 |
| FMD (%) | 1 | 12 | 5.72 ± 3.74 | 4.81 ± 4.55 | 0.70 |
| | 2 | 11 | 3.89 ± 4.61 | 5.65 ± 5.43 | 0.56 |
| NTG (%) | 1 | 7 | 15.76 ± 3.22 | 16.47 ± 8.61 | 0.09 |
| | 2 | 12 | 16.62 ± 5.15 | 15.44 ± 8.59 | 0.25 |

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow mediated dilation; m/s, meter/second; mm, millimeter; mmHg, millimeters of mercury; SD, standard deviation

Table 4. Between-side Differences in PWV in Participants without Stroke

| Outcome | n | Dominant Side Mean ± SD | Non-Dominant Side Mean ± SD | Between-group *P*-value |
|---|---|---|---|---|
| crPWV (m/s) | 28 | 7.42 ± 1.47 | 7.03 ± 1.44 | 0.95 |
| cfPWV (m/s) | 27 | 10.74 ± 2.85 | 10.24 ± 2.40 | 0.48 |
| ffPWV (m/s) | 27 | 9.35 ± 2.06 | 8.99 ± 1.51 | 0.19 |

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; m/s, meter/second; SD, standard deviation

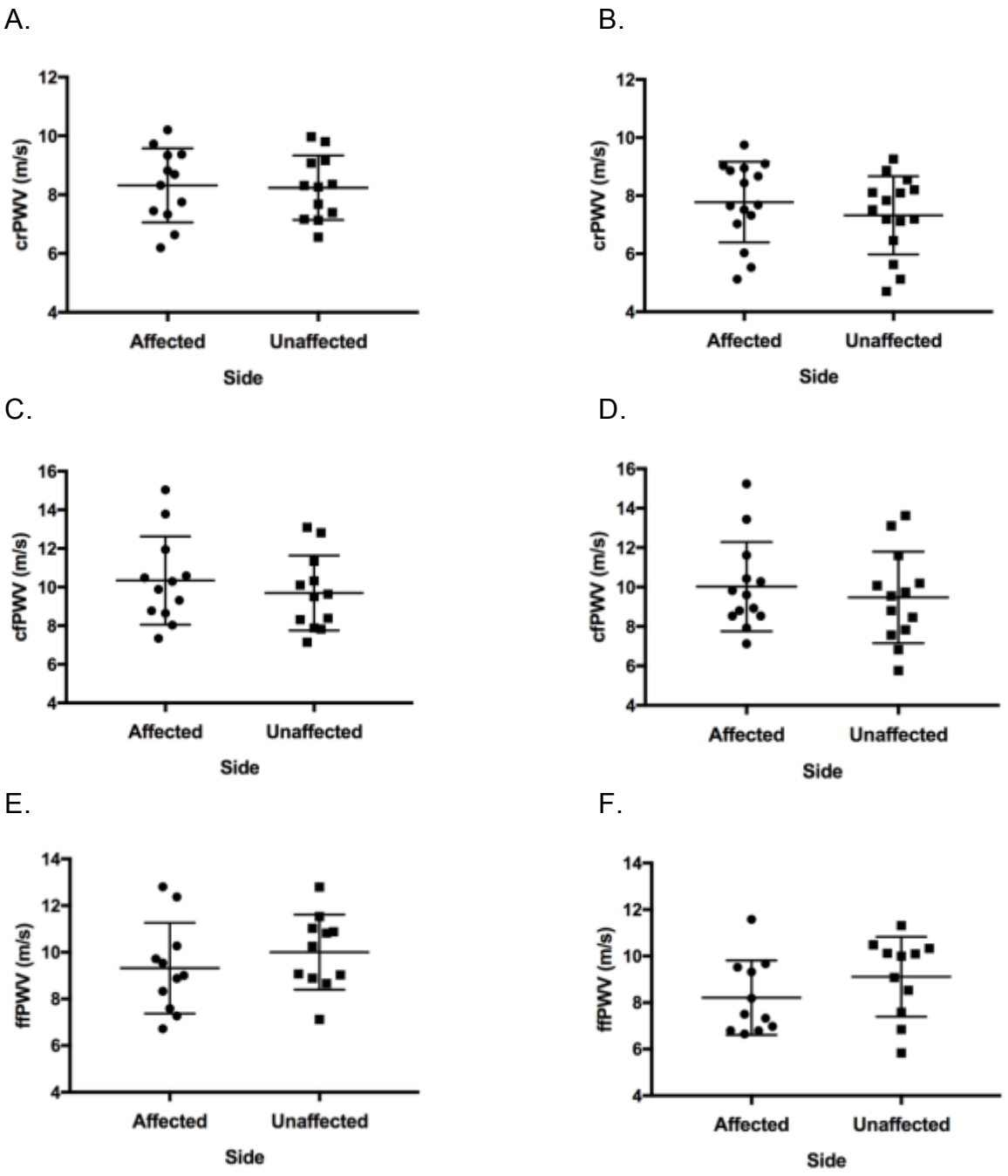



**Figure 11.** Between-side differences in A. carotid-radial pulse wave velocity (crPWV) visit 1 (n=12), B. crPWV visit 2 (n=15), C. carotid-femoral pulse wave velocity (cfPWV) visit 1 (n=12), D. cfPWV visit 2 (n=13), E. femoral-foot pulse wave velocity (ffPWV) visit 1 (n=11), and F. ffPWV visit 2 (n=11) in individuals with stroke. Horizontal line represents mean and error bars represent standard deviation.

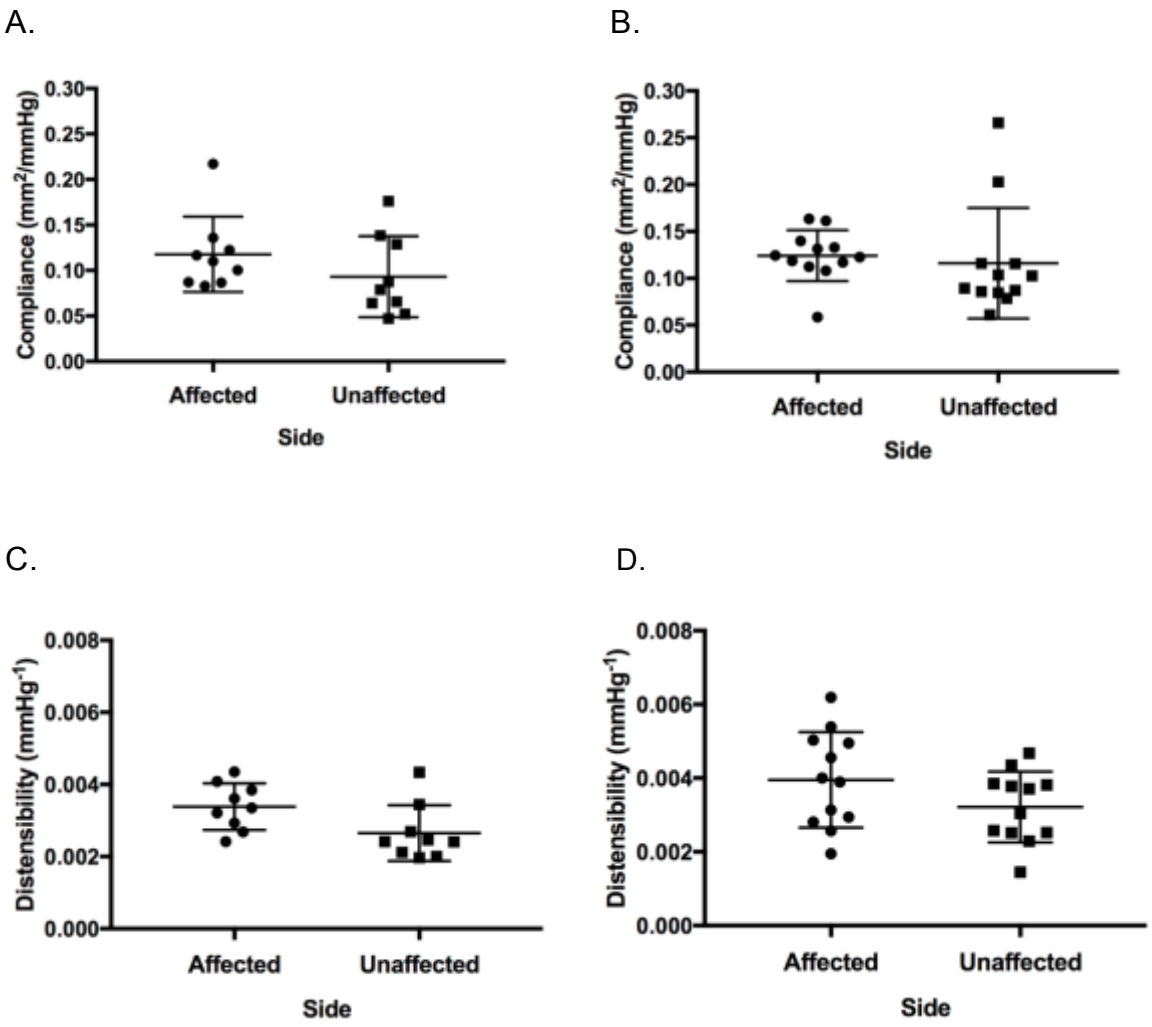



**Figure 12.** Between-side differences in A. compliance visit 1 (n=9), B. compliance visit 2 (n=12), C. distensibility visit 1 (n=9), and D. distensibility visit 2 (n=12) in individuals with stroke. Horizontal line represents mean and error bars represent standard deviation.

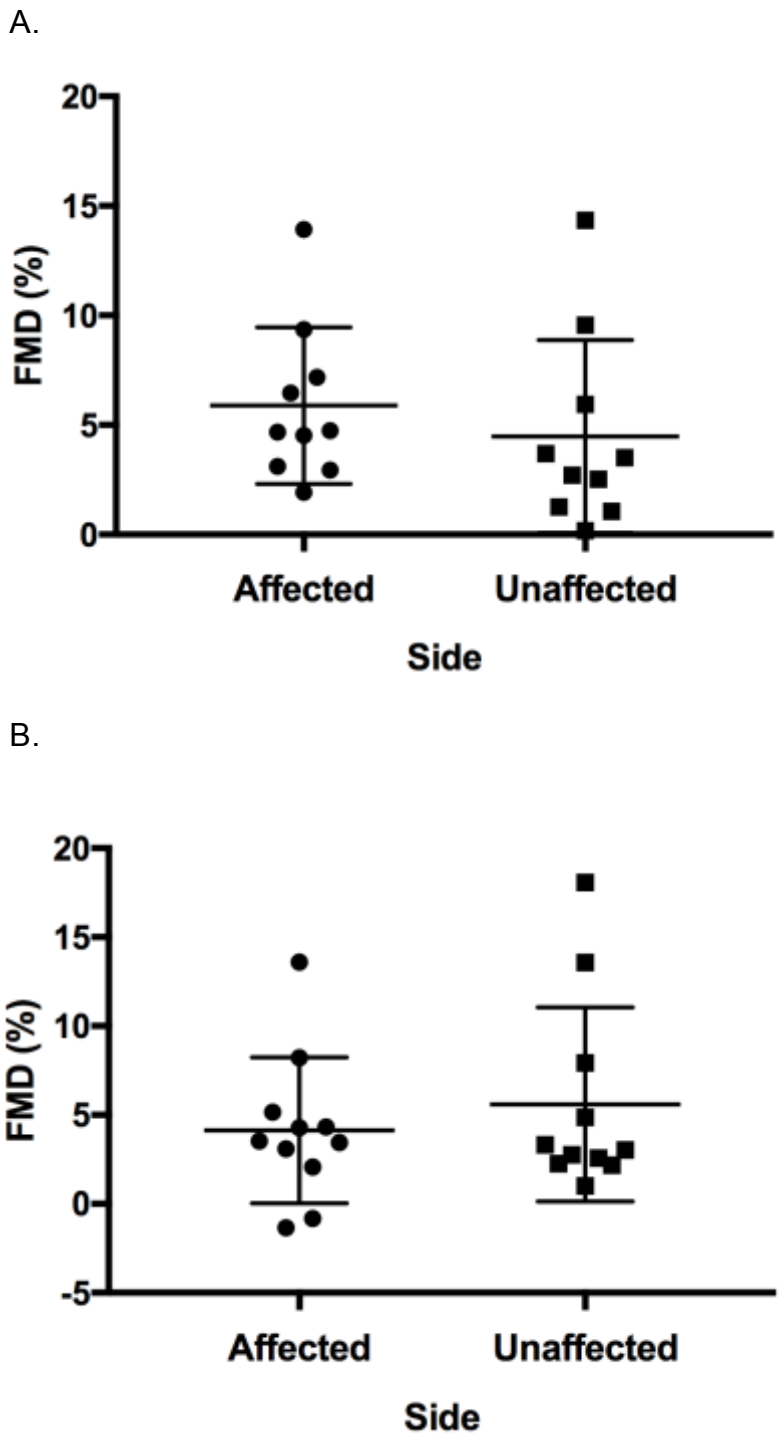



**Figure 13.** Between-side differences in A. flow-mediated dilation (FMD) visit 1, and B. FMD visit 2 in individuals with stroke (n=12). Horizontal line represents mean and error bars represent standard deviation.

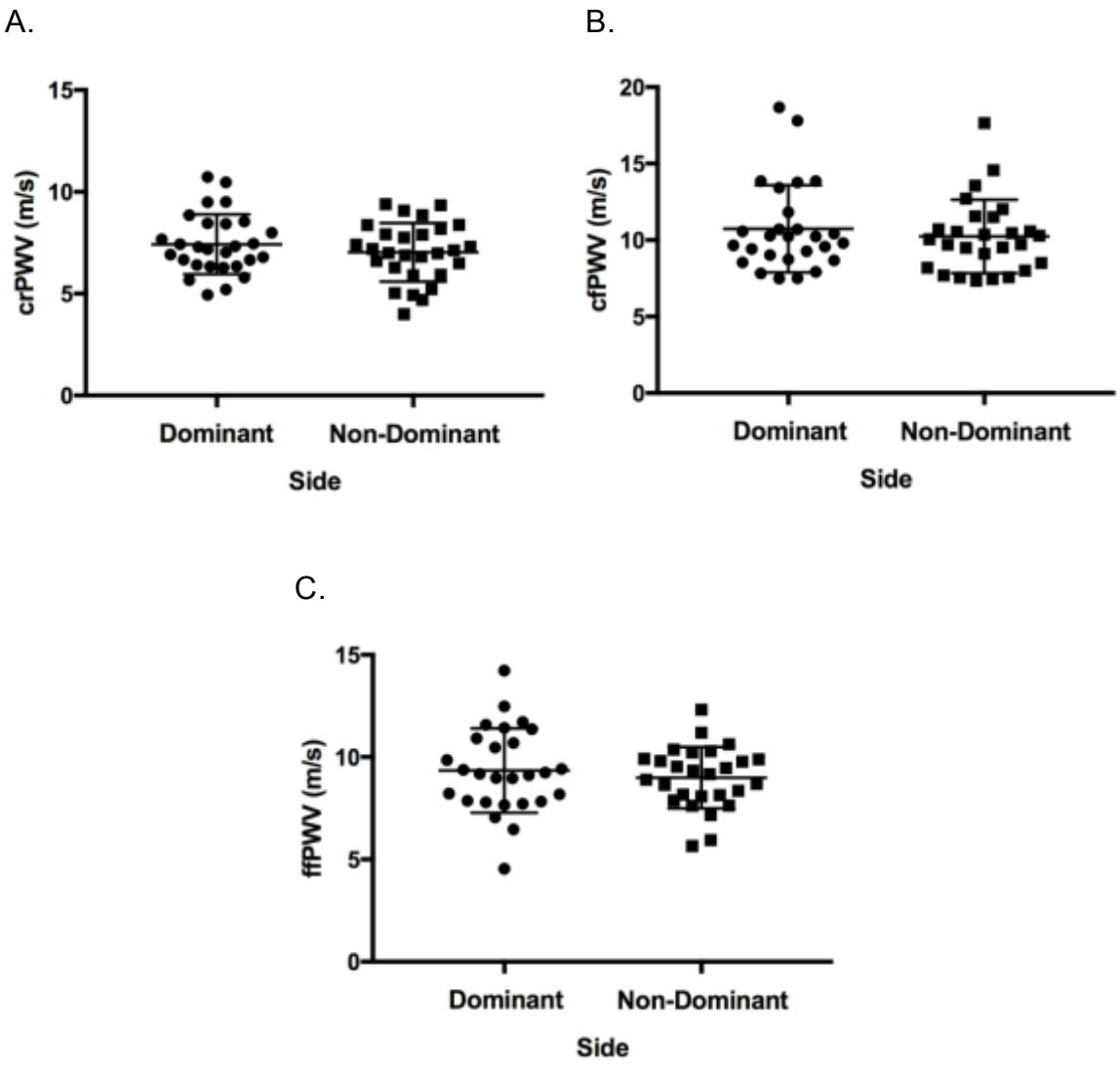



**Figure 14.** Between-side differences in A. carotid-radial pulse wave velocity (crPWV) (n=28), B. carotid-femoral pulse wave velocity (cfPWV) (n=27), and C. femoral-foot pulse wave velocity (ffPWV) (n=27) in individuals without stroke. Horizontal line represents mean and error bars represent standard deviation

the most well-established and commonly used non-invasive measure of endothelial function (Corretti et al., 2002). In the current study, substantial to almost perfect test-retest reliability was observed with these key measures of vascular function, supporting their use in the post-stroke population.
endothelial

Reasons for lower test-retest reliability in crPWV and ffPWV may in part be due to post-stroke impairments, such as increased muscular tone in the upper and lower extremities, which may have prevented full limb extension and thus hindered accurate, repeatable and standardized distance measurements from the carotid to radial arteries and femoral to dorsalis pedis arteries. Given the dependence of compliance and distensibility on pulse pressure, the low to moderate test-retest reliability of these measures may be partially attributed to the variability in blood pressure due to various autonomic, mechanical, myogenic and environmental factors (Floras et al., 1988; Korpelainen, Sotaniemi, Makikallio, Huikuri, & Myllyla, 1999).

The almost perfect test-retest reliability of cfPWV in the current study aligns with previous reports in healthy individuals (Bode 2012; Meyer et al., 2015) and in individuals with spinal cord injury (Currie et al., 2014; Miyatani et al., 2012). Similarly, moderate test-retest reliability of crPWV is consistent with a previous report of individuals with spinal cord injury (Miyatani et al., 2012). Reasons why test-retest reliability of upper extremity PWV was lower than central or lower extremity PWV in this past study were unknown, but was speculated to be in part

due to measurement error, given that results showed no variations in BP or RHR measures between the two days that would affect variations in the measure (Miyatani et al., 2012).

In contrast, test-retest reliability of ffPWV observed in the current study was lower than previous findings from a study focused on individuals with spinal cord injury (Miyatani et al., 2012). These inconsistent results may be partially attributed to differences in lower extremity impairment between studies. All participants in the study by Miyatani et al. (2012) presented with either paraplegia or tetraplegia (American Spinal Injury Association Impairment Scale classification A-D) but in the current study, all participants were ambulatory (able to walk 10 meters independently with or without an assistive device), and only few of our participants had lower extremity impairments (n=3 [17%] individuals with CMSA leg score ≤ 4). Previous findings suggest that physical activity 24 hours prior to a testing session may alter resting measures of PWV (Perdomo et al., 2016), thus it is possible that the reduced physical function of individuals with tetra- and paraplegia (Miyatani et al., 2012) may have resulted in lower variability of physical activity between visits, resulting in higher agreement.

The low to moderate test-retest reliability of carotid artery compliance and distensibility was lower than that observed in a previous report of older, healthy adults (Caviezel et al., 2013). Mixed model analyses in this previous study showed that less than 2.9% of the measurement variation was explained by the assessor or rater, and that the remaining variability of more than 74–81% was

explained by the subject (speculated to be attributed in part to day-to-day variations in blood pressure) (Caviezel et al., 2013). Given the dependence of compliance and distensibility on pulse pressure, the low to moderate test-retest reliability of these measures may be partially attributed to blood pressure variability, which may be due to a combination of various autonomic, mechanical, myogenic and environmental factors (Floras et al., 1988; Korpelainen et al., 1999). Given that blood pressure variability is associated with increased CV risk, (Korpelainen et al., 1999, Manning et al., 2014; Pierdomenico et al., 2006) compliance may not be an appropriate risk marker in the post-stroke population. However, future work should examine test-retest reliability of compliance in individuals with stroke with larger samples.

The substantial test-retest reliability of brachial artery FMD in the current study is lower than that previously observed in young, healthy adults (Meirelles et al., 2007; Welsch et al., 2002), but it is important to note that the test-retest reliability of FMD increased (ICC >0.88) following the removal of outlier values observed in Bland-Altman plots. Nonetheless, given that FMD is the most widely accepted non-invasive method used to quantify endothelial function, our results have positive implications for understanding vascular function in the post-stroke population. Further research is warranted to investigate test-retest reliability in larger samples of individuals with a greater range of functional abilities post-stroke.

Our findings reinforce that arterial stiffness and endothelial function, quantified using cfPWV and brachial artery FMD, respectively, may be used as early and sensitive markers of vascular risk progression, which may have important implications in establishing effective interventions to lower risk of future CV events.

*Between-side Differences*

There were no differences between the affected and unaffected sides in any vascular measures in individuals with stroke. These results were consistent with those observed in PWV in the participants without stroke, but in contrast to our *a priori* hypothesis.

Post stroke impairments, such as hemiparesis and spasticity, may lead to limited function and mobility of the affected limb. Thus, positioning for assessments of vascular function, particularly PWV and FMD, may be cumbersome and difficult to maintain for an extended period of time, and this may potentially affect the ability to obtain accurate measurements from the affected limb. Furthermore, limited function and mobility may contribute to unique unilateral adaptations in the affected limb, such as reductions in lean tissue mass and changes in fibre type distribution (Ivey et al., 2004). Previous studies have also found between-side differences in arterial structure in individuals with stroke, such as reductions in resting arterial diameter, resting arterial blood flow, and increased arterial wall thickness in the affected limb in individuals with stroke

(Billinger et al., 2009a; Billinger & Kluding, 2009b; Billinger et al., 2012; Ivey et al., 2004; Ivey et al., 2010). However, to our knowledge no studies have assessed between-side differences in arterial stiffness and only few have assessed between-side differences in endothelial function in the post-stroke population (Billinger et al., 2012; Ivey et al., 2004; Ivey et al., 2010).

Previous studies in individuals with stroke have inferred endothelial function by assessing changes in femoral artery blood flow following an increase in shear stress, and found greater reductions in reactive hyperaemic blood flow in the affected compared to the unaffected leg (Ivey et al., 2004, Ivey et al., 2010). Only one previous study (Billinger et al., 2012) has compared endothelial function between affected and unaffected limbs in individuals with stroke using FMD, the most well-established and commonly used measure of endothelial function (Corretti et al., 2002). In contrast to the findings from the current study, Billinger et al. (2012) reported reduced baseline brachial artery diameter and brachial artery FMD in the affected compared to the unaffected limb in individuals with stroke (Billinger et al., 2012). Inconsistencies in these results may be, in part, due to differences in time post-stroke (68.6 ± 40.1 days [Billinger et al., 2012] versus 4.5 ± 2.8 years post-stroke) as it is possible that in the early stages post-stroke, individuals may initially use the unaffected limb more often to compensate for the limited function in the affected limb, potentially explaining the greater between-side range in FMD values. There may be less disparity between sides in terms of limb use with time and continued rehabilitation, leading to reductions in between-

side differences in FMD.

Moreover, participants in the current study were living independently in the community and may thus have higher overall levels of physical activity. Indeed, compared to this previous report (Billinger et al., 2012) participants in the current study demonstrated higher distances walked on the 6MWT (304.1 ± 167.5 m versus 398.4 ± 108.5 m), a measure of walking ability that is commonly used as an indicator of physical activity following stroke (Pang et al., 2006; Saunders et al., 2016). Endothelial function can be improved with exercise training after stroke (Billinger et al., 2012; Ivey et al., 2010) and it may thus be possible that the higher FMD values observed in the current study are due to increased levels of activity seen in community-dwelling participants with stroke.

To our knowledge, this study was the first to investigate between-side differences in novel vascular risk markers in the chronic post-stroke population. Our findings suggest that between-side vascular measures are similar in individuals in the chronic stage of stroke recovery with mild to moderate impairment when measured across sides. Thus, it may be more feasible to assess vascular function on the unaffected side. This work is an important step in gaining insight into the feasibility of measurement in individuals with stroke, and provides background for future studies investigating vascular health after stroke.

*Limitations*

This study was limited by small sample size and the exclusion of participants

with severe stroke, which limits the generalizability of results. However, the subgroup included in this study is important to explore as it is reflective of the types of individuals who are living in the community post-stroke. This study is also limited by a portion of missing data (18% missing from entire cohort, 8% missing from older adult group, 25% missing from stroke group), although of the data missing from the stroke group, only 12% was due to participant-related factors, such as elevated resting blood pressure or unwillingness to participate in the assessment, indicating that a majority of the post-stroke may be able to well-tolerate these assessments. In addition, the software used in arterial diameter analysis is semi-automated but allows for individual manipulation, which may lead to observer bias. Appropriate steps were taken to minimize this risk of bias, such as adherence to standardized operating procedures, use of a consistent rater and analysis computer, and performance of quality checks on all outcomes.

## 2.6 Conclusion

Substantial to almost perfect reliability of cfPWV and FMD was observed among individuals with chronic stroke, providing support for the use of these measures in the post-stroke population for the assessment of vascular risk progression. Moreover, given no differences between affected and unaffected sides, it may be more feasible to assess these measures on the unaffected limbs in individuals with stroke. Future studies should continue to examine vascular risk

progression in larger samples and include individuals with severe post-stroke impairment.

# Chapter 3

## STUDY 2 – COMPARISON OF NOVEL VASCULAR RISK MARKERS ACROSS A PROPOSED CONTINUUM OF INCREASING VASCULAR RISK

### 3.1 Background

Following stroke, the risk of secondary cerebrovascular events is high. Five and 10 years after the first events, recurrence rates are 26% and 40%, respectively (Mohan et al., 2011) Despite healthcare efforts to manage CV risk factors in individuals with stroke, these strategies are not always successful. Previous research has reported that at least one sub-optimally controlled risk factor remained present in 99% of individuals with stroke, and two or more inadequately controlled risk factors were present in 91% (Kopunek et al., 2007). Reasons for high rates of sub-optimal risk factor management are complex but may be due to post-stroke physical impairments that result in deconditioning and predispose a sedentary lifestyle (Field et al., 2013).

Traditional cardiovascular risk factors, such as hypertension, dyslipidemia and diabetes, explain approximately 90% of the risk of stroke and other cardiovascular disease (CVD) in a population (Figure 2) (O'Donnell et al., 2010; Yusuf et al., 2004). Novel risk markers, such as **arterial stiffness** and **endothelial function**, may provide insight into the observed CVD risk that is not explained by the traditional risk factors alone. Arterial stiffness and endothelial dysfunction are important markers in the development of atherosclerosis, an arterial disease process that typically precedes CVD (Duprez & Cohn, 2007; Verma & Anderson, 2002). Consequently, these novel risk markers are associated with stroke and other CVD, such as CHD and heart failure (Blum et

al., 2012; Mattace-Raso et al., 2006; Ras et al., 2013), and may also provide earlier and more sensitive detection of future CVD risk.

*Arterial Stiffness.* Increased arterial stiffness alters the transmission velocity of pulse pressure waves throughout the body, ultimately resulting in increased left ventricular load and reduced coronary perfusion pressure, thereby predisposing the heart to increased workload and ischemia (Mac-Way et al., 2011; Safar et al., 2003). Furthermore, increased arterial stiffness exposes the arterioles in the brain to highly pulsatile pressure and flow, which may contribute to the pathogenesis of cerebral small vessel disease (SVD), with consequences such as lacunar infarcts, white matter lesions, hemorrhages and microbleeds (Cecelja & Chowienczyk, 2012).

*Endothelial Function.* The vascular endothelium regulates vascular tone through the release of factors in response to various stimuli (Paniagua et al., 2001). Endothelial dysfunction is characterized by an impairment of the endothelium to regulate vascular tone and blood flow due to a reduction of the bioavailability of vasodilators, predominantly nitric oxide (NO), or an increase in endothelial contracting factors. Endothelial dysfunction can result in endothelial cell damage and the promotion of atherosclerotic mechanisms, such as the upregulation of adhesion molecules and enhanced low-density lipoprotein oxidation (Verma & Anderson, 2002).

Previous research has suggested an inverse association between arterial stiffness and aerobic fitness (Tang et al., 2014a) as well as a positive benefit of

enhanced physical activity on reducing arterial stiffness in individuals with stroke (Lee et al., 2015; Woolley et al., 2015). In addition, physical activity and exercise appears to be effective in improving endothelial function after stroke (Billinger et al., 2012; Ivey et al., 2010). Measures of physical function, such as walking ability and balance, are commonly used as indicators of physical activity and fitness in people with stroke (Pang et al., 2006; Saunders et al., 2016), as limitations in physical function have been found to be associated with reduced cardiorespiratory fitness, muscle strength and muscle power (Flansbjer et al., 2012; Patterson et al., 2010; Saunders et al., 2008). However, the relationships between arterial stiffness, endothelial function and physical function have not yet been established in this population. Given that individuals with stroke are at high risk of recurrent events, examining the association between physical function and novel markers of CVD progression may provide insight into the role that physical function may play in mediating these risk markers.

Cognitive function may also be an important clinical marker of vascular disease progression. It is well-established that individuals who have experienced overt stroke commonly present with cognitive impairment (Levine et al, 2015; Rajan et al., 2014). Earlier research has also suggested that covert strokes, characterized by cerebral infarcts that fail to cause symptoms that are clinically recognized as stroke, may initially manifest as declines in cognitive function (Longstreth et al., 1998; Vermeer et al., 2007). These covert strokes share similar risk factors with overt stroke, and their presence more than doubles the risk of

subsequent stroke (Vermeer et al., 2007).

Covert stroke is a common manifestation of cerebral SVD, whereby pathological processes affect the cerebral microvasculature and result in vascular narrowing, endothelial damage, white matter lesions, and cerebral microbleeds (Pantoni, 2010). Indeed, earlier research has found associations between increased arterial stiffness, impaired endothelial function and incidence of cerebral SVD. A large scale, prospective study conducted by Ding et al., (2015) found that local increases in common carotid arterial stiffness were associated with incident cerebral microbleeds in older adults (n=2512; RR 1.04, 95% CI 1.05–1.24, $P<0.05$) (Ding et al., 2015). In addition, cerebral white matter hyperintensities were found to be positively associated with aortic arterial stiffness (n=208; OR 1.58, 06%CI 1.04-2.40, $P<0.05$) in older adults (Gustavsson, 2015), and with endothelial function in older adults with cardiovascular disease (n=25; r=0.63, p<0.01) (Hoth et al., 2007).

Although these earlier findings suggest an association between novel CV risk markers and cerebral SVD, a direct association between these novel risk markers and clinical assessments of cognitive function is less established. Earlier studies have found associations between aortic arterial stiffness and cognitive impairment in older adults (n=1820; $r^2=0.19$, p<0.02) (Cooper et al., 2015) and individuals with stroke (n=61; r=–0.45, p<0.01) (Lee et al., 2014), and between endothelial function and cognitive impairment in older adults (n=602; $r^2=0.73$, p<0.0001) (Vendemiale et al., 2013). However, differences in the degree of

progression of these novel risk markers have not previously been compared across a continuum of individuals with increasing vascular risk.

Given the association between cognitive impairment and both covert and overt stroke (Levine et al, 2015; Longstreth et al., 1998; Rajan et al., 2014; Vermeer et al., 2007), it may be suggested that the presence and severity of novel CV risk markers may increase across a proposed continuum of increasing vascular risk that is assessed through evaluations of the presence of cognitive impairment and stroke. Thus, a continuum of increasing cardiovascular (CV) risk may be created with subgroups of older adults 1) without cognitive impairment, 2) with cognitive impairment, and 3) with stroke (Figure 1). A comparison of novel risk markers across such a continuum may aid in our understanding of what is currently known regarding the progression of vascular disease and may also work to establish cognitive function as a clinical marker of vascular disease progression.

### 3.2 Objectives and Hypotheses

*Objective 1. To investigate differences in arterial stiffness and endothelial function across three subgroups in a proposed continuum of increasing vascular risk: older adults without cognitive impairment, with cognitive impairment and with stroke.* We hypothesized that arterial stiffness would be lowest and endothelial function would be least impaired in older adults without cognitive impairment and that arterial stiffness would be highest and endothelial function would be most

impaired in older adults with stroke.

*Objective 2. To investigate relationships between novel vascular markers (arterial stiffness and endothelial function) and physical function.* It was hypothesized that physical function would be negatively correlated with arterial stiffness and positively correlated with endothelial function.

## 3.3 Methodology

### 3.3.1 Study Design

This was a cohort study. This protocol was approved by the Hamilton Integrated Research Ethics Board (HIREB # 13-348). Informed written consent was obtained from all participants (participant information and consent displayed in Appendix 2).

### 3.3.2 Participants

Community-dwelling adults with or without stroke were recruited for this study and screened to fit the following criteria:

*Inclusion Criteria*

- 50-80 years of age
- ability to walk 10 meters independently with or without an assistive device
- for individuals with stroke, ≥ 12 months post stroke

*Exclusion Criteria*

- for individuals with stroke, stroke of non-cardiac origin

- significant cardiovascular, musculoskeletal or other neurological conditions
- significant cognitive or communication impairment or behavioural issues affecting ability to understand instructions for testing
- for individuals with stroke, stroke of non-cardiac origin

### 3.3.3 Assessments

Data were collected in the temperature-controlled Vascular Dynamics Laboratory at McMaster University. All participants were asked to abstain from food or drink for 4 hours, smoking, caffeine and alcohol for 12 hours and exercise for 24 hours before each testing visit.

*Participant Characteristics*

Participant characteristics were collected, including age, sex, body mass index (BMI), waist-hip ratio (WHR), resting SBP, DBP, and heart rate (RHR). Resting supine blood pressure was measured at the brachial artery from the dominant arm in participants without stroke and both affected and unaffected arms in participants with stroke (DINAMAP, Critikon Inc). Two readings were taken and averaged, but if values differed >5 mmHg, an additional 2 readings were taken and all 4 readings were averaged (Pickering et al., 2005). RHR was measured continuously using single-lead electrocardiography.

Self- and fast-paced walking speed were assessed using the 5 Meter Walk Test (5mWT), which provides a measure of the speed at which an individual is able to walk 5 meters, with or without an assistive device (Salbach et al., 2001). Walking endurance was assessed using the 6-Minute Walk Test (6MWT), which provides a measure of the distance walked over 6 minutes, with or without an assistive device and with rest breaks permitted (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002).

Balance ability was assessed using the 14-item Berg Balance Scale (BBS) (Berg Balance Scale, 2016; Berg et al., 1992). Items on this scale include functional balance activities, such as standing with eyes closed, retrieving an object from the floor and standing on one foot. Each item was scored on a 5-point scale (0-4), with the highest achievable score being 56 and higher scores representing better balance ability.

In addition, details of the stroke event were collected from participants with stroke (date of stroke, stroke location, type of stroke, stroke severity using the National Institutes of Health Stroke Scale (NIHSS) (NIH Stroke Scale, 2003) and limb impairment using the Chedoke-McMaster Stroke Assessment (CMSA) impairment inventory (Miller et al., 2008). The NIHSS is an 11-item assessment used to quantify stroke severity, with each item scoring between 0-4 and higher scores indicating greater severity (NIH Stroke Scale, 2003). The CMSA impairment inventory assesses motor impairment of the affected-side limbs (arm,

hand, leg and foot), scoring each on a seven-point scale, with lower scores indicating greater motor impairment (Miller et al., 2008).

*Cognitive Function*

Cognitive function was assessed using the 16-item Montreal Cognitive Assessment (MoCA) (McLennan et al., 2011), which assesses multiple cognitive domains including visual spatial and executive functions, naming, memory, attention, language, abstraction and orientation. The highest achievable score is 30, with higher scores representing better cognitive function and a score of <26 indicating mild cognitive impairment (Nasreddine, et al., 2005). MoCA scores were thus used to divide participants without stroke into two groups: those without cognitive impairment (score of ≥26) and those with cognitive impairment (score of <26). This measure has been shown to be valid and reliable in an older adult population for which it was developed, as well as a cardiovascular population (McLennan et al., 2011). The MoCA is displayed in Appendix 3.

*Novel Vascular Measures*

*Arterial Stiffness*

Arterial stiffness was evaluated using several of non-invasive measures. Pulse wave velocity (PWV), a systemic measure of arterial stiffness, was measured according to the most recent guidelines (Van Botdel et al., 2012). Simultaneous measurements of arterial pressure waveforms were obtained by

two trained assessors at two points along the arterial tree using applanation tonometry (Figure 5) (Model SPT-301; Millar Instruments Inc., Houston, TX, USA). A surface tape measure was used to assess distance between the two measurement sites. Central, or carotid-femoral, PWV (cfPWV), upper extremity, or carotid-radial, PWV (crPWV) and lower extremity, or femoral-foot, PWV (ffPWV) were assessed between the carotid and femoral, carotid and radial, and femoral and dorsalis pedis arteries, respectively.

PWV was calculated as:

$$\frac{d}{\Delta t}$$

where d is the distance between the two points along the arterial tree (meters [m]) and $\Delta t$ is the transit time of the pulse wave between these two points (seconds [s]) (Figure 6). Greater PWV values indicated increased arterial stiffness, and in turn, poorer vascular health. In accordance with recent PWV guidelines (Van Bortel et al., 2012), 80% of the direct measured distance between the carotid and femoral arteries was used for the calculation of cfPWV. Since the direct distance between the carotid and femoral arteries was not measured for the majority of participants with stroke (13/17 [76%] participants), a validated equation was used to convert subtracted distances (sternal notch to femoral artery minus sternal notch to carotid artery) to the direct distance (Vermeersch et al., 2009), and 80% of this calculated direct distance was used to

calculate cfPWV. The full distance was used between the other sites for calculation of crPWV and ffPWV.

In addition to PWV, common carotid artery compliance and distensibility, local measures of carotid artery elasticity, were used to assess local artery stiffness. Compliance and distensibility, which refer to the absolute and relative changes in artery diameter with pressure, respectfully, were measured using applanation tonometry and B-mode ultrasound (System FiVe; GE Medical Systems, Horten, Norway) to collect simultaneous carotid artery pulse pressures and images for 10 consecutive cardiac cycles (Currie et al., 2010).
Compliance was calculated as:

$$\frac{d_{max} - d_{min}}{PP}$$

Distensibility is calculated as:

$$\frac{d_{max} - d_{min}}{d_{min} \times PP}$$

where $d_{max}$ is the maximum lumen diameter of the artery (millimeters [mm]), $d_{min}$ is the minimum lumen diameter of the artery (mm) and PP is the pulse pressure (millimeters of mercury [mmHg]) (Currie et al., 2010).

Pulse pressure data were collected and analyzed offline using Powerlab acquisition equipment (Labchart 7; ADInstruments IC, Colorado Springs, CO,

USA). Carotid artery diameter was assessed from ultrasound images using semi-automated edge-detection software (Artery Measurement System; Image and Data Analysis, Gothenburg, Sweden).

*Endothelial Function*

Endothelial function (endothelial-dependent vasodilation) was assessed using brachial artery flow-mediated dilation (FMD) (Corretti et al., 2002). Participants were in the supine position with a pneumatic cuff positioned on the forearm distal to the antecubital fossa. The pneumatic cuff was inflated to suprasystolic pressure (200 mmHg) using a rapid cuff inflator (model E20 and AG101; Hokanson, Bellvue, WA) to occlude blood flow in forearm for 5 minutes. B-mode ultrasound was used to obtain images of the brachial artery for 30 seconds pre-occlusion to assess baseline arterial diameter and for 3 minutes post-deflation to assess maximum arterial diameter (Corretti et al., 2002). Figure 7 shows a schematic representation of the FMD assessment.

FMD (%) was calculated as

$$\frac{d_{max} - d_{min}}{d_{min}} \times 100$$

where $d_{max}$ is the maximum diameter of the artery (mm) and $d_{min}$ is the minimum diameter of the artery (mm). Lower FMD values indicate greater endothelial dysfunction, and thus poorer vascular health (Corretti et al., 2002).

In addition to FMD, endothelial-independent vasodilation was quantified in order to assess maximal arterial dilation capacity in response to agonists acting on vascular smooth muscle. At least 10 minutes following FMD assessment, a single dose of 0.4 mg nitroglycerin (NTG), a vasodilator that acts directly on smooth muscle cells to release NO (Corretti et al., 2002), was administered sublingually. B-mode ultrasound images of the brachial artery were obtained prior to NTG administration for 30 seconds in order to assess baseline arterial diameter, and for 30 seconds each minute thereafter for 10 minutes to assess maximum arterial diameter. NTG (%) was calculated using the same equation as FMD (%), where $d_{max}$ is the maximum diameter of the artery achieved during the 10 minutes following NTG administration (mm) and $d_{min}$ is the baseline diameter of the artery prior to NTG administration (mm).

For all ultrasound image analysis, the R-spike of the simultaneous ECG trace was used to determine end-diastolic image frames. These frames were extracted and stacked using commercially available software (Sante DICOM Editor, Version 3.0.12; Santesoft, Athens, Greece) to create an image file (Digital Imaging and Communications in Medicine [DICOM] file). Brachial artery diameter was then assessed from this file of end-diastolic ultrasound images using semi-automated edge-detection software (Artery Measurement System; Image and Data Analysis, Gothenburg, Sweden).

All vascular measures were assessed on the dominant side in participants without stroke. As part of a separate study examining day-day reliability and

between-side differences in vascular measures, participants with stroke attended two visits with bilateral vascular assessments. Our previous work demonstrated no between-visit or between-side differences in all vascular measures, and substantial to almost perfect test-retest reliability for cfPWV (ICC>0.88) and FMD (Chapter 2: Measurement Properties of Novel Vascular Risk Markers). For the purposes of this study, from participants with stroke, cfPWV and FMD assessments from the first visit and unaffected side were considered, and all other vascular assessments were considered from both visits and unaffected side.

### 3.3.4 Statistical Analyses

Statistical analysis was performed using Statistical Package for the Social Sciences software (version 20.0; IBM Corporation, Armonk, NY, USA). Descriptive statistics were performed on all measures (mean ± SD for continuous variables, n (%) for non-continuous variables). One-way analyses of variance were used to compare arterial stiffness and endothelial function across three subgroups (older adults without cognitive impairment, with cognitive impairment, and with stroke). Bivariate correlation analyses were used to determine relationships between novel risk markers (arterial stiffness and endothelial function) and physical and cognitive function in the entire cohort. Little's missing completely at random (MCAR) test was performed to determine if data was missing at random. Item mean substitution was used to impute missing data, for

which the imputed value was derived from the mean of the non-missing items for the case (Hawthorne & Elliott, 2005). Statistical significance was set at $p<0.05$.

## 3.4 Results

*Participant Characteristics*

Fifty-one participants (17 without cognitive impairment, 17 with cognitive impairment, and 17 with stroke) were included in this study. Participant characteristics are displayed in Table 5. The groups differed in a number of descriptive variables. As expected, participants without cognitive impairment had higher MoCA scores than those with stroke and cognitive impairment. There were also differences with respect to age, sex, health (BMI, WHR, antihypertensive medication use), and mobility (BBS, self- and fast-paced 5mWT, 6MWT).

*Missing Data*

Due to a number of different factors, 15% of the total data was missing (25% and 2% from participants with and without stroke, respectfully). Data was missing at random (Little's MCAR $chi^2$ 23.7, $P$=0.31) and pairwise deletion was performed for all analyses. Reasons for missing data are displayed in in Appendix 4.

*Between-Group Differences*

Group means and between-group $P$-values of all vascular measures are reported in Table 6, and between-group differences with individual participant

data points are represented in Figure 15. One-way analysis of variance revealed no between-group differences in any vascular measures. Bivariate Pearson correlation analysis revealed no associations between novel vascular measures and MoCA score (Table 7). These results were maintained following data imputation using the item mean substitution method (One-way analysis of variance results displayed in Table 8) (Hawthorne & Elliott, 2005).

*Associations Between Vascular Measures and Physical Function*

Bivariate Pearson correlation coefficients and *P*-values for all vascular measures and measures of physical function are displayed in Table 9. Bivariate Pearson correlation analyses revealed a positive association between cfPWV and fast-paced 5MWT. There were no other significant associations between novel vascular risk markers and measures of physical function (Table 9). These results were maintained following data imputation using the item mean substitution method.

Additional bivariate Pearson correlation analyses were performed within each of the three separate subgroups (participants without cognitive impairment, with cognitive impairment, and with stroke). Although no significant associations were observed, the strength of the positive association between cfPWV and fast-paced 5MWT increased across subgroups (from those without cognitive impairment to those with cognitive impairment to those with stroke). Bivariate Pearson

correlation coefficients and *P*-values for cfPWV and fast-paced 5MWT for each subgroup are displayed in Table 10.

### 3.5 Discussion

Contrary to our hypothesis, there were no differences in novel vascular risk markers across our continuum of increasing vascular risk and there were no associations between novel CV risk markers and MoCA score in the entire cohort. In addition, results suggest a positive association between cfPWV and walking ability in older adults with and without stroke.

*Between-Group Differences*

It is important to investigate novel markers of vascular risk progression, given that there a small portion of observed CVD risk (~10%) that is not explained by traditional risk factors alone (O'Donnell et al., 2010; Yusuf et al. 2004). Although earlier findings have suggested an association between novel vascular risk markers and cerebral SVD (Ding et al., 2015; Gustavsson, 2015, Hoth et al., 2007), direct associations between these novel risk markers and cognitive function are less established. To our knowledge, this is the first study to compare arterial stiffness and endothelial function across a continuum of increasing vascular risk that considers the presence of cognitive impairment and cerebrovascular disease.

Table 5. Characteristics for participants with and without stroke and cognitive impairment

| Characteristics | Individuals Without Cognitive Impairment | | | Individuals With Cognitive Impairment | | | Individuals With Stroke | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | n (%) or Mean±SD | Range | n | n (%) or Mean±SD | Range | n | n (%) or Mean±SD | Range | |
| Age (years) | 17 | 69.8 ± 3.4 | 66.0 – 78.0 | 17 | 71.7 ± 4.8 | 62.0 – 78.0 | 17 | 63.9 ± 8.6 | 50.0 – 80.0 | 0.001* |
| Sex (males/females) | 17 | 6(35) / 11(65) | | 17 | 11(65) / 6(35) | | 17 | 13(76) / 4(24) | | 0.04* |
| Systolic blood pressure (mmHg) | 17 | 127.3 ± 14.1 | 102.6 – 156.2 | 17 | 128.8 ± 12.7 | 100.8 – 150 | 16 | 136.1 ± 15.6 | 112.6 – 174.5 | 0.22 |
| Diastolic blood pressure (mmHg) | 17 | 72.6 ± 11.3 | 53.2 – 97.2 | 17 | 71.6 ± 7.9 | 62.0 – 89.2 | 16 | 79.5 ± 10.3 | 65.6 – 100.0 | 0.06 |
| Resting heart rate (bpm) | 17 | 60.3 ± 8.4 | 45 – 79.4 | 17 | 64.6± 11.0 | 47.6 – 91.4 | 16 | 65.7 ± 13.1 | 45.0 – 96.8 | 0.45 |
| Body mass index ($kg/m^2$) | 17 | 26.5 ± 4.7 | 19.4 – 14.2 | 17 | 24.8 ± 4.0 | 17.9 – 30.9 | 17 | 31.2 ± 5.5 | 23.1 – 41.6 | <0.001† |
| Waist-hip ratio | 17 | 0.9 ± 0.07 | 0.8 – 1.0 | 17 | 0.9 ± 0.07 | 0.8 – 1.1 | 17 | 1.0 ± 0.1 | 0.9 – 1.1 | 0.006* |
| Antihypertensive medication use (yes/no) | 17 | 5(29) / 12(71) | | 17 | 5(29) / 12(71) | | 17 | 13(76) / 4(24) | | 0.006* |
| Smoking (current/former/never) | 17 | 1(6) / 5(29) / 11(65) | | 17 | 0(0) / 7(41) / 10 (59) | | 17 | 3(18) / 9(53) / 5(29) | | 0.13 |
| Diabetes mellitus (yes/no) | 17 | 0(0) /17(100) | | 17 | 0(0) /17(100) | | 17 | 2(12) / 15(88) | | 0.38 |
| Number of chronic conditions (0/1-2/3-5) | 17 | 2(12) / 12(71) / 3(17) | | 17 | 0(0) / 7(41) / 10(59) | | 17 | 1(6) / 6(35) / 10(59) | | 0.07 |
| Montreal Cognitive Assessment | 17 | 27.6 ± 1.1 | 26 – 30 | 17 | 22.9 ± 2.9 | 13 – 25 | 17 | 21.8 ± 3.4 | 15 – 28 | <0.001† |
| Berg Balance Scale | 17 | 55.2 ± 1.1 | 52 – 56 | 17 | 55.1 ± 1.4 | 51 – 56 | 16 | 50.7 ± 4.2 | 42.0 – 56.0 | <0.001† |
| 5-Meter Walk Test | 17 | | | 17 | | | 17 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Self-paced (m/s) | | 1.1 ± 0.2 | 0.8 – 1.7 | | 1.3 ± 0.2 | 0.7 – 1.6 | | 1.0 ± 0.3 | 0.4 – 1.4 | 0.009* |
| Fast-paced (m/s) | | 1.5 ± 0.3 | 0.9 – 2.0 | | 1.8 ± 0.3 | 1.0 – 2.3 | | 1.3 ± 0.4 | 0.6 – 1.8 | 0.0004† |
| 6-Minute Walk Test (m) | 17 | 521.2 ± 88.6 | 367.2 – 749.5 | 17 | 521.1 ± 97.2 | 320.6 – 706.0 | 16 | 398.4 ± 108.5 | 203.4 – 549.0 | 0.009* |
| Time since stroke (years) | | | | | | | 17 | 4.8 ± 2.8 | 1.5 – 11.2 | |
| Stroke type | | | | | | | 17 | | | |
| Lacunar | | | | | | | | 4(24) | | |
| Infarct | | | | | | | | 7(41) | | |
| Hemorrhagic | | | | | | | | 2(12) | | |
| Unknown | | | | | | | | 4(24) | | |
| Stroke location | | | | | | | | | | |
| Cortical | | | | | | | | 6(35) | | |
| Subcortical | | | | | | | | 4(24) | | |
| Cerebellar | | | | | | | | 1(6) | | |
| Unknown | | | | | | | | 6(35) | | |
| Hemisphere affected (right/left) | | | | | | | 17 | 7(41)/10(59) | | |
| National Institutes of Health Stroke Scale | | | | | | | 17 | 1.8 ± 1.1 | 0 – 3 | |
| Chedoke-McMaster Stroke Assessment | | | | | | | 17 | | | |
| Arm | | | | | | | | 4.5 ± 1.7 | 2 – 7 | |
| Hand | | | | | | | | 4.8 ± 1.9 | 1 – 7 | |
| Leg | | | | | | | | 5.6 ± 1.2 | 3 – 7 | |
| Foot | | | | | | | | 4.6 ± 1.8 | 2 – 7 | |

Abbreviations: bpm, beats per minute; kg/m$^2$, kilogram/meter$^2$; m, meter; m/s, meter/second; mmHg, millimeters of mercury; SD, standard deviation

* p<005

† p<0.001

Table 6. Between-group Differences in Novel Vascular Risk Markers in Individuals with and without Stroke and Cognitive Impairment

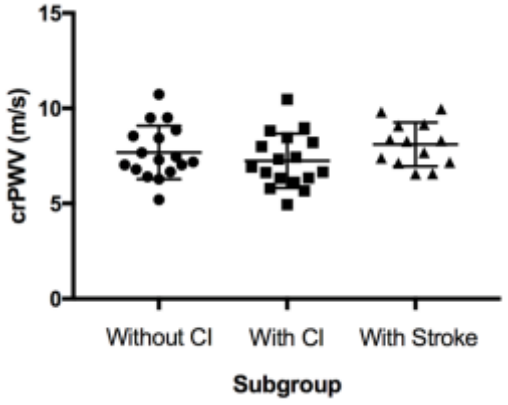
| Outcome | Individuals Without Cognitive Impairment | | Individuals With Cognitive Impairment | | Individuals With Stroke | | | *P*-Value |
|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | Visit | n | Mean±SD | |
| crPWV (m/s)* | 17 | 7.68 ± 1.40 | 17 | 7.24 ± 1.42 | 1 | 13 | 8.11 ± 1.15 | 0.22 |
| | | | | | 2 | 15 | 7.32 ± 1.35 | 0.62 |
| cfPWV (m/s)** | 16 | 10.26 ± 2.58 | 17 | 10.94 ± 2.65 | 1 | 12 | 9.70 ± 1.94 | 0.40 |
| ffPWV (m/s)* | 16 | 9.30 ± 2.12 | 17 | 9.25 ± 1.78 | 1 | 12 | 9.81 ± 1.69 | 0.99 |
| | | | | | 2 | 13 | 9.18 ± 1.74 | 0.99 |
| Compliance ($mm^2$/mmHg)* | 17 | 0.09 ± 0.03 | 17 | 0.11 ± 0.04 | 1 | 10 | 0.10 ± 0.05 | 0.23 |
| | | | | | 2 | 13 | 0.11 ± 0.06 | 0.21 |
| Distensibility ($mmHg^{-1}$)* | 17 | 0.003 ± 0.0008 | 17 | 0.003 ± 0.001 | 1 | 10 | 0.003 ± 0.001 | 0.64 |
| | | | | | 2 | 13 | 0.003 ± 0.0009 | 0.56 |
| FMD (%)** | 17 | 5.22 ± 4.07 | 17 | 5.01 ± 4.60 | 1 | 12 | 4.81 ± 4.55 | 0.97 |
| NTG(%)* | 17 | 16.76 ± 5.76 | 15 | 16.69 ± 6.95 | 1 | 10 | 14.66 ± 7.67 | 0.70 |
| | | | | | 2 | 9 | 14.60 ± 4.49 | 0.64 |

*Based on findings from Chapter 2, comparisons were made for unaffected side, visit 1 and 2

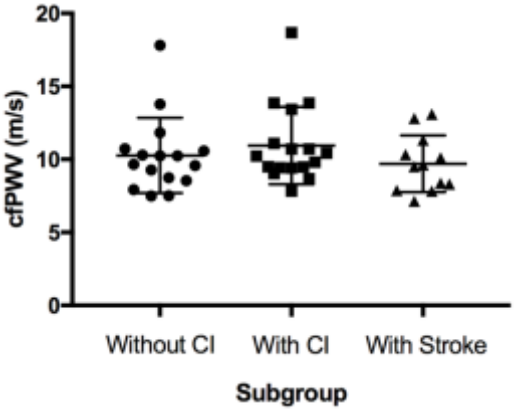
**Based on findings from Chapter 2, comparisons were made for unaffected side, visit 1 only

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; NTG, nitroglycerin assessment; SD, standard deviation

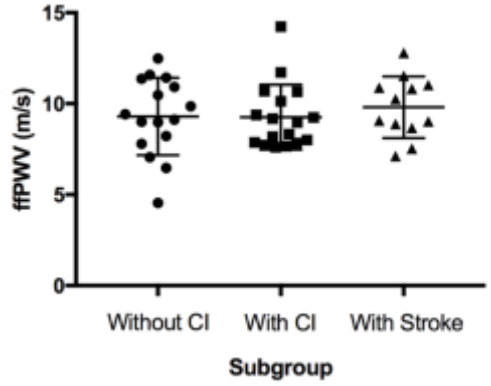
A.


crPWV (m/s)
15
10
5
0
Without CI
With CI
With Stroke
Subgroup

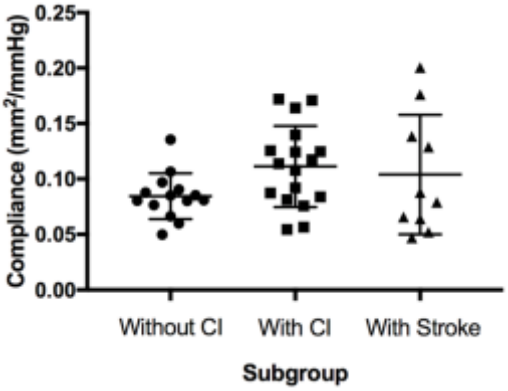
B.


cfPWV (m/s)
20
15
10
5
0
Without CI
With CI
With Stroke
Subgroup

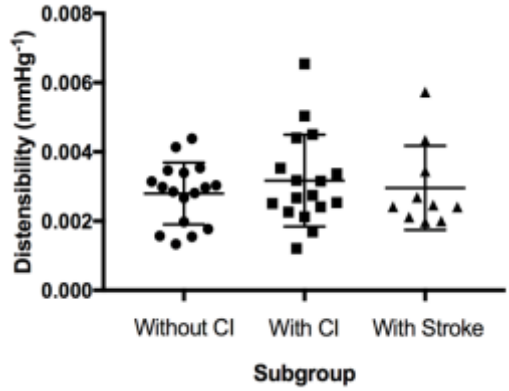
C.


ffPWV (m/s)
15
10
5
0
Without CI
With CI
With Stroke
Subgroup

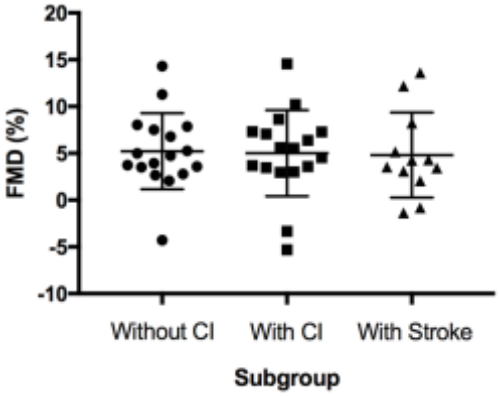
D.


Compliance (mm²/mmHg)
0.25
0.20
0.15
0.10
0.05
0.00
Without CI
With CI
With Stroke
Subgroup

E.


Distensibility (mmHg⁻¹)
0.008
0.006
0.004
0.002
0.000
Without CI
With CI
With Stroke
Subgroup

F.


FMD (%)
20
15
10
5
0
-5
-10
Without CI
With CI
With Stroke
Subgroup

G.

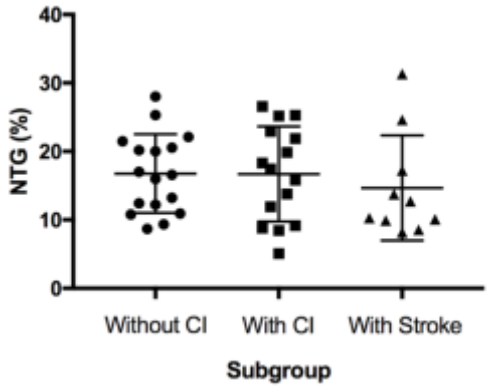



**Figure 15.** Between-group differences (individuals without cognitive impairment, with cognitive impairment, and with stroke) in A. carotid-radial pulse wave velocity (crPWV), B. carotid-femoral pulse wave velocity (cfPWV), C. femoral-foot pulse wave velocity (ffPWV), D. compliance, E. distensibility, F. flow-medicated dilation (FMD), and G. nitroglycerin administration (NTG). In stroke participants, measures were considered from unaffected side and visit 1. Horizontal line represents mean and error bars represent standard deviation.

Table 7. Bivariate Pearson Correlation Analyses Between Novel Vascular Risk Markers and Montreal Cognitive Assessment Score

| | Bivariate Pearson Correlation Analysis with MoCA Score | | |
|---|---|---|---|
| **Vascular Measure** | **n** | **r** | ***P*-value** |
| crPWV (m/s) | 47 | 0.13 | 0.38 |
| cfPWV (m/s) | 45 | 0.14 | 0.38 |
| ffPWV (m/s) | 45 | -0.02 | 0.88 |
| Compliance ($mm^2$/mmHg) | 44 | -0.02 | 0.90 |
| Distensibility ($mmHg^{-1}$) | 44 | -0.04 | 0.82 |
| FMD (%) | 46 | 0.01 | 0.95 |
| NTG (%) | 44 | 0.01 | 0.93 |

Vascular measures from unaffected side and visit 1 only participants with stroke

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; MoCA, Montreal Cognitive Assessment; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; NTG, nitroglycerin assessment

Table 8. Between-group Differences in Novel Vascular Risk Markers using Item Mean Data Imputation

| Outcome | Individuals Without Cognitive Impairment | | Individuals With Cognitive Impairment | | Individuals With Stroke | | | *P*-Value |
|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | Visit | n | Mean±SD | |
| crPWV (m/s)* | 17 | 7.68 ± 1.40 | 17 | 7.24 ± 1.42 | 1 | 17 | 8.20 ± 0.91 | 0.10 |
| | | | | | 2 | 17 | 7.32 ± 1.26 | 0.61 |
| cfPWV (m/s)** | 17 | 10.26 ± 2.50 | 17 | 10.94 ± 2.57 | 1 | 17 | 9.70 ± 1.66 | 0.29 |
| ffPWV (m/s)* | 17 | 9.30 ± 2.06 | 17 | 9.26 ± 1.73 | 1 | 17 | 9.80 ± 1.44 | 0.62 |
| | | | | | 2 | 17 | 9.18 ± 1.51 | 0.98 |
| Compliance ($mm^2$/mmHg)* | 17 | 0.09 ± 0.03 | 17 | 0.11 ± 0.04 | 1 | 17 | 0.10 ± 0.04 | 0.18 |
| | | | | | 2 | 17 | 0.11 ± 0.05 | 0.17 |
| Distensibility ($mmHg^{-1}$)* | 17 | 0.003 ± 0.0008 | 17 | 0.003 ± 0.001 | 1 | 17 | 0.003 ± 0.0009 | 0.60 |
| | | | | | 2 | 17 | 0.003 ± 0.0008 | 0.56 |
| FMD (%)** | 17 | 5.22 ± 4.07 | 17 | 5.01 ± 4.60 | 1 | 17 | 4.81 ± 3.77 | 0.96 |
| NTG(%)* | 17 | 16.76 ± 5.76 | 17 | 16.69 ± 6.49 | 1 | 17 | 14.66 ± 5.75 | 0.52 |
| | | | | | 2 | 17 | 15.18 ± 7.17 | 0.73 |

*Based on findings from Chapter 2, comparisons were made for unaffected side, visit 1 and 2

**Based on findings from Chapter 2, comparisons were made for unaffected side, visit 1 only

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; NTG, nitroglycerin assessment; SD, standard deviation

Table 9. Bivariate Pearson Correlation Analyses between Novel Vascular Risk Markers and Measures of Physical Function

| | Bivariate Pearson Correlation Analyses | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BBS | | | 5MWT-S | | | 5MWT-F | | | 6MWT | | |
| **Outcome** | **n** | **r** | ***P*-value** | **n** | **r** | ***P*-value** | **n** | **r** | ***P*-value** | **n** | **r** | ***P*-value** |
| crPWV (m/s) | 46 | -0.14 | 0.35 | 47 | 0.04 | 0.80 | 47 | 0.08 | 0.60 | 46 | 0.11 | 0.45 |
| cfPWV (m/s) | 44 | 0.10 | 0.52 | 45 | 0.21 | 0.18 | 45 | 0.30 | 0.04* | 44 | 0.21 | 0.17 |
| ffPWV (m/s) | 44 | -0.03 | 0.83 | 45 | 0.08 | 0.59 | 45 | 0.15 | 0.34 | 44 | 0.13 | 0.40 |
| Compliance ($mm^2$/mmHg) | 43 | -0.15 | 0.33 | 44 | 0.11 | 0.46 | 44 | 0.16 | 0.31 | 43 | 0.15 | 0.33 |
| Distensibility ($mmHg^{-1}$) | 43 | 0.04 | 0.81 | 44 | 0.09 | 0.55 | 44 | 0.07 | 0.65 | 43 | 0.03 | 0.83 |
| FMD (%) | 45 | -0.02 | 0.90 | 46 | -0.04 | 0.80 | 46 | 0.07 | 0.96 | 45 | -0.09 | 0.57 |
| NTG (%) | 41 | 0.31 | 0.06 | 42 | 0.06 | 0.72 | 42 | 0.02 | 0.99 | 42 | 0.026 | 0.87 |

Vascular measures from unaffected side and session 1 only in participants with stroke

* p<005

Abbreviations: BBS, Berg Balance Scale; cfPWV, carotid-femoral pulse-wave velocity; crPWV, carotid-radial pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; MoCA, Montreal Cognitive Assessment; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; NTG, nitroglycerin assessment; 5MWT-S, 5 meter walk test (slow); 5MWT-F, 5 meter walk test (fast); 6MWT, 6-minute walk test

Table 10. Bivariate Pearson Correlation Analyses between Carotid-Femoral Pulse Wave Velocity and Measures of Physical Function

| Outcome, Subgroup | Bivariate Pearson Correlation Analyses with 5MWT-F | | |
|---|---|---|---|
| | n | r | *P*-value |
| cfPWV (m/s), without cognitive impairment | 16 | 0.08 | 0.76 |
| cfPWV (m/s), with cognitive impairment | 17 | 0.20 | 0.43 |
| cfPWV (m/s), with stroke | 11 | 0.48 | 0.11 |

Vascular measures from unaffected side and visit 1 only in participants with stroke

* p<005

Abbreviations: cfPWV, carotid-femoral pulse-wave velocity; m/s, meters/second; 5MWT-S, self-paced 5-meter walk test; 5MWT-F, fast-paced 5-meter walk test; 6MWT, 6-minute walk test

Contrary to our hypothesis, there were no differences in novel CV risk markers across our continuum of increasing vascular risk and there were no associations between novel CV risk markers and MoCA score in the entire cohort. The current results conflict with previous findings that have demonstrated associations between novel risk markers and cognitive function in older adults and individuals with stroke. Earlier studies have reported associations between cfPWV and Mini Mental State Examination (MMSE) score in individuals with stroke ($r=-0.45$, $p<0.01$) (Lee et al., 2014), and cfPWV and immediate and delayed memory recall (California Verbal Learning Test) in older adults ($n=1820$; $r^2=0.19$, $p<0.02$) (Cooper et al., 2015). In addition, a previous study found an association between brachial artery FMD and cognitive impairment, assessed using an extensive cognitive screening method that considered specific memory domains, in older adults (Vendemiale et al., 2013).

Reasons for conflicting results may in part be due to differences in study participants, as unlike the previous study conducted by Lee et al. (2014), which only used individuals with stroke, the current study considered older adults with and without stroke. The lack of between-group differences in our cohort may also be attributed to the younger age of stroke participants compared to those without stroke, the inclusion of only mild to moderately impaired stroke participants, or the narrow age range of older adults without stroke.

Inconsistencies between our results and those of previous studies may also in part be due to differences in the cognitive assessments used. It is possible that

the Montreal Cognitive Assessment, a global screening measure of cognitive function, may not be sensitive enough to detect differences in CV risk in older adults. Unlike the Montreal Cognitive Assessment, the California Verbal Learning Test used by Cooper et al. (2015) is a specific neurophysiological test used to assess verbal memory (Woods, Delis, Scott, Kramer, & Holdnack, 2006). The assessment of cognitive function in the previous study conducted by Vendemiale et al. (2013) was extensive and composed of particular criteria, such as objective memory impairment and impairment of one or more non-memory domains. It may be possible that specific, as opposed to global, neurophysiological tests may be more appropriate to identify cognitive impairment in older adults. The cognitive assessments used in these earlier studies (Cooper et al., 2015; Vendemiale et al., 2013) included objective measures of memory, suggesting that impairments in this specific cognitive domain may be more indicative of cerebral SVD progression than global impairments in cognition. Indeed, previous findings suggest that impairments in in memory are associated with SVD progression in older adults with mild cognitive impairment (DeCarli et al., 2004), and are also present in individuals with acute ischemic stroke (Cho et al., 2014). Ongoing research should investigate relationships between cognitive impairment, particularly memory-specific domains, and novel risk markers in older adults with and without stroke.

*Associations Between cfPWV and Walking Ability*

Post-stroke limitations in physical function, such as walking and balance ability, have been previously associated with reduced cardiorespiratory fitness, muscle strength, and muscle power (Flansbjer et al., 2012; Patterson et al., 2010; Saunders et al., 2008), however, associations between post-stroke physical function and novel vascular risk markers have not yet been established. Results from the current study revealed an unanticipated positive association between cfPWV and walking speed. Even with further analyses, positive associations between cfPWV and walking speed in the individual subgroups were observed, though these associations were not significant. However, the strength of these positive associations increased across subgroups of decreasing cognitive function (without cognitive impairment, with cognitive impairment, with stroke).

These observed positive associations between walking speed and cfPWV were surprising as they suggest that individuals with better walking ability have poorer arterial health. Previous studies have reported negative associations between cfPWV and walking ability in young adults (Brunner et al., 2011), older adults (Gonzales, 2013), and individuals with peripheral artery disease (Watson et al., 2011). Reasons for the inconsistencies in results are unclear, but a possible explanation may be the relationship between cognitive impairment and fear of falling, which has been suggested to be an accurate indicator of balance and walking ability (Higuchi, Sudo, Tanaka, Fuchioka, & Hayashi, 2004; Manning, Neistadt, & Parker, 1998), in older adults. Cognitive impairment has been

associated with the absence of fear of falling in older adults (OR=0.04, 95% CI=0.00-0.50, p=0.01) (Shirooka et al., 2016), and a previous study also observed that the prevalence of fear of falling was lowest in older adults with global cognitive impairment (40.6%) compared to those with mild cognitive impairment and those without cognitive impairment (50.6% with mild cognitive impairment, 43.6% without cognitive impairment; p<0.001) (Uemura et al., 2014). These previous findings suggest a disparity between perceived and actual risk of falling in older adults with cognitive impairment, whereby those with cognitive impairment do not modify walking speed to compensate for fear and risk of falls. Future research should continue to investigate associations between novel CV risk markers and physical function in larger samples of individuals with stroke with a greater range of functional abilities.

*Limitations*

This study was limited by the exclusion of participants with severe stroke, which reduces the generalizability of results. However, the sample included in this study is important to explore, as it is reflective of the types of individuals who are living in the community yet are still at risk for recurrent events. Moreover, participants in the stroke group were of younger age compared to those without stroke, and this may have influenced the results given the well-established association between age and CV risk. While we did not deliberately match the subgroups by age, future research may do so in order to eliminate age as a

potential confounder. The use of the MoCA as a screening tool of global cognitive function may be a limitation, as it may not have been sensitive enough to divide the older adult group into those with high versus low CV risk. In addition, though this study is limited by a portion of missing data (15% missing from entire cohort, 2% missing from older adult group, 25% missing from stroke group), a small portion of missing data from stroke participants (12%) was due to participant-related factors, such as elevated resting blood pressure or unwillingness to participate in the assessment, suggesting that individuals with stroke may be able to well-tolerate assessments of novel vascular risk markers. Finally, the software used in arterial diameter analysis is semi-automated but allows for individual manipulation, which may lead to observer bias. Appropriate steps were taken to minimize this risk of bias, such as adherence to standardized operating procedures, use of a consistent rater and analysis computer, and performance of quality checks on all outcomes.

### 3.6 Conclusion

Results suggest no differences in novel CV risk marker progression between adults without cognitive impairment, with cognitive impairment, and with stroke. It may be possible that the MoCA, a global screening measure of cognitive function, may not useful in identifying differences in early vascular risk markers in older adults. Moreover, the positive associations between cfPWV and walking ability found in the current study conflict with previous reports, but may

highlight relationships between cognitive impairment and the absence of fear of falling in older adults. Future research should continue to investigate relationships between novel CV risk markers and memory-specific domains of cognitive function, as well as relationships between physical function and novel vascular markers in larger samples with greater ranges in functional ability.

# Chapter 4

# DISCUSSION

## 4.1 Discussion

The overall objective of this thesis was to increase understanding of novel vascular risk markers, namely arterial stiffness and endothelial function, in community-dwelling individuals with and without stroke. Individuals with history of stroke are at elevated risk of recurrent stroke and other adverse cardiovascular (CV) events (Dhamoon et al., 2006; Mohan et al., 2011). This increased risk is largely attributed to the suboptimal management of traditional, modifiable risk factors following stroke (Kopunek et al., 2007), however there remains approximately 10% of observed stroke risk that is not explained by traditional CV risk factors (O'Donnell et al., 2010). Arterial stiffness and endothelial dysfunction are important factors in the development of atherosclerosis (van Popele et al., 2001; Verma & Anderson, 2002) and hypertension (Franklin, 2005; Verma & Anderson, 2002), processes that typically precede cardiovascular disease (CVD) and stroke. Understanding these novel risk markers in individuals with and without stroke may provide information regarding early and sensitive CV risk detection.

*Study 1: Measurement Properties of Novel Vascular Risk Markers*

The first study aimed to investigate test-retest reliability of and between-side differences in novel vascular risk markers. Findings established almost perfect test-retest reliability for carotid-femoral pulse wave velocity (cfPWV) and substantial test-retest reliability for brachial artery flow-mediated dilation (FMD) in

individuals with stroke, and no between-side differences in any novel vascular risk markers in individuals with and without stroke.

To our knowledge, this was the first study to examine the reliability of arterial stiffness and endothelial function measures in the post-stroke population. The almost perfect test-retest reliability of cfPWV is aligned with previous reports in healthy adults (Meyer et al., 2015) and individuals with spinal cord injury (Currie et al., 2014; Miyatani et al., 2012). In contrast, the substantial test-retest reliability of brachial artery FMD in the current study is lower than that previously observed in young, healthy adults (Meirelles et al., 2007; Welsch et al., 2002), but it is worth noting that the test-retest reliability increased (ICC>0.88) following the removal of outlier values observed in Bland-Altman plots. Future research should examine test-retest reliability in larger samples of individuals with a greater range of functional abilities post-stroke.

Nonetheless, our findings are important, given that cfPWV and FMD are the recommended measures for arterial stiffness and endothelial function, respectively (Corretti et al., 2002; Van Bortel et al., 2012). Reasons for low to moderate test-retest reliability in the other vascular measures (crPWV, ffPWV, compliance, distensibility, and NTG) may be due to post-stroke impairments that may have affected the accuracy of distance measures required for peripheral pulse wave velocity (PWV) calculations, or blood pressure variability (Floras et al., 1988; Korpelainen et al., 1999) which may have affected compliance and distensibility measurement.

In contrast however, the absence of between-side differences in all vascular outcomes observed in this study conflicts with previous findings suggesting compromised endothelial function in the affected compared to the unaffected limb in individuals with stroke (Billinger et al., 2012; Ivey et al., 2004; Ivey et al., 2010). It is important to note that compared to individuals in the current cohort, those in previous reports had lower walking endurance (304.1 ± 167.5 m versus 398.4 ± 108.5 m) (Billinger et al., 2012) and slower self-paced walking speed (1.3 ± 0.6 m/s versus 1.0 ± 0.3 m/s) (Ivey et al., 2010), suggesting lower physical function, and those in one study (Billinger et al., 2012) were earlier post-stroke (<6 months post-stroke).

Our findings suggest that the presence of between-side differences in vascular health may be influenced by stroke characteristics, such as the time since event or level of post-stroke impairment, as shorter latency since stroke or greater level of post-stroke impairment may have contributed to unilateral physiological adaptations (i.e. differences in tissue composition (De Deyne, Hafer-Macko, Ivey, Ryan, & Macko, 2004), altered autonomic function (Herbaut, Cole, & Sedgwick, 1990), or enhanced sensitivity to endogeneous vasoconstrictor agents (Bevan, Clementson, Joyce, & Bevan, 1993). It is possible that these factors may have impaired vascular health in the affected limb to a greater extent than in the current report. Further research is needed to establish whether the presence or extent of between-side differences varies for different cohorts of stroke survivors, and to investigate the specific mechanisms involved

in the possible unilateral impairment of vascular health in this population.

### *Study 2: Comparison of Novel Vascular Risk Markers Across a Proposed Continuum of Increasing Vascular Risk*

The second study aimed to investigate differences in arterial stiffness and endothelial function across three subgroups of a proposed continuum of increasing vascular risk (older adults without cognitive impairment, with cognitive impairment, and with stroke), and to examine relationships between these novel vascular risk markers and physical function. Contrary to our hypotheses, findings suggest no between-group differences in novel vascular risk markers, and an unexpected positive association between cfPWV and walking ability was found in the entire cohort.

Previous findings have demonstrated associations between novel risk markers and cognitive function in older adults (Cooper et al., 2015; Vendemiale et al., 2013) and individuals with stroke (Lee et al., 2014). While no association was found in the current study, ours was the first to examine this relationship across a spectrum of individuals with and without cognitive impairment and stroke. The MoCA, a global screening measure of cognitive function, was used to delineate individuals with cognitive impairment whereas previous studies (Cooper et al., 2015; Vendemiale et al., 2013) tested specific neurophysiological domains, namely memory. It is possible that neurophysiological tests of specific cognitive domains may be more appropriate to detect differences in vascular risk progression. Indeed, impairments in memory have been shown to be associated

with SVD progression in older adults with mild cognitive impairment (DeCarli et al., 2004), and are present in individuals with acute ischemic stroke (Cho et al., 2014).

Furthermore, the positive associations observed between walking speed and cfPWV were surprising, and inconsistent with previous studies that have reported negative associations between cfPWV and walking ability in healthy adults (Brunner et al., 2011; Gonzales, 2013), and individuals with stroke (Lee 2015). The positive association observed in this study may in part be due to the association between cognitive impairment and the absence of fear of falling (Shirooka et al., 2016). Indeed, individuals with stroke in this study had global cognition scores indicative of cognitive impairment (mean Montreal Cognitive Assessment score <26 (Nasreddine, 2005), while those in a previous report (Lee et al., 2015) presented with scores that were greater than the established threshold indicative of cognitive impairment (mean Mini-mental Status Examination score ≥24 (Mitchell, 2009). Further research is needed to increase understanding about the relationship between physical function and vascular health in the older adults with and without stroke.

### 4.2 Clinical Significance

Given that individuals with history of stroke are at risk for experiencing recurrent events, identifying early markers of vascular risk is important to allow interventions for secondary prevention to be implemented in timely manner.

Findings from this thesis increase our understanding regarding measurement properties of these novel vascular risk markers in the post-stroke population, and provide insight into the use of these measures for early vascular risk detection.

Since substantial to almost perfect test-retest reliability of cfPWV and FMD was observed in the first study, these measures may have clinical utility in the assessment of early CV risk in the post-stroke population. This finding is important, given that cfPWV is the criterion standard of arterial stiffness (Laurent et al., 2001; Van Bortel et al., 2012), and FMD is the most well-established and commonly used non-invasive measure of endothelial function (Corretti et al., 2002).

With respect to cfPWV, though a standard cut-off value of 10 m/s has previously been suggested to be indicative of increased CV risk (Van Bortel et al., 2012), recent research suggests such a cut-off may vary in different populations depending on certain factors, such as age (Townsend et al., 2015). In healthy populations, expected FMD values range from 0.2–19%, and in from -1–14% in individuals with CHD (Bots, Westerink, Rabelink, & de Koning, 2005), and that a cut-off FMD value of 6% may be sensitive in detecting the presence of CAD (Teragawa et al., 2001). There is limited information surrounding PWV and FMD in individuals with stroke, but this thesis contributes to our understanding of typical values that may be observed in this population, which may assist to establish cut-off values that are indicative of increased CV risk. The application of these measures in individuals with stroke may be used to identify those with a

greater degree of vascular risk progression, and may inform future rehabilitation interventions geared towards reducing CV risk in this population.

Additionally, given that post-stroke impairments may lead to limited function and mobility of the affected side, positioning the limbs for assessments of vascular function, particularly PWV and FMD, may be cumbersome and difficult to attain and maintain for an extended period of time. This may affect the accuracy of these measurements in the affected limb. Since no between-side differences in novel vascular risk markers were observed, it may be more clinically feasible to perform novel vascular assessments on the unaffected limbs in individuals with stroke.

To our knowledge, this study was the first to compare arterial stiffness and endothelial function across a continuum of increasing vascular risk that considers the presence of cognitive impairment and cerebrovascular disease. Although we observed no differences in novel vascular risk markers across our proposed continuum, this work increases knowledge regarding vascular disease progression and the use of cognitive function as a screening tool for vascular disease risk in older adults with and without stroke. Our findings provide clinically relevant information regarding the use of the MoCA as a cognitive screening tool. Given that previous findings have established associations between vascular health and cognitive function in older adults using specific objective measures of memory (Cooper et al., 2015; Vendemiale et al., 2013), but current findings suggest no differences in all novel risk markers across our proposed continuum,

it may be possible that specific neurophysiological cognitive assessments may be more indicative of vascular disease progression than global cognitive screening tools, such as the MoCA. Our results may inform future research trials investigating the clinical use of cognitive assessment tools as indicators of vascular risk in older adults with and without stroke, which may lead to earlier detection of vascular risk.

Overall, this thesis advances knowledge surrounding the use of novel vascular risk markers as assessments of vascular disease progression in older adults with and without stroke. Collectively, our findings suggest that in the post-stroke population, cfPWV and FMD may be the most appropriate measures of vascular risk progression, and that it may be more clinically feasibly to assess these measures on the unaffected limb only. These results may inform future intervention studies aimed at improving vascular health in older adults with stroke as well as those who may be at increased risk of stroke, and in this way may provide important insight on how to optimize care and reduce the burden of stroke.

### 4.3 Limitations

Although generalizability of results was limited by the exclusion of participants with severe stroke, the sample included in this study is important to explore, as it is reflective of the types of individuals who are living in the community yet are still at risk for recurrent events. In addition, given the well-

established association between age and CV risk, the younger age of participants in the stroke group compared to the older adults may have influenced the results. Future research in this area should use age-matched comparisons.

Moreover, although the methodology of the vascular assessments performed in this study are well established, they may be subject to technical and interpretative limitations, such as equipment issues and poor signal and/or image quality. This thesis is limited by a portion of missing data (18% missing from entire cohort, 8% missing from older adult group, 25% missing from stroke group), although this proportion from our stroke group is consistent with a previous report examining arterial stiffness in individuals with stroke (27% missing data) (Tang et al., 2014a). It is, however, important to note that of the total missing data from the stroke group, only 12% was due to participant-related factors, such as elevated resting blood pressure or unwillingness to participate in the assessment, suggesting that individuals with stroke are able to well-tolerate these assessments.

### 4.4 Future Directions

Future research is warranted to establish whether our findings regarding test-retest reliability and between-side differences are consistent in cohorts of stroke survivors of different age, level of impairment, and time since stroke event. If between-side differences in novel vascular risk markers are established in other post-stroke cohorts, additional research should investigate the specific

mechanisms that may contribute to unilaterally impaired vascular health.

Additional research may also examine differences in novel vascular risk markers across a continuum of increasing vascular risk in order to increase knowledge regarding vascular disease progression. Future work should investigate relationships between novel vascular risk markers and memory-specific domains of cognitive function using specific neurophysiological tests. While we did not deliberately match the subgroups by age, future research should do so in order to eliminate age as a covariate. Further research is also needed to examine relationships between physical function and novel vascular risk markers in samples with greater ranges of functional ability.

Findings from this thesis form important pilot work that may inform longitudinal studies to examine the predictive capacity of these novel risk markers, as well as intervention trials to investigate the effects of exercise on novel vascular risk markers in older adults with and without stroke in order to reduce the risk of adverse CV events in these populations.

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# Appendix 1

## CUT-OFF VALUES FOR TRADITIONAL CARDIOVASCULAR RISK FACTORS

Table A1. Blood Pressure Values Indicative of Optimal Values, Prehypertension, Stage One Hypertension, and Stage Two Hypertension

| BP Category | Systolic BP (mmHg) | Diastolic BP (mmHg) |
|---|---|---|
| Normal | <120 | <80 |
| Prehypertension | 120–139 | 80–89 |
| Stage one hypertension | 140–159 | 90–99 |
| Stage two hypertension | ≥160 | ≥100 |

Abbreviations: BP, blood pressure; mmHg, millimeters of mercury

Adapted from American Medical Association (Chobanian et al., 2003)

Table A2. Serum Lipid Levels Indicative of Cardiovascular Risk Category

| CV Risk Category | TC (mmol/L) | HDL-c (mmol/L) | LDL-c (mmol/L) | TG (mmol/L) |
|---|---|---|---|---|
| Low CV risk (optimal levels) | <5.2 | ≥1.5 | <2.3 | <1.7 |
| Borderline CV risk | 5.2–6.2 | 1.0–1.5 (men)<br>1.3–1.5 (women) | 3.4–4.1 | 1.7–2.2 |
| High CV risk | ≥6.2 | <1.0 (men)<br><1.3 (women) | >4.1 | >2.2 |

Abbreviations: CV, cardiovascular; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglyceride; mmol/L, millimoles per litre

Adapted from American Association of Clinical Endocrinologists (Jellinger et al., 2012)

Table A3. Fasting Serum Glucose Levels Indicative of Pre-diabetes (hyperglycemia) and Diabetes

| Diabetes Category | FGB (mmol/L) |
|---|---|
| Pre-diabetes (hyperglycemia) | 6.1–6.9 |
| Diabetes Mellitus | >7.0 |

Abbreviations: FBG, fasting blood glucose; mmol/L, millimol per litre

Adapted from Goldenberg & Punthakee (2011)

Table A4. Body Mass Index, Waist Circumference, and Waist-hip Ratio Cut-Off Values for Anthropometric Category

| Anthropometric Category | BMI (kg/m$^2$) | WC (cm) | WHR |
|---|---|---|---|
| Underweight | <18.5 | | |
| Normal weight | 18.5–24.9 | | |
| Overweight | 25.0–29.9 | | |
| Obese | ≥30 | >102 (men)<br>>88 (women) | ≥0.90 (men)<br>≥0.85 (women) |

Abbreviations: BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; cm, centimeters; kg/m$^2$, kilogram per meter$^2$

Adapted from the World Health Organization: Waist Circumference and Waist-Hip Ratio, Report of a World Health Organization Expert Consultation (World Health Organization, 2008).

## Appendix 1 References

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## Appendix 2

## PARTICIPANT INFORMATION AND STUDY CONSENT FORM

# Participant Information Sheet

| | |
|---|---|
| **Title of Study** | Understanding the relationship between measures of vascular function with cardiovascular health and mobility in individuals living in the community after stroke |
| **Primary Investigator** | Ada Tang PT PhD, School of Rehabilitation Science |
| **Co-Investigators** | Maureen MacDonald PhD, Department of Kinesiology<br>Julie Richardson PT PhD, School of Rehabilitation Science |

**For study information and questions, contact Dr Ada Tang at (905) 525-9140 x27818**

You are being invited to participate in a research study that will look at the health of arteries of people with stroke, and how this relates to other tests of cardiovascular function and mobility.

To decide whether or not you want to be a part of this research study, it is important that you understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study and if you wish to participate, you will be asked to sign the form at the end of this information letter.

**WHY IS THIS RESEARCH BEING DONE?**

People with stroke often present with multiple health issues, such as high blood pressure, high cholesterol levels, or diabetes. These risk factors place them at higher risk for having another stroke. It is possible that increasing physical activity or exercising more after stroke may improve these risk factors but there is not enough research so far to know this for sure.

Other ways that we can evaluate cardiovascular risk is by evaluating vascular function. Assessing the health of arteries provides an indicator of cardiovascular disease (CVD) risk in people without stroke. This study will look at the degree of hardening of the arteries in people with stroke, and how well the blood vessels expand and contract. These tests

are novel in stroke research, as they haven't been used very much in previous studies involving people with stroke. These tests will be done on both sides to see if there are differences between the weaker versus stronger sides in people with stroke. We will also look at how these tests relate to each other, and how they related to more traditional risk factors (such as heart rate, blood pressure and fitness level), and with walking and balance.

**WHAT WILL I BE ASKED TO DO IF I DECIDE TO TAKE PART IN THE STUDY?**

If you agree to participate in this study, you will be asked to come in for 2 visits to the Vascular Dynamics Lab (Ivor Wynne Center Room E102) at McMaster University. At these visits, you will undergo baseline tests of vascular function, as well as other tests of cardiovascular function, fitness level, and walking and balance ability. All tests are described below.

**WHAT IS THE TIME COMMITMENT?**

The study will consist of 2 visits for a total time commitment of approximately 3.75 hours.

**WHAT IS INVOLVED?**

The tests that will be conducted, and the schedule for each of the 2 visits are outlined below. Day 1 will take approximately 2.25 hours; Day 2 will take approximately 1.5 hours. Both visits will be scheduled at the same time of day, and within 7 days of each other.

For both visits, we will ask you to abstain from food or drink for 4 hours, caffeine for 8 hours, smoking for 12 hours, caffeine and alcohol for 12 hours, and exercise for 24 hours.

| **Day 1** | **Time (min)** |
|---|---|
| Participant information and medical history | 10 |
| Arterial stiffness (both sides) | 20 (10 each side) |
| Artery function (both sides) | 40 (20 each side) |
| Resting heart rate and blood pressure in lying and sitting | 15 |
| Snack | 10 |
| Assessments of stroke recovery, balance | 30 |
| **Total** | **125** |
| **Day 2** (within 7 days of Day 1) | |

| | |
|---|---|
| Repeat arterial stiffness (both sides) | 20 (10 each side) |
| Repeat artery function (both sides) | 40 (20 each side) |
| Snack | 10 |
| Assessments of thinking and memory, arm and leg movement, walking | 40 |
| **Total** | **110** |

**Participant information and medical history**
We will ask you to complete a medical history questionnaire that will include information about your stroke (date of stroke, location, and type) and other medical information (such as diabetes and atrial fibrillation). We will also record the medications you are currently taking.

**Measurements**

*Arterial stiffness*
This is a measure of the speed that your pulse travels through your body that gives us an indication of the stiffness of your arteries. This test is non-invasive and painless, and will be done while you are lying down. We will measure one side at a time. Your pulse will be detected by probes that are placed on the surface of the skin on your neck and groin, and the distance between each site will be recorded. We will also use ultrasound to view the arteries in your neck. We will apply some gel on your neck, and then place the ultrasound transducer on the gel to look at the function of this artery.

*Artery function*
In this test, we will measure of how much an artery in your arm can expand. This test is non-invasive, and will be done while you are lying down. We will measure one side at a time. A special blood pressure cuff will be placed around your forearm, and will be inflated for 5 minutes to a pressure of approximately 200 mmHg, which stops blood flow to the hand. After 5 minutes, the cuff is deflated quickly and blood flow will return to your hand. Using the ultrasound, we will measure the size of an artery in your upper arms during and after the cuff is inflated. This procedure does cause some discomfort while the cuff is inflated (similar to pins and needles) but any discomfort is quickly relieved when the cuff is released. There is no danger from this experimental procedure unless the cuff is left inflated for very long periods (over 60 minutes).

We will also do a measure to look at the largest possible limit the arteries in your arm can expand. For this part of the test, we will give you a 0.4mg dose of sublingual (under

the tongue) nitroglycerine (NTG). We will measures of the size of the arteries in your upper arms after we spray the NTG under your tongue.

*Resting heart rate and blood pressure in lying and sitting*
Your heart rate and blood pressure will be measured while you are lying down. We will measure your heart rhythm using electrodes attached to your chest (they stick to your skin similar to a band-aid), and will measure your blood pressure using a blood pressure cuff. We will then sit you up, and measure your heart rate and blood pressure again.

*Assessments of stroke recovery, walking ability, and balance assessment*
We will measure your recovery from your stroke using 2 tests. You will asked to answer some questions and perform some simple movements and thinking tasks. We will measure your walking ability using 2 tests. For both tests, you will be permitted to use the walking aid that you normally used. First, we will measure your walking speed over a short distance (10 meters). Then, we will ask you to walk as far as possible over a 6-minute period. During the walk, your heart rate will be monitored. We will keep track of how far you walk, whether you use a walking aid, and if you need to rest during the walk. For this test, you may take a rest whenever you need one. Finally, a balance test will be performed, where you will be asked to perform a series of tasks such as standing and looking over each shoulder, reaching as far forward as you can, and reaching down to pick something up off the ground.

**WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?**

All procedures are non-invasive and offer minimal risk to you. If you are sensitive to adhesives, you may develop a minor rash from ultrasound gel or the electrodes. The test of artery function may cause brief numbness and/or tingling in the limb when blood flow is stopped (i.e. when the cuff is inflated) and immediately following release of the cuff. You may also notice the spasticity in your arm or leg increase temporarily, but this should reside after the cuff is released. There is no known medical risk associated with a 5-minute period of blood flow stoppage to the hand, and there is no lasting effect of this on the function of the limbs.

You might feel dizziness or lightheaded we sit you up after lying down for a long time. We will be monitoring your heart tracing and your blood pressure. There is also a small risk that you might fall during the walking or balance tests. To minimize the risk of falling during the 6-minute Walk Test, participants will use their typical walking aids. There is a small risk that participants might fall when being transferred to the assessment bed. We will monitor your balance carefully and provide safety supervision. You are asked to report any unusual symptoms during any of the tests. You may stop or rest whenever you wish because of feelings of tiredness or discomfort.

**WHAT ARE THE POSSIBLE BENEFITS OF THE STUDY FOR ME AND/OR SOCIETY?**

This study will contribute to our knowledge about the health and function of the arteries in people with stroke. Since these tests are novel in stroke research, we are taking some of the first steps in identifying ways to combine these new measures of the health of the arteries with traditional risk factors to classify who might be at higher or lower risk for having another stroke. Results will also be important for informing how we might design future research studies looking at the effects of exercise on cardiovascular health and lower the burden of stroke on society.

**WHAT IF I CHANGE MY MIND ABOUT PARTICIPATING IN THE STUDY?**

Your participation in this study is voluntary. If you volunteer to be in this study, you may withdraw at any time, even after signing the consent form or partway through the study, including during any of the tests. If you decide to withdraw, you have the option of removing your data from the study. You may also refuse to answer any questions you don't wish to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so or it becomes unsafe for you to continue.

**WILL I FIND OUT ABOUT THE STUDY RESULTS?**

All participants will be given the opportunity to contact the Primary Investigator (Ada Tang) at the end of the study to receive a summary of the study results.

**WILL THERE BE ANY PAYMENT OR REIMBURSEMENT IF I PARTICIPATE IN THIS STUDY?**

If you agree to take part in this study, you will receive an honorarium of $20.00 in order to cover parking and transportation expenses for the 2 visits involved in this study. If you decide to drop out of the study, your parking and transportation costs will be paid for the visits you attended.

**HOW WILL MY DATA REMAIN SECURE AND MY CONFIDENTIALITY BE MAINTAINED?**

Your data will not be shared with anyone except with your consent or as required by law. All personal information will be removed from the data and will be replaced with a number. A list linking the number with your name will be kept in a secure place, separate from your file. The data will be securely stored in a locked office. 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 without your specific consent to the disclosure.

**WHO CAN I CONTACT IF I HAVE ANY QUESTIONS OR PROBLEMS?**

At any time, if you have questions about the research, if you wish to withdraw from the study, or if you think you have a research-related injury, please contact the Primary Investigator, Dr Ada Tang by email at atang@mcmaster.ca or by phone (905) 525-9140 x27818.

This study has been reviewed and received ethics clearance through the Research Ethics Board of Hamilton Health Sciences/McMaster University Faculty of Health Sciences. If you have any questions regarding your rights as a research participant, you may contact the Office of the Chair of Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at (905) 521-2100 x42013.

# Consent Form

## SIGNATURE OF PARTICICIPANT/LEGALLY-AUTHORIZED REPRESENTATIVE

| | |
|---|---|
| **Title of Study** | Understanding the relationship between measures of vascular function with cardiovascular health and mobility in individuals living in the community after stroke |
| **Primary Investigator** | Ada Tang PT PhD, School of Rehabilitation Science |
| **Co-Investigators** | Maureen MacDonald PhD, Department of Kinesiology<br>Julie Richardson PT PhD, School of Rehabilitation Science |

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study involving the procedures described above, with an understanding of the known possible risks that might occur. I understand that I will receive a signed copy of this form.

_____

Participant Name Participant Signature Date

**Consent form administered and explained in person by:**
I confirm that I have explained the nature and purpose of the study to the participant name above. I have answered all questions. I believe the participant has the legal capacity to give informed consent to participate in this research study.

_____

Name and title Signature Date

**Signature of Principal Investigator**
I have delegated the informed consent discussion to _____

_____

Primary Investigator Name Primary Investigator Signature Date

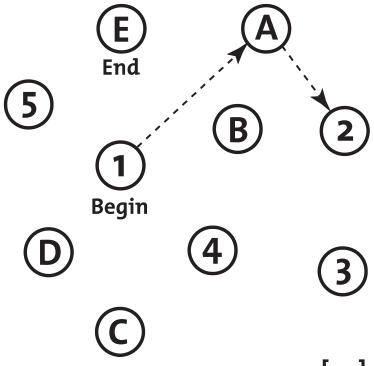
## Appendix 3

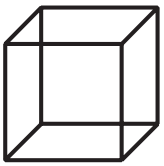
## MONTREAL COGNITIVE ASSESSMENT

## MONTREAL COGNITIVE ASSESSMENT (MOCA)

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

**VISUOSPATIAL / EXECUTIVE**



Copy cube

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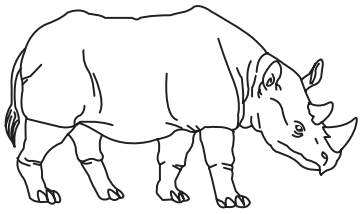
[ ] Contour [ ] Numbers [ ] Hands

POINTS __/5

**NAMING**

[ ]



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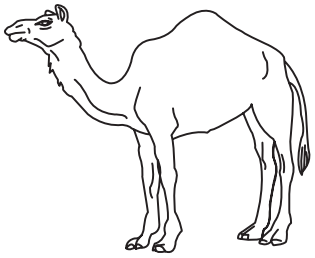


[ ]

__/3

**MEMORY** Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

| | FACE | VELVET | CHURCH | DAISY | RED |
|---|---|---|---|---|---|
| 1st trial | | | | | |
| 2nd trial | | | | | |

No points

**ATTENTION** Read list of digits (1 digit/ sec.).
Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 __/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

**LANGUAGE** Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) __/1

**ABSTRACTION** Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler __/2

**DELAYED RECALL**

| | Has to recall words WITH NO CUE | FACE [ ] | VELVET [ ] | CHURCH [ ] | DAISY [ ] | RED [ ] | Points for UNCUED recall only |
|---|---|---|---|---|---|---|---|
| Optional | Category cue | | | | | | |
| | Multiple choice cue | | | | | | |

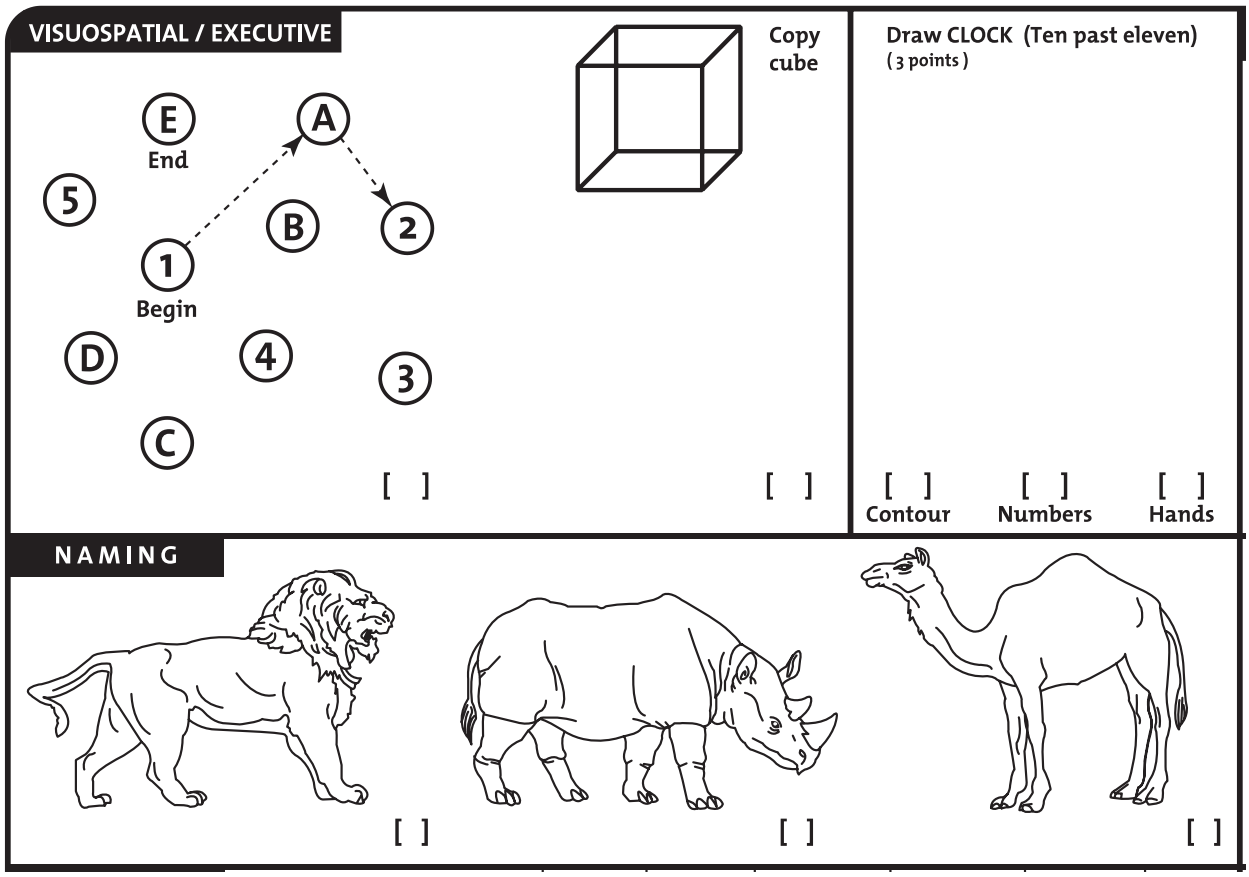
__/5

**ORIENTATION** [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

 Version November 7, 2004
www.mocatest.org

Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu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## Appendix 4

## MISSING OUTCOME DATA

**Table A5.** Missing Data for Participants with Stroke

| Participant | crPWV (m/s) | | | | cfPWV (m/s) | | | | ffPWV (m/s) | | | | Compliance ($mm^2/mmHg$) and Distensibility ($mmHg^{-1}$) | | | | FMD (%) | | | | NTG (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | V1 | | V2 | | V1 | | V2 | | V1 | | V2 | | V1 | | V2 | | V1 | | V2 | | V1 | | V2 | |
| | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US | AS | US |
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| 17 | | | | | | | | | | | | | | | | | | | | | | | | |

**Abbreviations** crPWV, carotid-radial pulse wave velocity; cfPWV, carotid-femoral pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; NTG, nitroglycerin; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; V1, visit 1; V2, visit 2; AS, affected side; US, unaffected side

**Table A6.** Missing Data for Participants without Stroke

| Participant | crPWV (m/s) | | cfPWV (m/s) | | ffPWV (m/s) | | Compliance ($mm^2/mmHg$) and Distensibility ($mmHg^{-1}$) | FMD (%) | NTG (%) |
|---|---|---|---|---|---|---|---|---|---|
| | V1 | V2 | V1 | V2 | V1 | V2 | | | |
| 1 | | | | | | | | | |
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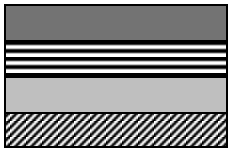
**Abbreviations** crPWV, carotid-radial pulse wave velocity; cfPWV, carotid-femoral pulse wave velocity; ffPWV, femoral-foot pulse wave velocity; FMD, flow-mediated dilation; NTG, nitroglycerin; m/s, meters/second; mm, millimeters; mmHg, millimeters of mercury; V1, visit 1; V2, visit 2

**Table A5 Legend**



Bad signal or image quality
Participant factor (physiological factors or did not wish to participate)
Equipment issues
Operator issues

**Table A6 Legend**

Bad signal or image quality
Participant factor (physiological factors or did not wish to participate)
Not assessed (testing took place prior to introducing between-side measurements in older adults without stroke as a study objective)