Vascular Aging:

Influences on cerebral blood flow and executive function

by

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Author’s Declaration

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

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Abstract

An age-related decline in cerebral blood flow (CBF) is widely acknowledged. However, uncertainty exists as to whether this reduction is the result of a reduced metabolic demand (cerebral atrophy) or an impaired delivery system (cerebrovascular disease). The purpose of these experiments was to examine the relationship of CBF and dynamic cerebrovascular regulation with changes in common carotid intima-media thickness (cIMT), brachial-ankle pulse wave velocity (baPWV) and common carotid distensibility. Additionally, we took an exploratory view into the effect of vascular aging and CBF reduction on brain function, as expressed through the performance of motor and cognitive tasks.

An important finding in elderly participants was that seated anterior CBF declined as a function of arterial stiffness, independently of age. Linear regression analysis developed a model that predicts CBF drops 22 ml/min (95% confidence interval (CI): 6, 38) for each 100 cm/s increase in baPWV and 8 ml/min (95% CI: 1, 15) for each additional year in age. The effect of baPWV appears to be mediated through an increase in cerebrovascular resistance ($r^2 = 0.84$, $p < 0.0001$). Additionally, CBF showed postural dependency and the volume of the drop in CBF between supine and seated positions was greatest in elderly participants (YOUNG: 65 ± 81 ml/min; ELDERLY: 155 ± 119 ml/min; $p = 0.001$). Despite this negative impact of vascular aging on steady state flow, dynamic regulation does not appear to be affected. Cerebrovascular responses to an acute drop in blood pressure or to activation of the motor cortex were not attenuated in the elderly participants. Finally, seated CBF had modest directionally-relevant relationships with perceptuo-motor and complex sequencing processes; while cIMT appeared to influence performance on initiation and inhibition tasks.

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List of Abbreviations

3MS	Modified Mini-Mental State Examination
ACT	active period of handgrip test
baPWV	brachial-ankle pulse wave velocity
CBF	cerebral blood flow
cDa	common carotid distensibility
cIMT	common carotid intima-media thickness
CO_2	carbon dioxide
CPP	cerebral perfusion pressure
cRT	choice reaction time
CVRi	cerebral vascular resistance index
D	dominant hand
DBP	diastolic blood pressure
gngRT	go-no go reaction time
GPT	Grooved Pegboard Test
HR	heart rate
ICA	internal carotid artery
MAP	mean arterial pressure
MBF	mean blood flow
MCA	middle cerebral artery
MCI	mild cognitive impairment
MFV	mean flow velocity
NAD	nadir (lowest point of MAP during sit test)
ND	non-dominant hand
PAD	peripheral artery disease
$P_{ET}CO_2$	end tidal partial pressure of carbon dioxide
PP	common carotid pulse pressure
PRO	prolonged active period of handgrip test
Q	cardiac output
REST	resting period of handgrip test
RMT	Recognition Memory Test
SBP	systolic blood pressure
SIT	seated posture
sRT	simple reaction time
SUP	supine posture
TCD	transcranial Doppler ultrasound
TRAILS	Trail Making Test
VA	vertebral artery
Xe^{133}	xenon 133

1.0 Literature Review

1.1 Introduction

Advancing age is a common factor in the development of numerous pathologies involving the vascular and cerebral domains. The incidence of hypertension (Franklin *et al.*, 1997), atherosclerosis (Ross, 1999), stroke (Williams, 2001), mild cognitive impairment (MCI) (Lopez *et al.*, 2003), and impaired motor function (Smith *et al.*, 1999;Houx & Jolles, 1993) all increase during old age. Recent literature has suggested that, in some cases, these changes are related.

Cerebral blood flow (CBF) is known to decline as humans age (Albayrak *et al.*, 2007;Scheel *et al.*, 2000). Some researchers have suggested that the age-related drop in CBF is a function of cerebral atrophy and reduced metabolic demand (Albayrak *et al.*, 2006)), while others have shown that vascular risk factors, such as hypertension, is associated with CBF decline beyond what is observed in the healthy elderly (Beason-Held *et al.*, 2007). Adding to the confusion is conflicting evidence with respect to the characterization of age-related changes in dynamic cerebrovascular regulation. While some have suggested that regulation is impaired (Orlandi & Murri, 1996), others suggest that it is maintained (Rosengarten *et al.*, 2003).

Changes in CBF and cerebrovascular health provide a promising paradigm from which to study brain function; however most studies to date have examined populations with overt vascular disease (Waldstein *et al.*, 2003) or cognitive impairment (Albayrak *et al.*, 2006) making it difficult to identify causation. Changes within the arterial system have been shown to have predictive capabilities with respect to the development of atherosclerosis (Oliver & Webb, 2003) and carotid plaques (Harloff *et al.*, 2006). This presents the possibility that structural and functional changes within the central vasculature could be used to identify

preclinical changes in the downstream cerebral vasculature prior to expression of cerebrovascular disease and any associated cognitive or motor dysfunction.

1.2 The Microvascular-Subcortical Syndrome of Aging

Pugh and Lipsitz (2002) have recently proposed a model that suggests cardiovascular risk factors affect brain function independently of age. Specifically, they hypothesize that cerebral microvascular disease leads to frontal lobe, sub-cortical lesions secondary to periodic bouts of ischemia. The assertion that the sub-cortical area is at particular risk is valid because much of it lies in a "watershed" region where capillary density is low, making it susceptible to even small reductions in perfusion.

This theory is consistent with findings associating long term hypertension with a drop in CBF (Beason-Held *et al.*, 2007) and cerebral atrophy (Firbank *et al.*, 2007). Beason-Held *et al.* monitored CBF over the course of 7 years in hypertensive patients and healthy controls as part of the Baltimore Longitudinal Study of Aging. They reported that hypertensives had larger decreases in regional CBF, as measured using positron emission tomography, to the prefrontal, anterior cingulate and motor areas compared to healthy controls over that time. Firbank *et al.* monitored hypertensive patients and normotensive controls over 4 years and found a direct relationship between baseline systolic blood pressure (SBP) and whole brain atrophy. Waldstein and colleagues (2003) reported on a relationship between peripheral artery disease (PAD) and performance on a range of neuropsychological tests. PAD patients demonstrated lower performances on manual dexterity, perceptuo-motor speed, executive function, non-verbal memory, and concentration tasks. A continuum was demonstrated such that PAD patients performed worse on these tasks than hypertensive patients, who in turn performed

worse than normotensive individuals. Still, few studies have examined pre-clinical measures of vascular health for relationships with CBF and brain function.

1.3 Cerebral Blood Flow

CBF has traditionally been a difficult and expensive variable to measure. Invasive procedures, such as xenon^{133} (Xe^{133}) clearance (Bishop *et al.*, 1986), and expensive equipment, such as that needed for functional magnetic resonance imaging (Du *et al.*, 2006), have limited the number and extent of studies in this area. Over the 25 years, many researchers have turned to the use of transcranial Doppler (TCD) ultrasound – a non-invasive procedure demonstrated by Aaslid *et al.* (1982). TCD can be used to measure mean blood flow velocity (MFV), but flow volume is unfeasible since vessel diameter cannot be determined non-invasively. Bishop *et al.* (1986) found that TCD-derived MFV did not correlate well with absolute CBF as determined using the Xe^{133} clearance technique. However, they did find that TCD was a valid method for studying relative changes in CBF under hypercapnic conditions. More recently, Serrador and others (2000) used magnetic resonance imaging to show that the diameter of the middle cerebral artery (MCA) does not change in response to simulated orthostasis (lower body negative pressure) or increased partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) despite changes in flow velocity. Additionally, Valdueza and colleagues (Valdueza *et al.*, 1997) have used a similar technique to show a constant diameter of the MCA during hyperventilation. These findings demonstrate that changes in MFV should reflect changes in blood flow within the MCA, under specific conditions, and validate TCD as a technique in the measurement of regional CBF.

Recently, Albayrak *et al.* (2007) and Scheel *et al.* (2000) have reported age-related declines in global supine CBF in healthy populations using a new, non-invasive protocol. This

protocol involves ultrasound imaging of the four extracranial blood vessels supplying blood to the brain – the right and left internal carotid arteries (ICA) and vertebral arteries (VA). This new methodology has a high potential for use because it is non-invasive, relatively inexpensive and portable, and measures quantitative flow rather than relative flow.

In addition to the previous findings that CBF declines with age, this technique has been used to find associations between CBF and MCI (Maalikjy *et al.*, 2005), as well as dementia (Albayrak *et al.*, 2006). In particular, Maalikjy and others (2005) reported that CBF disparities within a group of MCI patients had predictive information with respect to the progression of their condition to Alzheimer's disease. Of note, all of these studies have involved the participants lying supine during CBF measurement. CBF in the upright posture is clearly a void in the literature that needs to be addressed.

1.4 Dynamic Cerebrovascular Regulation

CBF accounts for a significant portion of the cardiac output (Q; ~15%) and whole body oxygen uptake (~20%) at rest (Mchedlishvili, 1986). This substantial flow is necessary because brain tissue does not have any significant energy store and must rely on the supply of carbohydrates and oxygen provided in the blood. A disturbance in blood supply, of even 1-2 minutes, has been associated with brain tissue damage (Mchedlishvili, 1986). Thus, not only is total CBF critical for the maintenance of brain function, but so is dynamic regulation of blood flow. Dynamic cerebral vascular regulation impairment has been related to brain lesions. Fu and others (2006) found a strong relationship between reduced acetazolamide-induced cerebral reactivity and white matter lesions in both periventricular and deep subcortical regions of the brain. However, this was a cross-sectional design and could not identify whether the cerebrovascular impairment led to the development of lesions or the contrary.

CBF is directly related to cerebral perfusion pressure (CPP) and inversely related to cerebrovascular resistance. CPP is the pressure gradient between mean arterial pressure (MAP) and intracranial pressure; however, because intracranial pressure cannot be measured non-invasively, a cerebral vascular resistance index (CVRi) is commonly used to relate CBF to MAP. CVRi relies on the assumption that intracranial pressure is negligible and changes in MFV reflect changes in CBF (Aaslid *et al.*, 1989).

$$CBF = MAP/CVRi \quad \text{(Equation 1.5.1)}$$

Thus, CBF relies on the systemic regulation of MAP and local regulation of resistance – affected through changes in vessel diameter.

From equation 1.5.1, we see that we can characterize dynamic regulation by modifying MAP and monitoring the response in CBF or MFV. One method of modifying MAP is through postural shifts. For example, moving from a supine to an upright position induces an orthostatic stress which pools blood in the lower extremities. The diminished venous return limits stroke volume and MAP subsequently falls – referred to as orthostatic hypotension (Rowell, 1993). Thus, the maintenance of CBF is regularly challenged throughout the day by changes in posture.

Cerebral autoregulation refers to the ability of the brain's vascular network to modulate resistance over a wide range of blood pressures in order to maintain flow (Paulson *et al.*, 1990). In healthy individuals, autoregulation allows for the maintenance of CBF with MAP ranging from ~60 to ~150 mmHg. As MAP drops, cerebral arterioles dilate to reduce resistance and maintain flow. As MAP rises, the reverse occurs – cerebral arterioles constrict to increase resistance and prevent increases in flow. If pressure rises acutely past the threshold for autoregulation, the ability of the arterioles to constrict is overridden and blood flow increases.

However, in hypertension, when blood pressure rises gradually, the limits of autoregulation are shifted upwards as well, so there is a new operating point (Paulson *et al.*, 1990). Studies that modify blood pressure to test the autoregulatory index typically monitor MFV through insonation of the MCA. Lipsitz and colleagues (2000) and Carey and others (2000) observed that elderly and hypertensive patients are able to maintain MFV in response to an acute drops in blood pressure. This finding suggests maintained cerebral autoregulation in elderly and hypertensive individuals. This autoregulation is achieved through changes in resistance of cerebral microvessels (Paulson *et al.*, 1990).

Cerebral vascular resistance is regulated locally by the vasomotion of the cerebral arterioles and capillaries. Vascular smooth muscle surrounding the arterioles, and contractile pericytes surrounding capillaries, are influenced by hemodynamic properties such as flow and pressure, as well as arterial carbon dioxide (CO_2) levels, and metabolites produced by surrounding neurons and glial cells (Girouard & Iadecola, 2006). Tight neurovascular coupling is present in healthy individuals, which ensures blood flow is adequately matched to the metabolic rate of the neighbouring neurons. Uncoupling of this relationship would result in impairment of substrate delivery and by-product removal which would be damaging to the brain tissue.

In functional experimental designs, insonation of the basal cerebral vessels are monitored for the MFV response to cerebral activation. Examination of MFV in the posterior cerebral artery, during a presentation of visual stimuli (Zaletel *et al.*, 2005), and the middle cerebral artery (MCA) during motor tasks (Orlandi & Murri, 1996;Kelley *et al.*, 1993), are examples. Zaletel and others (Zaletel *et al.*, 2005) reported an age-related attenuation of the cerebral MFV-to-evoked potential ratio in the posterior cerebral artery. Orlandi and Murri

(1996) noted that the elderly exhibited a smaller rate of increase and smaller amplitude change of MFV in the MCA while performing a light handgrip task as compared to young individuals. While these studies suggest an age-induced impairment in cerebrovascular responses to cerebral activation, Rosengarten and others (2003) reported no change in the MFV response during a visual stimulus with elderly, as compared to a younger population. Each study reported using healthy subjects but did not report specific exclusion criteria except for the presence of stenosis or prior cerebrovascular/cardiovascular events, making it difficult to suggest reasons for the discrepancy. Groschel *et al.* (2007) demonstrated reduced functional hyperemia in response to an arithmetic challenge in an elderly population with hypertension and hyperlipidemia but not in a healthy elderly group. This indicates that the presence or absence of cardiovascular risk factors must be clarified in the study sample, as they might confound the results.

1.5 Vascular Structure and Function

Age-related structural and functional changes occurring in the central vasculature have been shown to be predictive of future pathology. One structural indicator is common carotid intima-media thickness (cIMT). Thickness is defined as the distance from the luminal intima to the adventitial media of the vascular wall (Cheng *et al.*, 2002). This measure has been commonly used as an early detection tool for atherosclerosis and has been closely linked to the development of PAD (Allan *et al.*, 1997). In a longitudinal study of 1288 men, Salonen and Salonen (1991) reported intima-medial thickening was associated with 2.17-fold risk for myocardial infarction.

Arterial stiffness is a measure of vascular function and plays a role in how pressure and flow waves are transmitted along the arterial tree. Like cIMT, the link between arterial stiffness

and atherosclerotic risk has been demonstrated (Fujiwara *et al.*, 2004). There are multiple definitions with respect to how arterial stiffness is expressed in the literature (O'Rourke *et al.*, 2002). Distensibility is defined as the relative change in vessel area for a given pressure increment (typically pulse pressure). Pulse wave velocity is defined as the speed that a pulse wave travels along a specific arterial segment.

A decrease in distensibility and an increase in pulse wave velocity are consistent with increased vessel stiffness. Although the later is the hallmark measure of arterial stiffness (O'Rourke *et al.*, 2002), advances in the validity and reliability of non-invasive techniques to measure vessel diameter and local pulse pressure have resulted in an increased use of distensibility, particularly with the carotid artery (Laurent *et al.*, 1994;Steinback *et al.*, 2005). Carotid artery distensibility (cDa) holds special significance because of the role of local baroreceptors in the regulation of blood pressure. Pulse wave velocity is typically characterized using either the central vasculature (carotid-femoral pulse wave velocity), alone, or a combination of central and peripheral blood vessels (brachial-ankle pulse wave velocity; baPWV).

Many studies have focused on linking a solitary measure of vascular structure or function to target organ dysfunction. However, Kobayashi *et al.* (2004) have recently demonstrated the importance of incorporating multiple measures. When they classified individuals into tertiles for baPWV and cIMT, as well as flow-mediated dilation, individuals who were in the top tertile for all three variables were at a significantly higher risk for atherosclerosis and carotid plaques than individuals who were in none or only one of the categories.

Structurally, intima-media thickening impacts vessel properties by reducing lumen diameter. Functionally, arterial stiffening impacts vessel properties by speeding the return of reflected pressure waves from down stream resistance vessels and limiting the vessels ability to cushion high pressures. Clearly, these structural and functional alterations to central arteries would impact the dynamics of blood flow through the vessels.

In addition to their influence on hemodynamic properties, central vascular changes might be indicative of cerebrovascular health secondary to their association with inflammatory processes (Mattace-Raso *et al.*, 2004;Yasmin *et al.*, 2004). Inflammation is associated with endothelial dysfunction or impaired nitric oxide production (Vanhoutte *et al.*, 2005) – the primary mechanism controlling vasomotor action in the cerebral microvasculature (Girouard & Iadecola, 2006;Faraci & Heistad, 1991).

In a recent review on cardiovascular aging, Lakatta and Levy (2003) proposed that perhaps aging itself is not a disease, nor are the "age-associated" diseases solely a function of time-dependent exposure to pathological stimuli, but that normal "healthy" structural and functional changes that occur in the vasculature with aging provide a framework on which disease mechanisms can take root. While their hypothesis was aimed toward the development of cardiovascular disease, the same paradigm might be used to explain cerebrovascular disease and subsequent cognitive processing disorders. That is, age-related structural and functional changes might act as a catalyst for cerebral pathology to flourish if other conditions are appropriate. Importantly, these age-related vascular changes do not require the presence of atherosclerosis or hypertension, and therefore, should not be considered to be outright pathological in nature (Virmani *et al.*, 1991). However, the extent of these age-related changes is highly variable, and individuals who experience more extensive change are at higher risk for

development of cardiovascular pathology (Lakatta & Levy, 2003). This suggests that examination of the structural and functional characteristics of the central vasculature could provide insight into preclinical changes in the cerebrovascular system and relationships with the changes in motor and cognitive function that might follow.

2.0 Thesis Experiment

2.1 Purpose

The purpose of this study was to take an exploratory view into the relationship of vascular structure and function with cerebral blood flow (CBF), the dynamic regulation of CBF and the performance on motor and cognitive tasks requiring executive function.

Hypothesis

We hypothesized that age-related decline in CBF would be exaggerated in the upright position as a function of a change in vascular health, and that dynamic regulation of CBF in response to an acute drop in blood pressure and in response to an activation of the motor cortex would be attenuated in the elderly participants who had increased arterial stiffness and vessel wall thickness. Finally, we believed that, within the elderly participants, reduced CBF and elevated markers of vascular change would be associated with a decline in brain function. Specifically, a reduction in anterior CBF, supplying blood to the frontal lobe, would be associated reduction in cognitive and motor function. It is expected that more complex tasks (such as go-no go reaction time or Trail Making Test B) will be more greatly affected as they rely more on neuronal communication between multiple brain areas, which are joined by the susceptible sub-cortical networks in the “watershed” area of the brain.

2.2 Methods

Study Population

Thirty-four young and 20 elderly, functionally-independent men and women were recruited to participate in this study. Their physical characteristics are listed in Table 2.2.1. Young participants were recruited from the undergraduate student body in the Faculty of Applied Health Sciences at the University of Waterloo. Elderly participants were recruited from 2 separate retirement communities, as well as from the public community-at-large. One of the retirement communities was an Oakwood retirement community and linked with the University of Waterloo through the Research Institute of Aging. Each participant provided written, informed consent to participate in this study. Consent was obtained from the elderly candidates' power of attorney, when appropriate. All procedures and measurements were reviewed and cleared through the Office of Research Ethics at the University of Waterloo.

Participants were acquainted with the testing procedures in a preliminary orientation session at the time informed consent was obtained. Participants completed a Health Status Form to identify pre-existing cardiovascular, neurological, and psychomotor pathology and/or other chronic impairments, as well as their current medications and level of hormone supplementation. Grouped medical history and medications are listed in Table 2.2.2. Additionally, elderly participants completed the Waterloo Handedness Questionnaire – a 20-item survey assessing hand preference (Brown *et al.*, 2006) – and the Modified Mini-Mental State examination (3MS) – a general assessment of cognitive function (Teng & Chui, 1987). One elderly participant was left-handed and all scored at least 81 on the 3MS suggesting the absence of dementia (Bland & Newman, 2001).

Participants were given specific instructions to follow prior to the cerebrovascular testing to help eliminate confounding variables. They were asked to abstain from alcohol for 8 hours (Kawano *et al.*, 1992), as well as caffeine (Mahmud & Feely, 2001), nicotine (Kim *et al.*, 2005), food (Lipsitz *et al.*, 1983), and exercise (Kingwell *et al.*, 1997;DeVan *et al.*, 2005) for at least 3 hours prior to the experimental trial. Elderly participants were permitted to eat a light breakfast (e.g. piece of toast, fruit juice) the morning of the test for personal comfort. Young female participants were asked to schedule testing within the first 7 days of their menstrual cycle (Hayashi *et al.*, 2006).

For most subjects, testing was completed in the Cardiorespiratory and Vascular Dynamics Laboratory at the University of Waterloo. Elderly participants living in retirement communities were tested in a private room at their facility to accommodate travel concerns. Room temperature, barometric pressure, and relative humidity were monitored to maintain similar comfort levels between the 2 sites.

Experimental Protocols

The protocol was divided into 3 sessions, each taking place in the morning of a separate day. Cardiovascular/cerebrovascular testing, motor function testing and cognitive function testing were performed on separate days.

Cerebral Blood Flow and Vascular Characteristics

Upon arriving, participants were seated in a hi-back chair for measurement of seated CBF. After a period of acclimatization (~10 minutes) to a supine position, CBF, brachial-ankle pulse wave velocity (baPWV), common carotid distensibility (cDa), and common carotid intima-media thickness (cIMT) were measured.

Dynamic Cerebrovascular Regulation

Sit Test

The sit test measured the cerebrovascular system's ability to maintain CBF in response to an acute drop in perfusion pressure. As with the sit-to-stand protocol used by Lipsitz and colleagues (2000), this protocol is designed to simulate a practical orthostatic challenge. Participants completed a brisk transfer from a supine to a seated position. The transfer was researcher-assisted to help maintain similar effort and duration between trials and participants. Following the transition, participants maintained the seated position for 3 minutes. The postural change was repeated in triplicate, with each transition being separated by at least 3 minutes of supine rest.

Handgrip Test

The handgrip test measured the hemodynamic response to the neural activation associated with distal upper limb movements. The test was adapted from the handgrip protocol used by Orlandi and Murri (1996). Using their dominant hand, participants performed repeated contractions of a hand-held rubber bulb at 15 % of their maximum effort. The contraction rate was set at 60 Hz, which was maintained with the use of an auditory metronome. An analog scale provided visual feedback to the participants regarding the intensity of their grip. Each trial lasted 30 s and at least 30 s of rest was provided between trials. Participants were given a 3-s countdown at the beginning of each trial. This task was performed in triplicate.

Motor and Cognitive Function

A 5-item battery of neuropsychological tests was composed to test a variety of motor, cognitive and executive skills. The order of the battery was randomized for each participant.

Finger Tapping Test

Participants were instructed to repeatedly tap their extended index finger on a key as quickly as possible over a 10 s period. Finger tapping is an indicator of pure motor speed (Brown *et al.*, 2006). The task was repeated in duplicate on both the dominant and non-dominant hands with a 30-s rest period between each repetition to avoid fatigue. The starting hand was randomized to minimize the chance of a learning effect between trials. The number of keystrokes per second averaged over the 2 trials was used as the measure of performance.

Grooved Pegboard Tests

The standard "Place" task (Lafayette Instrument Co., 1989), as well as the alternate "Replace" task (Bryden & Roy, 2005) involving the Grooved Pegboard were used. The 2 tasks have been shown to measure different aspects of movement performance – manual dexterity and motor speed, respectively (Brown *et al.*, 2006). The "Place" phase involved inserting grooved pegs appropriately into slotted holes, as quickly as possible. The "Replace" phase involved returning the pegs to the receptacle, as quickly as possible. A modified version of the task was incorporated, such that only the first 2 rows (10 pegs) were used. This modification was used to eliminate possible confounding effects of fatigue and frustration with the elderly group. Each phase was repeated twice with the dominant hand and twice with the non-dominant hand. A 30-s rest period was provided between each trial. The starting hand was randomized to

minimize the chance of a learning effect between trials. The performance measure was the time taken to complete each trial. The lowest time of the 2 trials was used in analysis.

Reaction Time Tests

Simple (sRT), go-no go (gngRT) and choice (cRT) reaction time paradigms were tested using novel software programs (E-Prime, Psychology Software Tools Inc., Pittsburgh PA, USA). The 3 paradigms involved a familiar structure. Participants were instructed to respond to the target letter 'O' when it appeared on a laptop computer monitor. The target letter always appeared at some varied time interval following the appearance of the priming letter 'X'. The priming letter was present for 150, 300 or 450 ms prior to the appearance of the target letter. In the sRT paradigm, participants were asked to respond with their index finger each time the target letter appeared. In the gngRT paradigm, participants were instructed to respond with their index finger or withhold their response – depending on the colour of the target letter – each time the target letter appeared. Simple reaction time and gngRT were completed with the dominant and non-dominant hands in separate trials. In the cRT paradigm, participants were asked to respond with either their dominant or non-dominant index finger – depending on the colour of the target letter – each time the target letter appeared. Each reaction time test involved a set of 9 separate responses and was repeated in duplicate. An average of the second set of reaction times, which were preceded by a 300-ms priming signal, was used as the measure of performance.

Trail Making Test

The Trail Making Test (TRAILS) is a standardized neuropsychological test that assesses perceptuo-motor speed and mental flexibility. It is composed of 2 separate tests – A and B. TRAILS A required participants to draw a continuous line connecting a series of randomly

spaced numbers from 1 to 25 as quickly as possible. TRAILS B required a similar continuous line to be made, but with the connections alternating between a series of numbers and letters (e.g. 1-A-2-B-3…) (Spreen & Strauss, 1998). Each test was administered once. The amount of time needed to finish each test was used as a measure of performance.

Warrington's Recognition Memory Test

This Recognition Memory Test (RMT) is composed of 2 separate tasks: memory for words and memory for faces. Participants were required to remember sets of 50 words (verbal memory) or 50 faces (non-verbal memory) depending on the task. They were allowed to examine each item for 3 seconds and were asked to make a positive or negative association with each word/face. Participants were tested for retention by showing them each item again, paired with a similar distracter item, and asking them to identify the correct item. Each test was administered once. Performance is measured by the number of items participants were able to correctly identify (Warrington, 1984).

Data Acquisition and Analysis

Vascular Structure and Function

Common Carotid Intima-Media Thickness

Common carotid IMT was measured by brightness-mode (B-mode) echo ultrasonography with a 6-13 MHz, linear array transducer (HFL38, MicroMaxx, Sonosite Inc, Bothell WA, USA). Thickness was defined as the distance from the lumen-intimal border to the media-adventitial border. Longitudinal imaging of the common carotid artery allowed for cIMT measurement at a consistent site across participants. Each individual measurement took the average thickness

of a 1-cm long segment of the far vessel wall, 1-2 cm proximal to the carotid bulb (Figure 2.2.1). Echo ultrasonographic images were recorded in real time video (Sony, Tokyo, Japan) and digitally stored at 30 frames per second (Adobe Premiere, Adobe, San Jose CA, USA). Off-line analysis used a contrast-based edge detection software program (Dr. Hanif Ladak; Matlab, MathWorks, Natick MA, USA) to determine vessel wall thickness. Reported values were averages of triplicate measures from both the right and left vessels as suggested by Schmidt and Wendelhag (1999).

Common Carotid Distensibility

A combination of non-invasive ultrasound imaging and applanation tonometry was used to measure cDa. This allowed simultaneous measurement of pulse pressure (PP) in the left carotid artery and diameter changes in the right. Homogeneity between right and left vessels was assumed. M-mode imaging (a time-sensitive derivative of B-mode) tracked the diameter changes at a single point in the vessel wall over the course of a cardiac cycle (Figure 2.2.2). The vessel diameter was defined as the distance from the near media-adventitial border to the far media-adventitial border. Ultrasound images were collected and analyzed as described above. PP was measured by applanation tonometry using a hand-held probe with a Millar micromanometer incorporated into the tip (SPT-301, Millar Instruments, Houston TX, USA). Briefly, this technique flattens the curved surface of a vessel wall against a more dense underlying tissue. Flattening the vessel wall balances circumferential stresses so that the pressure recorded by the micromanometer reflects true intra-arterial pressure. To ensure a high quality signal, pulse wave contour recordings were assessed visually for PP and diastolic variation (O'Rourke *et al.*, 2001).

Heart rate (HR), arterial blood pressure and carotid pressures were sampled continuously at 1 kHz using data acquisition hardware (Powerlab, AD Instruments, Coloradio Springs CO, USA) and the associated software (Chart v5.4.2, AD Instruments, Colorado Springs CO, USA). Continuous data were blocked into beat-by-beat bundles (i.e., R-spike to R-spike). Raw carotid pressures were exaggerated secondary to the influence of hold down pressure. Therefore, mean and diastolic carotid pressures were calibrated against brachial mean and diastolic pressures (see Methodological Considerations). A corrected systolic carotid pressure was extrapolated from the calibrated slope. Pressure values and ultrasound images were collected on separate systems, thus R-R intervals from the 2 collection units were time-matched to ensure synchronicity of pressure and diameter fluctuations. Finally, cDa was expressed as the relative change in vessel area for a given change in pressure over the course of the cardiac cycle (O'Rourke *et al.*, 2002).

$$cDa = [(area_{sys} - area_{dia})/area_{dia}]/(pressure_{sys} - pressure_{dia}) \quad \text{(Equation 2.2.1)}$$

Reported values were averages of at least 10 consecutive beats.

Brachial-Ankle Pulse Wave Velocity

Arterial pressure wave contours were obtained directly from the brachial and posterior tibial arteries using applanation tonometry. Pulse waves at the antecubital fossa and posterior medial malleolus were measured sequentially. The maximum of the second derivative of the continuous pulse wave recordings was used to identify the foot of each wave (Kingwell *et al.*, 1997). For each site, the time from the R-spike in the QRS complex to the foot of the arterial pressure pulse wave was collected. The anatomical distance from the heart to each site was determined from a set of smaller measurements to obtain the most accurate path length of pulse wave travel for the calculation of velocity. The distances from the manubrium to the

antecubital fossa (dMB), from the manubrium to the xyphoid process (dMX), from the xyphoid process to the navel (dXN), and from the navel to the posterior medial malleolus (dNA) were measured using an inelastic tape. The estimated difference in vessel length between the brachial and ankle sites (baLENGTH) is equal to the distance from the manubrium to the medial malleolus minus the distance from the manubrium to the antecubital fossa.

$$baLENGTH = (dMX + dXN + dNA) - dMB \quad \text{(Equation 2.2.2)}$$

Brachial-ankle PWV was calculated as the estimated difference in vessel length divided by the difference in transit time between the brachial and posterior tibial sites.

$$baPWV = baLENGTH/time \quad \text{(Equation 2.2.3)}$$

Reported values were averages of at least 20 consecutive beats.

Global Cerebral Blood Flow

CBF values were obtained in seated and supine positions using non-invasive ultrasound imaging of the four extra-cranial vessels. The internal carotid artery (ICA) diameter and flow velocity were imaged 1-2 cm distal to the carotid bulb after the near and far vessel walls approached parallel (Figure 2.2.3) (Maalikjy *et al.*, 2005). The vertebral artery (VA) diameter and flow velocity were imaged at either the first or second inter-vertebral vessel segment (Figure 2.2.4) (Maalikjy *et al.*, 2005). Angle correction of velocity measures ranged from 55º to 70º for ICA measurements and 60º to 74º for VA measurements. Mean blood flow (MBF) through a vessel was equal to the product of the time-averaged mean of the mean flow velocity (MFV) and the vessel cross-sectional area.

$$MBF = MFV \times \pi(diameter/2)^2 \quad \text{(Equation 2.2.4)}$$

MFV values, averaged over ~10 consecutive cardiac cycles, were recorded in duplicate, and diameter measures in triplicate, for each vessel. These measures were then averaged for the

calculation of MBF. Anterior CBF is the sum of flow through the right and left ICA (equation 2.2.5), posterior CBF is the sum of flow through the right and left VA (equation 2.2.6), and total CBF is the sum of anterior and posterior CBF (equation 2.2.7).

Anterior CBF = Right ICA flow + Left ICA flow (Equation 2.2.5)

Posterior CBF = Right VA flow + Left VA flow (Equation 2.2.6)

Total CBF = Anterior CBF + Posterior CBF (Equation 2.2.7)

Dynamic Cerebral Vascular Regulation

During both the sit test and the handgrip test, HR, arterial blood pressure, MFV through the middle cerebral artery (MCA), cardiac output (Q), and partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) were collected continuously.

HR was monitored continuously by electrocardiogram (Pilot 9200, Colin Medical Instruments, San Antonio TX, USA) using a 3-electrode (MediTrace, Kendall LTP, Chicopee MA, USA) placement protocol (right and left arm, left leg).

MAP was estimated from continuous finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) on the middle finger of the participants' non-dominant hand. Briefly, a preprogrammed waveform filter estimated the brachial pressure waveform from the waveform observed within the distal finger artery. A glycerin column/pressure transducer corrected for any height-induced difference in hydrostatic pressure between the 2 sites. The height-corrected pressure was further validated by a systolic blood pressure-matching return-to-flow calibration using an automated arm cuff. The resulting estimation of brachial pressure has been shown to be accurate (mean difference ≤ 4 mmHg) and precise (standard deviation of the difference <8 mmHg) (Guelen *et al.*, 2003). MAP at the

level of the MCA when seated upright was calculated by subtracting an amount proportional to the distance between their heart and MCA (dHMCA).

$$MAP_{MCA} = MAP_{HEART} - dHMCA*0.78 \quad \text{(Equation 2.2.8)}$$

Where 0.78 is a factor equivalent to the ratio of the densities for blood and mercury (Finapres Medical Systems, 2003).

Q was estimated from a Modelflow algorithm, based on a self-adaptive model of aortic impedance and compliance, as well as peripheral resistance (Wesseling *et al.*, 1993). The algorithm used information derived from the arterial pressure wave contour in the distal finger to estimate Q (Finometer, Finapres Medical Systems, Amsterdam, the Netherlands). Estimation of aortic cross-sectional area and compliance relied on norms for weight, height, age, and gender, which reduces its accuracy (error = 19%), unless calibrated against thermodilution or another standard. However, this error is the result of a systematic overestimation, so the uncalibrated model is valid and reliable for detecting percent changes in cardiac output (Jansen *et al.*, 2001).

MFV through the non-dominant MCA was collected using transcranial Doppler ultrasonography (TCD) (Neurovision Transcranial Doppler Ultrasound Model 500V, Multigon Industries, Mt. Vernon, USA). Following TCD methods described by Aaslid and colleagues (1982), the MCA was insonated with a 2-MHz transducer. Briefly, the transducer was placed against the temporal window, above the skull's zygomatic arch and anterior to the ear's tragus, with a slightly forward orientation, which allowed for zero-angle insonation of the MCA. The MCA was insonated at a depth between 50 and 55 mm in all participants, consistent with the proximal M1 segment. Specific individual depths were based on the strength of signal. In accordance with Zwiebel and Pellerito (2005), traceability, velocity profile, strength of signal,

auditory pitch, and probe angle were used to confirm insonation of the MCA. The transducer was fixed in place using an adjustable headpiece (Marc 600, Spencer Technologies, Seattle WA, USA) to ensure that probe position and orientation was maintained over repeated trials. MFV collected from the MCA was a time-averaged mean of the outer envelope of the power spectrum, in accordance with normal practice.

Exhaled CO_2 was collected through a nasal canulla (Respan Products Inc., Erin, Canada) and measured using infrared absorption (Pilot 9200, Colin Medical Instruments, San Antonio TX, USA). $P_{ET}CO_2$ was calculated from the maximum CO_2 value of each breath.

HR, arterial blood pressure, Q, exhaled CO_2, and MFV_{MCA} were sampled continuously at 1 kHz using data acquisition hardware and software identified above. After blocking into beat-by-beat bundles, data were interpolated to provide second-by-second information (Matlab, MathWorks, Natick MA, USA). The repeated trials for each participant were then averaged together to create one data set for each participant.

Cerebral vascular regulation was examined differently in the sit test and handgrip test. In the sit test, the change in MFV for a given percent change in MAP at the level of MCA (Equation 2.2.9) was used as an indicator of how well flow is maintained in response to an acute pressure drop (Lipsitz *et al.*, 2000).

$$\text{Autoregulatory Index} = \Delta MFV_{MCA} / \%\Delta MAP_{MCA} \qquad \text{(Equation 2.2.9)}$$

In the handgrip test, the change in CVRi in response to activity was used as a criterion for evaluating cerebral vascular regulation. CVRi is defined by the relationship between MAP and CBF (or MFV) in the vessel of interest (Equation 1.5.1).

Three time points were selected for comparison in each test. In the sit test, supine (SUP), nadir (NAD) and sit (SIT) variables corresponded to the average of 30 s before

transition (-30 to -1 s), 3 s surrounding the MAP nadir, and 30 s at the end of recovery (91 to 120 s), respectively. Each participant's response was visually scanned to pick the appropriate NAD time point. In the handgrip test; resting (REST), active (ACT), and prolonged (PRO) variables corresponded to the average of 10 s before activity (-15 to -5 s), 3 s surrounding the time point immediately following the initial drop in CVRi, and 10 s at the end of the task (21 to 30 s), respectively. Each participant's response was visually scanned to pick the appropriate ACT time point.

Motor and Cognitive Function

Laterality

In motor testing, laterality provides an indicator of the relative differences between dominant (D) and non-dominant (ND) hands.

$$\text{Laterality} = (ND - D) / (ND + D) \times 100\% \qquad \text{(Equation 2.2.10)}$$

We have adapted the concept of laterality to fit both motor function and vascular health characteristics.

Measurement Success Rate

Data points for some variables were omitted from the analysis secondary to technological limitation. The success in obtaining specific vascular and hemodynamic measures is identified in Table 2.2.3. Estimated Q for 3 elderly participants exceeded the expected physiological range and was removed. A clear pulse wave signal was not obtained in the posterior tibial artery of 6 elderly participants, preventing the calculation of baPWV. Inaccurate measurement of flow velocity through the ICA due to plaque formation or tortuosity prevented anterior flow measurements in 5 elderly participants; while the VA was not clearly imaged in 8 elderly

participants. This limited the number of total flow data sets from the elderly participants to 12. Insonation of the MCA was successful in 60% of the elderly participants. The typical success rate for insonation of basal arteries in the elderly is ~66% (Ruitenberg *et al.*, 2005).

Statistical Analysis

All statistical analyses were completed using Statistical Analysis Software (SAS Institute, Cary NC, USA). Young and elderly participants' physical characteristics were compared using two-tailed t-tests. Within-group and between-group hemodynamic variables recorded over the course of the sit and handgrip tests were analyzed using two-way ANOVAs (age category x posture and age category x time, respectively). The least square means method was employed to determine main effects differences using the Tukey-Kramer adjustment for multiple comparisons. Multiple linear regression was used to examine the association between indicators of vascular aging on CBF. Simple linear regression was used to take a cursory view of the influence of vascular aging and CBF on the performance of tasks assessing motor and cognitive function. Differences were considered significant at $p < 0.05$ for all parameters.

Methodological Considerations

Applanation Tonometry

Applanation tonometry was a technique introduced to our laboratory with this research project and explained in further detail here. This technique relies on the principle that internal pressure of a container can be measured by placing a pressure transducer on a flattened portion of the container wall. Complete flattening beneath the head of the transducer is necessary to balance the circumferential force vectors created by the intra-arterial pressure (Kelly *et al.*, 1989).

Thus, placement of the transducer over the middle of the artery is important. Thick-walled arteries are somewhat challenging to applanate because they are more susceptible to rolling away when palpated (Perloff, 1990). To combat this, a free hand was used to stabilize the artery on either side of the Millar probe. Once the pulse was located, stabilizing pressure with the free hand was reduced. The carotid artery was palpated in the middle of the neck to avoid potential stimulation of the carotid baroreceptors, which could alter blood pressure through baroreceptor-mediated mechanisms (Perloff, 1990).

To obtain an appropriate measure of intra-arterial pressure, the external pressure applied through the transducer was adjusted to elicit the maximal systolic impact of the pulse wave. The amount of force required to applanate the vessel is dependent upon the surrounding anatomy, thus, values across participants will be subject to variable hold down pressure. Excessive hold down force was noted by a gradual increase in diastolic pressure or a deformation of the end-diastolic wave (i.e., sharp negative deflection) (Kelly *et al.*, 1989). The presence of this hold down force results in an over-estimation of the intra-arterial pressures. Consequently, the recorded data must be externally calibrated with photoplethysmograph data.

In traveling from the aorta to the radial artery, MAP and diastolic blood pressure (DBP) of the arterial pulse drop less than 1 mmHg (Kingwell *et al.*, 1997). This allows us to substitute the values of MAP and DBP estimated at the brachial artery for the signal representing these measures at the carotid artery. With this two-point calibration, systolic blood pressure (SBP) is obtained by extrapolating to the unknown peak on the carotid pulse wave. In a separate study, 8 participants were brought into the lab on 2 separate occasions to determine reliability of carotid PP measurements using this technique. Good correlation was demonstrated between the 2 sessions (CV = 4.23 %).

Doppler Ultrasonography

The ultrasound technique to measure MFV uses the Doppler shift theory. Briefly, ultrasonic waves transmitted through soft tissue are reflected, deflected, or absorbed depending on the properties of that tissue. When a wave hits a moving object, such as a red blood cell, the frequency of that wave is shifted in relation to the speed of the moving object. The system then compares the frequencies of the reflected waves to the transmitted waves and computes a velocity of the red blood cell using the following equation:

$$fR - fO = [2(fO)(Vel)(\cos(rad))]/c \qquad \text{(Equation 2.2.11)}$$

where, *fR* is the reflected frequency, *fO* is the transmitted frequency, *vel* is the velocity of the red blood cell, *rad* is the angle of insonation, and *c* is the speed of sound through human soft tissue (assumed to be 1540m/s) (Zwiebel & Pellerito, 2005).

Because the cos of the angle of insonation is used to compute velocity, this angle should ideally be below 60° to provide the most accurate measure of velocity (Zwiebel & Pellerito, 2005). As the angle approaches 90°, the stability of the "cos(rad)" decreases, thereby increasing the variability of the calculated velocity with only small changes in frequency shift.

Laminar blood flow is assumed in most vessels such that red blood cells near the vessel wall move slower, due to frictional forces, than do blood cells in the middle of the vessel (Zwiebel & Pellerito, 2005). As such, a range of Doppler-shifted frequencies will be received at any given time. The mean frequency shift provides a close approximation of the average velocity of the blood cells across the entire vessel. However, as the vessel lumen becomes smaller, the blood cells in the middle of the cell become closer to the wall and will subsequently be traveling at a velocity that is closer to the speed of those cells near the wall. In this case, the peak frequency shift provides a more accurate measure of the blood flow velocity

within smaller vessels. Common practice sees investigators use this peak value when quantifying blood flow velocity in the MCA.

Table 2.2.1 Participant Characteristics

	Young	Elderly	p-value
Age (years)	21 ± 2	79 ± 8 *	< 0.0001
Gender (male/female)	7/27	5/15	---
Height (cm)	167 ± 10	160 ± 9 *	0.0163
Weight (kg)	66 ± 12	66 ± 14	0.8221
HR (bpm)	60 ± 8	61 ± 11	0.6422
SBP (mmHg)	121 ± 8	149 ± 26 *	0.0001
DBP (mmHg)	66 ± 5	62 ± 11	0.2555
MAP (mmHg)	88 ± 6	94 ± 16	0.0968
PP (mmHg)	43 ± 8	73 ± 18 *	< 0.0001
Q (L/min)	5.2 ± 0.9	4.1 ± 1.3 *	0.0010
cDa ($mmHg^{-1}$)	$5.9x10^{-3} \pm 1.6x10^{-3}$	$1.7x10^{-3} \pm 0.5x10^{-3}$ *	< 0.0001
baPWV (cm/s)	783 ± 118	1153 ± 325 *	0.0006
cIMT (mm)	0.50 ± 0.07	1.00 ± 0.15 *	< 0.0001

HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; MAP – mean arterial pressure; PP – common carotid pulse pressure; Q – cardiac output; cDa – common carotid distensibility; baPWV – brachial-ankle pulse wave velocity; cIMT – common carotid intima-media thickness. Young (n = 34); Elderly (n = 20). All values are mean ± SD; * is significantly different from YOUNG, $\alpha = 0.05$.

Table 2.2.2 Participant Medical History and Medications

	Young	Elderly
DISEASE HISTORY		
High blood pressure	0	10
Heart disease	0	3
High cholesterol	0	3
Vascular	0	2
Diabetes	0	3
Transient ischemic attack	0	1
Tremors	0	2
Respiratory	5	6
MEDICATIONS		
ACE inhibitor	0	3
Diuretic	0	6
B-blocker	0	6
Vasodilator	0	2
Ca2+ channel blocker	0	5
Blood thinner	0	8
Anti-arrhythmic	0	3
Lipid lowering	0	1
NSAID	2	2
Proton pump inhibitor	0	4
Calcium antagonist	0	1
Hormone replacement	0	3
Anti-depressant	0	2
Cholinesterase inhibitor	0	1

ACE – angiotensin converting enzyme; NSAID – non-steroidal anti-inflammatory drug

Table 2.2.3 Success rate for cardiovascular measures in elderly population

Measure	Success Rate	
	Male	Female
baPWV	5/5 (100%)	10/15 (67%)
cDa	5/5 (100%)	15/15 (100%)
cIMT	5/5 (100%)	15/15 (100%)
ACBF	4/5 (80%)	11/15 (73%)
TCBF	3/5 (60%)	9/15 (60%)
MFV_{MCA}	4/5 (80%)	8/15 (53%)

baPWV – brachial-ankle pulse wave velocity; cDa – common carotid distensibility; cIMT – common carotid intima-media thickness; ACBF – anterior cerebral blood flow; TCBF – total cerebral blood flow; MFV_{MCA} – mean flow velocity in middle cerebral artery

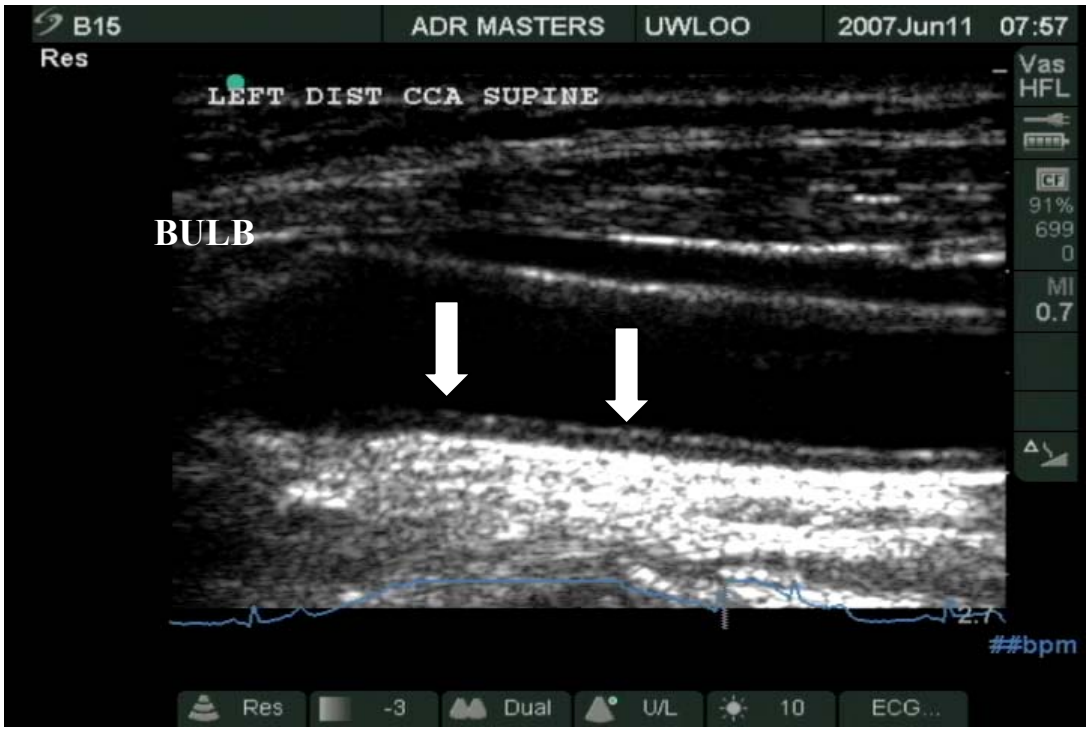

Figure 2.2.1 Longitudinal view of the common carotid artery
The distal segment of the common carotid artery was used for measurement of intima-media thickness. Longitudinal images were used to ensure measurement at a consistent location between participants. Values were calculated as the average thickness of an approximate 1-cm long segment of the vessel just proximal to the carotid bulb (between arrows). Thickness is measured as the distance from the luminal intima border to the adventitial media border. Right and left carotid vessels were measured in triplicate, and the mean of the 6 values was used for analysis.

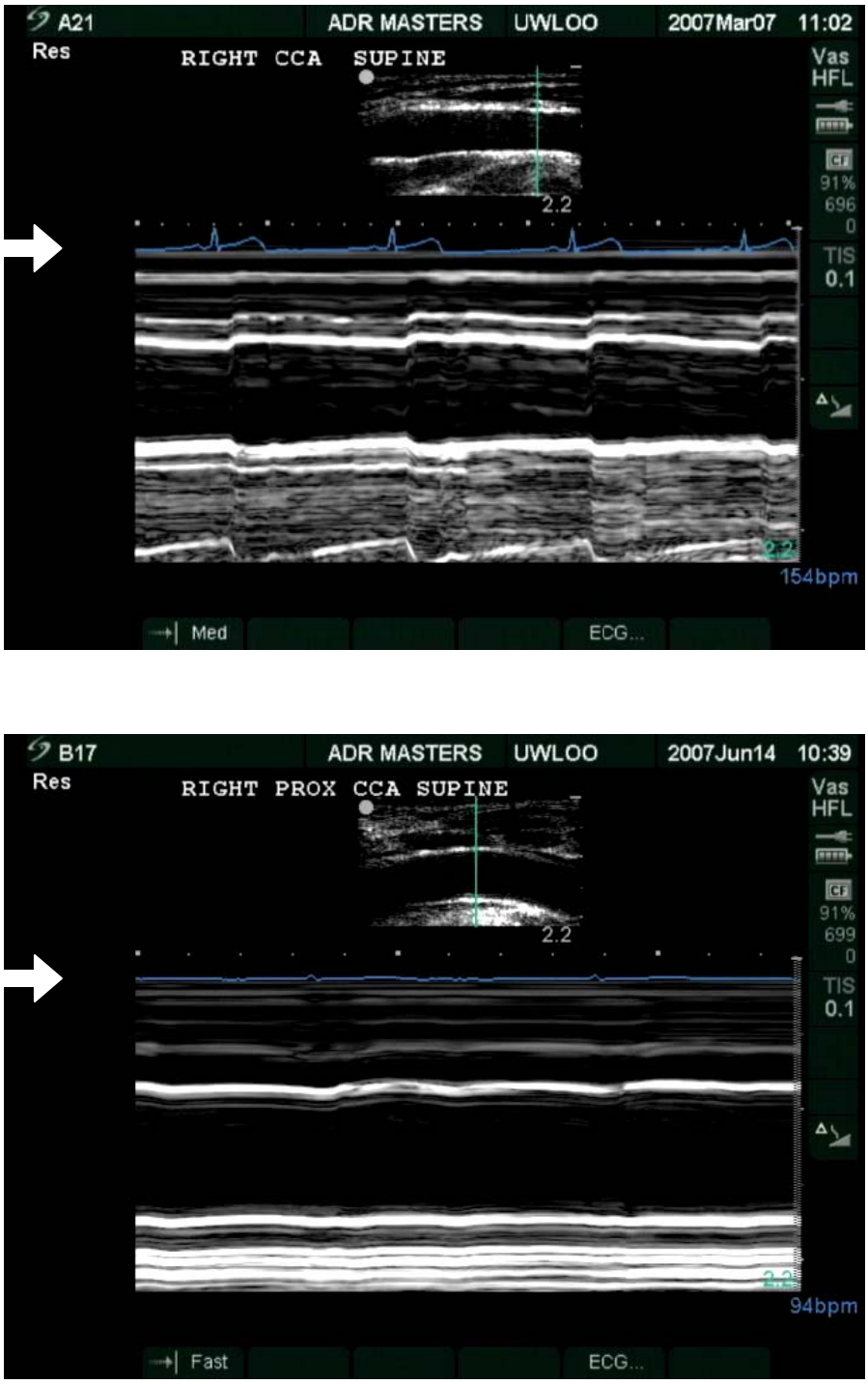

Figure 2.2.2 M-mode view of common carotid artery
M-mode ultrasound images of the distal common carotid artery of young (top) and elderly (bottom) individuals. M-mode ultrasound tracks the change in space of thin slice of pixels as a function of time, creating an image of the vessel's diameter change over the course of a cardiac cycle. Electrocardiogram trace is shown (arrow).

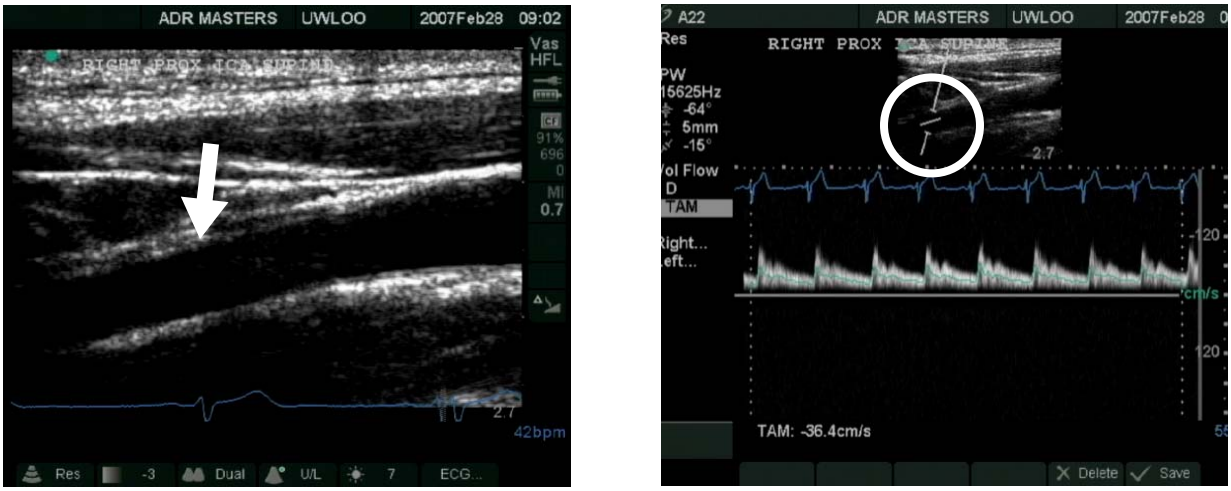

Figure 2.2.3 Diameter and velocity profile of an internal carotid artery
A longitudinal view (left) and velocity trace (right) of an internal carotid artery (ICA). Blood flow through the vessel is the product of cross-sectional area and mean flow velocity. Vessel diameter was measured 1 cm distal to the carotid bulb (arrow). The sample volume (circle) was placed such that velocity was obtained at the same location as vessel diameter was measured. The gate of the sample volume was increased to ensure that velocity was representative of the entire vessel. Blood flow through the right and left ICA were summed to obtain anterior cerebral blood flow.

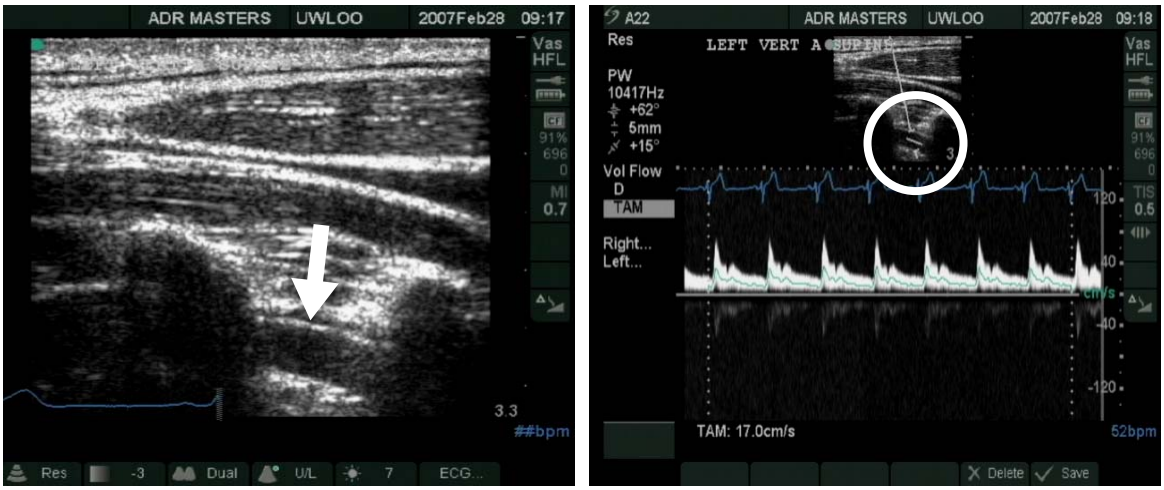

Figure 2.2.4 Diameter and velocity profile of a vertebral artery
A longitudinal view (left) and velocity trace (right) of a vertebral artery (VA). Blood flow through the vessel is the product of cross-sectional area and mean flow velocity. Vessel diameter was measured in the midpoint between two vertebrae (arrow). The sample volume (circle) was placed such that velocity was obtained at the same location as vessel diameter was measured. The gate of the sample volume was increased to ensure that velocity was representative of the entire vessel. Blood flow through the right and left VA were summed to obtain posterior blood flow.

2.3 Results

Subject Characteristics

Subject characteristics are listed in Table 2.2.1. Young and elderly participants were similar in weight, HR, MAP and DBP. Elderly individuals had reduced Q ($p = 0.001$) and cDa ($p < 0.0001$), as well as elevated SBP ($p = 0.0001$), PP ($p < 0.0001$), baPWV ($p = 0.0006$) and cIMT ($p < 0.0001$). Nine elderly participants were taking multiple medications for cardiovascular-related diagnoses (Table 2.2.2).

Global Cerebral Blood Flow and Postural Change

Steady state CBF values amongst young and elderly participants in supine and seated positions are listed in Table 2.3.1 and depicted in Figure 2.3.1. CBF was similar between young and elderly participants while lying supine (YOUNG: 937 ± 128; ELDERLY: 887 ± 191 ml/min; $p = 0.1173$). The percentage of total flow through the bilateral ICA to the anterior brain was ~75% in both the young and elderly participants ($p = 0.7781$). However, young participants had equal flow to the right and left hemispheres and the elderly participants tended to have more lateralized flow (percent of total flow to right-hemisphere – YOUNG: 50 ± 7; ELDERLY: 46 ± 8 %; $p = 0.0200$).

Total CBF was noted to drop in both groups after moving to a seated position. In young participants, this change was the result of a drop in anterior flow between supine (SUP) and seated (SIT) positions (SUP: 703 ± 108; SIT: 646 ± 102 ml/min; $p = 0.0051$). In the elderly, a drop was noted in both anterior flow (SUP: 666 ± 164; SIT: 552 ± 134 ml/min; $p = 0.0001$) and posterior flow (SUP: 220 ± 68; SIT: 173 ± 66 ml/min; $p < 0.0001$). The drop in anterior blood

flow was significantly greater in the elderly (YOUNG: -56 ± 95; ELDERLY: -114 ± 89 ml/min; $p = 0.0481$), which helped to induce a distinct difference in total CBF between young and elderly participants (YOUNG: 872 ± 129; ELDERLY: 732 ± 168 ml/min; $p < 0.0001$) in the seated position.

Breakdown of the anterior CBF data shows that age-related differences exist in both ICA velocity and cross-sectional area. MFV was slower in the elderly participants (seated – YOUNG: 32 ± 6; ELDERLY: 23 ± 7 cm/s; $p < 0.0001$), while cross-sectional area was enlarged (seated – YOUNG: 17 ± 4; ELDERLY: 20 ± 6 mm^2; $p < 0.0001$). Trends within each group towards a slower MFV (YOUNG: $p = 0.2335$; ELDERLY: $p = 0.3191$) and smaller cross-sectional area (YOUNG: $p = 0.4394$; ELDERLY: $p = 0.0363$) were noted as participants moved from a supine to a seated position.

When the elderly group was stratified – with individuals exhibiting a vascular trait (cIMT, cDa, or baPWV) in the top quartile of the respective category being placed into a separate group – differences within the elderly population were noted (Figure 2.3.2). Thus, individuals in the high quartile (HIGH) exhibited at least one of the following traits compared to the rest of the elderly participants (LOW): high cIMT, high baPWV, low cDa. Individuals in the high quartile had significantly reduced total and anterior blood flow compared to the rest of the elderly participants in the supine position (Total CBF – LOW: 940 ± 146; HIGH: 782 ± 249 ml/min; $p = 0.0043$; Anterior – LOW: 726 ± 93; HIGH: 576 ± 213 ml/min; $p = 0.0013$). These differences persisted in the seated position (Total CBF: $p = 0.0003$; Anterior CBF: $p = 0.0004$).

Factors Influencing Cerebral Blood Flow

Linear regression analysis examined the relationships of age and specific vascular factors with CBF within the group of elderly participants. Seated anterior CBF was used in the analysis to take advantage of the most complete data set. Scatter plots suggested strong relationships for anterior CBF with age ($r^2 = 0.34$; $p = 0.0273$), baPWV ($r^2 = 0.55$; $p = 0.0037$) and cDa ($r^2 = 0.43$; $p = 0.0146$); however, no relationship appeared to exist for cIMT ($r^2 = 0.03$; $p = 0.5666$) (Figure 2.3.3). Age, baPWV and cDa were entered into a stepwise multiple regression procedure to determine the independence of these factors with respect to their influence on anterior CBF. Common carotid distensibility was dropped, leaving a model with baPWV and age that accounted for 71% of the variance in anterior CBF ($p = 0.0022$).

$$\text{Anterior CBF} = 1.441 - 0.0002238\text{baPWV} - 0.00832\text{age} \qquad \text{(Equation 2.3.1)}$$

Q was also considered as it had a moderate direct relationship with anterior CBF ($r^2 = 0.29$; $p = 0.0589$); however, when multiple linear regression analysis was completed, both Q and age were deemed not significant and a weaker model resulted. It is felt that the strong relationship between Q and anterior CBF is maintained by keeping a model that includes age, which itself has a strong relationship with Q ($r^2 = 0.56$; $p = 0.0032$).

From equation 1.5.1, CBF is directly proportional to MAP and inversely proportional to CVRi. A linear regression analysis of the relationship between baPWV and CVRi showed a very strong correlation ($r^2 = 0.84$; $p < 0.0001$; Figure 2.3.4). The relationship between anterior CBF and CVRi ($r^2 = 0.78$; $p < 0.0001$) is illustrated in Figure 2.3.5.

Dynamic Cerebrovascular Regulation

Sit Test

Cerebrovascular regulation in response to the sit test was examined by identifying the change in hemodynamic and cerebral vascular measures between supine (SUP) and the nadir (NAD) point following the postural shift. The supine-to-sit transition was completed over a 2-3 s period and the nadir point was reached ~7-8 s following the start of the maneouvre in both young and elderly participants. Additionally, a visual comparison of individual responses within the elderly participants (Figure 2.3.6) revealed no apparent stratification between individuals in the highest quartile for vascular measures compared to other elderly individuals. Representative arterial blood pressure and velocity recordings over the course of a single transition are plotted in Figure 2.3.7 and Figure 2.3.8, respectively. MAP, MFV, CVRi, and HR responses to the orthostatic stress are listed in Table 2.3.2.

While lying supine, elderly participants had significantly higher MAP than their younger counterparts (YOUNG: 88 ± 7; ELDERLY: 107 ± 15 mmHg; $p < 0.0001$). Immediately after sitting, MAP in the MCA dropped a similar degree in both groups (YOUNG: -39 ± 9; ELDERLY: -44 ± 13 mmHg; $p = 0.1236$). Within 2 minutes of sitting (SIT), MAP had risen in both groups, but still remained below their supine values (YOUNG – SIT: 72 ± 8; SUP: 88 ± 7 mmHg; $p < 0.0001$; ELDERLY – SIT: 80 ± 15; SUP: 107 ± 15 mmHg; $p < 0.0001$).

The drop in blood pressure following the postural transition was accompanied by a group-dependent MFV response. In the young individuals, MFV dropped further than was seen in the elderly (YOUNG: -12 ± 7; ELDERLY: -2 ± 4 cm/s; $p < 0.0001$). In fact, MFV in the elderly group was actually maintained throughout the protocol (SUP: 49 ± 12; NAD: 46 ± 12

cm/s; p = 0.5713). As such, the elderly group exhibited a lower autoregulatory index than the young group (YOUNG: 0.28 ± 0.17; ELDERLY: 0.05 ± 0.14; p = 0.0002).

The elderly participants compensated with a larger change in vascular resistance. CVRi decreased significantly in both young and elderly groups (YOUNG – SUP: 1.30 ± 0.25; NAD: 0.87 ± 0.20 mmHg/cm/s; p < 0.0001; ELDERLY – SUP: 2.36 ± 0.62; NAD: 1.43 ± 0.51 mmHg/cm/s; p <0.0001). However; even when adjusting for a higher starting point by looking at the percent change, the elderly group had a larger drop (YOUNG: -32 ± 12; ELDERLY: -40 ± 10%; p = 0.0415). The lower autoregulatory index in the elderly occurred despite a lower HR response. HR was elevated at NAD in each group, but the younger participants exhibited a larger increase (YOUNG: 22 ± 7; ELDERLY: 9 ± 5 bpm; p < 0.0001).

Handgrip Test

Active cerebrovascular regulation was examined through the hemodynamic and vascular responses to the onset of a light gripping task. The initial drop in CVRi reached a plateau ~6-8 s after the onset of the task in both young and elderly groups. This was the reference point used for comparison of variables between active (ACT) and resting (REST) states. MFV and CVRi responses are identified in Table 2.3.3 and Figure 2.3.9.

Resting MFV was higher in the young participants at rest (YOUNG: 64 ± 10; ELDERLY: 46 ± 12 cm/s; p < 0.0001) and remained distinct throughout the task. The handgrip activity was associated with a significant increase in MFV through the contralateral MCA in young and elderly individuals (YOUNG – REST: 64 ± 10; ACT: 69 ± 10 cm/s; p < 0.0001; ELDERLY – REST: 46 ± 12; ACT: 51 ± 14 cm/s; p < 0.0072). The CVRi response showed a similar relative reduction in cerebrovascular resistance in both groups (YNG: -7 ± 6; ELD: -7 ± 4 %; p = 0.9959). A small elevation in MAP became apparent over the course of the task

in both groups (YOUNG: $p = 0.0492$; ELDERLY: $p = 0.0253$). $P_{ET}CO_2$ was unchanged over the course of the task (YOUNG: $p > 0.9712$; ELDERLY: $p > 0.9245$).

Motor, Cognitive and Executive Function

Within the elderly pool, select linear regression analyses were executed on the neuropsychological test performances with vascular indices of structure and function, as well as CBF. These relationships are depicted in figures 2.3.10 – 2.3.19. Common carotid distensibility had only weak relationships with performance on any of the tests. These relationships are not shown.

Finger Tapping Test

Although performance on the finger tapping test had only a weak association with age ($r^2 = 0.04$; $p = 0.4221$; Figure 2.3.10A), modest relationships were apparent with arterial stiffness, CBF and vessel wall thickness. An inverse relationship with baPWV ($r^2 = 0.20$; $p = 0.0930$; Figure 2.3.10D) was observed with dominant hand finger tapping frequency. Additionally, not only was there a direct relationship between dominant hand finger tapping performance and total anterior CBF, but the relationship remained when only blood flow to the contralateral, non-dominant hemisphere was considered ($r^2 = 0.14$; $p = 0.1682$; Figure 2.3.10B). A modest inverse relationship was noted with bilateral cIMT ($r^2 = 0.18$; $p = 0.0660$), but this relationship was not sustained when assessing thickness in only the contralateral, non-dominant carotid artery ($r^2 = 0.02$; $p = 0.2752$; Figure 2.3.10C).

Grooved Pegboard Test

Modest relationships were observed for the performance of GPT-Place phase with age, anterior CBF and vascular factors. A modest direct relationship was observed between age and dominant hand performance, while an indirect relationship was noted between completion time and anterior CBF. The relationship with CBF was strongest when flow through the non-dominant ICA was considered ($r^2 = 0.11$; $p = 0.2357$; Figure 2.3.11). A direct relationship was also noted between performance and cIMT; however, the relationship between cIMT and GPT-Place phase performance was stronger with vessel thickness on the dominant side ($r^2 = 0.24$; $p = 0.0316$) than on the non-dominant side ($r^2 = 0.02$; $p = 0.5964$; Figure 2.3.11C). Despite an apparent relationship with age, there was essentially no relationship for blood flow or vascular characteristics and completion time of the GPT-Replace phase (Figure 2.3.12).

Reaction Time Tests

Despite an apparent direct relationship between cRT and age ($r^2 = 0.16$; $p = 0.0916$), virtually no correlation was present between vascular measures or anterior CBF (Figure 2.3.13). A relationship was noted between vascular factors and gngRT (Figure 2.3.14); however, this was limited to the presence of cIMT ($r^2 = 0.15$; $p = 0.1127$). A closer examination of this relationship involved looking at the laterality of each variable (Figure 2.3.15). The inverse relationship between reaction time laterality and cIMT laterality was modest ($r^2 = 0.23$; $p = 0.0463$).

Trail Making Test

Modest relationships were noted between the performance of TRAILS A with age ($r^2 = 0.39$; $p = 0.0228$), anterior CBF ($r^2 = 0.16$; $p = 0.1695$), baPWV ($r^2 = 0.13$; $p = 0.2322$) and cIMT

($r^2 = 0.30$; $p = 0.0526$); however, a relationship between performance and age was the only apparent association with TRAILS B (Figure 2.3.16). Interestingly, if the focus was restricted to left-sided anterior CBF, there was a modest, indirect association with performance on TRAILS B ($r^2 = 0.11$; $p = 0.2189$; Figure 2.3.17A).

Warrington's Recognition Memory Test

Linear regression plots for the RMT – Words (Figure 2.3.18) and RMT – Faces (Figure 2.3.19) appeared to demonstrate relationships with age only. The exception being a weak relationship between right cIMT and performance on the RMT – Faces ($r^2 = 0.12$; $p = 0.1435$; Figure 2.3.19C).

Table 2.3.1 Postural change in cerebral blood flow of young and elderly individuals

Location – Posture	Young	Elderly	p-value (between groups)
Total – Supine (ml/min)	937 ± 128	887 ± 191	0.1173
Total – Seated (ml/min)	872 ± 129 #	732 ± 168 * #	< 0.0001
Δ Total (ml/min)	-65 ± 81	-155 ± 119 *	0.0279
%Δ Total (%)	-7 ± 9	-17 ± 12 *	0.0151
Anterior – Supine (ml/min)	703 ± 108	666 ± 164	0.2883
Anterior – Seated (ml/min)	646 ± 102 #	552 ±134 * #	< 0.0001
Δ Anterior (ml/min)	-56 ± 95	-114 ± 89 *	0.0481
%Δ Anterior (%)	-7 ± 14	-16 ± 13 *	0.0427
Posterior – Supine (ml/min)	234 ± 44	220 ± 68	0.4479
Posterior – Seated (ml/min)	226 ± 60	173 ± 66 * #	< 0.0001
Δ Posterior (ml/min)	-9 ± 33	-47 ± 54 *	0.0336
%Δ Posterior (%)	- 4 ± 14	-20 ± 24	0.0588

Young (n=34); Elderly (n=12 for total and posterior measures, n=15 for anterior measures). All values are mean ± SD; * is significantly different from YOUNG; # is significantly different from SUPINE; $\alpha = 0.05$.

Table 2.3.2 Hemodynamic, cerebrovascular and heart rate changes in response to sit test

	Young	Elderly	p-value (between groups)
MAP supine (mmHg)	88 ± 7	107 ± 15 *	< 0.0001
MAP nadir (mmHg)	50 ± 10 #	62 ± 14 * #	< 0.0001
Δ MAP (mmHg)	-39 ± 9	-44 ± 13	0.1236
Δ MAP (%)	-44 ± 10	-41 ±10	0.4074
MFV supine (cm/s)	70 ± 12	49 ± 12 *	< 0.0001
MFV nadir (cm/s)	58 ± 9 #	46 ± 12 *	< 0.0001
Δ MFV (cm/s)	-12 ± 7	-2 ± 4 *	< 0.0001
Δ MFV (%)	-17 ± 8	-6 ± 12 *	0.0154
CVRi supine (mmHg/cm/s)	1.30 ± 0.25	2.36 ± 0.62 *	< 0.0001
CVRi nadir (mmHg/cm/s)	0.87 ± 0.20 #	1.43 ± 0.51 * #	< 0.0001
Δ CVRi (mmHg/cm/s)	-0.43 ± 0.23	-0.94 ± 0.27 *	< 0.0001
Δ CVRi (%)	-32 ± 12	-40 ± 10 *	0.0415
HR supine (bpm)	62 ± 9	62 ± 9	1.0000
HR nadir (bpm)	84 ± 11 #	71 ± 10 * #	0.0485
Δ HR (bpm)	21 ± 7	8 ± 5 *	< 0.0001
Δ HR (%)	36 ± 14	14 ± 9 *	< 0.0001

Young (n=34); Elderly (n=17 for MAP and HR, n=12 for MFV and CVRi). MAP – mean arterial pressure; MFV – mean flow velocity through the middle cerebral artery; CVRi – cerebral vascular resistance index; HR – heart rate. Nadir – lowest point MAP (occurred ~7-8s after postural transition). All values are expressed as mean ± SD. * is significantly different from YOUNG, # is significantly different from SUPINE, $\alpha = 0.05$

Table 2.3.3 Hemodynamic and cerebrovascular response to handgrip activity

	Young	Elderly	p-value (between groups)
MFV_{REST} (cm/s)	64 ± 10	46 ± 12 *	< 0.0001
MFV_{ACT} (cm/s)	69 ± 10 #	51 ± 14 * #	< 0.0001
ΔMFV (cm/s)	4 ± 4	5 ± 3	0.7016
ΔMFV (%)	8 ± 7	11 ± 5	0.1045
$CVRi_{REST}$ (mmHg/cm/s)	1.14 ± 0.26	1.93 ± 0.53 *	< 0.0001
$CVRi_{ACT}$ ((mmHg/cm/s)	1.06 ± 0.22 #	1.80 ± 0.52 * #	< 0.0001
ΔCVRi (mmHg/cm/s)	-0.08 ± 0.08	-0.13 ± 0.08	0.1607
ΔCVRi (%)	-7 ± 6	-7 ± 4	0.9959

Young (n=30); Elderly (n=8). MFV – mean flow velocity through the middle cerebral artery; CVRi – cerebral vascular resistance index. REST – resting state; ACT – ~6-8 s after beginning task. All values are expressed as mean ± SD. * is significantly different from YOUNG, # is significantly different from REST, $\alpha = 0.05$

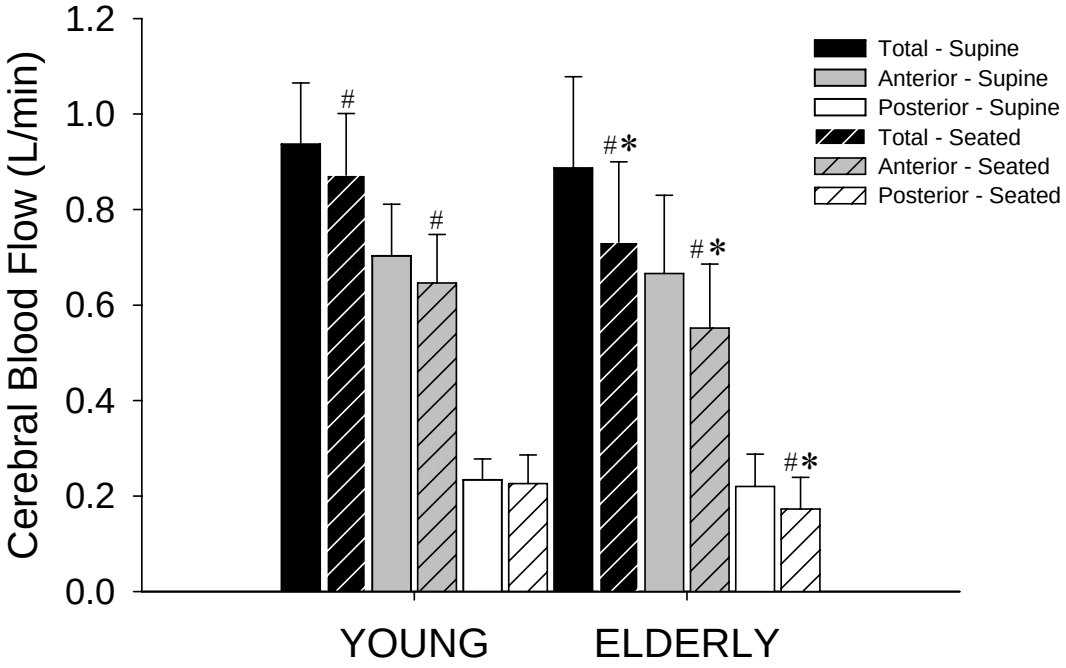

Figure 2.3.1 Cerebral blood flow is lower when in an upright posture
Cerebral blood flow of young and elderly participants in supine (solid bars) and seated (hashed bars) positions. Total flow (black), as well as anterior (grey) and posterior (white) divisions, is shown. * indicates significantly different from YOUNG in the same position; # indicates significantly different from SUPINE within the same group; $\alpha = 0.05$.

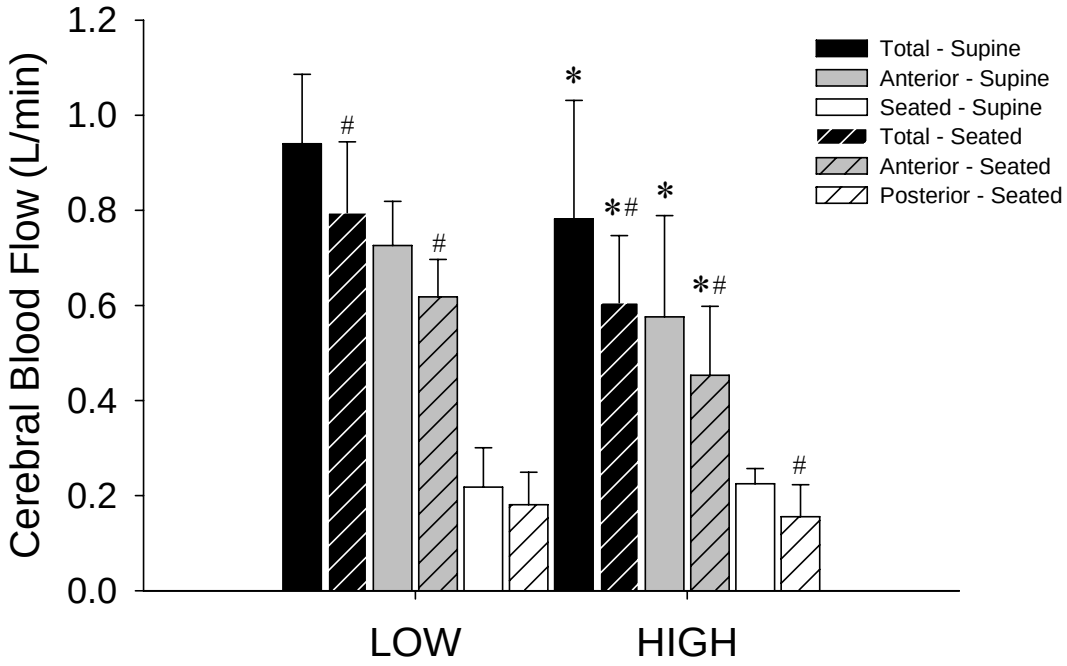

Figure 2.3.2 Cerebral blood flow is influenced by vascular factors
Cerebral blood flow of elderly participants with vascular indices in the lower 3 quartiles of the participant pool (LOW) compared to the highest quartile (HIGH) between supine (solid bars) and seated (hashed bars) positions. Total flow (black), as well as anterior (grey) and posterior (white) divisions, is shown. * indicates significantly different from LOW in the same position; # indicates significantly different from SUPINE within the same group; $\alpha = 0.05$.

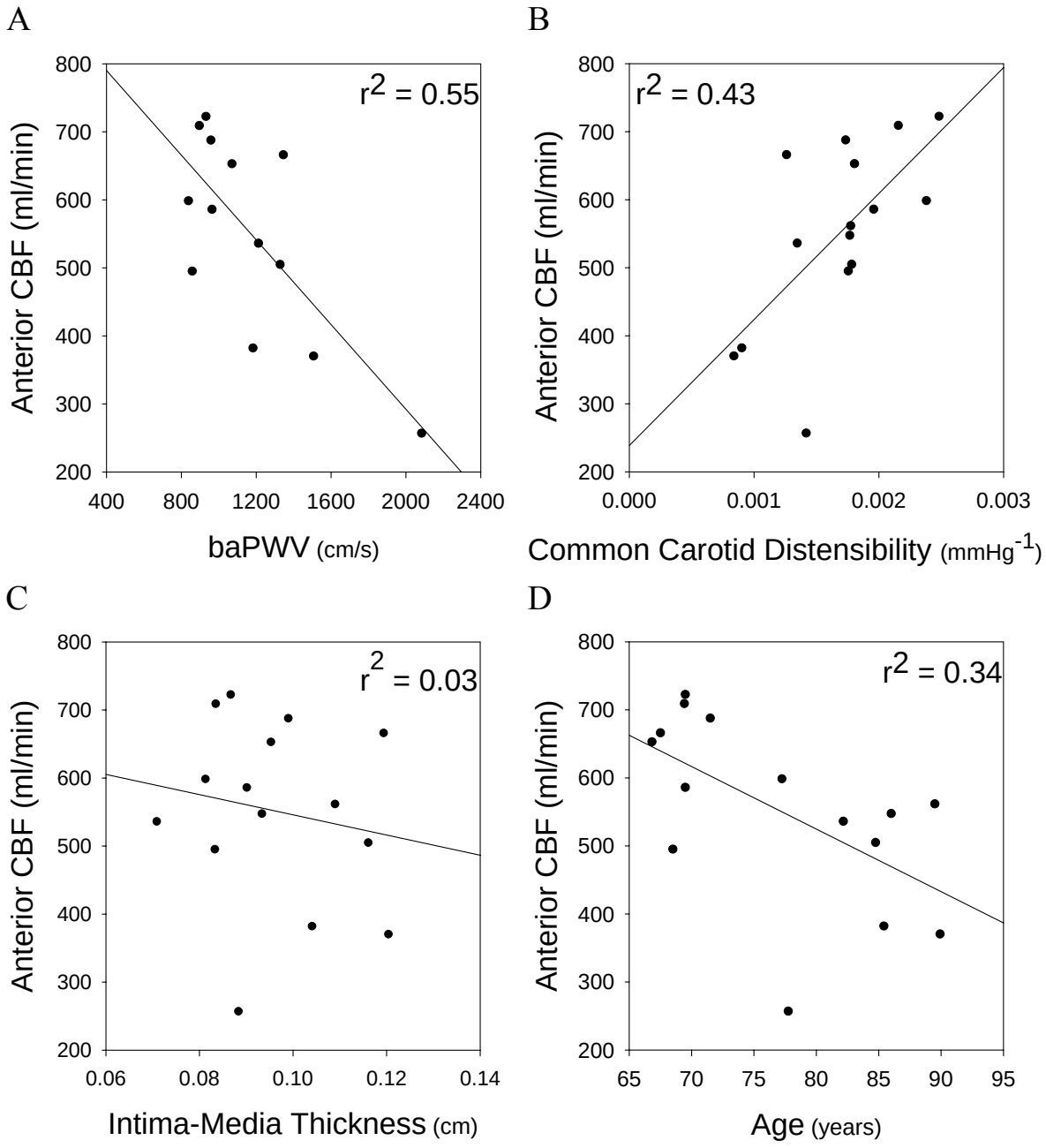

Figure 2.3.3 Relationships of age and vascular factors with cerebral blood flow
Regression scatter plots demonstrating the influence of brachial-ankle pulse wave velocity (baPWV) (A), common carotid distensibility (B), common carotid intima-media thickness (C) and age (D) on seated, anterior cerebral blood flow (CBF).

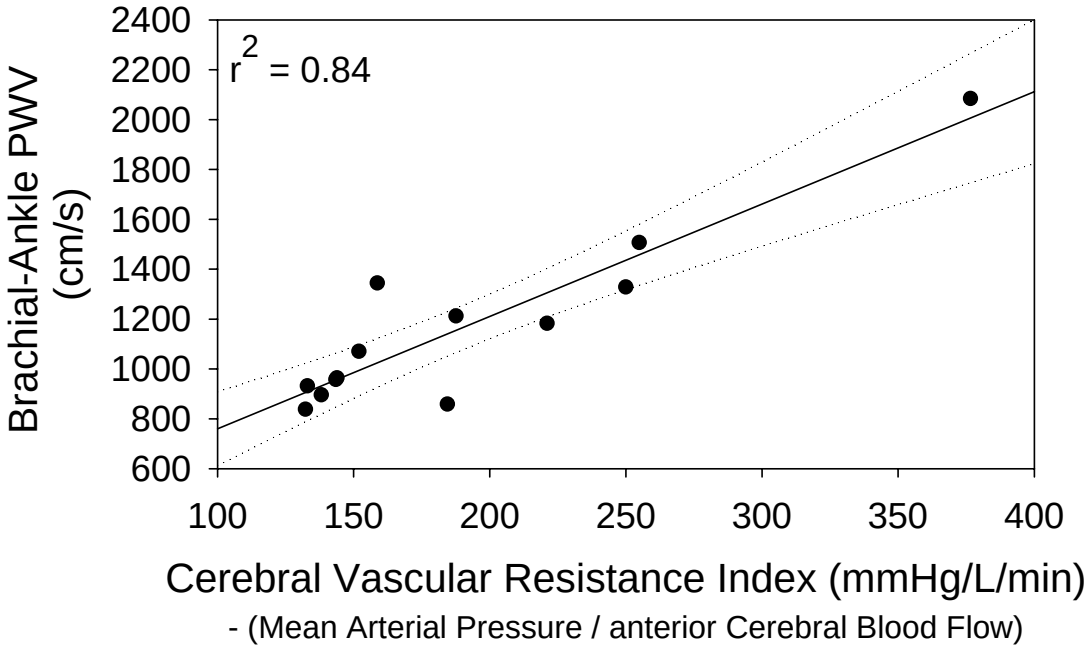

Figure 2.3.4 Arterial stiffness is directly related to cerebrovascular resistance
A linear regression scatter plot and 95% confidence intervals (dotted lines) suggesting a very close relationship between brachial-ankle pulse wave velocity (PWV) and cerebral vascular resistance index.

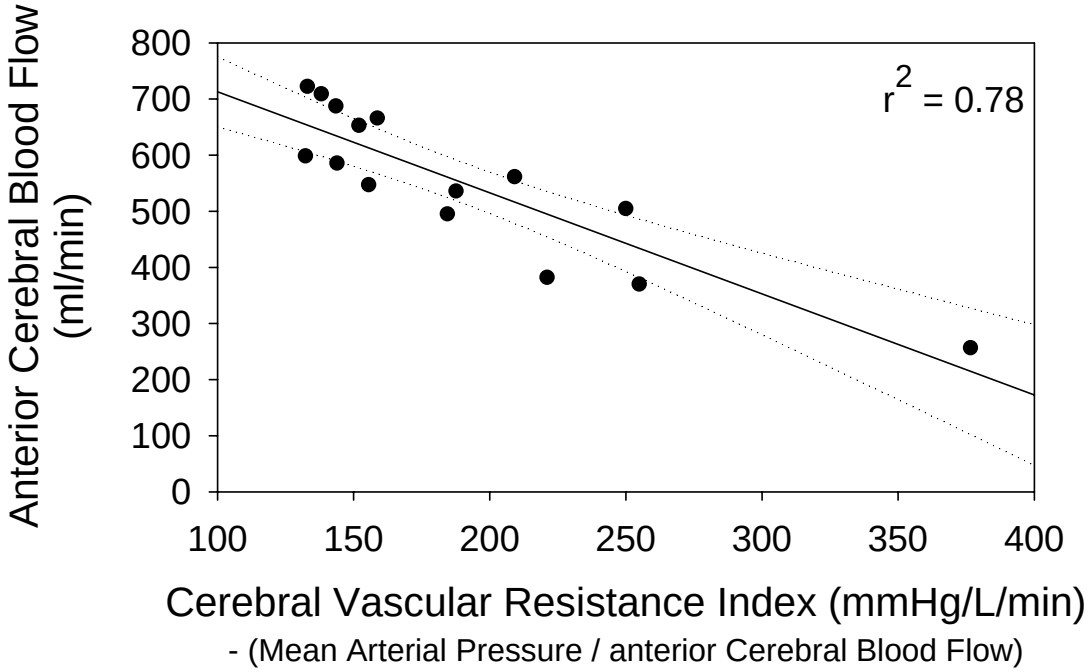

Figure 2.3.5 Cerebral blood flow is inversely related to cerebrovascular resistance
A linear regression scatter plot and 95% confidence intervals (dotted lines) suggesting a very close relationship between anterior cerebral blood flow and cerebral vascular resistance index.

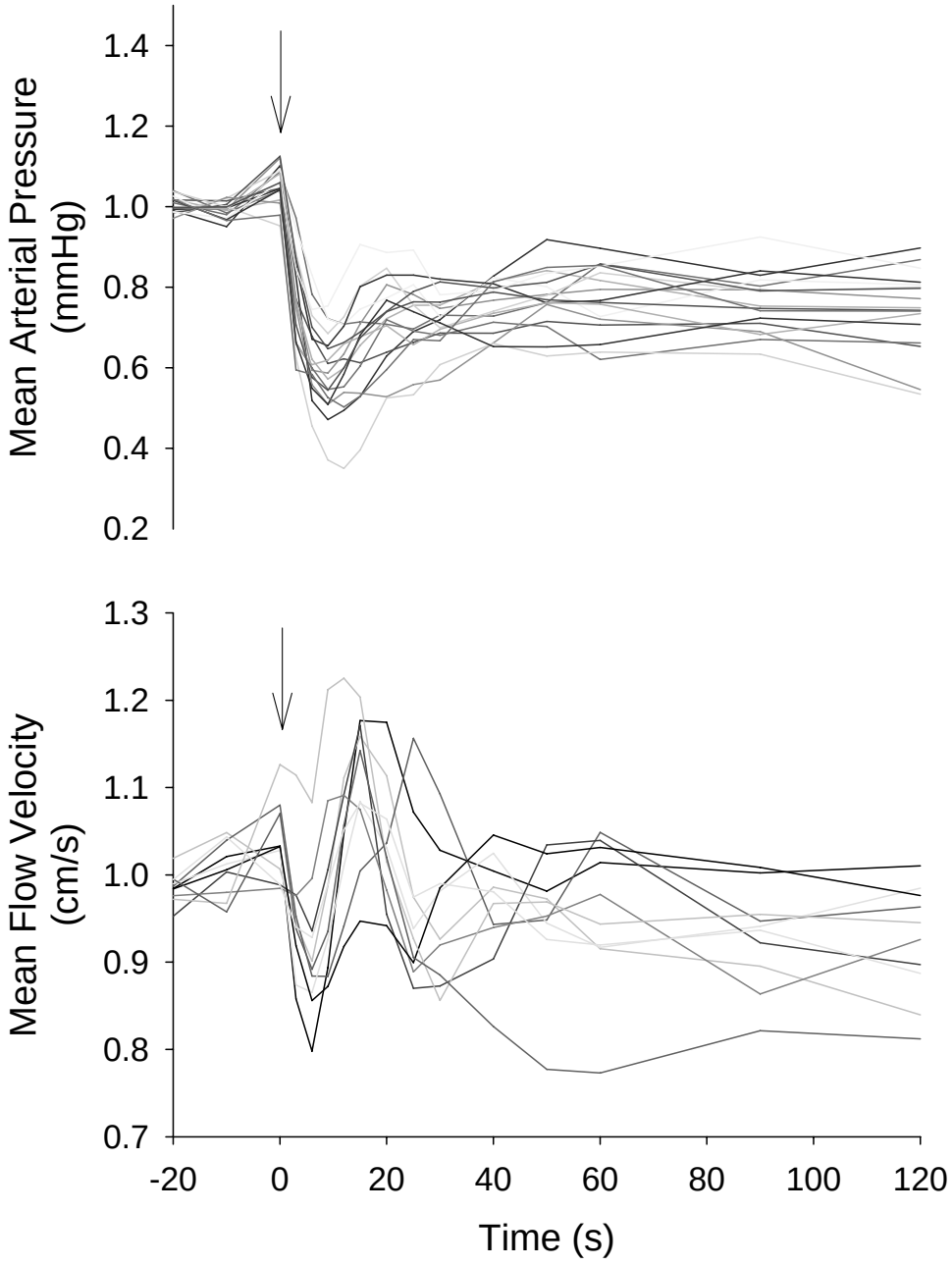

Figure 2.3.6 Mean hemodynamic responses to sit test of individual elderly participants
Mean arterial pressure (top) and mean flow velocity (bottom) in the middle cerebral artery in response to a transition from a supine to a seated posture (arrow) for individual elderly participants.

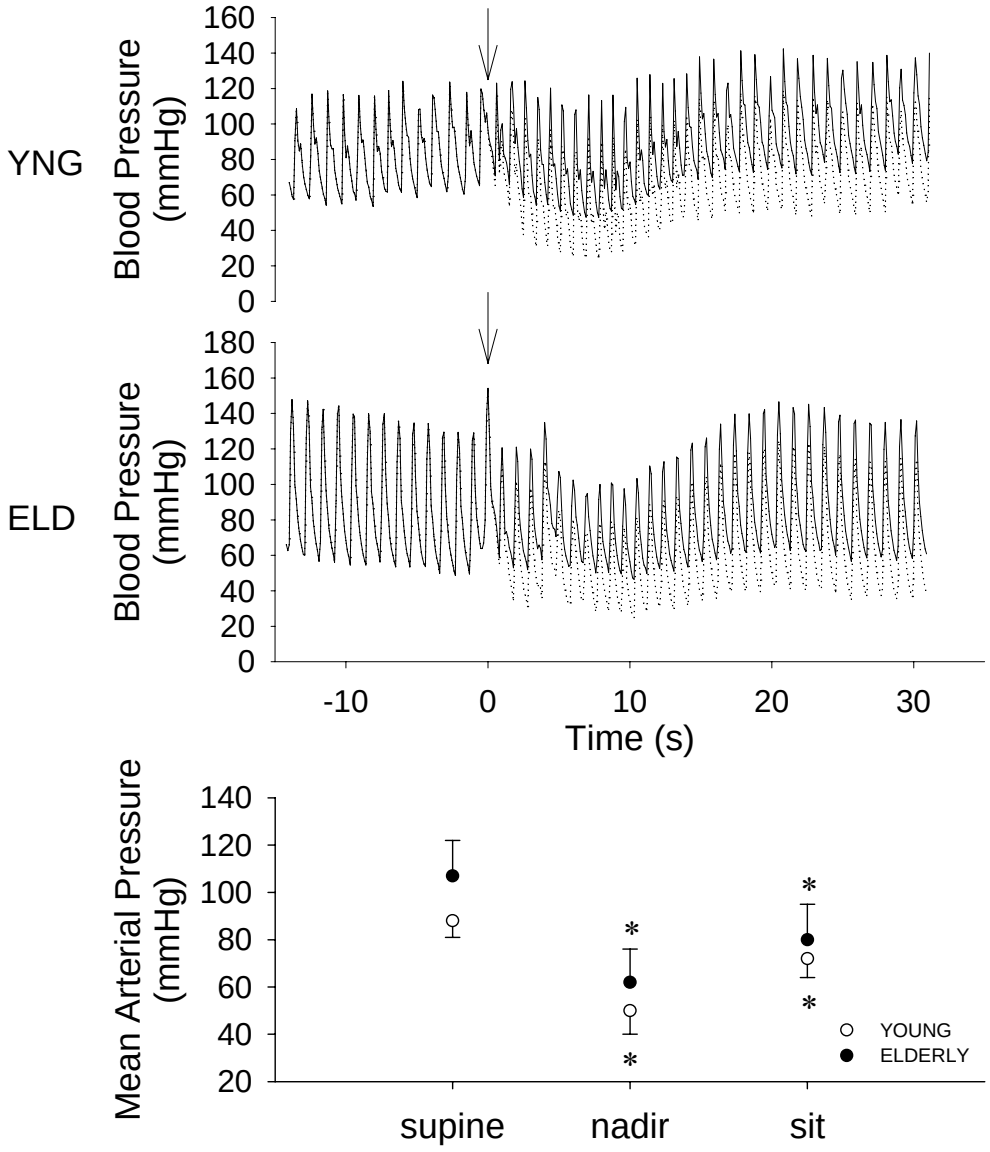

Figure 2.3.7 Representative individual and group blood pressure during the sit test
Continuous blood pressure at the heart (solid line) and middle cerebral artery (MCA) (dotted line) during sit test (transition at arrow) in representative young (YNG) and elderly (ELD) participants. Change in MCA mean arterial pressure over the course of the transition (bottom). * indicates significantly different from SUPINE; $\alpha = 0.05$.

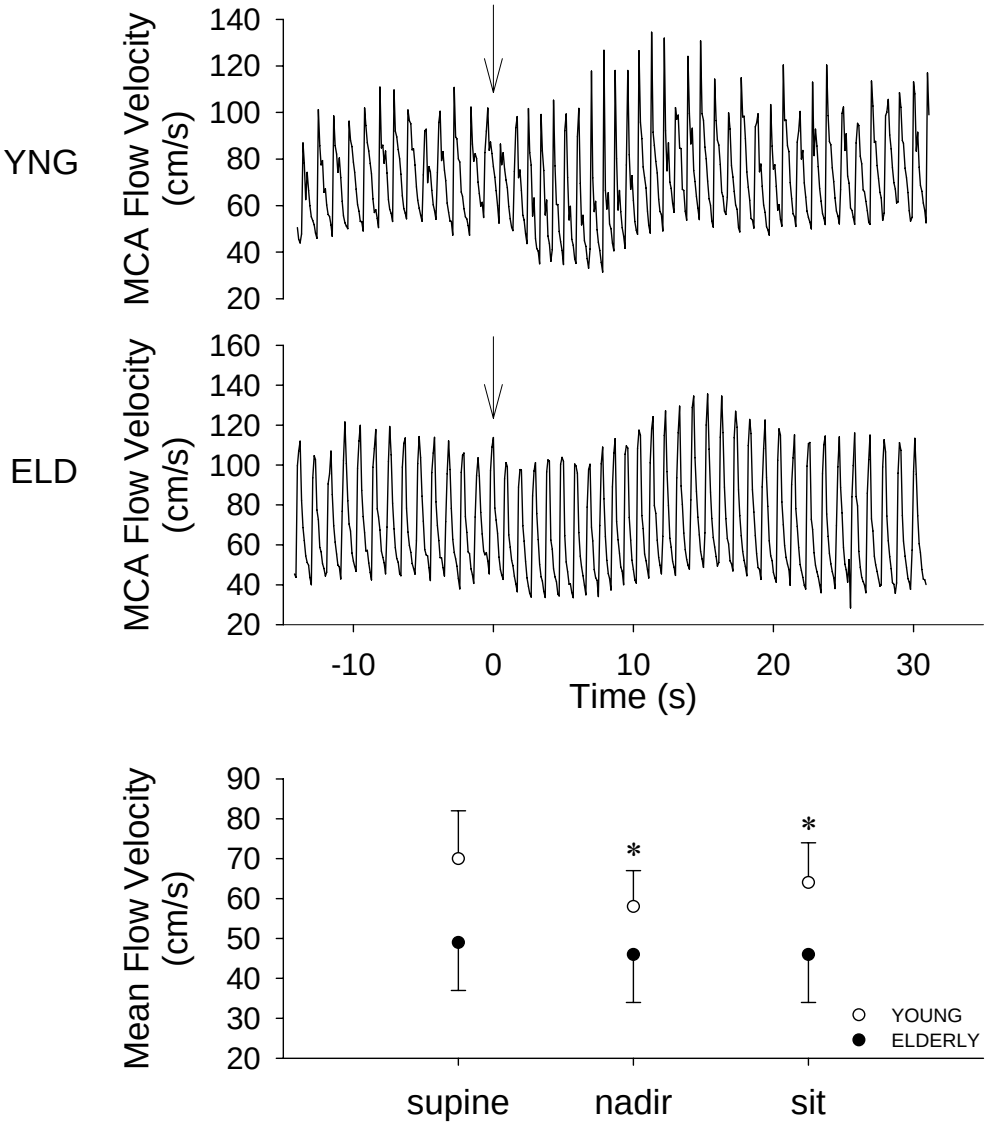

Figure 2.3.8 Representative individual and group blood velocity during the sit test
Continuous flow velocity through the middle cerebral artery (MCA) during the sit test (transition at arrow) in representative young (YNG) and elderly (ELD) participants. Change in MCA mean flow velocity over the course of the transition (bottom). * indicates significantly different from SUPINE; $\alpha = 0.05$.

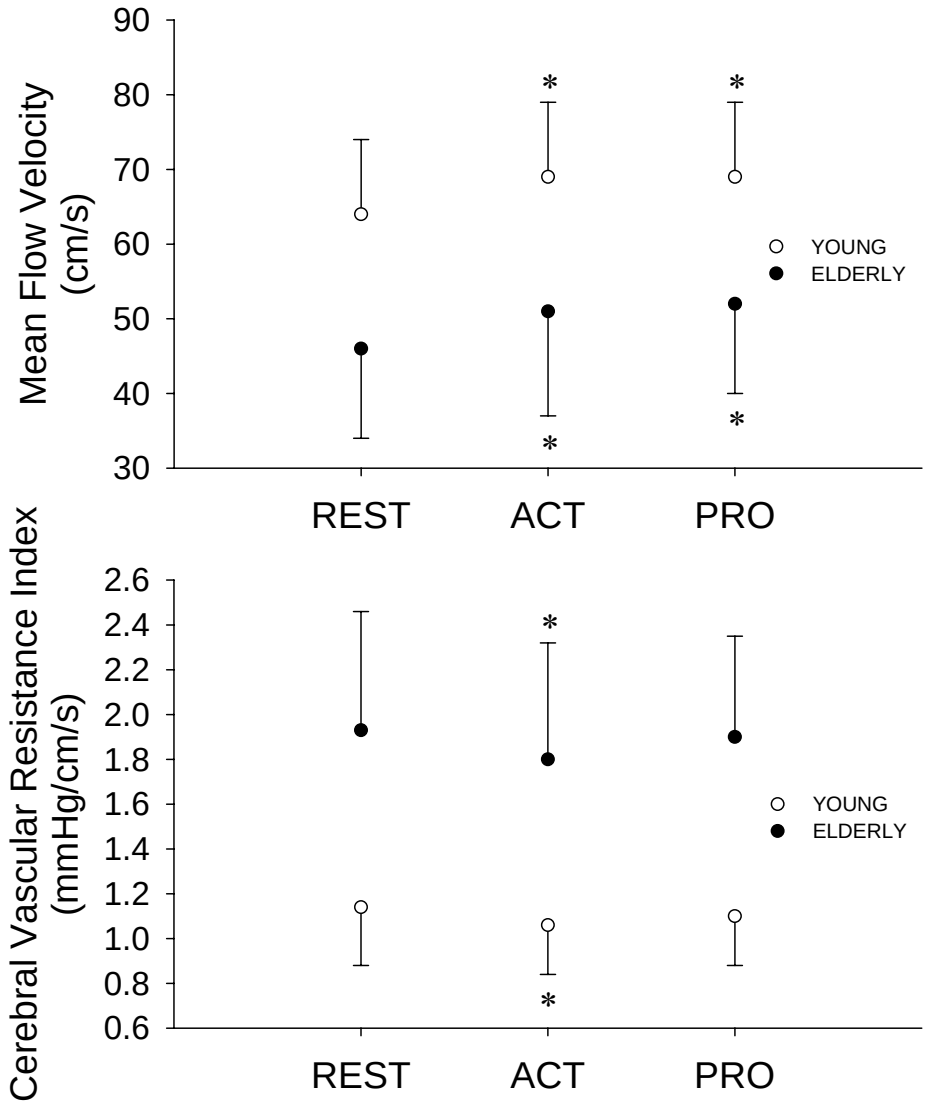

Figure 2.3.9 Group mean hemodynamic responses to handgrip test
Change in the middle cerebral artery mean flow velocity (top) and cerebral vascular resistance index (bottom) over the course of the handgrip test. REST – resting state: ACT - ~6-8s after beginning task; PRO – 30s after beginning task. * indicates significantly different from REST; $\alpha = 0.05$.

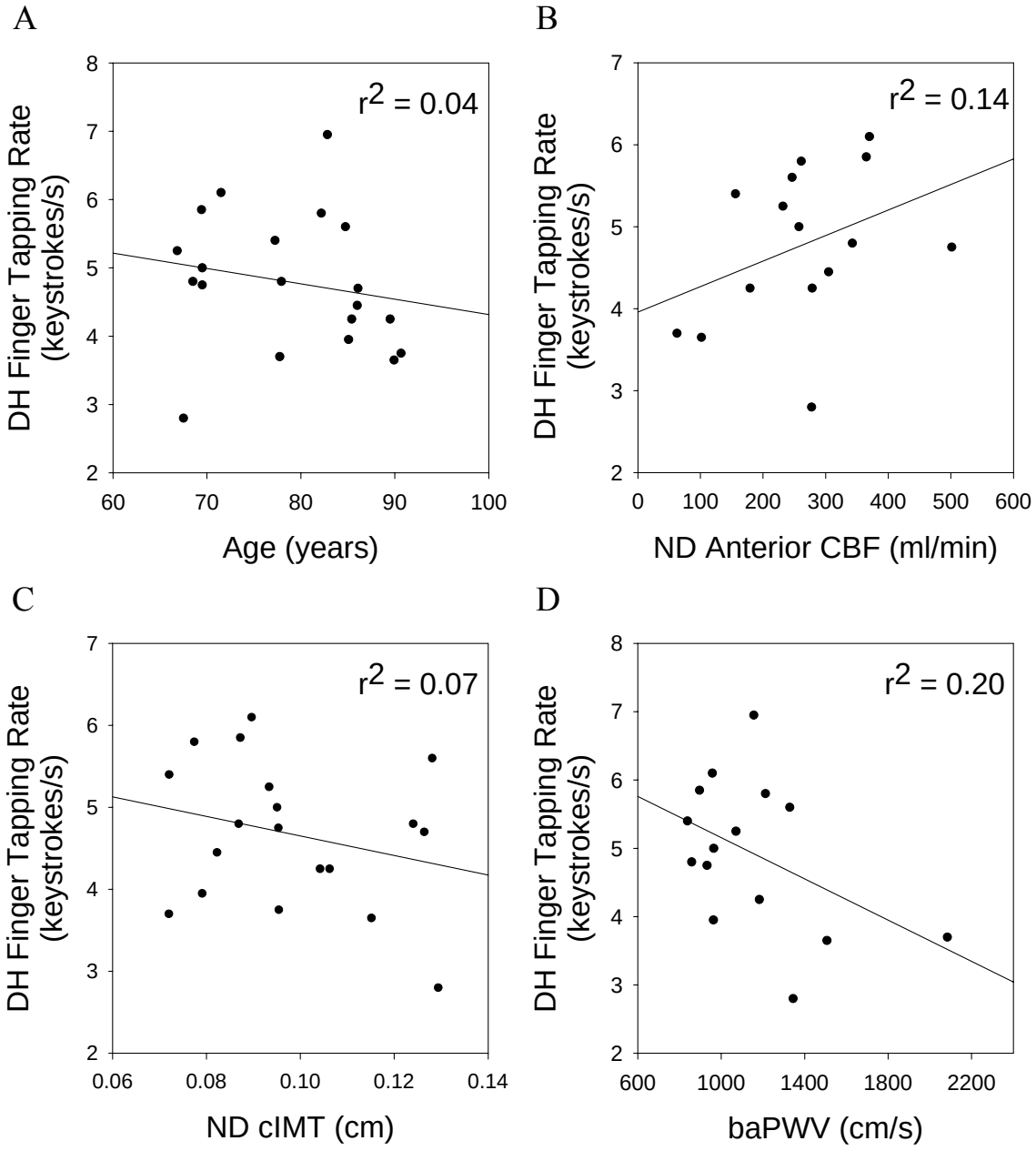

Figure 2.3.10 Relationships of age, cerebral blood flow, and vascular factors with performance on finger tapping test

Linear regression scatter plots demonstrating the relationships of age (A), non-dominant side (ND) anterior cerebral blood flow (CBF) (B), ND common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with dominant hand (DH) finger tapping rate.

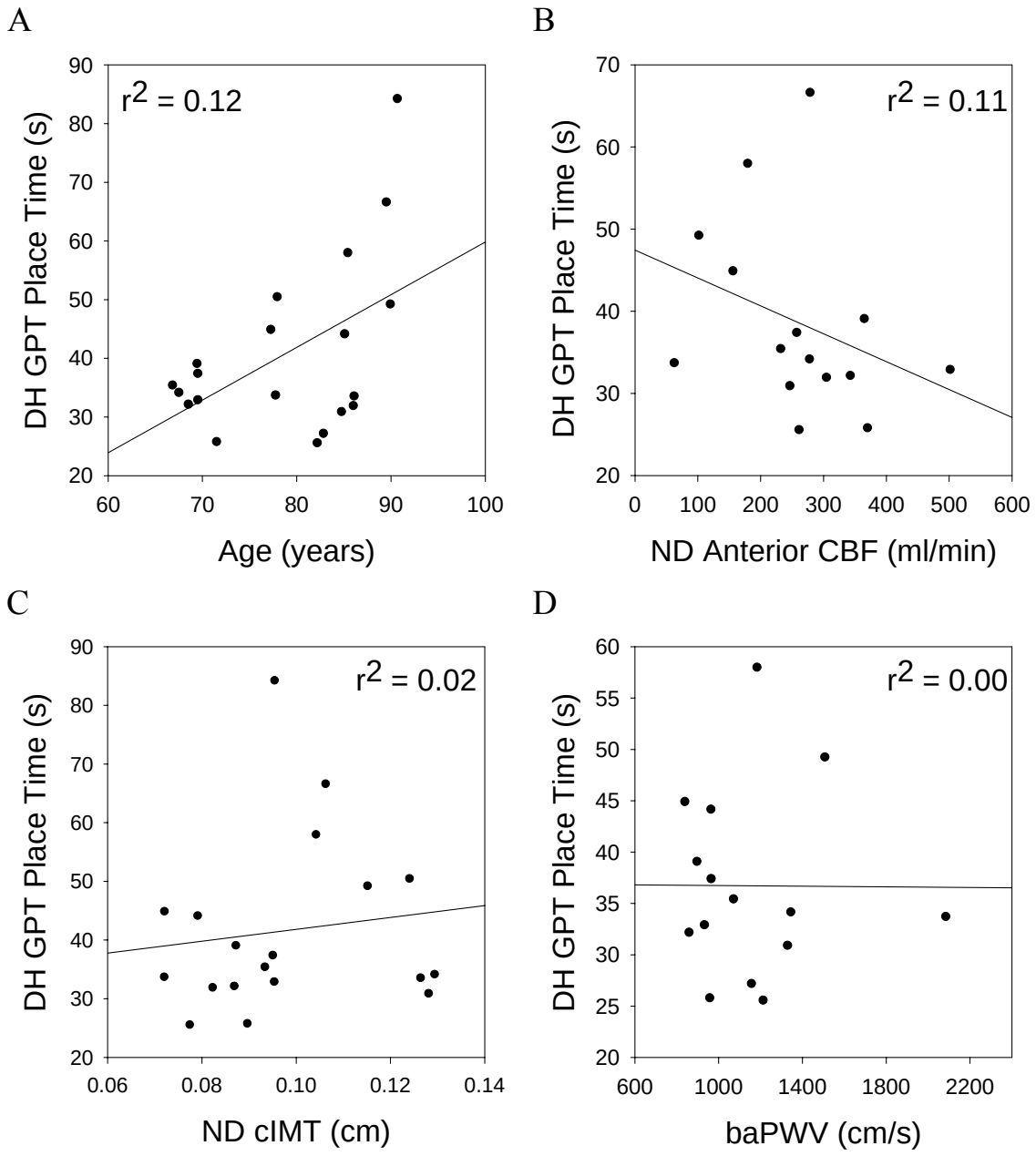

Figure 2.3.11 Relationships of age, cerebral blood flow and vascular factors with performance on the Grooved Pegboard Test - Place phase

Linear regression scatter plots demonstrating the relationships of age (A), non-dominant side (ND) anterior cerebral blood flow (CBF) (B), ND common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with dominant hand (DH) performance on the Grooved Pegboard Test (GPT) – Place phase.

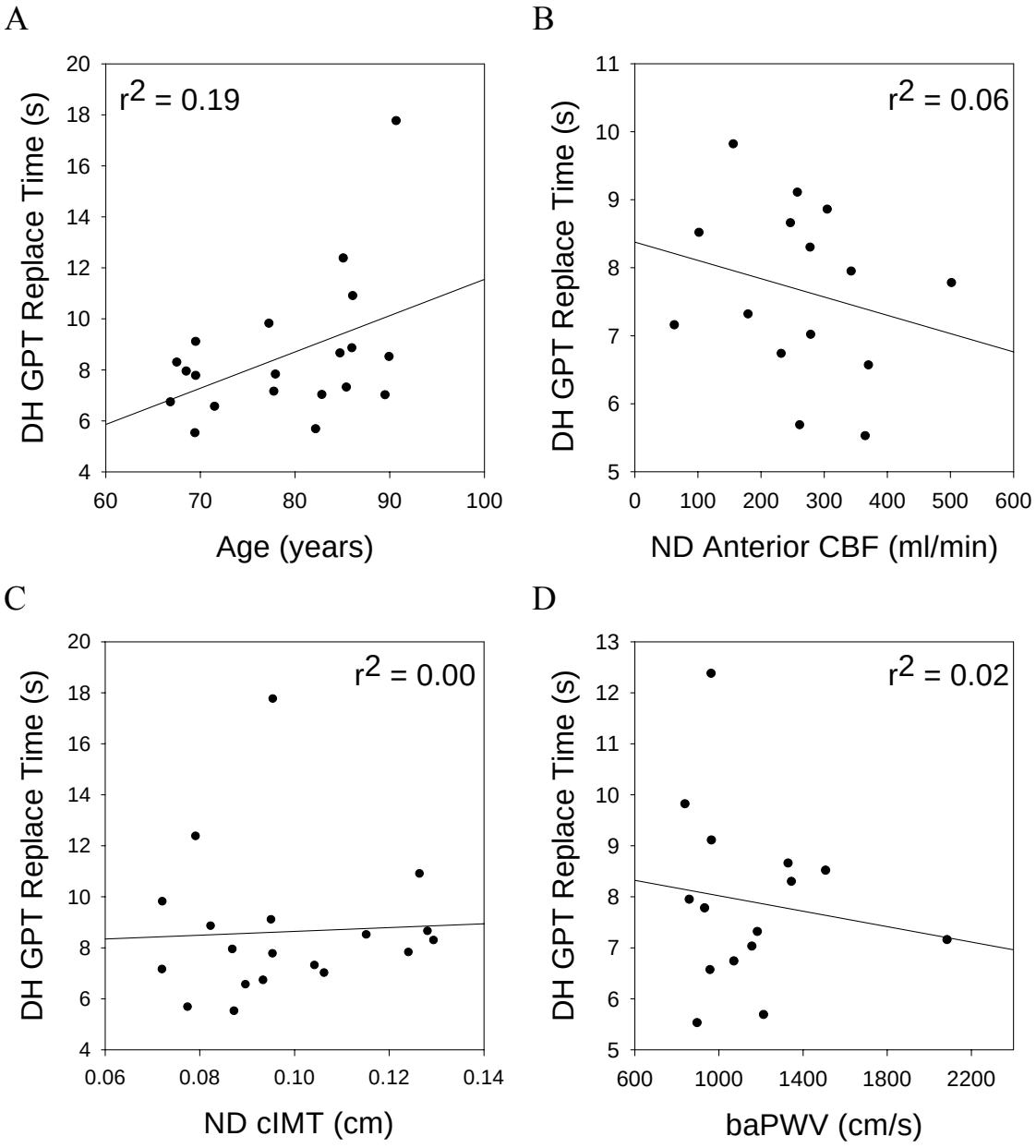

Figure 2.3.12 Relationships of age, cerebral blood flow and vascular factors with performance on the Grooved Pegboard Test - Replace phase
Linear regression scatter plots demonstrating the relationships of age (A), non-dominant side (ND) anterior cerebral blood flow (CBF) (B), ND common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with dominant hand (DH) performance on the Grooved Pegboard Test (GPT) – Replace phase.

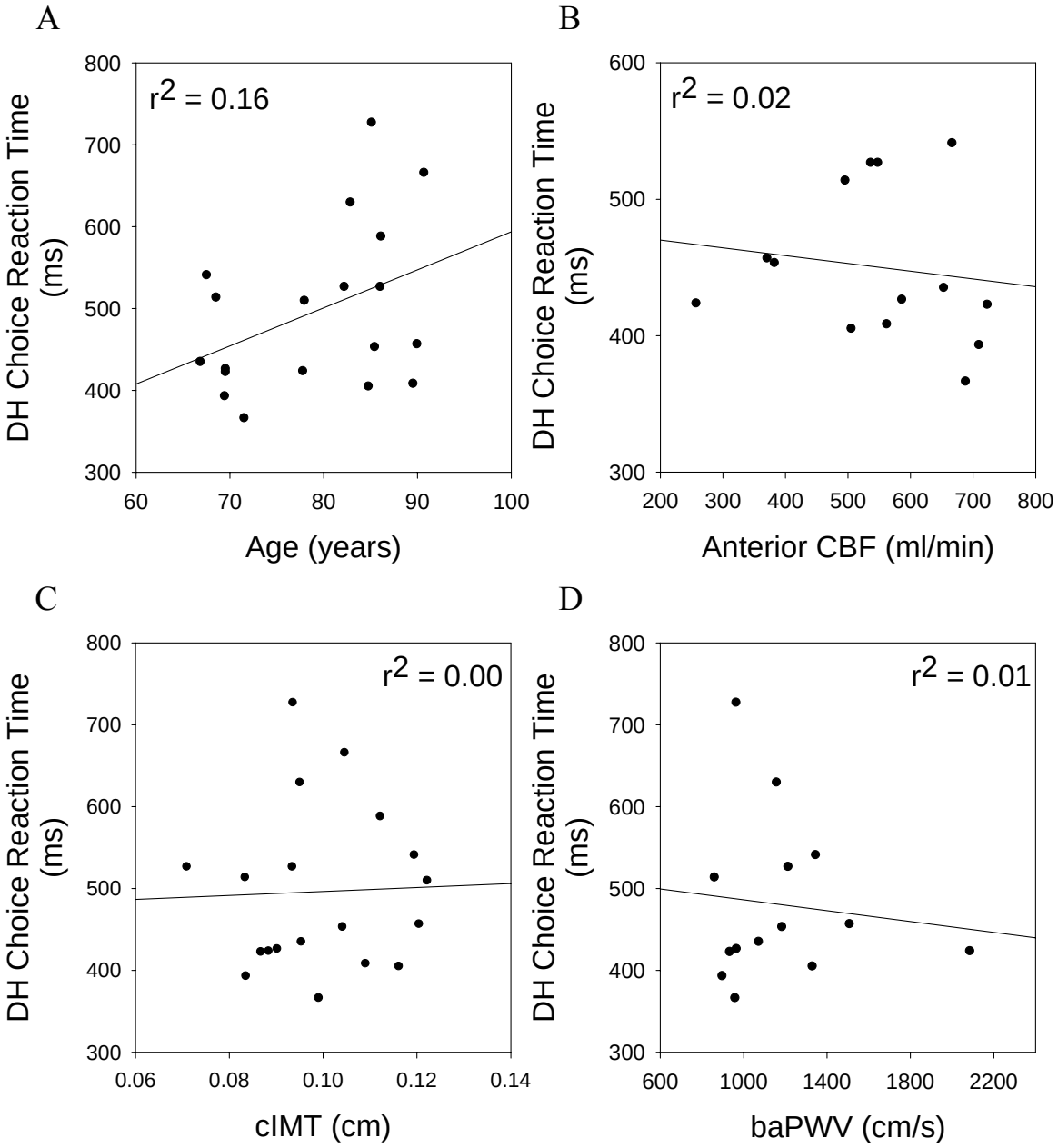

Figure 2.3.13 Relationships of age, cerebral blood flow, and vascular factors with performance of choice reaction time

Linear regression scatter plots demonstrating the relationships of age (A), anterior cerebral blood flow (CBF) (B), common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with dominant hand (DH) performance on the choice reaction time test.

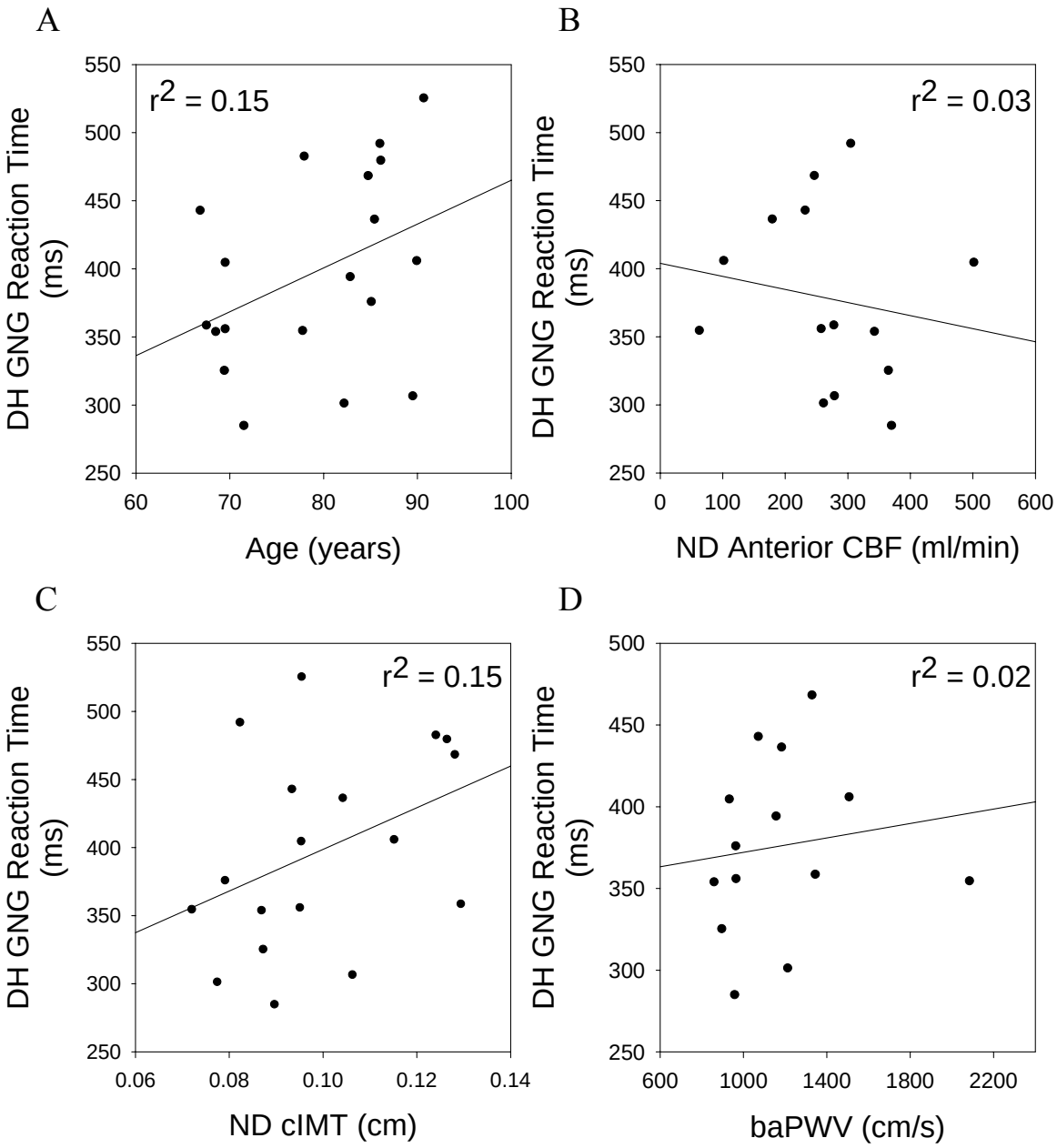

Figure 2.3.14 Relationships of age, cerebral blood flow, and vascular factors with performance of go-no go reaction time

Linear regression scatter plots demonstrating the relationships of age (A), anterior cerebral blood flow (CBF) (B), common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with dominant hand (DH) performance on the go-no go (GNG) reaction time test.

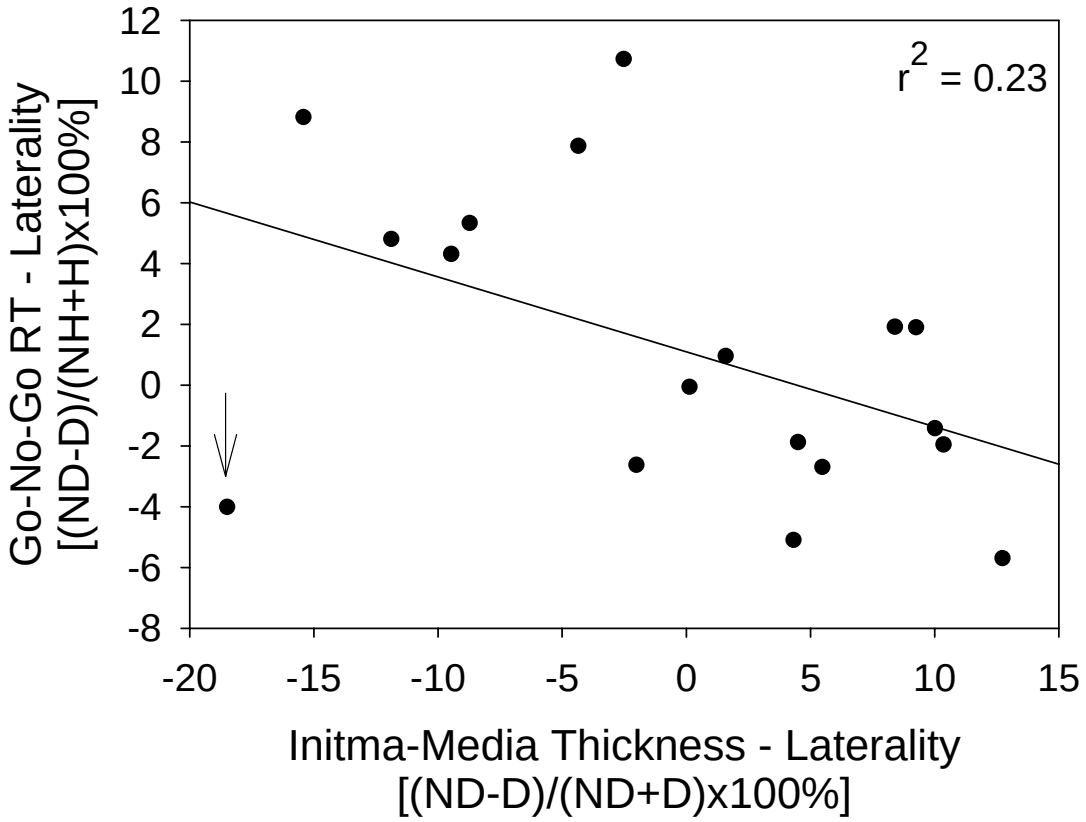

Figure 2.3.15 Laterality index of go-no go reaction time and hemispheric differences in intima-media thickness

Linear regression scatter plot of laterality measures for go-no go reaction time (RT) and common carotid intima-media thickness (cIMT). A positive score indicates better performance (quicker RT) and better health (less cIMT) for the dominant side on their respective scale. The negative slope indicates that a smaller cIMT on the left side is consistent with faster RT on the right side. $R^2 = 0.51$ without possible outlier (arrow). ND – non-dominant side; D – dominant side.

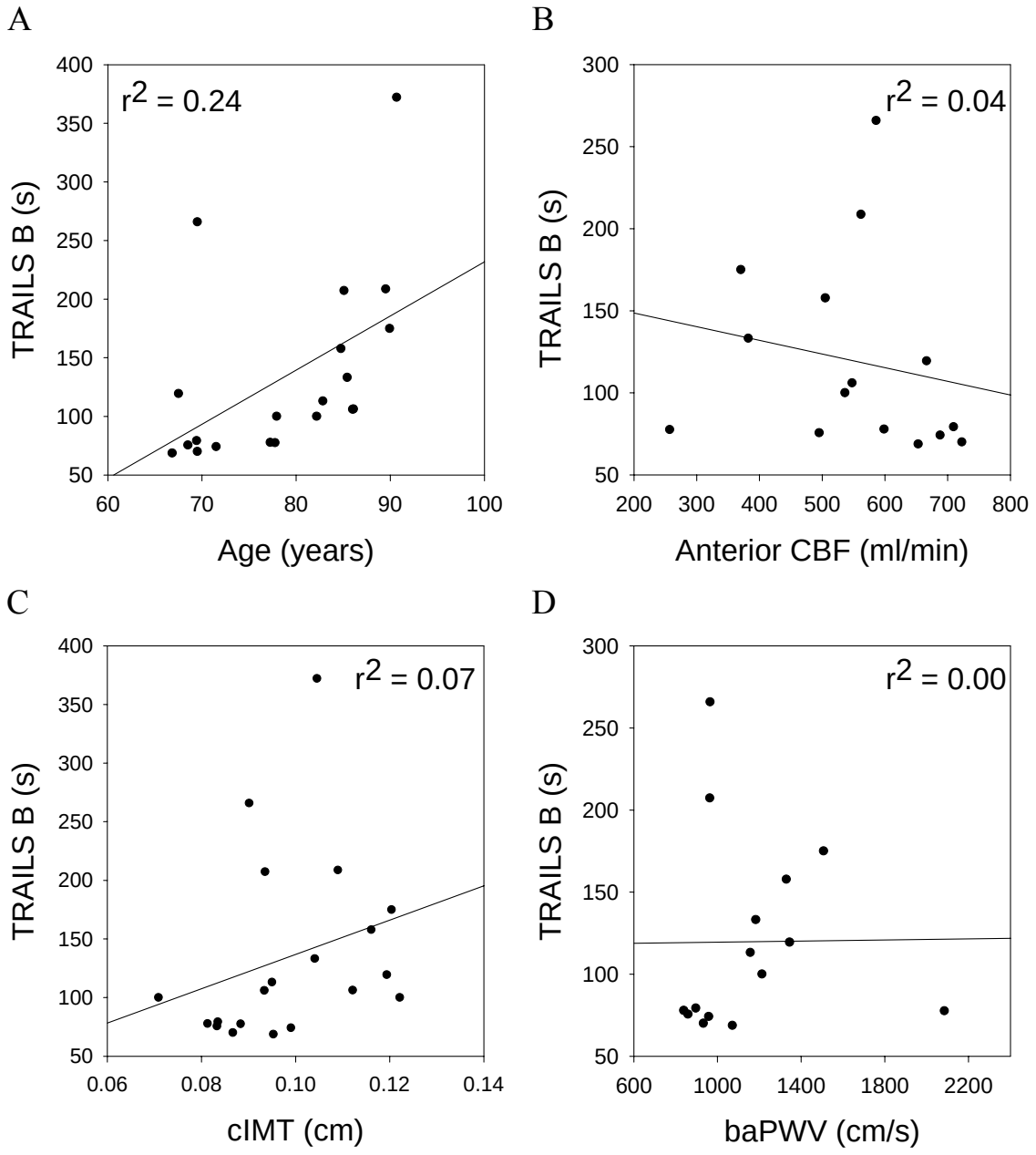

Figure 2.3.16 Relationships of age, cerebral blood flow, and vascular factors with performance on Trail Making Test - B

Linear regression scatter plots demonstrating the relationships of age (A), anterior cerebral blood flow (CBF) (B), common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with performance on the Trail Making Test – B (TRAILS B).

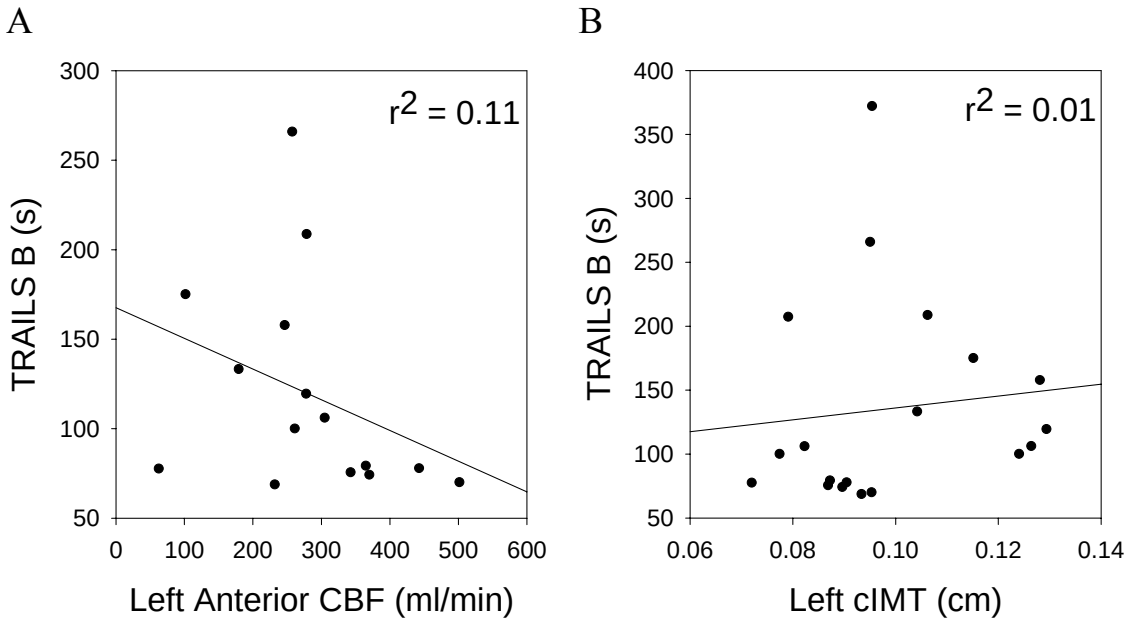

Figure 2.3.17 Relationships of left cerebral blood flow and left intima media thickness with performance on Trail Making Test - B
Linear regression scatter plots demonstrating the relationships of left anterior cerebral blood flow (CBF) (A) and left common carotid intima-media thickness (cIMT) (B) with performance on the Trail Making Test – B (TRAILS B).

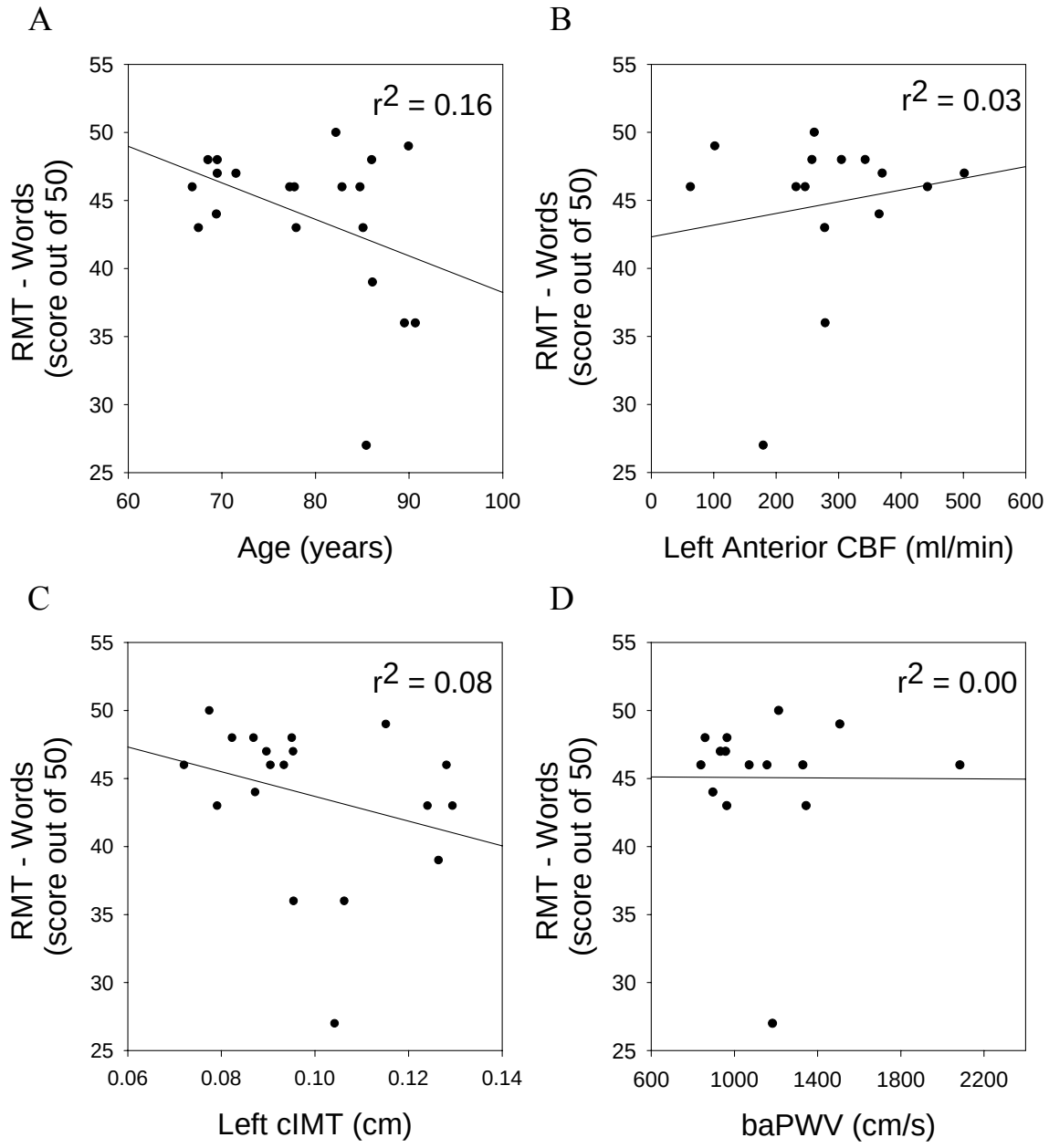

Figure 2.3.18 Relationships of age, cerebral blood flow, and vascular factors with performance on Recognition Memory Test for Words

Linear regression scatter plots demonstrating the relationships of age (A), left-side anterior cerebral blood flow (CBF) (B), left-side common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with performance on the Warrington's Recognition Memory Test (RMT) for Words.

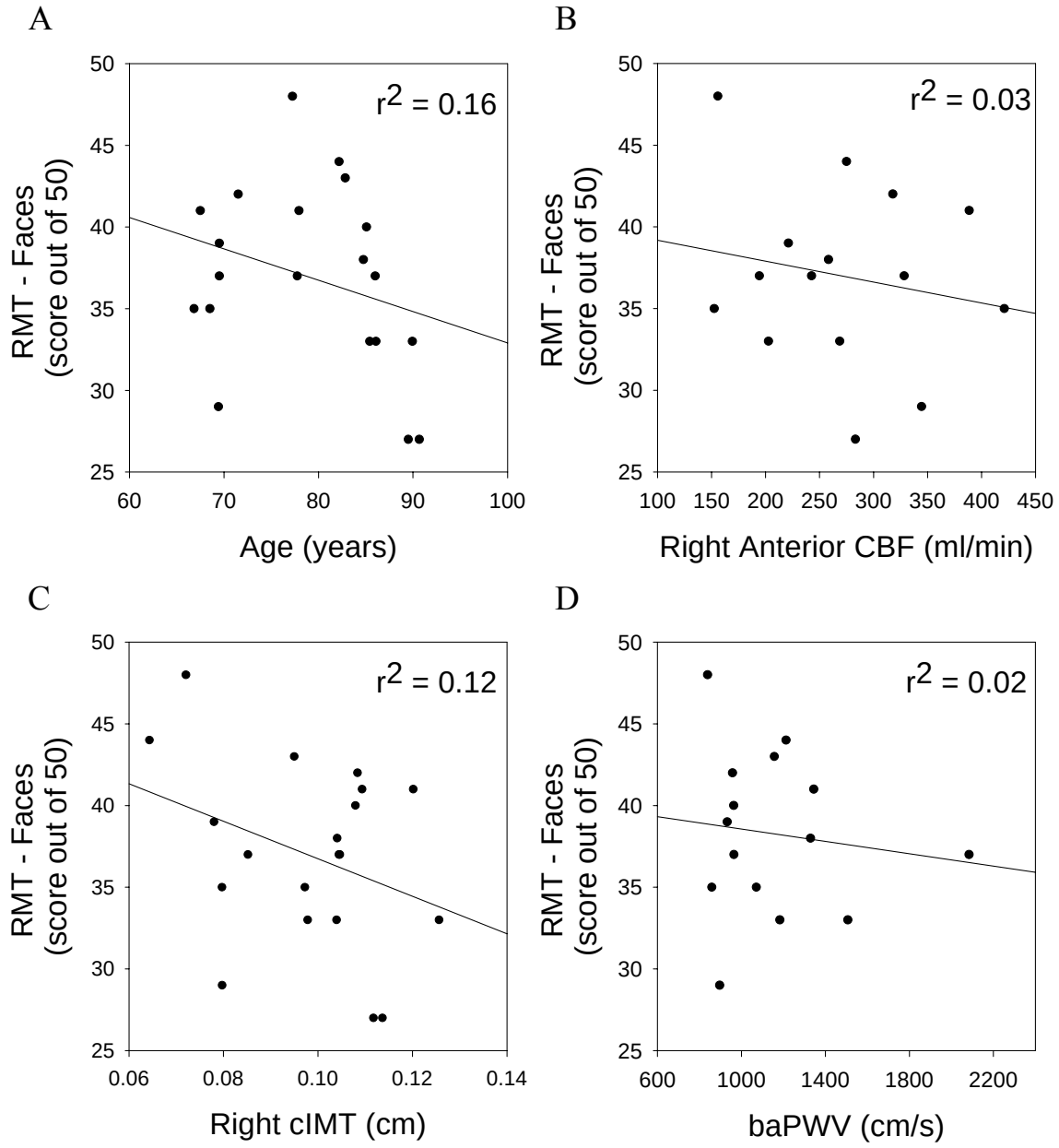

Figure 2.3.19 Relationships of age, cerebral blood flow, and vascular factors with performance on Recognition Memory Test for Faces

Linear regression scatter plots demonstrating the relationships of age (A), right-side anterior cerebral blood flow (CBF) (B), right-side common carotid intima-media thickness (cIMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) with performance on the Warrington's Recognition Memory Test (RMT) for Faces.

2.4 Discussion

This thesis research set out to take an exploratory view into the impact of vascular aging on CBF regulation and the potential consequences of reduced flow on the performance of motor and cognitive tasks requiring executive function. An important finding in the elderly population was that seated CBF, and specifically that to the frontal lobe, declined with increasing age and arterial stiffness. This finding counters the argument that the age-related reduction in CBF is solely the result of a reduced metabolic demand, but suggests rather that increased cerebrovascular resistance, as a result of vascular-mediated changes, is also a factor. Age and arterial stiffness were also related to the finding that the drop in CBF, which occurs when both young and elderly individuals move from a supine to an upright posture, was exaggerated in the elderly. Despite this impact on steady state blood flow, neither age nor changes to blood vessel structure or function influenced dynamic cerebrovascular regulation in response to acute drops in blood pressure or increases in brain activity. Setting the stage for further exploration, the decline in anterior CBF and the presence of intima-media thickening showed modest relationships with declines in performance of motor and cognitive tasks.

Vascular Characteristics

Each measure of vascular health followed the expected differences between young and elderly participants Table 2.2.1. In general, values were in accordance with the literature. Specifically, cIMT was greater in the elderly than young participants ($p < 0.0001$), and our reported elderly values (~1.00 mm) were similar to those presented by other larger scale studies (males (65-75 yrs): ~0.91 mm; females (65-75 yrs): ~ 0.84 mm) (Allan *et al.*, 1997). Eight elderly participants were noted to have a cIMT greater than 1.0 mm, which is considered to be a threshold for

moderate to severe risk of atherosclerotic disease (Salonen & Salonen, 1991). Brachial-ankle PWV was also elevated in the elderly compared to young individuals ($p = 0.0006$). Our reported values for the young (~783 cm/s) and elderly (~1153 cm/s) were on the low end of velocity ranges previously cited for similar age groups (young: ~800-1300 cm/s; elderly: ~1200–2000 cm/s) (Sugawara *et al.*, 2005). In contrast to cIMT, only one elderly participant was noted to have a baPWV in the range typically associated with high atherosclerotic risk (> 2000 cm/s) (Fujiwara *et al.*, 2005). Also, cDa values were significantly lower in the elderly participants as compared to young ($p < 0.0001$), as expected. Young values (~0.0059 $mmHg^{-1}$) were consistent with previous literature (~0.006 $mmHg^{-1}$) (Steinback *et al.*, 2005); however, methods of calculating distensibility have varied greatly between studies and a comparison with elderly values using our methodology could not be found.

Global Cerebral Blood Flow and Postural Change

When lying supine, the total CBF observed in this study was slightly higher than what has been reported in other studies. Our young group had a flow of 937 ± 128 ml/min compared with 716 ± 75 ml/min as observed in 20-39 year-olds by Albayrak and colleagues (2007). As well, we did not see the age-related decline in supine CBF that they reported in their healthy participants. Albayrak and colleagues reported a difference of ~132 ml/min in the total blood flow between 20-39 year old and 60-79 year old age groups; whereas, we only report a difference of ~50 ml/min ($p = 0.1173$). Compared to young and elderly values reported by Albayrak and colleagues, we noted slower ICA velocities (YOUNG: ~32 cm/s vs. Albayrak *et al.*: ~38 cm/s; ELDERLY: ~23 cm/s vs. Albayrak *et al.*: ~30 cm/s) and larger ICA cross-sectional areas (YOUNG: ~17 mm^2 vs. Albayrak *et al.*: ~11 mm^2; ELDERLY: ~20 mm^2 vs. Albayrak *et al.*:~13 mm^2). Importantly, our flow measurements were taken 1-2 cm distal to the

carotid bulb, similar to Albayrak and colleagues. Our diameters were recorded in conjunction with the R-spike of the QRS complex on the electrocardiogram indicating that the vessel would be near its smallest diameter, while Albayrak and colleagues reported taking measurements during the maximal diameter at systole, making the difference in cross-sectional area even more curious. Albayrak and colleagues did not indicate their method of measurement which might be the cause of the discrepancy.

Interestingly, differences in supine CBF between young participants and a sub-group of the elderly participants did become apparent when changes in vascular structure and function were considered. In fact, differences were even noted between the two elderly sub-groups. Individuals exhibiting vascular characteristics at the upper end of the baPWV and cIMT scales, and/or lower end of the cDa scale, were noted to have lower supine CBF than the rest of their elderly counterparts. This implies that vascular factors, and not just age, impact CBF. Additionally, a difference in CBF between the young and elderly groups (all participants) did become apparent when measurements were taken in a seated position – something that, to the best of our knowledge, has not been shown before using ultrasound technology.

Some studies have suggested that age-related decline in CBF is a function of cerebral atrophy and reduced metabolic demand (Albayrak *et al.*, 2006). However, general correlations from an elderly population, with a wide array of brain impairments, have shown only weak relationships, especially when only mild atrophy is present (Kitagawa *et al.*, 1985). Our results suggest that vascular health is a major contributing factor to the age-related drop in CBF. Increasing arterial stiffness was strongly correlated with the reduction of CBF independent of age. Linear regression analysis involving elderly participants between the ages of 67 and 92 showed that seated anterior CBF was primarily influenced by age and baPWV, which is an

index of stiffness for the main conduit and peripheral arteries. The model predicts that anterior CBF drops 22 ml/min (95% confidence interval (CI): 6, 38) for every 100 cm/s increase in baPWV, as well as 8 ml/min (95% CI: 1, 15) for every additional year (Equation 2.3.1). Subsequent analysis suggested that the impact of increased arterial stiffness on CBF is mediated by increased cerebrovascular resistance. Even given the small sample size (15) used to determine this relationship, the model has impressive statistical power ($\beta = 0.01$).

The association of arterial stiffness and blood flow is not novel; although this is believed to be the first time it has been demonstrated in the cerebral vasculature. Kizu and colleagues (2003), as well as Suzuki and others (2001), have demonstrated associations between arterial stiffness and peripheral circulation in the lower extremities of a diabetic population. Both groups reported that the reduction in leg and/or foot blood flow, was negatively correlated with stiffness of upstream vessels ($r^2 = 0.35$ and 0.30, respectively). Interestingly, our findings ($r^2 = 0.55$) suggest a stronger relationship in the cerebral vasculature. Suzuki *et al.* showed that the highest risk group (most elevated baPWV) had lower blood flow through the popliteal artery near the end of the cardiac cycle compared to lower risk groups, which is indicative of increased arterial resistance in the high risk group. It is recognized that these studies were performed in a circulation bed which is exposed to different hydrostatic stressors than would be seen in the cerebral circulation. Still, we found that baPWV and resistance downstream of the internal carotid arteries had a very strong relationship (Figure 2.3.4). Increasing cerebral vascular resistance was strongly correlated with reduction of CBF in the elderly participants (Figure 2.3.5).

In healthy conditions, the brain circulation has relatively low resistance, compared to other vascular beds. This is observed in flow and velocity profiles showing substantial high

flow throughout the diastole phase. Consequently, flow and pressure pulsations travel well into the arterial tree, similar to kidney circulation, and reach smaller more fragile, blood vessels. As put forth by O'Rourke and Safar (2005), it is reasonable to expect that changes to central vasculature, such as increased arterial stiffness, would be a greater detriment to these vascular beds than to blood vessels in skeletal muscle, for instance, whose highly-resistant arterioles prevent large pulse pressures from reaching the microcirculation.

In spontaneously hypertensive rats (SHR), an animal model used to study hypertension and brain damage, Sabbatini and colleagues (2001) reported arterial wall hypertrophy and luminal narrowing in the frontal cortex vasculature compared to age-matched Wistar-Kyoto controls. In a human population, the retinal artery is often examined to infer changes to cerebral vessels. Liao and others (2004) reported an association between retinal artery narrowing and arterial stiffness; however, these findings were regarded with caution because measures of arterial stiffness and retinal narrowing were taken three to six years apart. These structural changes reported in the animal and human literature are consistent with increased resistance in the cerebral vascular bed. It is possible that arterial stiffness increases the exposure of smaller arterioles to high pressure and velocity fluctuations, triggering a vascular growth response which leads to increased resistance. Despite these reported changes in steady state CBF and cerebrovascular resistance, dynamic autoregulation of blood flow appeared to be maintained or even enhanced in the elderly participants.

Dynamic Cerebrovascular Regulation

Dynamic cerebrovascular regulation was examined by looking at regional CBF in response to a sit test and a handgrip test. Structural and functional alterations within the central vasculature are related to atherosclerotic development (Fujiwara *et al.*, 2004) which were thought would

have negative impact on cerebral hemodynamic responses to an orthostatic challenge and a handgrip task. Interestingly, neither aging nor the degree of arterial stiffening or cIMT appeared to influence regulation. In fact, contrary to our hypothesis, elderly participants presented with enhanced autoregulation in response to the sit test compared to the young participants, and variability in the response of elderly individuals did not appear to be related to vascular change.

During the sit test, the acute orthostatic challenge mediated by a transition to a seated posture resulted in similar blood pressure change in young and old participants. This is consistent with what has been demonstrated using other methods to induce a hypotensive challenge including thigh cuff release, lower body negative pressure in healthy elderly (Carey *et al.*, 2000) and sit to stand transitions in hypertensive elderly (Lipsitz *et al.*, 2000). In agreement with the findings by Carey *et al.* and Lipsitz *et al.*, we reported that the elderly participants did not show a diminished cerebral autoregulatory response. In fact, it appeared to be enhanced. While Carey *et al.* carefully selected young and elderly participants who were MAP-matched, Lipsitz and colleagues compared hypertensive elderly to normotensive elderly, but still found no attenuation of cerebral autoregulation. Despite varying degrees of arterial stiffness and cIMT, the responses for our elderly participants were homogeneous. The only difference between young and elderly groups was a reduced heart rate response in the elderly group, which is consistent with a decrease in the cardiovagal baroreflex sensitivity that is associated with aging and arterial stiffening (Kingwell *et al.*, 1995;Laitinen *et al.*, 1998).

Whereas the sit test examined the autoregulatory component of dynamic CBF regulation, the handgrip task examines a functional regulatory component of CBF. Functional hyperemia describes the blood flow response to an increase in neuronal metabolic activity

(Paulson *et al.*, 1990). A power-grip task is associated with strong contra-lateral activation of the primary sensorimotor and motor areas (Ehrsson *et al.*, 2000), an area primarily supplied by the MCA. In both young and elderly groups, CVRi decreased in the contralateral MCA immediately upon beginning the task. The same relative drop was observed in the elderly and young participants. For the majority of the task, changes in resistance were associated with similar changes in MFV, suggesting that it is regional vasodilation accounting for the increase in flow. Our finding of similarities in the responses of young and elderly participants was in contrast to the findings by Orlandi and Murri (1996) who found the hemodynamic response in elderly to be attenuated. Orlandi and Murri did not measure MAP and thus cannot definitively say that the additional increase in MFV that they observed in the young participants was not secondary to an increase in MAP; however, this does appear unlikely given that the observed rise in MAP in the current study was minimal. Groschel *et al.* (2007) demonstrated differences in the functional hyperemic response to arithmetic and language tests between elderly with "vascular risk factors" and healthy elderly. Vascular risk in their study was determined by the presence of hypertension, hyperlipidemia, and diabetes. Orlandi and Murri (1996) did not provide a detailed description of their participant pool. It is possible that heterogeneity between the elderly populations of these studies and the current study accounts for the discrepancy in the findings.

The results of these cerebral activation tasks have to be viewed with caution since we cannot be confident of similar neuronal activation patterns in the young and elderly participants, despite using a similar task between groups. In his review on motor system aging, Ward (2006) notes that there appears to be greater activation over a wider area for a similar motor task in the elderly, compared to younger individuals. The similar increases in MFV seen

in the elderly group might have been the result of more widespread neuronal activation, rather than a similar activation in a specific location. If greater overall activation is present in the elderly participants, then perhaps the vasodilation per unit activation is still impaired in this population; however, this impairment would be masked secondary to our methodology. Using functional magnetic resonance imaging, Jennings and colleagues (2005) identified that hypertensive individuals had a muted blood flow response compared to normotensives, but that the response was dependent on the region of interest. Some individuals actually demonstrated compensatory increases in other areas. The age-related changes in organizational structure of the brain make it difficult to ascertain any conclusion from TCD data in response to functional activation unless a measure of brain activity (e.g. electroencephalogram) is measured concurrently.

Functional hyperemia is primarily mediated through the release on nitric oxide from endothelial tissue (Girouard & Iadecola, 2006). One experimental technique to test endothelial function in the cerebral vasculature is to examine vascular responses to increases in the partial pressure of arterial CO_2. Lipsitz and colleagues (2000), and more recently Groschel and others (2007), have demonstrated a reduced reactivity in the elderly, with and without hypertension.

Motor, Cognitive and Executive Function

Age showed to have modest correlations with most motor and cognitive tasks such that increasing age was associated with reduced performance, as expected. Finger tapping rate had the lowest correlation; however this appeared to be mostly do to the effect of a single outlier (Figure 2.3.10A). Apparent relationships were identified between performances on specific aspects of the neuropsychological battery of tests and CBF, as well as cIMT.

Finger tapping rate, the GPT-Place task and TRAILS B are all tasks evaluating a component of motor speed, with the GPT-Place task also involving manual dexterity traits (Ruff & Parker, 1993), and TRAILS B involving visuoperceptual processing traits (Spreen & Strauss, 1998). All three involve activation of the frontal lobe. Interestingly, anterior CBF had a modest relationship with each, such that performance tended to decrease as CBF tended to reduce. With the finger tapping task and GPT-Place task, the relationship was with blood flow through the non-dominant internal carotid artery. This is relevant because motor tasks are primarily controlled by the contralateral hemisphere (Stavrinou *et al.*, 2007). Therefore, the modest relationship observed between CBF and motor tasks, is not only directionally appropriate given our hypothesis, but the relationship remains when hemispheric differences are considered. The relationship between TRAILS B and CBF, although modest, was only apparent on the left side.

It was our initial belief that changes in cIMT would be related to changes in flow through a reduction in the lumen diameter of cerebral vessels as atherosclerosis develops. However, the correlations of vascular factors and CBF with cognitive and motor performance appear to suggest that cIMT has an influence on function, independent of anterior CBF. Common carotid IMT had a modest direct relationship with gngRT, whereas anterior CBF did not. The relationship between cIMT and reaction time was appropriate with respect to directional and hemispheric associations. Examination of the laterality of these two measures demonstrated that as vessel wall thickness increases in a carotid artery, reaction time for the contralateral limb increases (i.e., performance is reduced). Additionally, a relationship between right-side cIMT and the performance of the RMT-Faces was observed, apparently independent of anterior CBF. This was somewhat unexpected since our hypothesis proposed that functional

changes would be limited to executive function. RMT-Faces challenges non-verbal memory which involves processing on the right hemisphere (Warrington, 1984), so the relationship demonstrated appropriate directional and hemispheric associations. The RMT-Words task, which challenges verbal memory, identifies lesions selective to the left hemisphere. We did not observe any relationship with respect to the vascular indices or anterior CBF with this task. It is interesting to note that Waldstein *et al.* (2003) reported that individuals with stage II peripheral arterial disease performed worse than hypertensive or normotensive individuals on non-verbal memory tasks, but not on verbal memory tasks.

The findings reported here are interesting, but puzzling. Anterior CBF shows modest relationships with some executive tasks (GPT-Place task), but not with others (reaction time). Common carotid IMT appears to have an effect, independent of CBF, including a modest relationship with gngRT, but not cRT. Non-verbal memory appears to be effected more than verbal memory. These discrepancies might be explained by the hypothesis that blood flow disturbances and inflammation processes affect brain function through separate mechanisms and that certain areas of the brain might be more susceptible to damage than others.

Common carotid IMT is a common marker for the presence of inflammation (Nishida *et al.*, 2007). Yaffe *et al.* (2004) recently demonstrated that inflammation increases the risk of cognitive decline in individuals with metabolic syndrome. Even in the presence of multiple vascular risk factors presented by metabolic syndrome (i.e. obesity, hypertriglyceridemia, hypertension, low high-density lipoprotein level, and hyperglycemia), inflammation provided an additional risk as indicated by a greater decline in the performance on the 3MS over a four-year period (Yaffe *et al.*, 2004). This raises the possibility that the presence of cIMT could have a dual effect against brain function. That is, the structural atherosclerotic changes, as

predicted by elevated cIMT, could reduce lumen cross-sectional area, thereby restricting blood flow in the cerebrovasculature; but elevated cIMT could also be indicative of the presence of inflammatory processes, effecting brain function through a hormonal involvement.

The secondary contributing hypothesis to the varied relationships is that specific areas in the frontal lobe have varying susceptibility to vascular-related changes. The vascular bed within the brain is such that blood flow is abundant in the cortical areas, but deep brain structures lie within a "watershed" area where capillary density is low (Pugh & Lipsitz, 2002). Recently, reductions in CBF have been related to lesions in the periventricular white matter, but not deep white matter (ten Dam *et al.*, 2007). It is likely that areas lying within this watershed region, which are already receiving a limited blood supply would be most at risk to further impairments. Following this hypothesis, the individual development of the brain and cerebrovascular network would have a major impact on the specificity and likelihood of the development of brain dysfunction in later years.

Limitations

This exploratory study was taken on to identify specific areas in which to focus further research. Consequently, the sample sizes are too small to make any firm conclusions regarding the relationships of CBF and vascular health with motor and cognitive function. Additionally, technological limitations prevented complete data sets for all participants (Table 2.2.3). Even still, our findings seem to strongly hint at these relationships discussed above. Sample size calculations were performed to identify statistically powerful ($\beta = 0.20$) relationships from the associations for CBF and cIMT with motor and cognitive function discussed above. The required samples sizes are listed in Table 2.4.1.

Again, secondary to the exploratory nature of this study, exclusion criteria were very limited. Any elderly volunteer who could demonstrate functional independence was accepted into the study. Consequently, there are a number of confounding variables that were not controlled. Participants' health history was voluntarily provided and could not be confirmed in all cases. In any event, the elderly participants' medical history was quite heterogeneous (Table 2.2.2). Half of the elderly participants reported being hypertensive and were taking at least one anti-hypertensive medication. Lipsitz *et al.* (2005) demonstrated that anti-hypertensive medication reduces arterial stiffness and increases blood flow, thus relationships between arterial stiffness and executive function might have been masked by the effects of medication. The extent of the masking would likely depend on length of medication use and extent of arterial stiffness and/or hypertension prior to medication use. As well, two of the participants were taking medications related to depression and one of the participants was taking a cholinesterase inhibitor, commonly prescribed for dementia.

Practical Implications

Results from the 2001 Census by Statistics Canada indicate that 13% of the Canadian population is over the age of 65 and that the size of this elderly population grew 10% from 1996 to 2001 – more than double the growth rate of the 20 to 64-year-old population (Statistics Canada, 2003). Clearly our population is growing older, meaning more people will be at risk for the development of cerebrovascular disease.

The most important finding of the current study was the identification of the role of arterial stiffness in lowering CBF. Knowledge of the link between brachial-ankle PWV and cerebrovascular resistance provides a further understanding of how cerebrovascular disease develops. Increasing the knowledge surrounding the mediating factors of reduced CBF opens

new avenues through which we can tackle cerebrovascular disease with rehabilitative and preventative strategies. Additionally, the knowledge of postural-related alterations in CBF in the elderly population will allow long-term care facilities to implement strategies to safeguard against periodic episodes of low CBF. These strategies could include rules with respect to post-meal activities and patient-transfer guidelines.

As we move forward, there is great potential because of the predictive capabilities of the vascular measures that are being examined. If links to cerebrovascular disease can be demonstrated and specific stages of vascular health identified, then, these non-invasive measures will be a valuable tool for medical practitioners to monitor the health of their patients. Identification of pre-clinical risk factors allows the appropriate preventative measures to be taken so that lifestyle changes can be made and independence maintained for a longer period.

Future Directions

The identification of the postural-related alterations in CBF has practical implications for researchers examining the relationship of CBF with executive function. To date, many studies have attempted to link CBF and cognitive health. However, to the best of our knowledge, all of these studies have assessed blood flow in the supine position. Considering that brain function is being assessed by neuropsychological tests when individuals are in upright posture, we believe that assessment in seated or standing postures would be a more sensitive tool for observing relationships. Continuation with the current investigation into relationships between upright CBF, vascular characteristics and indices of motor, cognitive and executive function is needed. With these preliminary data, we will be able to target specific areas on which to focus – including tasks with emphasis on executive function, such as gngRT and GPT-Place phase. As

well, we now know the sample sizes needed to reach statistically powerful results. Developing longitudinal studies to examine these factors would also be beneficial to identify time-course and causal relationships.

Using the information we now know with respect to arterial stiffness and cerebrovascular resistance, future studies can look at the effectiveness of reducing arterial stiffness to treat cerebrovascular disease. Arterial stiffness has been shown to be modifiable by simple lifestyle changes such as moderate aerobic activity (Cameron & Dart, 1994;Edwards *et al.*, 2004) or antihypertensive medication (Agabiti-Rosei & Muiesan, 2007;Lipsitz *et al.*, 2005). The result on cerebrovascular resistance as a consequence of modifications of arterial stiffness has not been examined. Clearly, from this exploratory study, there are many branches from which to extend.

Table 2.4.1 Sample sizes needed to demonstrate powerful relationships (β = 0.20)

Behavioural Activity	Cerebrovascular Variable	Expected r^2	Sample Size
Finger Tapping	Anterior CBF	0.14	**56**
GPT-Place phase	Anterior CBF	0.11	**70**
GNG Reaction Time	cIMT	0.15	**50**
TRAILS B	Left Anterior CBF	0.11	**70**

GPT – Grooved Pegboard Test; GNG – go-no go; TRAILS B – Trail Making Test B; CBF – cerebral blood flow; cIMT – common carotid intima media thickness

2.5 Conclusions

We investigated the relationship of steady state and dynamic CBF control with structural and functional measures of vascular health. Also, we took an exploratory look into how CBF and these vascular measures relate to neuropsychological measures of brain function. Interestingly, we demonstrated that arterial stiffness, and specifically baPWV, is an independent contributor to CBF, along with age. It appears to have an effect through increasing cerebrovascular resistance, such that as baPWV increases, cerebrovascular resistance increases and CBF decreases. This finding shines light on the debate of whether CBF is reduced in the elderly because of a reduced demand or impaired delivery. According to this model, impaired delivery occurs, at least in part, due to elevated resistance restricting flow.

In addition, we found that CBF is reduced in an upright posture as compared to supine. Global flow measurements indicated a drop in flow, even in young individuals. Notably, the drop in CBF is much greater in elderly individuals. Clearly from this study, we have seen that vascular characteristics influence CBF. These relationships appear to be related to steady state flow only, since cerebrovascular dynamics were maintained in the elderly participants compared to the young.

Finally, modest relationships were identified for CBF and cIMT with performance of tasks involving aspects of motor speed, coordination, visuomotor perception, initiation and inhibition, as well as non-verbal memory. The study presented here has helped to create a platform for further research.

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