
UNIVERSITY OF BIRMINGHAM

# A MULTIMODAL INVESTIGATION OF BRAIN HEALTH: CEREBRAL BLOOD FLOW, COGNITIVE PERFORMANCE AND QUALITY OF LIFE

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# DEDICATION

I would like to dedicate this work to anyone who has experienced the devastating effects of neurological and psychiatric conditions. From my closest loved ones, friends and extended family; patients and families I have worked with in the NHS and private hospitals; and the many inspirational colleagues with whom I have had the pleasure to work with. I hope that this research will be just the beginning of a long-term valuable contribution to health-related sciences, dedicated to minimising the incidence of neurological and psychiatric conditions and the distress that they cause.

# ABSTRACT

Understanding brain health is crucial in diagnosing, preventing and treating neurocognitive conditions (e.g., dementia). However, the literature reveals discrepancies around the interpretation of brain health and differences between populations. This thesis investigates brain health measures from different disciplines, including: resting cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) (using transcranial Doppler (TCD) ultrasound and magnetic resonance imaging (MRI)); cognition (including attention and memory); and quality of life (QoL) questionnaires. Differences between age (younger versus older) and cardiorespiratory fitness (fit versus unfit) groups were also investigated. Importantly, these multimodal brain health measures were completed in the same cohort.

Declines were observed between younger and older groups in resting CBF measures (derived using TCD and MRI), and cognitive performance measures (attention-switching, learning and memory). In the older group, higher fitness offset declines in resting CBF and improved markers of cognition. In both groups, fitness significantly positively correlated with better QoL. However, no differences between age or fitness groups were observed in CVR measures. Further, CVR differed significantly depending on the imaging and analysis approach used. Future research is required to elucidate the cause of discrepancies and determine differences between groups (i.e., age/fitness/disease). Further, robust approaches to assess brain vascular health are needed.

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# PUBLICATIONS TO DATE

**Journal Publications related to this thesis**

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**Journal Publications from additional work completed alongside PhD**

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Burley CV, Mullinger K, Francis ST, Phillips AC & Lucas SJE (2017). *Magnetic Resonance Imaging meets Doppler: Contradictory or complementary measures of cerebrovascular reactivity?* Presented at the combined 64th Annual Meeting of American College of Sports Medicine (ACSM) and 8th World Congress on Exercise is Medicine, Denver, Colorado. Published in World Congress on the Basic Science of Exercise and the Brain, 49:5 and Medicine and Science in Sports and Exercise 49:825.

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Burley CV, Lucas SJE, Mullinger K & Whittaker AC (2017). *Revisiting the cross-stressor adaptation hypothesis: effects of ageing and aerobic fitness on stress reactivity.*

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Burley CV, Mullinger K, Francis ST, Phillips AC & Lucas SJE (2016). *Magnetic Resonance Imaging meets Doppler: Contradictory or complementary measures of cerebrovascular reactivity?* Presented at the Physiological Society Annual Meeting, Dublin, Ireland. Published in Proceedings of the Physiological Society 37, PCB293.

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Lucas SJE, Thomas K, Wright RL, Ramsey JRE, Medwell AL & Burley CV (2017). *Effect of Aquatic-Treadmill Training on Cerebrovascular Function in Community-Dwelling Stroke Survivors: A Pilot Study*. Poster presentation at the combined 64th Annual Meeting of American College of Sports Medicine (ACSM) and 8th World Congress on Exercise is Medicine, Denver, Colorado. Published in World Congress on the Basic Science of Exercise and the Brain, 49:5 and Medicine and Science in Sports and Exercise 49:30.

Thomas K, Burley CV, Wright RL, Ramsay JRE, Lucas SJE (2017). *Effect of aquatic-treadmill training on cerebrovascular function and gait in community-dwelling stroke survivors: A Pilot Study*. Poster presentation at the Congress on NeuroRehabilitation and Neural Repair, Maastricht, The Netherlands.

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# ABBREVIATIONS

| | |
|---|---|
| ACA | Anterior carotid artery |
| ANOVA | Analysis of variance |
| ASL | Arterial spin labelling |
| AST | Attention switching task |
| BOLD | Blood-oxygen-level dependent signal |
| CANTAB | Cambridge neuropsychological test automated battery |
| CBF | Cerebral blood flow |
| $CO_2$ | Carbon dioxide |
| CVR | Cerebrovascular reactivity |
| CVCi | Cerebrovascular conductance index |
| CVRi | Cerebrovascular resistance index |
| CVR | Cerebrovascular reactivity |
| DABS | Double acquisition background suppression |
| ECA | External carotid artery |
| FAIR | Flow-sensitive alternating inversion recovery |
| GLM | General linear model |
| GM | Grey matter |
| HVLT | Hopkins verbal learning task |
| ICA | Internal carotid artery |
| MAP | Mean arterial blood pressure |
| MCA | Middle cerebral artery |
| MCAv | Middle cerebral artery blood velocity |
| MPRAGE | Magnetisation prepared rapid gradient echo |
| MRI | Magnetic resonance imaging |
| NIRS | Near-infrared spectroscopy |
| NZPARQ-SF | New Zealand physical activity readiness questionnaire - short form |
| PAL | Paired associates learning |
| PASAT | Paced auditory serial addition task |
| PASL | Pulsed arterial spin labelling |
| $P_{ET}CO_2$ | End-tidal carbon dioxide |
| PCA | Posterior carotid artery |

| | |
|---|---|
| PC angiography | Phase contrast angiography |
| $PCO_2$ | Arterial content of carbon dioxide |
| RAND-36 | Research and Development 36-item short form survey |
| RoI | Region of interest |
| RT | Reaction time |
| RTI | Reaction time task |
| SPSS | Statistical package for the social sciences |
| SWM | Spatial working memory |
| TCD | Transcranial Doppler |
| tDCS | Transcranial direct current stimulation |
| QoL | Quality of life |
| VE-$CO_2$ | Ventilatory sensitivity to carbon dioxide |
| V̇O2 max | Maximum oxygen consumption |
| WHOQOL-BRIEF | World Health Organisation Quality of Life questionnaire, abbreviated version |

# 1. INTRODUCTION

Optimal functioning of the human brain is crucial in living a healthy lifestyle and avoiding neurological and psychiatric conditions that are associated with impaired brain health (e.g., dementia and depression). Complications arise when considering what the optimal approaches are for measuring brain health, as there is no singular universally agreed measure. Rather, there are countless outcome measures that have developed from a range of different scientific disciplines, each with very different and complex theoretical underpinnings. The overall aim of this PhD was to investigate a number of different approaches in obtaining brain health outcome measures; specifically, cerebral blood flow (CBF) (resting CBF and cerebrovascular reactivity (CVR) using transcranial Doppler (TCD) ultrasound and magnetic resonance imaging (MRI)), cognitive performance and quality of life. Further, associations with ageing and fitness were investigated.

Chapter 2 introduces several approaches in measuring brain health that are present in the scientific literature, and includes their theoretical underpinnings, populations they have been investigated in, advantages and disadvantages of the chosen technique, and further questions that warrant investigation. This leads to a summary of research questions this thesis aimed to address.

Chapter 3 investigated transcranial Doppler (TCD) ultrasound methodological approaches in assessing brain health by calculating the common outcome measure of CVR (i.e., the cerebrovascular response to breathing room air with increased carbon dioxide ($CO_2$)). Specifically, the $CO_2$ stimulus duration and method of data extraction (i.e., the defined steady state time-point) were investigated to see whether different methodological

approaches affected the TCD-derived CVR outcome measure. The stimulus duration and method of data extraction did indeed alter the outcome measure. Therefore, caution should be taken when comparing results between studies that use different stimulus durations of steady state time-points. This led to the question of whether different imaging modalities (e.g., TCD versus MRI) would also affect outcome measures involving CBF (either resting CBF or CVR).

Therefore, Chapter 4 investigated different brain imaging modalities and analysis approaches in obtaining resting CBF outcome measures. Brain imaging modalities were TCD (middle cerebral artery blood velocity (MCAv), cerebrovascular conductance (CVCi), and cerebrovascular resistance (CVRi)), and MRI (cerebral perfusion and transit times obtained from the whole grey matter and specific regions of interest (RoIs)). Chapter 5 investigated different brain imaging modalities and analysis approaches in obtaining CVR outcome measures. Brain imaging modalities were TCD (extracting 30-seconds of MCAv and end-tidal $CO_2$ data from the stimulus duration) and MRI (calculating CVR from a linear regression across the whole stimulus duration, again obtained from the whole grey matter and RoIs). For both Chapter 4 and 5, differences between age and fitness groups were also investigated. Further, associations were calculated between outcome measures obtained using different approaches.

Resting CBF measures showed similar differences between age and fitness groups, though none of the fitness effects were significant. These findings indicate that although resting CBF measures obtained using TCD and MRI are not technically measuring the same physiological phenomenon (i.e., TCD is targeting the centre of the MCA to obtain velocity whereas MRI is targeting perfusion in the microvasculature), they do show similar patterns

between age and fitness groups. In contrast, no patterns were observed between age and fitness groups in CVR measures obtained using different modalities, illustrating that responses are different between the macro (i.e., MCA) and micro vasculature. Further TCD-derived and MRI-derived measures of CVR may be differentially affected by healthy ageing and aerobic fitness.

Chapter 6 investigated whether differences in cognitive performance in several tasks (attention switching, memory, learning and mental arithmetic) and differences in quality of life (QoL) were observed between different age and fitness groups. The findings from these data showed that cognitive performance declines across the lifespan though can be offset by physical fitness. The fitness effect was specifically evident when looking at performance in the attention switching task and mental arithmetic, though fitness effects from the other cognitive tasks (memory and learning) were less clear. Quality of life measures significantly correlated with fitness in both the younger and older groups, indicating that being physically active improves mood and well-being and may protect against negative affect. Finally, Chapter 7 gives an overall conclusion of all the chapters included in this thesis.

# 2. BACKGROUND AND EXISTING LITERATURE

## 2.1. Overview

The aim of this chapter is to give an overview of the various brain health outcome measures that are used across different scientific disciplines and in clinical contexts, and consider how outcome measures are interpreted to reveal brain changes that occur during healthy ageing, in response to clinical conditions (e.g., dementia, stroke, depression), and in response to a targeted intervention (e.g., non-pharmacological interventions including cognitive training and physical exercise). This review will also consider how methodological approaches between research centres may change the outcome measures and interpretations. Further, recent studies will be discussed that combine different methodological approaches, and consider how they can be appropriately developed into multi-disciplinary brain health assessments that are individualised and person-centred.

An exhaustive inclusion of all brain health outcome measures is beyond the scope of this review, here focus will be on: brain health outcome measures that assess structural and functional changes from different neuroimaging techniques (including Doppler ultrasound, magnetic resonance imaging (MRI) and near-infrared spectroscopy (NIRS)); brain health measures assessing performance in cognitive domains (including attention, memory, learning, emotional processing and mental arithmetic under time pressure); neuropsychological measures of psychological function used in a clinical context to determine severity of symptoms and therapeutic outcomes (via questionnaires), and quality of life (QoL) questionnaires that assess well-being.

## 2.2. Introduction and Background to Measuring Brain Health

The incidence of cognitive decline and neurodegenerative diseases (e.g., stroke and dementia) is increasing. Numerous studies show that regular exercise has beneficial effects on brain health in healthy and clinical populations. However, the underlying mechanisms are poorly understood and the way in which 'brain health' is measured varies considerably between studies and disciplines. There is also a growing interest in optimising brain health in healthy individuals to improve cognitive function, emotional well-being and quality of life in an increasingly ageing population. Consequently, a robust measurement of brain health is necessary to: (a) assist with the diagnosis of conditions that are linked with impaired brain function; (b) determine whether a clinical or lifestyle intervention has been effective, and if so, by what mechanism(s), and (c) to understand and predict healthy ageing in relation to brain health for the general population.

There is currently no universally agreed measure of brain health, and no single methodology that can adequately address any research question relevant to brain health. Therefore, the aims of this review are to: i) introduce the main methods that are currently used in the scientific and clinical literature; ii) consider the context in which they are used and the research questions that are being explored; iii) assess the strengths and weaknesses of each method considering the research question, and finally iv) consider how a combination of measures may be used to develop a multi-modal measurement of brain health. This will provide a much more informed and sophisticated overall picture of brain health. I will also address whether various methods are complementary or contradictory, particularly when assessing the same outcome measure (e.g., Doppler versus MRI measures of cerebrovascular reactivity).

Firstly, this review will introduce and discuss the theory and development of measures currently in use. These include imaging methods to measure cerebral blood flow (CBF) including: Doppler ultrasound (spanning transcranial Doppler (TCD), Duplex Doppler and transcranial colour Doppler (TCCD)), magnetic resonance imaging (MRI) (particularly arterial spin labelling (ASL) and blood-oxygen-level-dependent (BOLD) signal measures), and near-infrared spectroscopy (NIRS). They also include cognitive and neuropsychological measures such as: cognitive tasks (e.g., attention, memory, learning, emotional processing and mental arithmetic); clinical and neuropsychological batteries, and measures of well-being and quality of life. Next, these methods will be put into context by discussing the research questions in which they are used and consider the methodological strengths and limitations of each. Conflicts between and within different techniques including variations of use within the same technique will also be considered. Finally, the use of these measures specifically to assess the effects of physical exercise on the brain and natural variability of such measures across the lifespan due to healthy ageing will be reviewed. The overall purpose of this review is to shed light on the most appropriate measures of brain health to use that assess the effects of an exercise intervention in both clinical and healthy ageing populations. A summary of key studies using different brain health outcomes measures is presented at the end of this chapter (Table 2.1).

### 2.3. Neuroimaging Brain Structure and Function

The division between structural and functional imaging is difficult to define because they are often intertwined in the brain (Symms et al., 2004). Although definitions vary and are sometimes broad, structural imaging tends to be described as providing static anatomical information about the brain whereas functional imaging provides dynamic physiological

information, often in response to a stimulus. The basis of functional neuroimaging measures involves the measurement CBF either at rest or during function (i.e., in response to a stimulus). This section will include a brief history of the measurement of CBF and the development of tools commonly used today, followed by focused sections on Doppler ultrasound technology, MRI and NIRS.

### 2.3.1. Measuring Cerebral Blood Flow and Metabolism – A Brief History

Over one hundred years ago, German physician and physiologist, Adolf Fick introduced the principle based on the conservation of mass; 'that the quantity of a substance taken up by an organ per unit of time is equal to the blood flow through that organ multiplied by the difference between its arterial and venous concentrations' (Fick, 1870). Since then the well-established 'Fick principle', originally proposed as a method of measuring cardiac output, has been translated to the assessment of CBF and has influenced the on-going development of this outcome measure.

During the 1940s, cardiac catheterisation became available and the technique could be tested in humans. In 1945, Seymour Kety and Carl Schmidt applied the Fick principle to CBF measures in humans by using 15% nitric oxide ($N_2O$) in air (Kety and Schmidt, 1944). They then estimated blood flow across the whole brain from repeated sampling of arterial and jugular venous blood during inhalation of $N_2O$ (in air) until approaching tissue saturation (this would take approximately 10 minutes) (Figure 2.1). This became the first quantitative method of measuring CBF in humans.

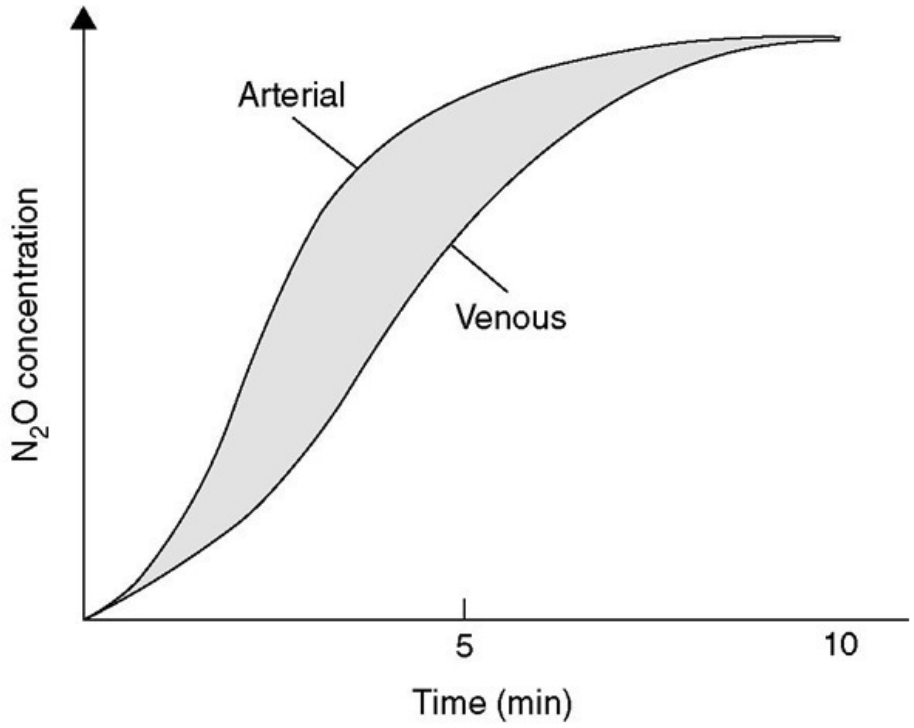



Figure 2.1. The Kety-Schmidt technique for measuring cerebral blood flow using nitric oxide ($N_2O$) as a diffusible tracer. After approximately ten minutes of inhalation, brain tissue is reaching saturation and the arterial and venous concentrations of $N_2O$ are almost equal. The grey area is proportional to hemispheric blood flow (Kety, 1951).

In 1951, the Fick principle was extended to account for the exchange of diffusible gases in the lungs and tissue and the convolution integral to CBF method was first applied in animals (Kety, 1951). This method, unlike earlier work, did not require brain tissue to meet saturation because the convolution integral yields tissue concentration at any time point. In these early animal studies, trifluoroiodomethane was inhaled or intravenously infused for one minute and post-mortem slices were used to determine cerebral uptake. This allowed for highly localised CBF measurements and clearly established differences in brain blood flow between grey and white matter (Pinsky, 2002).

In 1961, human studies involving intra-carotid injections were introduced where the internal carotid artery (ICA) was injected with Xenon-133 ($^{133}$Xe) a highly diffusible gas, and gamma radiation monitored by extracranial detectors (Ingvar and Lassen, 1961). Transit times were then implemented into a height divided by area analysis where the first quantitative regional assessment of CBF was possible in humans. Later developments involved less invasive inhalation methods rather than injections into the ICA (Mallett and Veall, 1965). Kety's equation was first applied to human studies by using least squares curve fitting, which provided separate estimates of grey and white matter flow and $^{133}$Xe inhalation became the most widely used technique.

Alongside these developments, brain imaging technologies such as Doppler ultrasound were evolving. In 1842 Austrian mathematician and physicist Christian Doppler described what is now known as the Doppler effect. This principle expresses how the observed frequency of a wave depends on the relative speed of the source and the observer. In the 1950s, physicians used this principle to develop the first two-dimensional compound ultrasound scanner by incorporating an immersion tank using a cattle-watering container with an ultrasound transducer mounted on a wooden rail (described in Goldberg et al., 1993; Figure 2.2). By the 1980s portable Doppler ultrasound technology was developed for use in hospitals and today can be used on hand-held equipment (e.g., tablet).

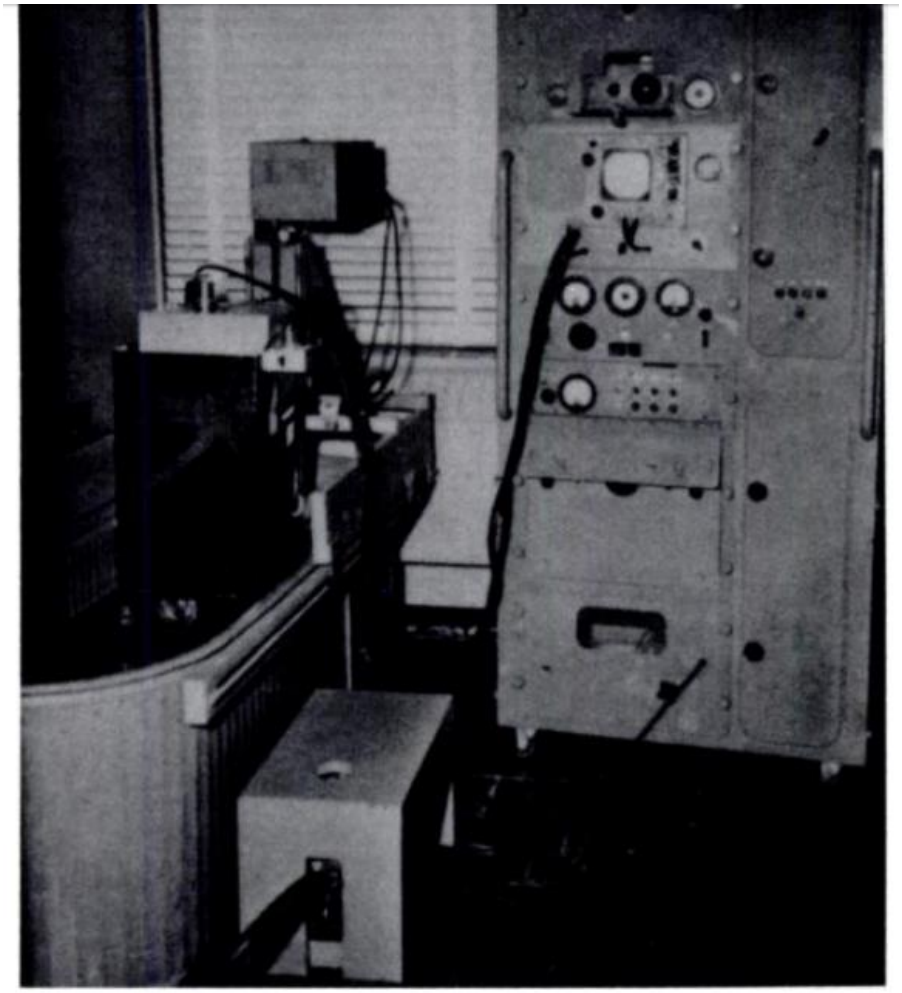


Figure 2.2. Colorado group's earliest successful cattle-tank scanner. The transducer was mounted on a wooden rail that ran outside of the tank (Goldberg et al., 1993; reprinted from Medical Diagnostic Ultrasound: A Retrospective on its 40th Anniversary).

The transducer uses the piezoelectric effect (pressure-electric) to create an ultrasound beam that is reflected to the transducer. Longitudinal waves bounce in and out of the tissue to produce a 2D grey scale ultrasound image. The beam continues through liquid densities and bounces back in response to air and bone, making it ideal for detecting soft tissue biological structures including the heart and arteries. When acquiring an image using ultrasound, identification of the acoustic window is required where there is a lot of liquid that allows the sound area to travel through so that the beam can reach biological structures. One application of this is to use Doppler ultrasound technology to measure

CBF, and in 1982 Rune Aaslid developed and introduced the transcranial Doppler (TCD) method providing measurements of blood flow velocity in the middle cerebral artery (MCA), anterior cerebral artery (ACA) and posterior cerebral artery (PCA) (Aaslid et al., 1982) located through the temporal bone above the zygomatic arch.

The Circle of Willis provides the blood supply to the brain by connecting the internal carotid and vertebral artery (VA) circuits together (Figure 2.3). The ICA further divides into the MCA and ACA, and the MCA feeds the lateral sulcus. It is important that repeat measurements are taken from as close to the same location as possible to ensure that the same artery has been measured. Further, velocity measures may differ depending on where in the vessel they are taken. This is particularly evident in a clinical context, for example, in intracranial arterial disease where there is a focal increase in velocity at the site of luminal narrowing, and decreased velocity immediately downstream from the lesion (Sarkar et al., 2007).

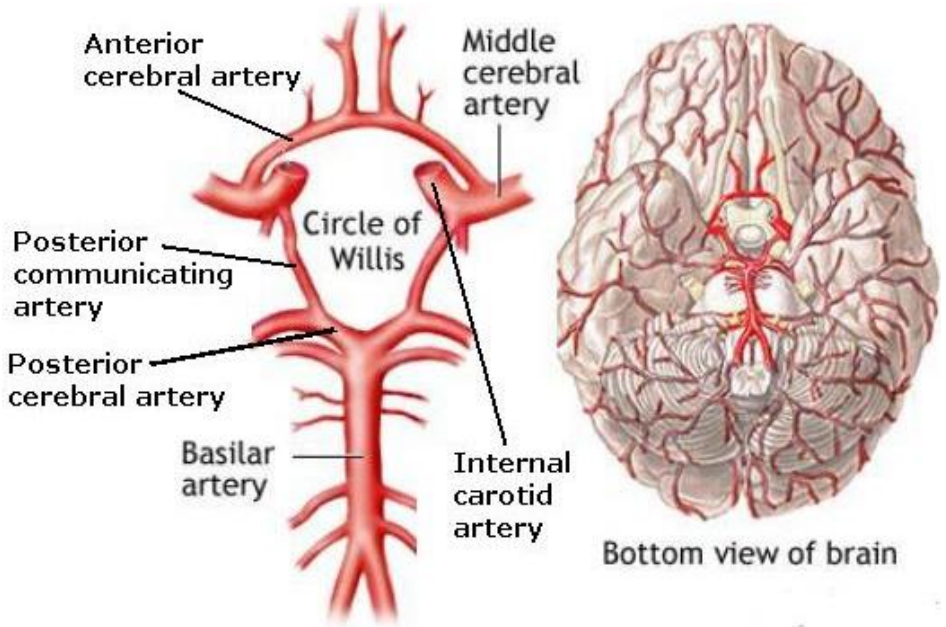



Figure 2.3. The main components of the Circle of Willis: the anterior cerebral artery, anterior communicating artery, the internal carotid arteries, posterior communicating artery, and the middle cerebral arteries (taken from American Accreditation HealthCare Commission (ADAM) Inc., www.urac.org).

The application of Doppler ultrasound allows the methodological approach of calculating resting brain blood velocity (using TCD) as well as measures of vessel diameter and flow (using Duplex Doppler) and blood flow responsiveness (i.e., CBF response to a stimulus such as carbon dioxide or oxygen, or from a rapid change in blood pressure) outcome measures commonly acquired today, and these will be discussed in more detail in the next section.

However, techniques using external detectors do not allow measurements of structure (i.e., hippocampal volume) or function (i.e., CBF) from deeper areas of the brain. During the 1970s and 1980s, a form of MRI and computed tomography (CT) were developed, shortly followed by single-photon emission computed tomography (SPECT) and positron emission tomography (PET), allowing non-invasive neuroimaging that can now be used to reveal anatomical and CBF information from brain structures deep beneath the surface as well as the surface measures achieved with Doppler ultrasound. Each of these methods have strengths and limitations in what can be done with the methods, which will be discussed later in this chapter.

### 2.3.2. Doppler Ultrasound

The following section will describe Doppler ultrasound techniques in more detail including TCD, linear Duplex Doppler and TCCD. Current literature will be discussed including methodological development and considerations, and ageing and fitness effects on the outcome measures that these techniques provide.

#### 2.3.2.1. Transcranial Doppler (TCD)

Transcranial Doppler is a non-invasive technique used to measure CBF velocity within the cerebral vessels through ultrasound (described in more detail in Section 2.3.1). In the context of TCD, resting CBF velocity is used to index CBF. Responsiveness measures, where CBF changes in response to a stimulus, are often taken as well as resting CBF measures. General tests within this field include: cerebral autoregulation (CA) (Sorond et al., 2005, 2013; Meel-van den Abeelen et al., 2014), neurovascular coupling (NVC) (Sorond et al., 2011; Yam et al., 2005), and blood flow velocity response to increased carbon dioxide ($CO_2$) inhalation (known as cerebrovascular reactivity/ responsiveness (CVR)) (Bailey et al., 2013; Barnes et al., 2013). These are discussed in more detail below after a brief overview of the main cerebral arteries that can be imaged using TCD.

The MCA (Figure 2.3) has received the most attention in the literature because of its vital role in feeding the brain (total CBF perfusion distribution: MCA, 21%; distal MCA, 6%; ACA, 12%; distal ACA, 4%; ophthalmic artery, 2%; PCA, 8%; basilar artery (BA), 20%; and vertebral artery (VA), 28%; Zarrinkoob et al., 2015), its relative ease to locate using TCD, and that it has a high baseline velocity. For a more detailed review of this technique see a review by Willie and colleagues (Willie et al., 2011). The posterior cerebral artery (PCA) has also received attention and it had been assumed that the CVR outcome measure was similar within both posterior and anterior regions (Ainslie and Duffin, 2009), though other studies have challenged this assumption (Skow et al., 2013; Sorond et al., 2005). For example, Skow and colleagues reported that the absolute (though not relative) CVR measure was larger in the MCA compared to the PCA. Further, differences in both CA and CVR have been observed between the MCA and PCA territories (Sorond et al., 2005). Differences in anterior and posterior circulation have also been shown in response to

exercise (Sato et al., 2011), heat (Bain et al., 2015) and hypoxia (Willie et al., 2012). Cerebral blood flow is measured with Doppler ultrasound in different ways depending on the technology used. Transcranial Doppler ultrasound measures CBF velocity in centimetres per second through the section of the artery being imaged. In contrast, Duplex Doppler measures of CBF involve a calculation of flow using measurements of CBF velocity and vessel diameter. Poiseuille's Law describes the flow of fluids through an intravenous catheter. It states that the flow (Q) of fluid is related to a number of factors: the viscosity of the fluid (i.e., blood), the pressure gradient across the tubing (i.e., vessel), and the length and diameter (r) of the tubing. With regard to Duplex Doppler, the flow volume of an artery is determined using the formulae: flow volume = time-averaged velocity x area (Q = TAV x A) (Schöning et al., 1993).

Transcranial Doppler has been used to measure brain health by assessing both absolute measures (i.e., cerebral blood flow velocity at rest) and functional measures in response to a stimulus (e.g., CVR). Measures of resting CBF velocity are often examined where participants are asked to lie down in a supine position, relax and breathe as naturally as possible whilst TCD measurements are taken (e.g., Ainslie et al., 2008; Brown et al., 2010; Bailey et al., 2013; Barnes et al., 2013). A key study by Ainslie and colleagues (2008) related to this thesis topic used this approach to quantify age-related and physical fitness effects on CBF (velocity). Their findings clearly demonstrated the age-related decline in MCA velocity independent of training status in healthy sedentary and endurance trained men between the ages of 18 to 79 years, and that MCA velocity was consistently elevated in those who were endurance trained (Figure 2.4).

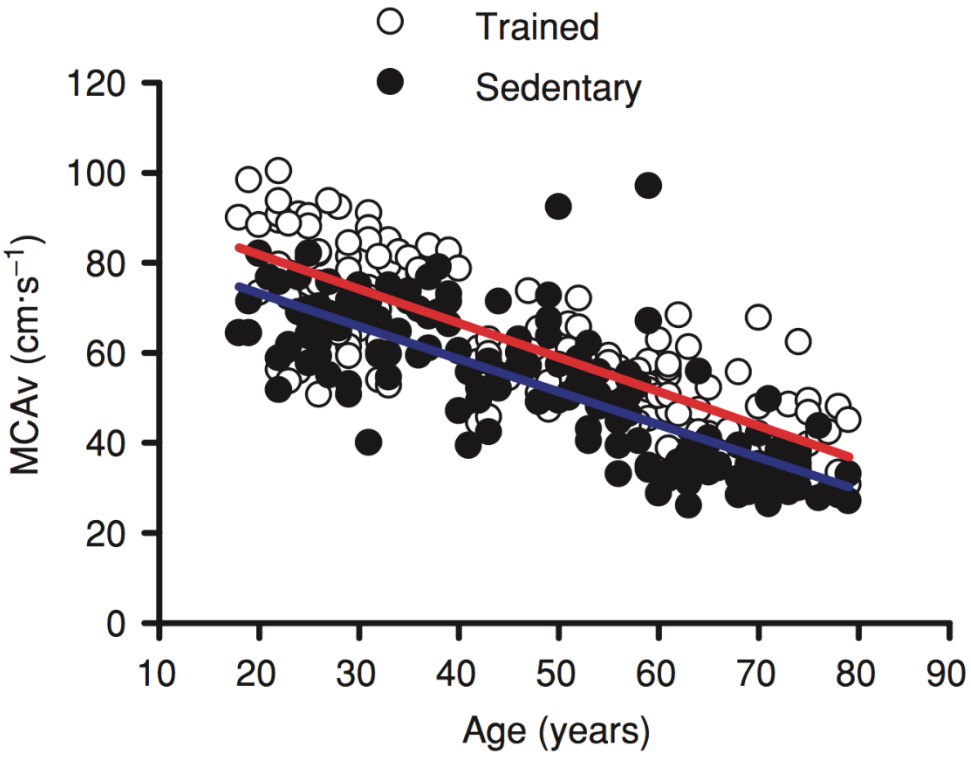



Figure 2.4. Graph showing the relationship between age, CBF velocity (obtained from the middle cerebral artery (MCAv)) and physical fitness. The red line represents the linear regression for the endurance-trained group. The blue line represents the linear regression for the sedentary group. MCAv was consistently elevated by 9.1 ± 3.3 cm · $s^{-1}$ (CI = 2.7-15.6, $p$ = 0.006 (17%)) in endurance-trained men throughout ageing (Ainslie et al., 2008).

Other studies have reported resting CBF values corrected for blood pressure and found similar age-related patterns (Brown et al., 2010; Barnes et al., 2013; Bailey et al., 2013) (Figure 2.5, A,C). Concurrent recordings of mean arterial blood pressure (MAP) enable researchers to calculate cerebrovascular conductance indices (CVCi) calculated as MCAv (cm·$s^{-1}$) per MAP (mm Hg), and cerebrovascular resistance (CVRi), which is the inverse of CVCi (i.e., MAP/ MCAv). These measures have the additional benefit of accounting for changes in CBF velocity driven by blood pressure (Harper et al., 1966).

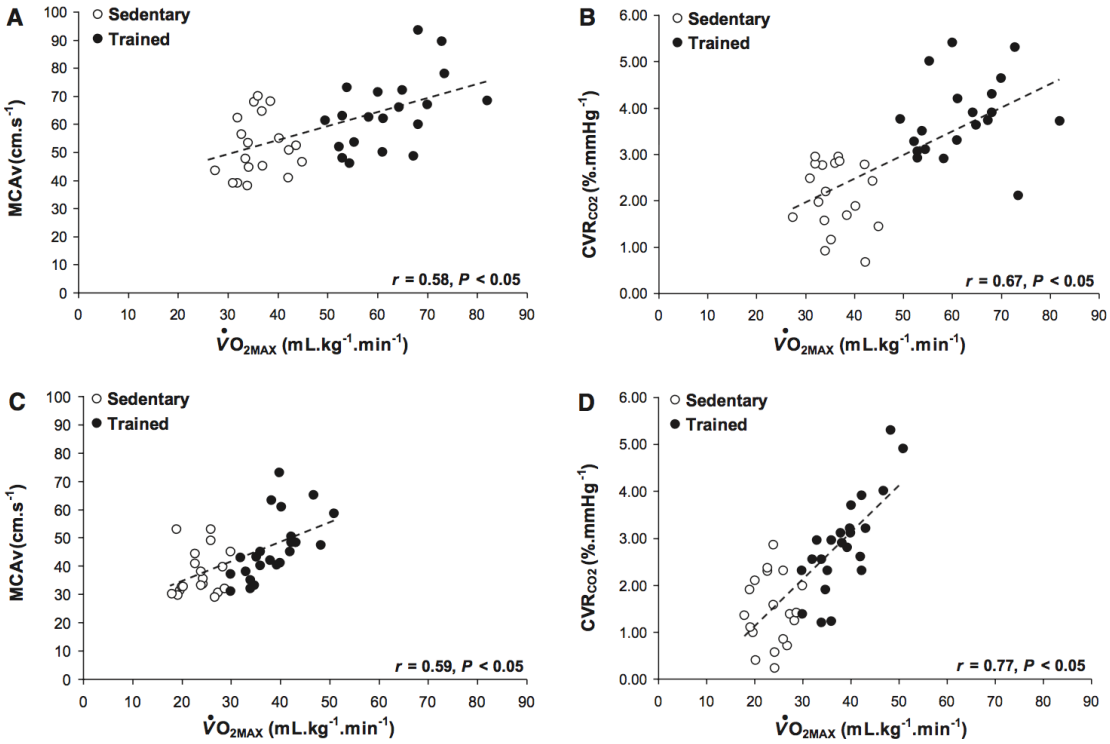



Figure 2.5. Graphs showing the relationships between cardiorespiratory fitness and cerebrovascular haemodynamics in younger (A and B) and older (C and D) adults as a function of physical activity status. $CVR_{CO2}$ indicates CVR to carbon dioxide (5% in air); MCAv, middle cerebral artery blood velocity; and V̇O2 max indicates maximal oxygen uptake (i.e., an individual's peak aerobic capacity) (Bailey et al., 2013).

Brown and colleagues reported similar patterns in older women (Brown et al., 2010); specifically, physically fit women had higher CVCi compared to their sedentary counterparts. Further, overall cognition was negatively correlated with age and positively correlated with fitness (as measured by V̇O2 max). In this study, fitness was calculated as a percentage of age-predicted V̇O2 max values (Tanaka et al., 1997) so that measures could be calculated across all participants without being confounded by effects of age. However, it could be argued that because the raw quantitative values derived from the fitness test are no longer being used, the fitness score is less representative due to the subjective nature that participants are put into one of three groups (determined by level of activity assessed via a questionnaire) to determine their age-predicted percentage, therefore making the measure more subjective.

Overall, these TCD-derived findings complement similar findings reported from MRI studies investigating baseline perfusion, where ageing is associated with a decrease in whole brain and region specific cerebral perfusion (Parkes et al., 2004; Chen et al., 2011; Clark et al., 2015); discussed in detail below.

#### 2.3.2.2. Cerebral blood flow regulation and Doppler Ultrasound

High metabolic demands of the brain involving large amounts of oxygen over a relatively small mass are required to support the high rate of ATP production and glucose delivery, so that neuronal signal transmission is maintained (reviewed by Hoiland et al., 2016 & Bailey at al., 2009). This high rate of oxygen consumption is associated with a high 'vulnerability for failure' and adequate delivery of oxygen to the brain via precise CBF regulation is vital to maintaining optimal function and avoid cellular damage (reviewed in Bailey et al., 2009).

Cerebral autoregulation refers to the regulatory process of the brain vasculature that responds to maintain cerebral perfusion relatively constant during changes in blood pressure (BP) (Lassen 1959; Paulson et al., 1990; Van Beek et al., 2008; Lucas et al., 2010; Willie et al., 2014) (Figure 2.6). Traditionally, a rise in MAP was considered to accelerate CBF, and a fall to slacken it (Bayliss et at., 1895). However, Lassen (1959) plotted average BP and total CBF from seven studies including 11 different patient groups (Figure 2.6), that revealed a plateau region where CBF appears to be stable across a wide range of blood pressures (~60-150 mm Hg). Physiological studies support the idea of hysteresis in the dynamic pressure-flow relationship (reviewed by Ainslie and Brassard, 2014), where the brain effectively buffers acute hypertension better than hypotension.

The physiological relationship between reflex adjustments in cerebrovascular resistance and BP was termed static CA (Willie at al., 2014). Static CA (sCA) describes this CBF response over a longer period (i.e., minutes of BP change), whereas dynamic CA (dCA) traditionally refers to the alterations in CBF in response to rapid changes in BP (i.e., over seconds). Of note, a change in thinking regarding the distinction between sCA and dCA has emerged within the scientific literature where dCA may simply reflect a faster static function (Tan, 2012). This lack of clarity around the division between sCA and dCA parallels the MRI structural versus functional distinctions described above.

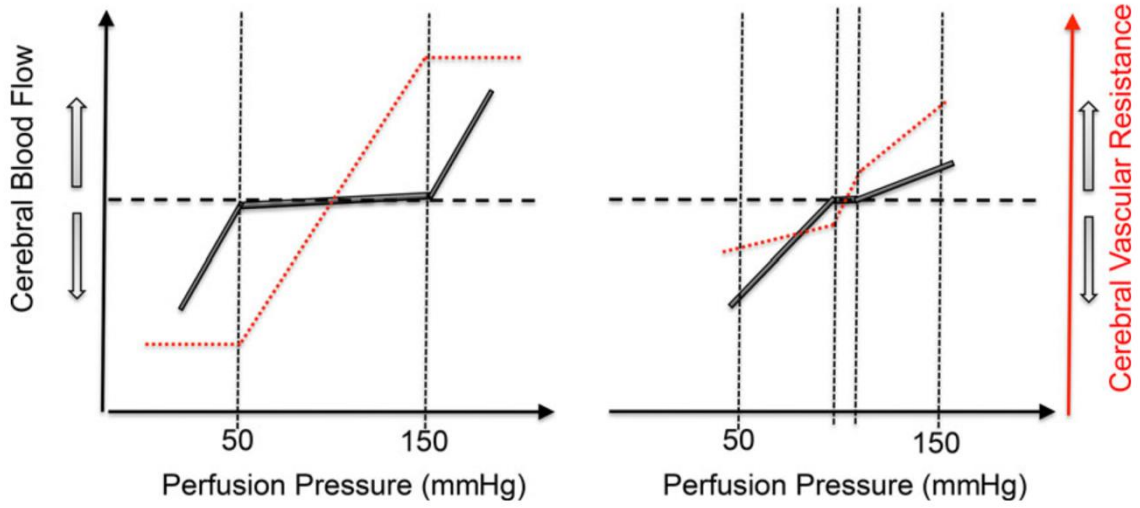



Figure 2.6. Representation of the classical (left) and contemporary (right) relationships between mean arterial blood pressure (MAP) and cerebral blood flow (CBF), i.e., cerebral autoregulation (CA). Right panel indicates a small plateau region and CA hysteresis, and a far more pressure-passive CBF then is conventionally believed (taken from Willie, 2014).

Given the transient nature of the process being assessed during CA (particularly dynamic), TCD is an ideal imaging approach to use due to its high temporal resolution. More recently, CA has also been assessed using MRI and compared with TCD, where similarities were observed between the techniques in acute ischaemic stroke (AIS) patients (Panerai et al., 2016). In this study, the method used to manipulate BP was the thigh cuff

manoeuvre, where a sudden drop in CBF velocity is induced and dCA assessment is the rate of return to baseline.

Dynamic CA has been shown to be impaired in several age-related clinical conditions, including ischemic stroke (Eames et al., 2002), carotid stenosis (White and Markus, 1997), and more recently, Alzheimer's disease (Meel-van den Abeelen et al., 2014). Healthy ageing is generally associated with preserved CA in the MCA, in response to postural change using a sit-to-stand protocol, though there may be increased vulnerability of the posterior cerebral autoregulation to blood pressure (Sorond et al., 2005).

Cerebral metabolic demands and increases in CBF are also linked with NVC. Neurovascular coupling is defined here as the functional and spatial relationship between neuronal activation and CBF, and ensures adequate oxygen and glucose to the activated neurons (Purkayastha and Sorond, 2012). Regional increases in CBF velocity are seen during cognitive and motor tasks due to increases in metabolic demand. NVC is a transfer function between neural and CBF responses. Therefore, TCD can be used to measure the output of NVC by recording CBF velocity during cognitive or motor tasks. Further, TCD is non-invasive and has high temporal resolution (see Phillips et al., 2016 for review of methods used to assess NVC and measurement guidelines). Impaired NVC has been associated with significant pathology (e.g., ischaemic stroke patients; Lin et al., 2011) though remains unaffected during normal ageing (Yam et al., 2005), in contrast to other Doppler-derived cerebrovascular measures (i.e., resting CBF and CVR). Further, physical activity and dietary modifications have been linked with altered NVC, and NVC has been associated with performance in cognitive tasks (Sorond et al., 2011; 2013 respectively). In Sorond and colleagues' 2011 study, they found impaired NVC, assessed in bilateral MCA

using TCD during the N-Back task, in slower walkers compared to their fast walking counterparts, indicating improved NVC was associated with physical activity. In 2013, Sorond and colleagues showed that four weeks of controlled cocoa consumption had significant effects on NVC in participants with impaired coupling at baseline, and this was also positively associated with cognitive performance, again assessed with the N-Back task.

#### 2.3.2.3. Cerebrovascular reactivity

The CVR to a stimulus such as changes in arterial content of $CO_2$ or $O_2$ can also be assessed by using TCD, and is emerging as a key marker of brain health that changes across the lifespan and in response to disease (investigated in Chapters 3 and 5, described in more detail in Sections 2.3.3.2, 3.2 and 5.2.1). Carbon dioxide is commonly used to induce this response, commonly referred to as CVR, cerebral vasoreactivity or CBF-$CO_2$ responsiveness. The term CVR will be used primarily throughout this thesis. Unfortunately, researchers have used different methodological approaches to assess this marker of vascular function. These difference approaches include the stimulus duration (e.g., 1, 2, 4 or 5 minutes) and gas concentration (e.g., 4, 5, 7% $CO_2$) used; the brain imaging modalities used to collect data (i.e., MRI: BOLD and ASL, discussed in Section 2.3.3.; and TCD: MCAv); and the analysis approaches used to calculate these measures. Such differences may in part explain some of the inconsistent findings in the literature to date, as discussed below.

Based on TCD-derived measures, CVR is emerging as a potential indicator of brain vascular health in both general and clinical populations. For example, impaired CVR and impaired CA has been observed in heart failure (Alosco et al., 2014) and Alzheimer's

Disease (Meel-van den Abeelen et al., 2014). In addition, CVR has been shown to predict the risk of stroke and transient ischaemic attack (TIA) in patients with carotid occlusion (Markus et al., 2001), and has also been linked with increased mortality (Portegies et al., 2014). Further, CVR has been linked with healthy ageing and cognitive performance outcome measures (discussed below and in Section 2.5).

Outcome measures of CVR have also been used to explore the effects of physical activity on brain health. Cross-sectional studies have shown that sustained aerobic fitness throughout the lifespan is associated with improved cerebral haemodynamics determined by CVR (Bailey et al., 2013) (Figure 2.5, right panel). In addition to this, Bailey and colleagues suggest age-related impairment in CVR may be an outcome of the poorer fitness effect they observed in those with lower CVR. Therefore, in this healthy population, ageing may be a risk factor for disease that responded well to aerobic fitness training. These findings are supported by other studies that have explored the effects of an exercise intervention on brain vascular health. Murrell and colleagues investigated the effects of a 12-week aerobic-based training programme on resting MCA velocity and CVR. They found a greater CVR in both younger and older sedentary individuals following the training (Murrell et al., 2013). Cross-sectional studies have also shown that higher CVR and cardiorespiratory fitness in older adults were significantly correlated (Barnes et al., 2013). Though, this association was not observed in the younger group.

However, other studies report conflicting results, where higher aerobic fitness has been associated with reduced CVR, though these tend to be MRI-based studies (Thomas et al., 2013; DuBose et al., 2016). Group comparison studies have investigated ageing and fitness effects on CBF outcome measures (Zhu et al., 2013). Zhu and colleagues compared a

group of older Masters athletes with a sedentary older and sedentary younger group, finding no differences between the older groups, in contrast to the younger group who had significantly lower CVR than the Masters athletes. They concluded that life-long aerobic training has minimal effects on the age-related differences in cerebral haemodynamics, though this is perhaps due to subtle fitness effects being less pronounced than the large age effects (i.e., the younger group had a mean age of 27 years and the older group 72 years); further, this study had a small sample size (10 sedentary elderly and 11 Masters athletes). The relatively subtle fitness effects may mean that there is more variability in the measure as a result of other factors (e.g., diet, lifestyle, genetics, etc.), such that significant differences between fitness groups are only seen with larger sample sizes or well defined and differentiated fitness groups. Indeed, this latter point was emphasised in the Bailey et al., (2013) study where clear effects were apparent between fitness groups (used TCD-derived measures).

An important point to consider is the relatively low sample sizes in the studies examining CVR fitness effects published to date. Consequently, conflicting findings thus far may reflect this noise. For example, a blunted CBF-$CO_2$ response was reported in a group of Masters athletes (Thomas et al., 2013) relative to sedentary age-matched controls when measured using MRI techniques. Further, in another study of older adults, higher cardiorespiratory fitness was associated with lower CVR (DuBose et al., 2016). This leads us to consider methodological limitations in the utility of these techniques and whether CVR measures obtained with Doppler ultrasound are the same as those obtained with MRI acquisition, as well as whether imaging modality related differences will expand to fitness related differences.

### 2.3.2.4. Strengths and limitations

The following section provides a summary of the strengths and limitations of using TCD compared with other techniques, and the various CVR-derived outcome measures used as tools to measure brain vascular health.

Measures of CBF using TCD can show changes in real-time so that we can observe immediate cerebrovascular responsiveness to functional stimuli including standing, physical activity, cognitive tasks and stress. In contrast, the extent to which participants' can be observed whilst moving is limited with other neuroimaging approaches, such as MRI, PET and magnetoencephalography (MEG). Some studies have investigated exercising whilst in a MR scanner, examples include using a hand-grip (Park et al., 2008) and recumbent cycling (Gusso et al., 2012; Figure 2.7).

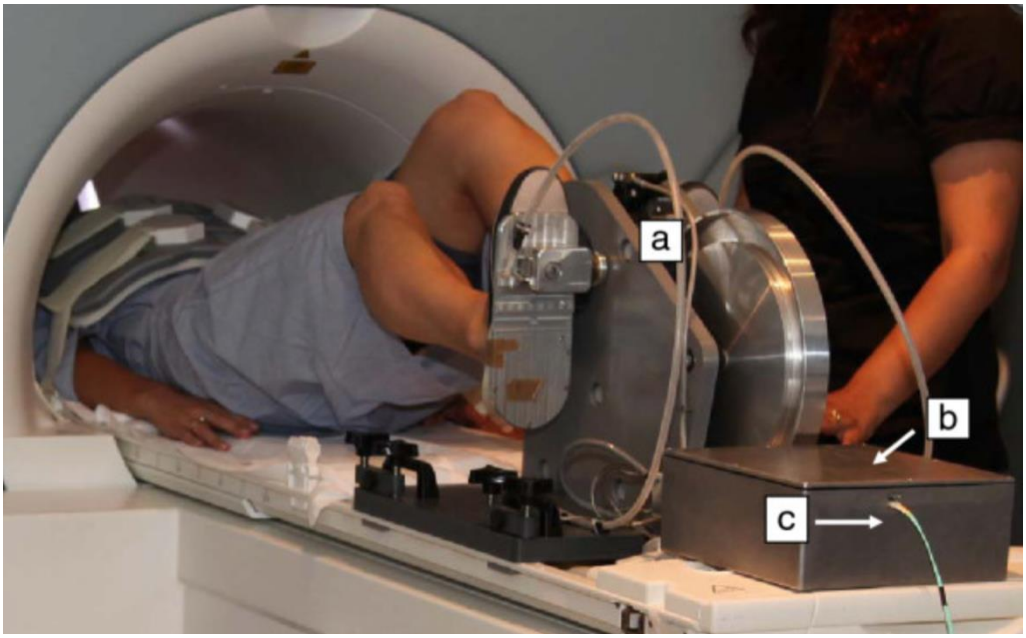



Figure 2.7. MRI cycle ergometer setting with: (a) cycle ergometer, (b) electronic box, and (c) optical fibre line (Gusso et al., 2012).

However, these are less representative of real life due to restricted movement, making it difficult to monitor dynamic and physiologically challenging adaptations involved in CBF

regulation. Further, these adaptations often involve standing whereas most MRI, PET and MEG scanners require participants to be sitting or lying down. In contrast, TCD can be worn whilst participants are undergoing common and real life behaviours, including intense whole-body physical activity such as rowing (Faull et al., 2014), being 'dropped' into water (Button et al., 2015), or simple repeated postural shifts (Phillips et al., 2016). It is important to note that studies sometimes report dysfunction that is revealed during functional tests that require movement that can reflect the benefits of regular habitual activity (i.e., optimal function maintained in individuals with higher fitness). For example, in a study examining CBF velocity at rest and during different intensities, differences between fitness groups were only revealed during the exercise stress testing and not whilst recording resting values (Bruginaux et al., 2014).

CBF velocity measures are also useful for examining changes that may occur in response to a clinical intervention, for example, taking measurements at baseline and following an 8-week exercise intervention. Measurements using the TCD modality have the potential to be used more routinely in a clinical setting as they are more cost effective than other imaging methods such as MRI, PET and MEG (Alexandrov et al., 2012) and can be taken to the patient. All these factors put TCD at a distinct advantage related to the other imaging tools, but there are important limitations of this technique that need to be considered.

Limitations of Doppler measurements of CBF velocity using TCD include velocity versus flow for indexing measures of CBF, the potential for vessel diameter change (especially with $CO_2$ stimulus), and limited depth within the brain that can be reached using this technology. Issues with this CBF measurement come mainly from methodological inconsistencies across studies. This leads us to question the interpretation of results and

whether the same results and conclusions would be drawn had a slightly different methodological approach been used. For example, the measurement of resting CBF velocity is generally consistent across studies where the mean blood flow velocity (mostly reported from the MCA) is the primary outcome measure, and is often reported along with MAP. In contrast, the way in which CVR is determined using TCD has many different approaches, including rebreathing versus steady state (e.g., Brothers et al., 2014; Skow et al., 2013), stimulus duration (i.e., 1, 2, 4 or 5 minutes) and concentration (i.e., 4, 5 or 7% $CO_2$), and the time-point where steady state is defined (determining the point of data extraction).

Steady-state methods involve participants breathing premixed gases similar to room air though with higher $CO_2$ concentration. In contrast rebreathing methods involve participants rebreathing their own expired gas mixture continuously via a Y-valve connected to a rebreathing bag. Duffin and colleagues describe in more detail the chemoreceptor responses that occur throughout ventilation that affect these outputs (Ainslie and Duffin 2009; Duffin 2011), but extensive coverage of this area is beyond the scope of this review. Of relevance here is that although steady-state methods have the benefit of being more reflective of real life breathing conditions, the interaction between the vascular and ventilatory responses may cause the final measured values of CBF velocity (and therefore the CVR outcome measure) to vary depending on the time course from which they are taken (i.e., ventilation changes throughout the stimulus will likely alter CVR values due to altering inhaled quantities of $CO_2$. This is investigated in Chapter 3).

There are also methodological variations within each of these techniques. For example, the open-circuit steady state inhalation of $CO_2$ has been administered for a stimulus duration of 1.5 (Vernieri et al., 2009), 3 (Murrell et al., 2013; Bailey et al., 2013), 4 (Guiney et al., 2015; Kastrup et al., 2001) or 5 minutes (Lavallee et al., 2009). Also, the determined time-point of steady state (i.e., beginning or end of stimulus duration) and the duration of data extraction (ranging from 10-60 seconds) differs between studies (e.g., Vernieri et al., 2009; List et al., 2015). Further, how the TCD-derived CVR measure is presented (i.e., relative vs. absolute values) may also alter the interpretation. For example, Murrell and colleague's study (2013) reported improved CVR in response to exercise training though this was only revealed by absolute, and not relative CVR (Murrell et al., 2013). Consequently, the question of whether the CVR outcome measure would have been the same and whether the same interpretations and conclusions would have been drawn between approaches arises. The first study of this thesis addresses this question; by comparing the different stimulus durations that are used as well as the time-point of steady state and duration of data extraction to determine whether the CVR outcome measure is altered depending on such methodological inconsistencies (see Chapter 3).

#### 2.4.3.2. Doppler Technologies and Neuroimaging

As previously mentioned, one complication with CBF measurements using TCD is the interpretation of MCA vessel diameter change in response to changes in $CO_2$ concentrations (Ainslie and Hoiland, 2014), and whether changes in CBF velocity in response to hypercapnia (increased arterial $CO_2$ content) and hypocapnia (lowered arterial $CO_2$ content) are accurate representations of changes in cerebral blood flow *per se* (Coverdale et al., 2014). Recently, developed Duplex Doppler technology allows the

concurrent measurement of vessel diameter in addition to blood flow velocity that can be combined to estimate absolute CBF in the insonated vessel (e.g., ICA, VA, CCA).

Duplex Doppler techniques are not without limitations. Doppler sonography in general is a particularly specialised technique that takes relatively extensive training compared to other brain health measurement methods (e.g., cognitive function and questionnaire based measures) and despite this, data collection and consequential interpretation of the analysis are highly operator-dependent (Pinto et al., 2013). Technical recommendations have been developed to try and reduce inconsistent methodological approaches (e.g., Thomas et al., 2015 for Duplex ultrasound; Willie et al., 2011 for TCD and Phillips et al., 2016 for NVC) and similar approaches to develop guidelines may be beneficial for other technologies that are influenced by experimenter abilities as well as data collection and analysis approaches (e.g., CVR). Another challenge with creating guidelines and ensuring they are up to date is that technologies are rapidly evolving. These developments improve data and imaging quality (for example) but also present challenges in ensuring consistent and reliable methodological approaches are maintained.

Traditionally, the insonated vessels (e.g., ICA, VA, MCA) assessed using Doppler approaches were thought to be largely conduit in nature, with vasomotor regulation occurring further downstream in the pial vessels (Willie et al., 2014). However, recent findings have indicated that there are discrepancies between patterns of responsiveness in the middle cerebral artery (MCA) and internal carotid artery (ICA), and that 'conduit' vessels do have a role in flow regulation via changes in vasomotor tone (Willie et al., 2011 and 2012; Willie et al., 2014). Further, TCD studies incorporating MRI approaches to

investigate the cross-sectional area of the MCA during mild hypercapnia using a 6% $CO_2$ stimulus have found increases of approximately 8% (Coverdale et al., 2015).

Recent developments in the transcranial approach have enabled the concurrent measurement of vessel diameter and blood velocity, via TCCD in healthy adults at altitude (Willie et al, 2013) and in clinical conditions including stroke and encephalic circulatory arrest (Bonvin et al., 2010; Tanzi et al., 2015). TCCD measures blood flow velocity similarly to TCD, though also displays a 2-dimensional image of the vessel and can provide concurrent measures of vessel diameter. Studies investigating changes in CBF (including measures of velocity and flow) in humans at high altitude have reported variable time courses in blood flow increases both within individuals and between studies using other approaches (i.e., TCD) (Willie et al., 2013). This variation has been partly attributed to inter-individual variation in the ventilatory and cerebrovascular responses to hypoxia, and likely reflects proportional differences from central peripheral and chemoreceptor drives (Hirshman et al., 1975). This may be one reason why different brain imaging modalities and approaches result in different findings.

Willie and colleagues (2013) study aimed to overcome this issue by using TCCD technology in co-ordination with linear Duplex Doppler ultrasound so that CBF could be measured in three distinct sites; the MCA using TCCD, the ICA and VA using linear Duplex. They found that following gradual ascent over 5-9 days to 5,050 m, CBF continued to increase over the first sixty hours at 5,050 m and then began to decrease. They observed sustained MCA dilation using the TCCD and no dilation in the VA using the linear duplex. Further they found significant inter-individual variability in CBF during

ascent and decent, accounting for approximately 40% of the statistical variation. These findings demonstrate that intracerebral vessel dilation facilitates increased oxygen delivery to the brain and should be considered in studies only using blood flow velocity. An additional consideration is that results from different Doppler ultrasound imaging approaches (TCD versus Duplex Doppler) show regional and anatomical differences (i.e., MCA versus VA) in vessel response (Willie et al., 2013).

As discussed in Section 2.3.2.3, lower CVR has been associated with healthy ageing and with neurodegenerative disease, thus, in these instances lower CVR is considered to demonstrate an impaired CBF responsiveness. However, a lower CVR may not necessarily reflect maladaptation in all individuals, particularly when considering directional changes for responsiveness measures for disease states and individuals with high fitness (e.g., Thomas et al., 2013, discussed in Section 2.3.3). Further, whether CVR is altered similarly across the cerebrovasculature is unclear. Recently, Braz et al., (2017) compared Duplex and TCD outcome measures, finding that age and fitness effects for baseline CBF were different depending on the Doppler technique used (i.e., Duplex Doppler showed fitness but not age effects, whereas TCD showed age but not fitness effects). Perhaps lack of MCA diameter constancy accounts for differential findings in Braz and colleagues study (including the trend for a differential effect with CVR)) as well as operator consistency in recording measures.

Taken together, this field of research is divided in terms of whether MCA diameter changes during alterations in arterial blood pressure, and whether this affects the CVR measure. The debate highlights recent technological advancements in Doppler ultrasound and MRI that allow us to measure brain vessel function with higher spatial resolution

(Brothers and Zhang, 2016; Hoiland and Ainslie, 2016). These different techniques have revealed conflicting findings for key markers of brain vascular health, including resting 'flow' and/or CVR (Bailey et al., 2013; Thomas et al., 2013); perhaps resulting from how such measures are derived (i.e., blood flow velocity, blood flow or changes in MRI-derived BOLD signal intensity), methodological variations between and within studies, and/or pertinent study population characteristics. This highlights a need for more research into this area, particularly across different modalities where several measures of brain health are compared in the same cohort. Further, research is required to investigate whether similar ageing and fitness effects are observed across measures derived using different methodologies.

### 2.3.3. Magnetic Resonance Imaging

The following section will describe MRI techniques in more detail and will mainly focus on ASL, BOLD and PCA acquisition. Current literature will be discussed including methodological development and considerations, as well as ageing and fitness effects.

Recent studies have incorporated MRI assessment of MCA diameter to investigate changes in response to manipulated $CO_2$ concentrations (Verbree et al., 2014), though this area is in its early infancy, and rapidly developing due to technological advancement. In Verbree and colleagues study, MCA diameter was assessed using a 7-Tesla (7T) ultra-high-field MRI, which affords a higher signal-to-noise ratio and contrast–to-noise ratio, allowing high resolution acquisition and contrast-rich images compared to 3-Tesla (3T) MRI. Verbree and colleagues concluded that MCA diameter remains constant during 'small' changes in $CO_2$ of -1 or +1 kPa (7.5 mm Hg or Torr), though changes in diameter of 7% were observed during higher $CO_2$ increases of +2 kPa (15 mm Hg). While the

advancement of 7T MRI technology from more commonly used 3T is exciting and will likely improve brain vascular health understanding and clinical applications (van der Kolk et al., 2013), studies so far have found similar patterns between age and fitness groups regardless of the MRI field strength (i.e., 7- versus 3-Tesla) (Bhogal et al., 2015; Driver et al., 2010; Hare et al., 2015). In contrast, comparative methodological research is needed that compares different modalities (i.e., MRI versus Doppler ultrasound) as well as different typical disciplinary approaches of analysis to determine brain health outcome measures. This will be explored in Chapters 4 and 5 of this thesis.

#### 2.3.3.1. Arterial Spin Labelling for Measures of Resting Cerebral Blood Flow

Arterial spin labelling (ASL) is a commonly used MRI method to assess steady-state CBF and is also used to examine changes in CBF in response to cognitive challenges (Ye et al., 1998), ageing (Bastos-Leite et al., 2008; Zimmerman et al., 2014) and clinical conditions including cerebrovascular disease (Detre et al., 1998), Alzheimer's disease (Alsop et al., 2000; Zhang et al., 2017) and traumatic brain injury (TBI) (Kim et al., 2010). Advantages of ASL include that it is non-invasive (i.e., no introduction of tracer fusion or other instruments into the body) and provides quantifiable measures of CBF. Further, improved spatial resolution of MRI, compared with Duplex Doppler, allows the accurate representation of specific regions of interest, as well as grey and white matter.

Measures of resting CBF in the context of ASL include cerebral perfusion and arterial transit times. Cerebral perfusion refers to the rate of delivery of arterial blood to the capillary bed in brain tissue in millimetres per 100 grams per minute. Transit time refers to the time for water to reach the tissue from a tagged location, usually in the neck, and is measured in seconds. ASL utilises the blood water molecules by magnetically tagging

them to create an endogenous tracer by applying a 180-degree radiofrequency inversion pulse to the blood vessels in the neck. The tracer then flows from where it has been tagged in the neck region into an identified region of interest (RoI), with the time it takes known as the transit time. Importantly, oxygen is paramagnetic, and in the RoI, the inverted spins within the blood water reduce the magnetic properties of the tissue, thus changing the MR signal and image intensity. These changes in blood oxygenation concentration and magnetism are what allow the brain and cerebral blood flow to be imaged. The image obtained is known as the tag image. This procedure is then repeated without tagging the arterial blood and another image is created known as the control image. The images are subtracted to produce a perfusion image showing the amount of blood to reach each voxel within the slice from which the transit time can be calculated (Figure 2.8). The difference between the label and control images is in the order of 1% of the baseline images (Wintermark et al., 2005). Therefore, multiple image pairs are acquired to achieve an adequate signal-to-noise ratio.

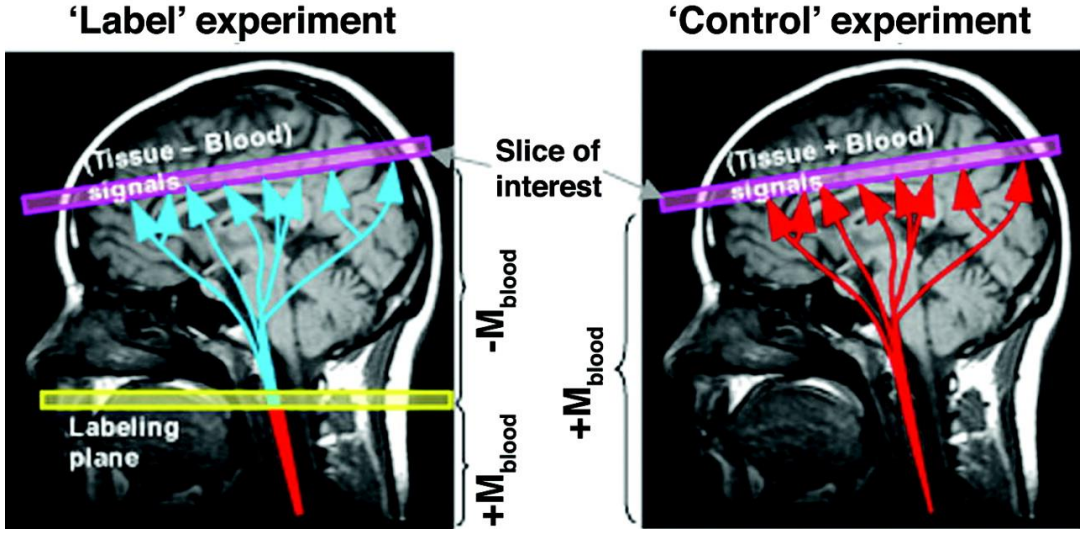



Figure 2.8. The calculation of cerebral perfusion and transit time using arterial spin labelling. Arterial blood is tagged (labelled; shown in yellow) by magnetic inversion and then travels to region of interest (slice; shown in purple) and the tag image is acquired (left). This is repeated without the tag to acquire a control image (right). The difference in magnetisation between control and tag image is proportional to cerebral blood flow (Wintermark et al., 2005).

Previous studies show cerebral perfusion declines during healthy ageing (Parkes 2004), and that it is attributable to more than just decreased tissue volume (Chen et al., 2011). Chen and colleagues found reductions in cerebral perfusion in distinct RoIs that were different to the regional effects observed when looking at grey matter atrophy. Specifically, they found age-related reductions in cerebral perfusion involving the superior-frontal, orbito-frontal, superior-parietal, middle-inferior temporal, insular, precuneus, supramarginal, lateral-occipital and cingulate regions. In another study by Clark et al., resting blood measures were measured using ASL in two older groups. They found reduced resting CBF and elevated cerebrovascular resistance (CVRi) in the younger-old group (60-75 years) compared to the very-old group (80+ years). However, MAP measurements used to calculate CVRi were not taken during the scan and CVRi was calculated post data processing. They observed that verbal fluency performance was related to CVRi in the thalamus, with higher CVRi being associated with poorer performance (Clark et al., 2015), indicating that cognition may be in part mediated by measures of resting blood flow and blood pressure.

Discrepancies are present in the literature between studies in the approach used to report ASL-derived resting perfusion measures, including whether absolute or relative values are reported, and whether resting perfusion in the whole brain should be analysed together, or separated into distinct RoIs. For example, Kim and colleagues suggested that although perfusion is a viable surrogate for an indirect index of structural or functional alterations of the brain, resting perfusion is less confounded by differences between individuals and over time, and therefore is less likely to be a noisy measure of pathology (Kim et al., 2010). Further, they report that RoI approaches have been criticised by many authors because of

limited spatial resolution, labour intensiveness, poor objectivity and low reproducibility, therefore warranting whole-brain mapping as a more appropriate approach for use in traumatic brain injury (TBI) populations. This work highlights that may be different approaches are preferential depending on the population; for example, absolute whole brain analysis of resting perfusion should be used in populations with focal damage such as TBI and stroke, while quantitative perfusion analysis is better in healthy and ageing populations, and RoI analysis a logical option for populations where specific parts of the brain and cognitively associated functions and/or behaviour are affected (e.g., dementia).

Reproducibility studies have shown that cerebral perfusion measures remain stable in the short term, though large variation exists between participants. Parkes and colleagues (2004) examined this reproducibility question by acquiring repeated ASL measurements over the course of a day and a week in ten healthy participants, and five participants returned several months later (Parkes et al., 2004). They found that the mean difference in standard deviation of perfusion between repeats was 7.1 % in the grey matter and 10.7 % in the white matter. Further, grey matter perfusion was significantly lower in males compared to females. Higher CBF velocity in females has also been reported in studies that have measured blood perfusion using Doppler technologies. CBF velocity measured using TCD has also been reported to be lower in the morning than later in the day (Conroy et al., 2005) and observed to follow a circadian rhythm where day-to-day variation is less than 10 $cm \cdot s^{-1}$ in 95% of individuals (Moppett and Mahajan, 2004). Therefore, studies utilising these techniques and taking multiple measurements across different days aim to measure at the same time of day to avoid this confound.

In general, and demonstrated by the studies discussed in this section, resting cerebral perfusion declines across the lifespan and reduced cerebral perfusion is associated with impaired cognitive performance. Hypoperfusion has been shown to occur in neurodegenerative conditions including stroke, dementia and hypertension in some studies (Figure 2.9), however, contrary to this, increased hippocampal blood flow has been observed in sedentary adults at genetic risk for Alzheimer's disease (Zlater et al., 2014).

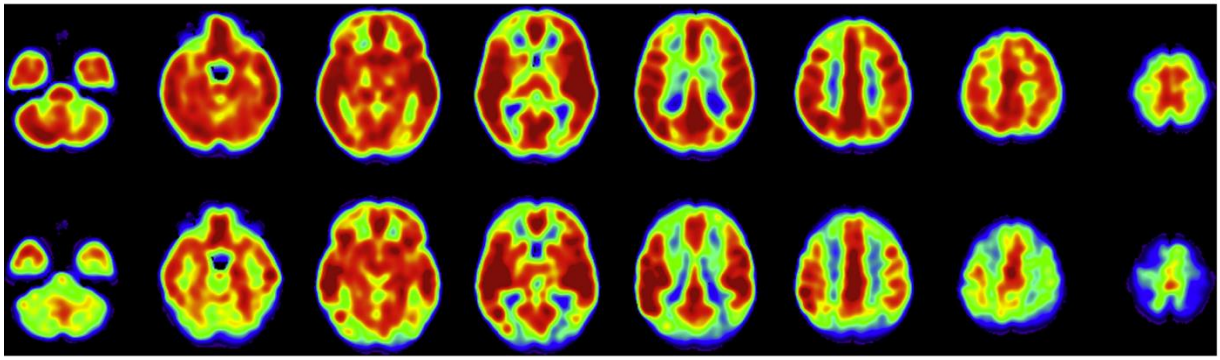


Figure 2.9. Processed ASL CBF images of a 32-year old (top row) and an 80-year old (bottom row). The reduction in CBF is readily observed in widespread brain areas of the older compared to the younger individual (Zhang et al., 2017).

#### 2.3.3.2. Blood-Oxygen-Level Dependent (BOLD) Signal to Measure Cerebrovascular Reactivity

Magnetic resonance imaging (MRI) measures brain activity by detecting changes in blood oxygenation and flow that occur alongside neural activation. When a brain area is activated it consumes more oxygen so blood flow towards the active area will increase (i.e., neurovascular coupling). MRI does not directly measure neuronal activation but instead records the BOLD signal that is dependent on the difference in the magnetic properties of oxygenated or deoxygenated haemoglobin and the relative levels of these two forms of haemoglobin (Hare et al., 2013; Gauthier et al., 2015; Bhogal et al., 2014).

During an MRI brain scan the person lies as still as possible whilst activation maps are produced. These maps can illustrate how different areas of the brain respond to stimuli including cognitive tasks or visual stimulation such as a checkerboard (Mullinger et al., 2017), so that areas of the brain involved in certain cognitive processes can be studied (e.g., attention, memory, emotional processing, learning, visual processing, etc.).

Magnetic resonance imaging can also be used to measure CVR, similar to TCD discussed in Section 2.3.2.3. However, the methodological approaches between these two imaging modalities are markedly different. Measures of CVR from MRI use the blood-oxygen-level dependent signal (BOLD) as a surrogate marker of CBF, in contrast to TCD or Duplex Doppler that use blood flow velocity/flow, respectively, through a specific artery such as the MCA or the ICA (though this can also be done with MRI using phase-contrast (PA) angiography, discussed in more detail below). The percentage change in BOLD signal intensity between a $CO_2$ stimulus compared with normal room air is taken from either the whole grey matter of the brain (Frosch et al., 2017), or from specific RoIs and is then divided by the change in end-tidal $CO_2$, similar to Doppler approaches to calculate the CVR outcome measure. However, this BOLD-derived CVR outcome measure is generally determined through linear regression in contrast to Doppler analysis approaches that extract 30 or 60 seconds of steady-state data whilst the participant is at rest and during the stimulus duration. An alternative MRI method to investigate CVR is arterial spin labelling (ASL). ASL is a direct measure of CBF in the capillaries, often termed perfusion. However, few studies have used ASL to assess CVR and instead use BOLD (Ekstrom et al., 2010; Bhogal et al., 2014, 2015, 2016; Duffin et al., 2017) due to the inherently low SNR of ASL data (Buxton 2013).

In healthy participants, the CVR in response to hypercapnia is assumed to be linear or sigmoidal. However, other response patterns occur, particularly in cerebrovascular disease (Duffin et al., 2017). Different response patterns will affect the CVR outcome measure, particularly as most MRI studies use linear fits to calculate the response and TCD approaches will extract approximately 30 or 60 seconds of data from a defined steady state, which can also differ between studies (e.g., after 90 s of stimulus vs. end of stimulus duration). When considering whether $CO_2$ responses are predominantly linear or sigmoidal, the choice of fit does not seem to affect the CVR outcome measure when end-tidal $CO_2$ values are not going above a certain threshold (around 55 mm Hg). These observations of appropriate fitting models have been discussed and investigated in both TCD (Battisti-Charbonney et al., 2011) and MRI (Bhogal et al., 2014, 2015 and 2016) modality literature (as shown in Figure 2.10).

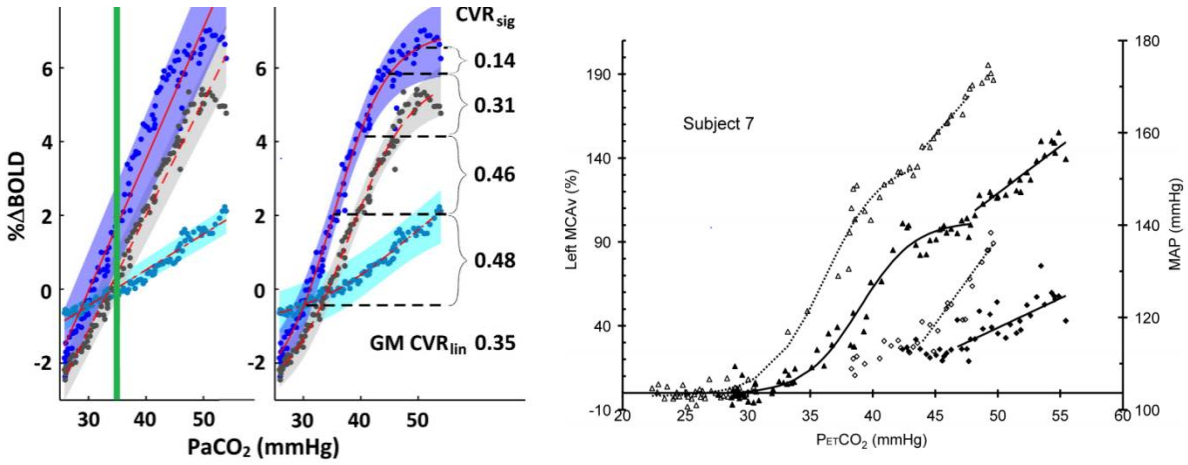



Figure 2.10. Linear and sigmoidal fits for CVR (left: BOLD, Bhogal 2014; right: MCAv, Battisti-Charbonney et al., 2011). Left graph shows BOLD 7T data. Dark blue shows grey matter, light blue shows white matter, grey shows whole brain and green line shows baseline $PaCO_2$. Right graph shows MCAv (triangles) and MAP (diamonds) responses to hyperoxic (continuous lines; filled symbols) and hypoxic (dotted lines; open symbols) rebreathing test.

Therefore, studies employing these methods, as is typical for MRI BOLD-derived CVR and rebreathing approaches using TCD, need to be cautious and consider that particularly high end-tidal carbon dioxide ($P_{ET}CO_2$) values can potentially further confound the CVR outcome measure if linear fit models are being utilised for the calculation.

More recently, Duffin and colleagues have modelled and investigated new MRI BOLD approaches that include sigmoidal reduction in resistance at the vascular beds in response to a progressive rise in $CO_2$ in order to determine the CVR outcome measure (Duffin et al., 2017). Developing more sophisticated models to determine CVR may lead to more accurate interpretations of the outcome measure and reduce inconsistent findings between studies, particularly those considering complex interactions with underlying disease pathology.

Some studies have examined different analysis approaches for measures of blood flow within the same neuroimaging modality. For example, BOLD and ASL techniques have been reported to complement one another, with the strongest correlations between BOLD and CVR outcome measures in the grey matter, and with greater responses occurring in the occipital lobe than the frontal and parietal lobes (Zhou et al., 2015). Despite these associations, only decreases in resting CBF were associated with increased ageing, but there was no association with age during mild hypercapnia (5% $CO_2$). Studies have also compared different methods for the inhalation of gases to induce the response. For example, Hare and colleagues compared carbogen gas (containing only 5% $CO_2$ and 95% oxygen) with more typically used mixtures of 5% $CO_2$ in air (21% oxygen balanced with Nitrogen). They compared CVR outcome measures acquired from BOLD and ASL imaging. Both BOLD and ASL images were acquired in two separate scanning sessions,

one where a 5% $CO_2$ in air stimulus was used and one where carbogen gas was used. Similarly to Zhou and colleagues study, Hare et al., found significant correlations between the CVR outcome measures derived using the BOLD modality with measures using the ASL modality when the 5% $CO_2$ in air stimulus was used. However, this correlation between the different modalities was not present when the carbogen gas was used (Hare et al., 2013). They suggested that this was due to the high oxygen content making it impossible to identify the extent to which increased CBF is driving the BOLD signal, rather than an increase in venous oxygen saturation. Therefore, caution should also be taken when comparing studies that use different stimuli to induce the response.

The signals of different neuroimaging techniques, as well as the techniques themselves have different physiological underpinnings that also warrant consideration when designing studies and comparing results between studies. In contrast to TCD approaches that only use MCAv as a metric of CBF (though some studies do use MAP also), the BOLD signal is driven by changes in CBF, blood volume and oxygen metabolism, and the balance of these three factors will largely influence the signal (Buxton, 2013). Therefore, the BOLD signal provides an arbitrary measure, and is not a direct measure of neuronal activation. ASL specifically measures CBF, however, it has worse spatial and temporal resolution than what BOLD currently provides. Therefore, a combination of BOLD and ASL data are sometimes acquired to provide additional quantitative information and a more complete assessment (i.e., using double acquisition background suppression (DABS).

As previously discussed, higher CVR is traditionally associated with younger age and higher fitness. However, inconsistencies are also present within the MRI literature in terms of how the CVR outcome measure is associated with ageing, disease and fitness, similarly

to the TCD literature. In some instances, these discrepancies are due to whether the whole brain or a specific RoI was investigated. For example, Gauthier and colleagues investigated measures of vascular brain health, aerobic fitness and cognitive ageing in two groups of younger and older adults (Gauthier et al., 2015). They found that age was negatively associated with BOLD-derived CVR calculated from the frontal brain region. However, fitness (determined by $\dot{V}O2$ max) was positively associated with BOLD-derived CVR, but only when taken from the periventricular watershed region, postcentral gyrus. In contrast, a negative association was found between fitness and BOLD-derived CVR when derived from the frontal regions. These findings indicate that there is not a simple one-way linear relationship between BOLD-derived CVR and fitness that is consistent across the whole brain, rather that responsiveness may differ between brain regions. Further, Gauthier and colleagues also found age-related changes in executive function and fitness, which may modify the region specific CVR observed. In this study, CVR was assessed using a sequential gas delivery circuit (RespirActTM, Thornhill Research Inc., Toronto, Canada) and the hypercapnic manipulation consisted of two, 2-minute blocks of hypercapnia, with 2-minutes of air before and after each block. It is important to note that participants were not fully familiarised to the gas challenge prior to the testing day, rather just once outside the MRI scanner before the scanning session on the same day. Other studies using similar techniques will typically fully familiarise participants to procedures involving breathing gases, before obtaining data used for CVR measures (Barnes et al., 2013; Murrell et al., 2013; Lucas et al., 2012).

An MRI study using ASL acquisition at rest and during mild hypercapnia of approximately 1 kPa (+5-7 mm Hg) has shown that reduced ASL-derived CVR is significantly associated with insulin sensitivity (Frosch et al., 2017), supporting the theory

that vascular abnormalities may be associated with an increased risk and development of clinical conditions including insulin resistance, diabetes and dementia.

Chapman and colleagues used MRI techniques to investigate and compared the effect of cognitive and physical exercise training interventions in 36 healthy sedentary adults (aged 56-75 years). Outcome measures included resting perfusion using ASL, CVR using BOLD, and cognitive performance measures on higher executive function (Chapman et al., 2016). They found that the cognitive training group showed increased resting perfusion within the prefrontal and middle/ posterior cingulate cortex (PCC), but no change in CVR. Improvements in complex abstraction were associated with increased resting perfusion in the dorsal anterior cingulate cortex (dACC) for the cognitive training group. Participants doing the physical training who had higher resting perfusion in hippocampi regions showed better immediate memory. In this study, it was concluded that resting perfusion measures implicate increased neural health rather than improved vascular response determine by CVR.

Neither BOLD or ASL measures of blood flow include measures of blood vessel cross-section or diameter, or possible changes in response to a stimulus that may influence the outcome measure, a similar limitation of the TCD-derived CVR approach. Therefore, the next section will focus on different neuroimaging approaches using phase-contrast (PC) angiography that addresses this.

### 2.3.3.3. Phase Contrast Angiography for Measuring Cerebral Blood Flow

As with linear Duplex and TCCD ultrasound approaches, MRI can be used for measures of blood flow that also consider changes in vessel diameter in addition to blood flow velocity, with PC angiography (Figure 2.11).

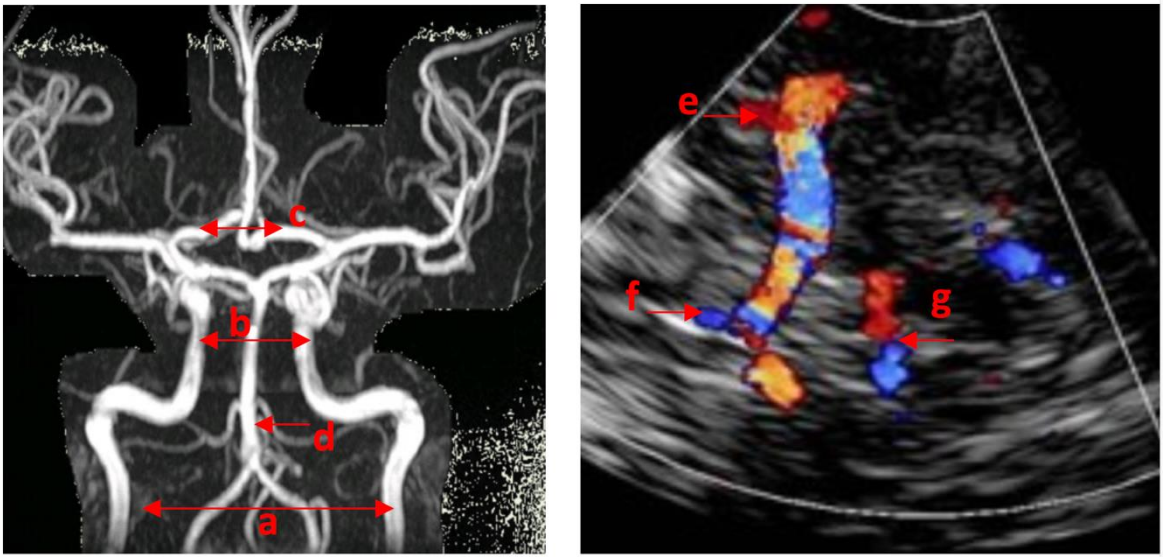



Figure 2.11. Circle of Willis cerebral blood vessels imaged using phase-contrast (PC) angiography (left) and transcranial colour Doppler (TCCD) (right). PC angiography image shows the carotid artery (a), internal carotid arteries (b), middle cerebral arteries (c), basilar artery (BA) and connecting macro- and micro-vessels. The TCCD image shows the middle cerebral artery (e), anterior (f) and posterior (g) cerebral artery.

A study compared CBF measures between MRI and Doppler ultrasound techniques by comparing blood flow velocity measures between TCCD and PC angiography (Balédent et al., 2006). Although measures of diameter were not included in this study (as they are more recent developments in these measurement approaches), this study is still a rarity in its approach to directly compare different imaging modalities. Blood flow velocities between modalities were compared at different levels of the arterial tree for the right and left ICA, VA, and the BA. They found significant correlations between modalities for the right ICA, VA and BA, with Spearman's *r* correlation coefficients ranging from 0.23 - 0.84. However, whether the PC angiography approach underestimated values or whether

the TCD approach overestimated values is unclear, though poorer correlations between modalities were found for arteries of the circle of Willis in extra cranial arteries. As discussed in Section 2.2.2.2, MCA diameter changes have been reported in hypercapnia when values are at least +2 kPa (15 mm Hg). The advancement in technology and analysis now offers better opportunity to image and quantify such changes (see Verbree et al., 2014; Figure 2.12).

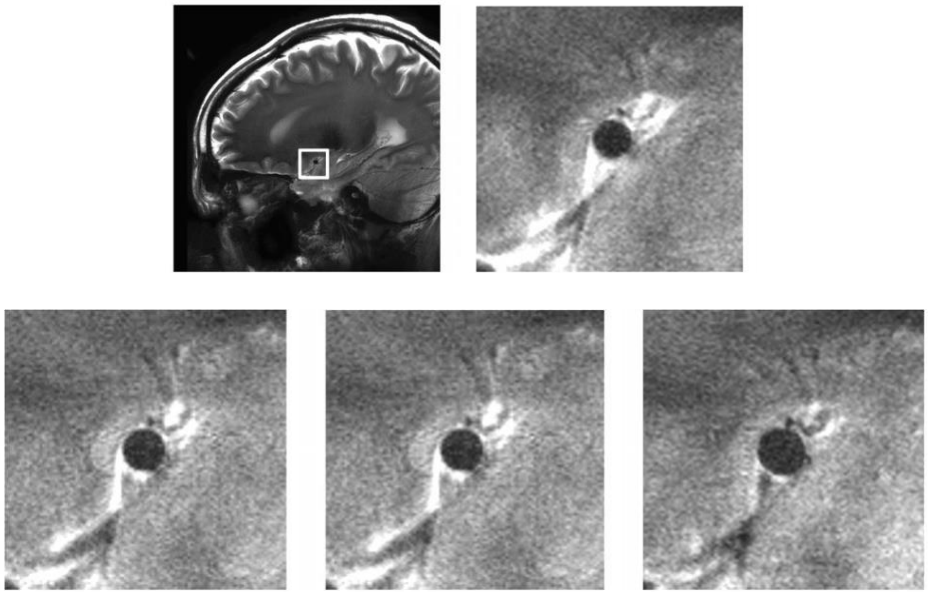


Figure 2.12. High-resolution 7-Tesla MRI scans planned perpendicular in the middle cerebral artery (MCA). Upper: the white square shows the location of the following zoomed images, zoomed image at hypocapnia (-1 kPa). Lower: zoomed imaged at baseline normocapnia (0 kPa), and hypercapnia (+1 and +2 kPa end-tidal $CO_2$ respectively) (Verbree et al., 2014).

More recently phase-contrast MRI from the Rotterdam population-based study in older adults (mean age 61 years) showed that lower resting cerebral perfusion was significantly associated with risk for dementia. Of 4759 participants who were scanned between 2005 and 2012, 123 developed dementia. Further, increasing severity of white matter hyperintensities increased risk of dementia with hypo-perfusion (i.e., low cerebral perfusion), and this was also linked to accelerated cognitive decline (Wolters et al., 2017).

In this study, cognition was assessed using the letter-digit substitution task, verbal fluency test, Stroop test and a 15-word learning test (immediate and delayed recall).

Participant data from the Rotterdam study has also been collected and examined from measures of CVR using TCD (Portegies et al., 2014) to investigate risk of mortality, independent of stroke. In this analysis 1,695 participants aged 55+ years initially completed the CVR challenge. In a fully-adjusted model, the hazard ratio per standard deviation decrease in CVR was 1.10 for all-cause mortality, 1.09 for cardiovascular mortality, and 1.10 for non-cardiovascular mortality, meaning a 10% increased risk of mortality with every SD decrease in CVR. In this study, similar approaches of the gas challenge were employed as previously discussed (i.e., 5% $CO_2$ in air inhalation for 2-minutes) and lower responsiveness was concluded to reflect an impaired vascular system and be associated with increased risk of death.

#### 2.3.3.4. Other Imaging Approaches

Other imaging approaches (e.g., examining brain structure volumes, diffusion tensor imaging and white matter hyper-intensities), have been used to assess brain health with regards to ageing and disease. Although thorough investigation of these measures is beyond the scope of this thesis, a brief overview is given in the following section that includes associations with cognition and aerobic fitness in human and animal studies.

Structural magnetic resonance imaging (sMRI) produces images of the structure of the brain (e.g., hippocampus, entorhinal cortex), and closer analysis of the size of various structures can reveal abnormalities due to brain atrophy. Decreased hippocampal volume

have been associated with mild cognitive impairment (Nathan et al., 2017) and poorer memory performance (Erickson et al., 2011). Levels of physical exercise have been shown to increase hippocampal volume in healthy adult humans (Erickson et al., 2011; Killgore et al., 2013) and increase hippocampal neurogenesis in animal models (Nokia et al., 2016). Further, in a cross-sectional study investigating hippocampal change in cognitively healthy older adults, physical activity has been shown to reduce atrophy in those at genetic risk for Alzheimer's disease (Smith et al., 2014).

Several other MRI techniques are used to examine the brain, including analysing white matter hyperintensities (WMI) (Camicioli and Verghese, 2015), diffusion tensor imaging (DTI) (Madden et al., 2009) and magnetization transfer imaging. WMH are areas of high intensity on MRI scans that reflect lesions within the white matter produced by demyelination and axonal loss. They are seen in healthy ageing though are also associated with clinical conditions. DTI is a technique that allows the mapping and characterisation of the diffusion of water in tissue as a function of spatial location (Alexander et al., 2007). These measures are often explored alongside cognitive tasks that expose age-related cognitive decline, particularly in episodic memory, executive function and information processing speed.

In a clinical and research context, structural and functional MRI are commonly used in tandem to assess changes in brain structure and function in the ageing brain and in clinical conditions such as dementia. Cerebral white matter ageing is associated with declines in episodic memory, executive function and information-processing speed, revealed by several MRI approaches including DTI and WMI (Gunning-Dixon, Brickman, Cheng, and Alexopoulos, 2009). However, few investigations have been done comparing outcome

measures of resting CBF or CVR between different brain imaging modalities. Further, no known investigations have directly compared MRI with TCD measures in the same individuals, to determine whether outcome measures derived using different modalities will lead to similar/ different associations with ageing and fitness. Dementia is a neurodegenerative disease associated with cognitive decline, particularly memory, and white matter integrity is associated with cognitive decline. One challenge that has often been acknowledged is establishing what brain changes revealed by MRI are a result of natural ageing, and to what extent these are caused by modifiable lifestyle factors such as physical activity, nutrition and other lifestyle choices.

Changes in cognitive performance in domains such as processing speed, reasoning, memory and executive functions across the lifespan as a result of healthy ageing have been investigated including the role of lifestyle factors such as exercise and diet (Deary et al., 2009). A number of publications have acknowledged the beneficial effects of exercise on brain health by using MRI as an outcome measure (Burzynska et al., 2014; Camicioli and Verghese, 2015; Hayes et al., 2014; Hötting and Röder, 2013; Voss et al., 2011, Kramer et al., 1999; Colcombe et al., 2006). Hötting and Röder reviewed several studies using a range of designs (cross-sectional, intervention, animal and human studies) and suggested that physical activity may play a role in facilitating neuroplasticity and cognition (Hötting and Röder, 2013). The well recognised limitation with such cross-sectional and cohort studies is the difficulty in making cause and effect inferences about physically active people performing better in cognitive tasks and academically because there is always the possibility of reverse causation. This means that people with higher cognitive performance may be more likely to engage in physical activity, rather than regular physical activity producing improved cognitive performance. Nevertheless, these research designs allow us

to investigate changes over time and also explore a number of variables including: amount of physical activity, cardiovascular fitness as well as measures of cognition, emotion and well-being. Thus, they can provide us with a greater overall picture of brain health across the lifespan.

In a prospective cohort study, Rovio and colleagues interviewed people 50 years of age about their lifestyle and physical activities (Rovio et al., 2010). Twenty-one years later, structural and functional MRI scans (i.e., T1-weighted images to investigate grey matter (GM)) density and FLAIR-images for white matter lesions (WML)) were taken from the same individuals and differences were found between individuals who exercised twice a week or more compared to those who exercised less. Specifically, in frontal brain regions associated with higher executive functioning more active individuals had larger grey matter volumes compared to their less active counterparts. In further support of these findings, another population study looking at Swedish twins found evidence that individuals who engage in light exercise such as gardening or walking, or regular exercise had a reduced risk for dementia compared to those who did very little exercise (Andel et al., 2008).

Camicioli and colleagues measured activity in individuals using actigraphy and scanned them using MRI. They found that the harmful effects of white matter hyperintensities were less pronounced in people who engaged in more active lifestyles. This finding is consistent with previous studies showing that white matter hyperintensities are associated with impaired global motor function and indicates a protective effect of physical activity on brain function and mobility (Camicioli and Verghese, 2015). There is clearly a substantial

amount of research illustrating the beneficial effects of physical activity on brain health by using MRI to investigate the brain.

Studies using animal models also show conflicting conclusions with regard to the effects of exercise on resting measures of CBF and CVR, and introduce further imaging approaches to determine these measures. In a study investigating five weeks of voluntary running in transgenic mice, a 70% diminished response to a hypercapnic challenge was observed in the running compared to the non-running, whereas no difference in resting haemodynamics were observed between groups (Dorr et al., 2017). This study used two-photon fluorescence microscopy to examine functional changes in cerebral vasculature by following the passage of a fluorescent dextran bolus through the microvasculature bed beneath the cortical surface. The inhalation of 10% $CO_2$ was used to induce the cerebral vasodilation and increases in blood flow. It is often debated in the literature the relevance of animal studies to human functioning though researchers are beginning to integrate these methodologies to 'bridge the gap' between different approaches (Voss et al., 2013).

#### 2.3.3.5. Strengths, Limitations and Challenges of MRI

The following section provides a summary of the strengths and limitations of using MRI as a tool to measure brain health. MRI data can reveal a large amount of information from a relatively small sample size, in contrast to an observational or cohort study that requires much larger sample sizes and across long periods such as the lifespan. As demonstrated by the studies described above, these quantitative outputs can be compared with other quantitative outputs such as behavioural performance measures revealed by cognitive tasks. We can combine these methodologies to investigate the interplay between brain activity, behaviour and cognitive processing in response to an experimental manipulation.

For example, studies can investigate whether an increase in BOLD signal intensity (as a surrogate for neuronal activation) in the substantia nigra is correlated with better performance in a cognitive task involving valence manipulation (cognitive processing) (Guitart-Masip et al., 2012). This indicates that this area of the brain plays a role in reward and punishment processing and is responsible for behaviour that may lead to receiving a reward. The substantia nigra nests deep within the brain and is not accessible using other techniques used to measure brain health. Therefore, a major advantage in this methodology is that we can measure deep inside the brain, which contrasts many other neuroimaging methodologies that have relatively less spatial penetration depth and resolution (e.g., Doppler and NIRS, the latter discussed below).

However, MRI also has several drawbacks. In terms of practicality, they are large machines that have limited portability (although mobile versions have been developed), so have less flexibility in terms of measuring real life movement behaviour that other methodologies do so more readily (e.g., transcranial Doppler (TCD) and NIRS, as discussed above (TCD) and below (NIRS)). Participants being scanned in an MRI machine are required to keep their head as still as they can so the quality of data is maintained, though physiological post-processing includes correcting the data to account for artefacts caused by movement and linear drift. Interpretations of the acquired data also rely on assumptions. As discussed previously, BOLD is not a direct measure of neuronal activity though is considered to reflect peri-synaptic activity in the local field potential, thus reflecting neural processing. However, models have been discussed where the underlying relation with BOLD may be more complex than a direct correspondence (Ekstrom, 2010).

MRI is becoming a more popular tool to be used in a clinical setting, particularly in dementia as the quality of scans are becoming more technologically advanced. Further, mobile scanners housed in trucks have been developed that can allow wider use in the field or community (e.g., brain (and muscle) structural changes studied during a running endurance study across Europe (Schütz et al., 2012)). However, MRI technology is expensive and has high costs associated with its use (e.g., £500/ hour), so is unlikely to be a dominant measure in a current clinical (especially community) setting. Also, from a clinical perspective, it may be very difficult for some patient groups to lie still in an MRI scanner for extended periods. Populations who are likely to find this difficult include: children, particularly those with attention deficit hyperactivity disorder (ADHD), people with anxiety or claustrophobia, patients with movement disorders and patients with disorders affecting cognitive function such as mild cognitive impairment (MCI) and dementia. Further, extra caution is required in patients and individuals who have metal implants and in many cases, they cannot be scanned for safety reasons.

Another consideration that also applies to other imaging modalities, is the consistency of methodological procedures and protocols across studies. For example, 'resting state' scans are often used to examine the brain at rest. However, MRI studies have shown large individual differences in inner phenomena revealed through descriptive experience sampling methods whilst scanning resting-state networks (Hurlburt et al., 2015), and it could be argued that the brain is never really at rest. Therefore it is important to ensure that approaches of defining, measuring and interpreting these 'resting' measures are consistent. In existing research studies, the resting state protocol requires the participant to lie still with their eyes either closed or open depending on the preferences of the research group, yet studies have revealed that this alone can affect brain activation (Zou et al., 2009).

However, there are protocols with control versions of the task to account for this, such as congruent versus incongruent tasks to increase the cognitive load (e.g., Fernandez-Duque et al., 2008).

Another important consideration, particularly when looking at measures of blood flow and volume, is reliability and re-testing when $P_{ET}CO_2$ values are not taken (or considered) during scans, as this will affect the BOLD signal. This is important given that participants are often quite anxious about going into the scanner, particularly if it is their first time, so they are likely to hyperventilate. Hyperventilation in the context of the CVR functional test will lower CBF and therefore infer a lower cerebral perfusion in this 'rested' state. More research is needed to investigate how the BOLD signal is affected by breathing patterns and participants' general state of mind (particularly levels of anxiety), which may not always be obvious or acknowledged by both participants and/or researchers/clinicians.

Given the limited portability and practicality of MRI, as well as its high cost, it may be beneficial to use more transportable and cost effective techniques to measure CBF, particularly if we can be confident that they predict the same outcome measure. Though MRI techniques will be beneficial in some contexts, where more detail of specific regions of interests or deep structures need investigation, for example, in disease states.

### 2.3.4. Functional Near-Infrared Spectroscopy

Infrared radiation was first described in 1800 by Sir William Herschel when he was looking at sunlight using a red filter through a prism and deduced that the invisible portions of the electromagnetic spectrum were responsible for the localised rise in temperature. Functional near-infrared spectroscopy (NIRS) measures changes in

oxygenated and deoxygenated haemoglobin (($HbO_2$) and (HHb), respectively) from cortical tissue. Concentration changes are calculated using an attenuation change of the measurement of light and by solving a linear equation based on the modified Lambert-Beer law. NIRS can be used to monitor outcomes in clinical populations including cerebrovascular disease, epilepsy, migraine and traumatic brain injury (TBI) (Davies et al., 2015).

Near-infrared spectroscopy has been used to investigate the relationships between prefrontal oxygenation, cognitive performance and cardiorespiratory fitness in cross-sectional studies (Albinet et al., 2014; Fabiani et al., 2014). NIRS has also been used to assess changes in $HbO_2$ and HHb during and following acute bouts of exercise (Lucas et al., 2012; Giles et al., 2014). In these acute studies, bouts of physical activity involving cycling at various intensities increased oxygenated and deoxygenated haemoglobin in the prefrontal cortex. Further, in Lucas and colleagues' study they found that regardless of age, cognitive performance in a modified Stroop task improved during exercise. Albinet and colleagues cross-sectional study revealed improved oxygenation response measures using NIRS in both the left and right dorsal lateral pre-frontal cortex (DLPFC) in older fit women compared to their unfit counterparts. These effects were mediated by executive performance, evaluated by using the auditory random number generator (RNG) task, designed to utilise inhibitory control (Albinet et al., 2006). Collectively, these studies demonstrate that both acute bouts of physical activity and general higher levels of fitness improve cerebral haemodynamics and cognition function. Other studies have investigated associations between cerebral haemodynamics using NIRS and physical activity and found similar results (Cameron et al., 2015) where higher anterior frontal oxygenated haemoglobin was associated with better performance for the most difficult cognitive task,

and this was mediated by higher activity levels. Further, this study investigated young adults who were at the peak of development.

More recently NIRS measured regional cerebral tissue oxygen saturation has been investigated in response to CVR gas challenges (Miller and Mitra, 2017), similar to MRI and TCD approaches described in Sections 2.3.2.3 and 2.3.3.2. NIRS approaches can also determine CVR by assessing regulatory responses to hypercapnia through administration of several hypercapnic challenges of 5 or 10 minute durations whilst continuously monitoring partial pressure of end tidal $CO_2$. The parameter for flow change using NIRS is regional cerebral tissue oxygen saturation. In the Miller and Mitra (2017) study, it was concluded that exercising more than ten hours a week may produce a higher resting CVR to $CO_2$ inhalation

Near-infrared spectroscopy is an attractive tool for assessing the brain due to its haemodynamic measurement basis. Other advantages of NIRS technology when compared with other neuroimaging modalities such as MRI, include its: portability, high temporal resolution, ability to record in natural settings, and cost. Long periods of recording time are achievable and it is less sensitive to head motion. Further, it is safe to use and can be used easily on infants, children and patients. However, NIRS has lower spatial resolution and can only record near the brain surface; although more recently research centres have developed the technology to allow recording deeper (though still relatively superficial compared to MRI) within the brain and shown these measures can be associated with MRI (Fabiani et al., 2014). NIRS may also be criticised for not having standard analysing packages, though this drawback also applies to other imaging modalities, as mentioned above. Finally, activation and registration procedures may be inaccurate and lead to poor

localisation. Despite these drawbacks, the advantages of NIRS are making this technology an increasingly useful tool to assess brain health in disease states and consider haemodynamic associations with cognitive function.

### 2.3.5. Summary and Integration of Neuroimaging Measures

To date, all three Doppler techniques and MRI have not been directly compared to examine whether they provide comparable outcome measures in populations that are thought to have different cerebrovascular regulation (e.g., younger versus older or levels of aerobic fitness). Further research is needed to explore how outcome measures may correlate with haemodynamic measures acquired from MRI techniques, and assess whether they are complementary or contradictory to Doppler-derived measures. In addition, research can be carried out to investigate how these measures may change in response to a clinical intervention such as one involving physical activity. Examples of populations where investigating further the effects of exercise on brain health may be those at risk for developing dementia (e.g., older adults, patients with hypertension, or individuals genetically profiled as APOE-E4 carriers, a gene associated with Alzheimer's disease). Given that we know exercise has beneficial effects on these populations, we need to better understand the underlying mechanisms that drive these positive changes, and indeed, how to effectively *measure* changes in brain health, so that we can prescribe tailored individual approaches.

## 2.4. Assessing Cognitive Performance, Neuropsychology and Quality of Life

Behavioural measures and questionnaires are also used to measure brain health; measures spanning performance in cognitive tasks, responses to neuropsychological assessments and questionnaires that assess quality of life and well-being. The following section will review

pertinent literature on this extensive area of research with the specific focus on measures that will be applied in this thesis.

### 2.4.1. Cognitive performance tasks

The following section will describe cognitive approaches that mainly focus on performance in tasks that target specific cognitive domains, including: attention, memory, learning, mental arithmetic and emotion recognition. Current literature will be discussed including methodological development and considerations, as well as ageing and fitness effects.

Cognitive performance is often used to determine brain function and health. Behavioural measures including reaction times and percentage of correct responses to a behavioural task, usually administered on a computer, which provide quantitative outputs that can easily be compared. Researchers can observe differences between and within individuals either at the same time point or across several time points. For example, in large scale population studies such as Whitehall II, a decline in cognitive performance involving executive function across the lifespan in the general population has been reported (Shiroma and Lee, 2010). Differences in response to an experimental manipulation (e.g., pharmacological) on task performance has also been examined (Guitart-Masip et al., 2012). Further, cognitive measures have also been used to show how cognitive performance is affected after stroke and how improvements can be seen across time through clinical intervention such as neuro-rehabilitation (Bickerton et al., 2014; Demeyere et al., 2015).

There are several cognitive tasks used in clinical and research tools such as the Oxford Cognitive Test (Oxford University Innovation Ltd., Oxford, UK; http://www.ocs-test.org, previously the Birmingham University Cognitive Screen) (Demeyere et al., 2015; Bickerton et al., 2015), CogState (Cogstate Ltd., Melbourne, Australia; www.cogstate.com) and CANTAB (Cambridge Cognition Ltd., UK; http://www.cambridgecognition.com). These cognitive assessments focus on different cognitive modalities including: attention, memory, language, mathematical skills, spatial processing and verbal reasoning. Disentangling these different cognitive domains has been shown to be extremely useful in the assessment, diagnosis and treatment of disorders where cognitive health is affected. In establishing the domain that has been compromised, clinicians are now better placed to reach a more accurate diagnosis with such assessment tools. Examples of clinical conditions where regional and cognitive domain specific considerations are particularly informative include: Parkinson's Disease, hemi-spatial neglect, different subtypes of dementia (semantic dementia, frontotemporal dementia, Lewy-Body dementia and Alzheimer's disease) and other conditions where specific parts of the brain and different cognitive domains may be affected at different points in time depending on the nature and progression of the disease. However, although these tools have been used extensively for diagnosis and to monitor patient outcomes, less research has focused on examining the effects of an exercise intervention on these various cognitive modalities.

Other types of cognitive measures have been designed to explore emotional processing, which can be useful in clinical disorders where mood is affected (e.g., depression (Browning et al., 2012)); and may be more in line with research exploring stimuli that has emotional meaning to the individual. An example of this is a facial processing dot-probe

task, where participants are required to respond as quickly and as accurately as they can to faces expressing various emotions on the screen. Patients with mood disorders will typically respond differently compared to healthy individuals, taking longer to react to negatively expressed faces (Browning et al., 2012). Interestingly, these measures have been correlated with MRI outcome measures to identify specific brain regions involved with emotional processing such as the anterior cingulate and the amygdala (Etkin et al., 2010). Another example of cognitive tasks being used to explore attentional biases is a variation in the Stroop task, where the meaning of words is purposefully manipulated. Individuals with eating disorders will typically take longer to respond to words associated with foods (emotional words) (Epp et al., 2012), in a similar way to how individuals with depression will take longer to respond to words or faces that have negative associations (Wilson and Wallis, 2013).

An interesting question may be to explore the effects of a lifestyle intervention, such as physical activity, on negative cognitive biases and the interpretation of ambiguous scenarios. For example, whether we view the world in a more negative or positive light and whether we predict positive or negative outcomes from future experiences. Existing research has explored the effects of exercise on cognition in healthy and clinical populations. These are included in Hötting and Roder's review on the beneficial effects of exercise on neuroplasticity and cognition (Hötting and Röder, 2013). Typically, the studies discussed have either explored the effects on cognition immediately after a single session of exercise or they have addressed chronic effects of an intervention that may last either several weeks or longer (e.g., 8 or 12 weeks). Other studies that have utilised cognitive measures in response to an exercise intervention will be discussed more in the following

section titled ‘Combining neuroimaging methodologies with cognitive performance’, as they tend to incorporate other measures such as neuronal activation or cerebral blood flow.

As with the previously discussed tools to assess brain health, there are strengths and drawbacks of using cognitive tasks as a tool to measure brain health. Cognitive tasks often involve relatively meaningless stimuli to the individual, which may affect their motivation and overall performance; particularly if a monetary incentive has been given to encourage them to take part. It is therefore important to question whether the task is really measuring what it is intending to (i.e., measurement validity). Cognitive tasks tend to rely on assumptions that the cognitive domain being tested in the experimental condition reflects how it would function in a real-life setting and transferability across other cognitive domains. Indeed, an improvement in cognitive performance may be observed in a domain in response to an intervention, but this may not mean that improvements will be seen in other similar tasks (Owen et al., 2010).

While cognitive measures provide fast detailed and quantitative information, they may also be affected by a large amount of inter-individual variability that is difficult to control for (Li et al., 2001). Therefore, it could be argued that they are more useful in determining differences in the same individual across time (i.e., within-individual), either through disease progression, clinical outcomes or lifestyle changes (not withstanding repeat testing learning effects). Further, when considering the effects of acute exercise on cognition (e.g., Lucas et al., 2012, discussed below), the time that the participant is tested after the exercise bout will also affect performance in the task due to the potential of chronic exercise effects and short-term arousal (Chmura el al., 1994; McMorris et al., 2011). The question of

whether cognitive performance provides meaningful information in the context of real life situations leads to the consideration of measures focusing on quality of life and well-being.

### 2.4.2. Questionnaires for quality of life and well-being

Another crucial approach in determining brain health is via measures that aim to assess well-being and quality of life. These are generally questionnaire based and include those that are designed to recognise clinical symptoms related to mood disorders such as anxiety and depression. Examples of questionnaires that have been rigorously studied in terms of their validity and reliability include the Beck Depression Inventory (BDI) (Beck, 1988), Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983).

Questionnaires have also been developed to assess the subjective health and well-being in the general population (for example, the World Health Organisation Quality of Life questionnaire; abbreviated version (WHOQOL-BRIEF); Skevington et al., 2004), though further explorations of these in the scientific literature are much more difficult to interpret. This is due to the complexities of human nature on a whole and the definition of 'normal' everyday life stresses and experiences, as well as additional influences of culture, religion and moral reasoning. Questionnaires that measure well-being and quality of life may also be used to assess the outcome of an intervention either at the clinical, community or population level. One issue with these measures is that they tend to be very subjective and rely on the individual's interpretation of themselves (Gough et al., 2012). Experience sampling methods (ESM) may provide an attractive solution to this where people are randomly prompted throughout the day to record their current feelings and circumstances

(Moberly and Watkins, 2008; Myin-Germeys et al., 2009). However, it could be argued that this is still quite intrusive and interrupts the natural flow of a person's day.

Responses in neuropsychological questionnaires may sometimes rely on someone else's interpretation of an individual's experience. This is particularly relevant in conditions like dementia, where a family member or member of care staff who works closely with the individual will answer questions on their behalf (Whitaker et al., 2013). This is due to the communication difficulties that arise because of the condition and a perceived lack of mental capacity as defined by the Mental Capacity Act in 2005 (Greaney et al., 2005). After appropriate assessment by a qualified clinician or researcher, it may be decided that the person's next of kin make important decisions on their behalf, as well as communicate information for them (Whitaker et al., 2014).

Despite the limitations of subjective questionnaires, they are invaluable as they are directly tapping into the real life and experience of the individual (Moberly and Watkins, 2008). Other measures are more likely to make inferences or assumptions around cause and effect. For example, observing a specific neural or physiological response often leads to assumptions that this represents a particular level of brain health and this is further complicated by individual variability. The ability to directly tap into an individual's experience is particularly important in populations where we may be more interested in quality of life, well-being and general levels of contentment, rather than cognitive or physiological performance. The priority for these patients is to reduce their distress and support them in being happier and healthier. In contrast, physiological and cognitive performance may be prioritised more highly in competitive elite athletes or Nobel Prize winners. Further, there are many athletes and scholars who suffer with psychological

conditions including depression and anxiety; providing examples of the uncoupling between brain health, physical or academic (i.e., cognitive) performance and well-being. Though in these cases, poor psychological outcomes may be more related to injury or social pressure (Kontos et al., 2012 and Hammond et al., 2013 respectively). Populations where quality of life may be considered particularly important include people who are suffering significant psychological distress because of a clinical condition (e.g., dementia, anxiety and depression) as well as in the general population. After all, a society that is in good health, functions well both physically and psychologically, and consists of people who are generally content in their existence and their interactions with others, is one that is likely to function much more successfully.

### 2.5. Combining Neuroimaging Methodologies with Cognitive Performance

Some studies have already been discussed that use multimodal approaches including neuroimaging and cognition. The aim of this section is to focus on more studies like these, summarise the main conclusions and consider future directions.

More recently, studies have incorporated a combination of methodological approaches either to compare directly to one another and establish associations (Boots et al., 2015) or to give a broader understanding of the effects of an intervention. Vaughan and colleagues undertook a randomised controlled trial of community-dwelling healthy women aged 65-75 years and randomly assigned 25 to an intervention group and 24 to a no exercise control group (see Vaughan et al., 2012 for methods). Neurocognitive functioning was measured via tasks related to processes of working memory, inhibition shifting, verbal fluency and reaction times (all components of higher executive functioning). Physical functioning was assessed by a 6-minute walking test, the ‘timed up and go’ test (Shumway-Cook et al.,

2000), the 1-leg test of balance (Fregly et al., 1973), waist and hip measures, resting heart rate and blood pressure. They also assessed brain derived neurotropic factor (BDNF), a considered biomarker of brain plasticity (Martinowich et al., 2007). They found that the 16-week exercise intervention led to improved cognitive functioning and improved physical functioning (Vaughan et al., 2014). Although this study clearly illustrates the benefits of an exercise intervention in older adults and incorporates several measures, more research is needed to understand the underlying mechanisms that drive these improvements in cognitive and physical function.

Boots and colleagues selected 315 participants from the Wisconsin Registry for Alzheimer's Prevention (WRAP); the WRAP is a longitudinal cohort of approximately 1,500 cognitively healthy adults aged 40 to 65 years (Boots et al., 2015). They found significant associations between cardiorespiratory fitness and a range of brain health measures, including: MRI measures of brain structure (grey matter volumes) and measures of cognition and mood. Studies like these provide evidence that supports a range of promising measures to assess brain health, particularly due to the large sample sizes they employ. However, they do not reveal the underlying mechanisms by which improvements in outcome measures are mediated or include functional measures of brain activity. More specific studies are required to assess these mechanisms in more detail, though unfortunately these types of studies often have smaller sample sizes for practical reasons.

A promising area of research has combined cognitive outcome measures with functional physiological measures of CBF velocity measured by TCD. Studies have demonstrated that the regulation of CBF velocity is not only related to cognitive function and physical activity, but can mediate cognition improvements through exercise (e.g., Guiney et al.,

2015). In this study by Guiney and colleagues, performance in cognitive control was examined by using tasks focusing on inhibition and switching. In addition, they examined CVR to a $CO_2$ stimulus (open circuit, steady-state method: 4 min of 5% $CO_2$ in air) in 55 healthy young adults. They found that the measures of cognitive performance and cerebrovascular health were related to habitual physical activity levels. Those who were more physically active had better CBF regulation (as indexed by CVR) and better cognitive inhibitory control. They also found that frequency of activity indicated improved cognitive control mediated by this measure of CBF regulation. Their findings complemented those from a study examining the effects of exercise on cognition in older women (Brown et al., 2012; discussed in 2.3.2.1, Vaughan et al., 2014) and cerebral haemodynamics assessed with NIRS (Cameron et al.2015). Future studies like this are required that adapt a multimodal approach to explore the relationship between blood flow and cognition and other measures associated with brain health.

The effects of healthy ageing and acute exercise on cognitive function and cerebral perfusion have also been investigated in a combined TCD and NIRS study (Lucas et al., 2012). In this study, cognitive function improved whilst exercising in both age groups (young 24 ± 5 y; older 62 ± 3 y), though imaging measures of cerebral perfusion were differentially affected between imaging modalities. Specifically, increases in MCAv observed by the TCD modality in response to exercise were similar during cognitive tasks for both age groups, whereas prefrontal cortical haemodynamic NIRS measures showed smaller increases in the older group between exercise intensities. Interestingly, the exercise-induced improvement in cognition was similar between the age groups. As such, while exercise reveals improvements in cognition in both younger and older participants, the differential effects on cerebral perfusion values between modalities poses questions

about how perfusion may differ across the vascular tree (i.e., conduit versus capillary flow) and could indicate potential regional differences (prefrontal versus global flow) as well as how measurements during acute exercise differ to resting perfusion changes during cognitive tasks. Against this background, whether these measures are derived using blood flow velocity and/or vessel diameter in an isolated region such as the MCA, or across the whole GM or relatively larger RoIs (in contrast to the centre of the MCA cross-section) using the BOLD signal, will likely affect reported outcome measures of resting CBF and CVR.

Measures of CVR and associations with cognitive function have also been investigated in clinical populations (Hajjar et al., 2014). In hypertensive patients, lower CVR was associated with a worse executive function test. In this study by Hajjar and colleagues, executive function was assessed using the Trail-Making test and measures of memory and attention. CBF was assessed using TCD measures of MCAv with a hypercapnic challenge of five minutes 5% $CO_2$ inhalation. In contrast to other studies using gas challenge protocols whilst participants are in a supine position, in this study they were sat upright. According to other research findings, postural differences do not affect this measure (Tymko et al., 2016), though this is another example of methodological inconsistencies. Further, this study also did not observe regional differences in CBF between anterior and posterior regions found previously in the literature (as discussed in Section 2.3.2.1).

Other studies have combined MRI measures of BOLD-derived CVR with executive function using the Stroop task (Gauthier et al., 2015; discussed in 2.3.3.2) and complex abstraction (Chapman et al., 2016; discussed in 2.3.3.2) to investigate ageing and fitness effects. The Gauthier study found associations between Stroop task performance and aortic

arch elasticity, though there was no significant association between performance on the Stroop task and BOLD-derived CVR. However, CVR in the frontal regions involved in executive function were determined to be negatively associated with fitness, indicating that fitness may mediate cognitive performance via decreased CVR in the prefrontal cortex (Figure 2.13). Finally, fNIRS and cognition have also been investigated in fitness and ageing studies, reporting links between fitness, DLPFC oxygen supply and cognitive performance (Albinet et al., 2014; Cameron et al., 2015; Bierre et al., 2016).

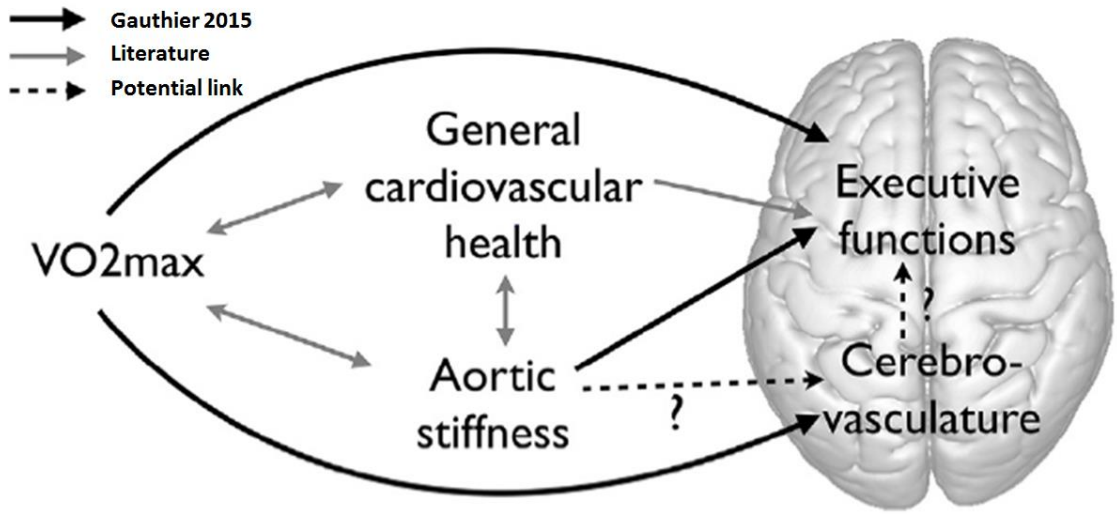



Figure 2.13. Conceptual framework for links between vascular health, cardiorespiratory fitness, and executive functions. The black arrows represent the links supported by data in Gauthier and colleagues' study, including V̇O2 max and preserved executive function, V̇O2 max and CVR, and aortic pulse wave velocity and executive function and general cardiovascular health (e.g., blood pressure). The grey arrow shows links supported by the literature. The broken arrows show missing causal links likely to underlie the association between cerebrovascular health, cardiorespiratory fitness and cognitive function (Gauthier et al., 2015).

Finally, it is also important to consider mechanistic and translational aspects of this area of research. Although beyond the scope of this thesis, other areas of research have focused on exercise-induced physiological stress on cells, tissues and organs within the body, that lead to molecular adaptations (see Burley et al., 2016 for review). These translate to haemodynamic and structural brain modifications, such as improved CBF and CVR (discussed previously), angiogenesis, neuroplasticity (Voss et al., 2013) and cognitive functioning (Kramer et al., 1999), as well as changes in clinical presentation, quality of life and well-being. Possible underlying mechanisms that mediate these adaptations in the brain have been reviewed (Lucas et al., 2015; Lautenschlager et al., 2012; Voss et al., 2013) and are briefly summarised in Figure 2.14. It is crucial that we improve understanding of these mechanisms and their relative contribution to help optimise intervention strategies (Burley et al., 2016).

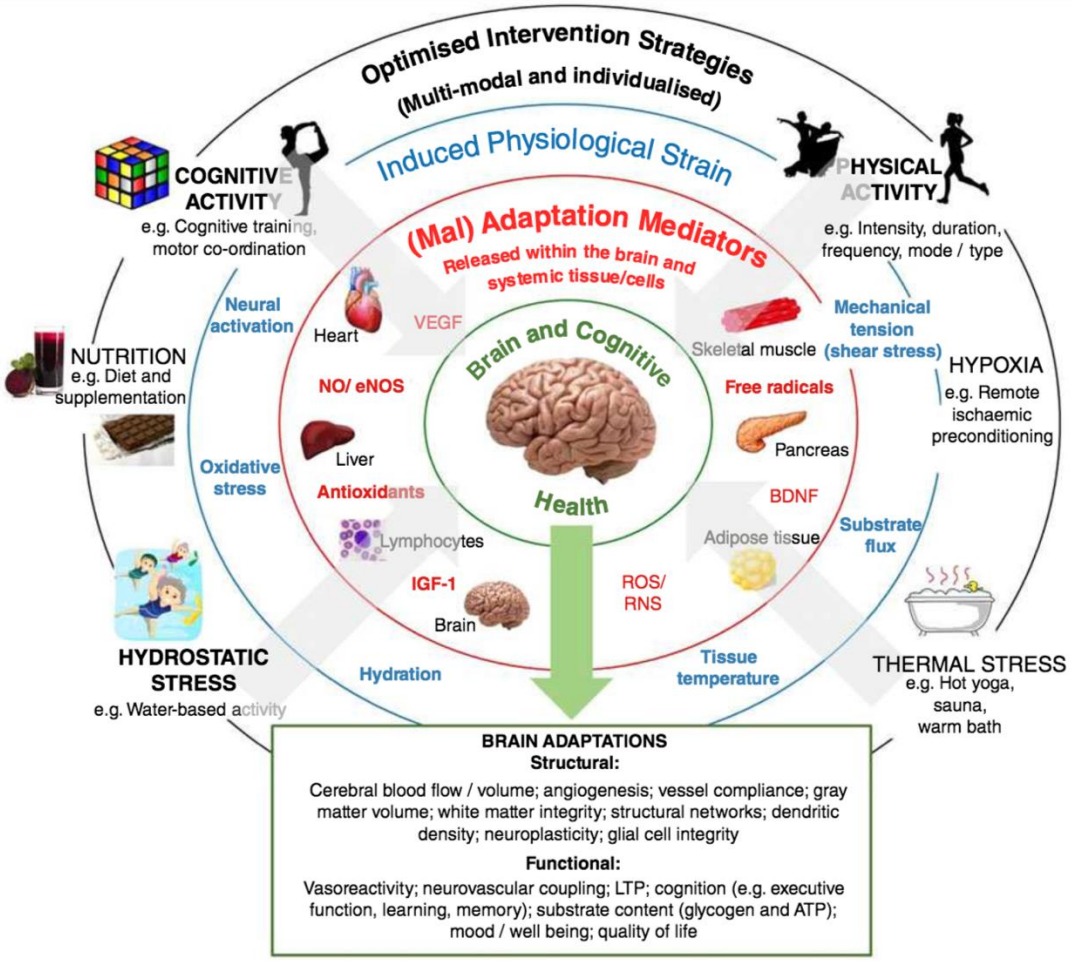



Figure 2.14. Potential mechanistic pathways through which the components of optimised intervention strategies may lead to beneficial brain adaptations of structure and function, and ultimately, improve brain and cognitive health. Strategies can occur concurrently to create multi-modal and individualised interventions. They include: physical activity; cognitive activity; nutritional supplementation; hydrostatic stress; thermal stress, and hypoxia. Such strategies induce physiological stress on cells, tissues and organs that facilitate stimulus-strain responses within the brain and systemic organs, tissues and cells and involve a number of mediators that can be adaptive or maladaptive depending on their concentrations. Thus, emphasising the potential hormesis effect for dose-response requirements to avoid toxicity (which may lead to dysfunction) and optimise physical and cognitive health. Mediators include: NO (nitric oxide); eNOS (endothelial nitric oxide synthase 3); BDNF (brain-derived neurotrophic factor); free radicals; ROS (reactive oxygen species); IGF-1 (insulin-like growth factor) and antioxidants (Burley et al., 2016).

## 2.6. Summary

Although the measures discussed vary quite substantially in terms of the process through which they measure brain health and exactly what they are measuring, the vast majority have demonstrated a positive role for regular exercise and greater fitness in improving the efficiency of the brain in some way (although some have led to reconsideration of what is considered impaired (i.e., studies using MRI-derived CVR measures showing the opposite fitness association to studies using TCD-derived CVR measures)). Whether that be through an increase in cerebrovascular responsiveness as seen with TCD, an improved cognitive performance determined by faster reaction times and increased number of correct responses in behavioural tasks, an improved subjective interpretations of emotional well-being revealed through questionnaires, an increased neuronal activation and improved integrity revealed by MRI, or increased oxygenation levels in brain tissue measured with the NIRS.

However, when used in isolation they all possess weaknesses that force us to question how confident we can be in the way that we interpret the results. For example, it may be possible that a patient with mild cognitive impairment may present a significantly improved score on a cognitive task that measures their attention and ability to ignore irrelevant stimuli, have an excellent cerebrovascular response measured with TCD, show increased neural activation in frontotemporal regions associated with higher executive functioning, yet, through clinical and psychological questionnaires measuring well-being and symptoms of depression, present as significantly depressed and suicidal. This is an incredibly over-simplified scenario though illustrates the dangers of relying on any measure in isolation, no matter how ‘good’ a measure it may be. If clinicians were to end this patient’s care based on the first measures of brain health that revealed very favourable

outcomes, then they would have failed to help this patient. Therefore, one measure cannot quantify all the effects associated with brain health. Further, it is important that we adopt a broader multi-disciplinary approach that utilises a range of measures on a person-centred basis.

Research studies are already beginning to utilise a more multimodal approach, where findings revealed from a combination of measures may either complement one another and strengthen the observed findings or contradict one another and evoke further exploration. Neurovascular coupling is an example of a more holistic brain-body approach where we can combine neuronal response measures in the brain with physiological measures in the cerebral vasculature (e.g., Phillips et al., 2016). We can further expand on this holistic approach by incorporating the various other methods introduced in this review. However, this needs to be done in a manner that is not going to lead to an excessive overload of measurements as this is neither therapeutic nor practical. This review demonstrates that more research is needed to better understand how we can most effectively utilise measures of brain health with the ultimate goal of confidently providing reliable, clinically relevant outcomes to any therapeutic intervention.

No known studies to date have directly compared TCD approaches to MRI approaches for outcome measures of resting CBF or CVR, yet both these measures are commonly used (in isolation) to predict health and brain targeted intervention. Considering the various issues discussed in this chapter within the different neuroimaging modalities, in addition to the different underlying mechanisms that determine the CVR measures (i.e., BOLD intensity signal or MCA velocity), and the discrepancies between ageing and fitness affects reported recently, this highlights an essential area and number of research questions that need to be

addressed. Further, although some studies have investigated cognitive performance associations with cerebrovascular health measures, this area of research is in its early infancy and no study thus far has explored whether these measures translate to, or are associated with, improved quality of life and well-being.

Therefore, this thesis will address the following research questions:

1. Do different methodological approaches ($CO_2$ stimulus duration and method of data extraction) affect the TCD-derived CVR outcome measure? (Chapter 3)
2. Will differences in resting CBF measures be observed between different age and fitness groups? (Chapter 4)
3. Do different neuroimaging modalities (TCD versus MRI) and analysis approaches alter the resting CBF outcome measure? (Chapter 4)
4. Will differences in CVR measures be observed between different age and fitness groups? (Chapter 5)
5. Do different neuroimaging modalities (TCD versus MRI) and analysis approaches alter the CVR outcome measure? (Chapter 5)
6. Will differences in cognitive performance, quality of life and well-being be observed between different age and fitness groups? (Chapter 6)

Table 2.1. Summary of key studies assessing measures of cerebral blood flow and multimodal approaches (i.e., with cognition).

| Study | Index of brain health | Modality | Participants | Fitness and age categorisation/ Intervention groups | Results |
|---|---|---|---|---|---|
| | *Resting Cerebral Blood Flow (CBF)* | | | | |
| Ainslie (2008) | Resting CBF (MCA velocity) | TCD | 153 healthy sedentary and 154 endurance-trained men aged 78-79 years | Lifelong physical activity or inactivity to determine group. Confirmed by cycle/treadmill V̇O2 max test (endurance mean V̇O2 max: 52.4 mL·kg$^{-1}$·min$^{-1}$; sedentary mean = 34.9 mL·kg$^{-1}$·min$^{-1}$ | Age-related decline in MCAv (-0.76±0.04 cm·s$^{-1}$·year$^{-1}$) independent of training status. MCAv elevated in endurance-trained (9.1±3.3 cm·s$^{-1}$). Approximately 17% difference approximate to 10 year reduction in MCAv 'age' |
| Bailey (2013) | Resting CBF (MCAv, CVCi and CVRi) (CVR below) | TCD | 81 healthy males. 4 groups: younger/ older and trained/ sedentary | Younger, ≤30 years, and older≥60 years. Lifetime PA level: trained, ≥150 minutes aerobic exercise (confirmed with V̇O2 max test), and sedentary, no activity | PA attenuated age-related decline in resting MCAv (older group: trained, 46 cm·s$^{-1}$ versus sedentary, 37 cm·s$^{-1}$) and CVCi (older group: trained, 0.54 cm·s$^{-1}$·mm Hg$^{-1}$ versus sedentary, 0.42 cm·s$^{-1}$·mm Hg$^{-1}$). Linear relationship between V̇O2 max and MCAv ($r$=0.58-0.77, $P$<0.05) |
| Barnes (2013) | Resting CBF (MCAv, CVCi and CVRi) (CVR below) | TCD | 29 healthy volunteers aged 55-77 years | Younger: mean age 26 years; mean V̇O2 max 38 mL·kg$^{-1}$·min$^{-1}$, and older: mean age 64 years; mean V̇O2 max 27 mL·kg$^{-1}$·min$^{-1}$ | MCAv and CVCi were lower in older adults compared to younger adults (MCAv: 39 cm·s$^{-1}$ versus 55 cm·s$^{-1}$; CVCi: 0.47 cm·s$^{-1}$·mm Hg$^{-1}$ versus 0.68 cm·s$^{-1}$·mm Hg$^{-1}$ respectively) |
| Chen (2011) | Cerebral perfusion | MRI ASL | 86 healthy cognitively and physically active adults (23-88 years) | Young (age<40 years), middle-aged (40 ≤age<60 years), and older (age≥60 years) groups | Observed age-related reductions in perfusion involving: superior-frontal, middle-inferior temporal, insular, precuneus, supramarginal, lateral-occipital and cingulate regions |
| Murrell (2013) | Resting CBF (MCAv, CVCi and CVRi) (CVR below) | TCD | 12-week aerobic-based training on resting MCAv. | Ten younger (23±5 years) and 10 older (63±5 years) | Effects of training on MCAv at rest were unclear in both age groups |

| | | | | | |
|---|---|---|---|---|---|
| | *Cerebrovascular Reactivity (CVR)* | | | | |
| Bailey (2013) | CVR (see resting CBF above) | TCD | 81 healthy males. 4 groups: younger/ older and trained/ sedentary | Younger, ≤30 years, and older, ≥60 years. Lifetime PA level: trained, ≥150 minutes aerobic exercise (confirmed with V̇O2 max test), and sedentary, no activity | PA attenuated age-related decline in CVR (older group: trained, 0.54 %·mm $Hg^{-1}$ versus sedentary, 0.42 %·mm $Hg^{-1}$). Linear relationship between V̇O2 max and CVR ($r$=0.58-0.77, $p$<0.05) |
| Barnes (2013) | CVR | TCD | 29 healthy volunteers aged 55-77 years | Younger: mean age 26 years; mean V̇O2 max 38 mL·$kg^{-1}$·$min^{-1}$, and older: mean age 64 years; mean V̇O2 max 27 mL·$kg^{-1}$·$min^{-1}$ | MCAv-CVR and CVCi -CVR were lower in older adults compared to younger adults (MCAv-CVR: 1.61 cm·$s^{-1}$ · mm $Hg^{-1}$ versus 1.06 cm·$s^{-1}$·mm $Hg^{-1}$; CVCi-CVR: 0.007 cm·$s^{-1}$ ·mm $Hg^{-1}$ versus 0.015 cm·$s^{-1}$·mm $Hg^{-1}$, respectively) |
| Thomas (2013) | Baseline CBF and CVR | MRI BOLD | 10 Masters athletes (MA) (8 male, 2 female), 10 sedentary elders 9 (SE) and sedentary younger control (YC) participants | MA consistent age-group place winners for 23 years with weekly mileage of 32 miles of equivalent cycling or swimming | MA had higher resting CBF in posterior cingulate, and lower CVR compared to SE. Dose-response relationship shown by V̇O2 max negatively correlating with CVR |
| DuBose (2016) | CVR | MRI BOLD | 32 healthy older adults (50-71 years) | V̇O2 max values were correlated with CVR values for RoIs using lobular approach | In entire cohort, V̇O2 max inversely correlated with frontal ($r$=-0.43, $p$<0.05), occipital ($r$=-0.46, $p$<0.05), parietal ($r$=-0.46, $p$<0.01) and temporal ($r$=-0.44, $p$<0.05) lobe CVR using lobular, not voxel-wise approach. Correlations strengthened when subset of 5 highest and lowest fit used ($r$=-0.56, $p$<0.78) |
| Murrell (2013) | CVR (see resting CBF above) | TCD | 12-week aerobic-based training on MCAv response to exercise and CVR to 5% $CO_2$ | Ten younger (23±5 years) and 10 older (63±5 years) | Absolute MCAv response to exercise was greater in the younger (9 and 9 cm·$s^{-1}$) than older (5 and 4 cm·$s^{-1}$) ($p$<0.05) and was similar following training. After 12-week training, CVR was elevated at rest (2.87±0.76 vs. 2.54±1.12 cm·$s^{-1}$·mm $Hg^{-1}$, $p$=0.01) and during exercise, irrespective of age |

| | | | | | |
|---|---|---|---|---|---|
| | *Multi-modal approaches (Cerebrovascular health and cognitive function outcome measures)* | | | | |
| Chapman (2016) | Resting CBF and CVR; cognition | MRI BOLD; complex abstraction | 36 healthy sedentary adults aged 56-75 years | Intervention study: cognitive (CT) versus physical (PT). 3hrs/ week for 12 weeks. Measures taken pre, mid and post | CT showed improved executive function, increased resting CBF in PCC, and no change in CVR compared to PT group. Complex abstraction associated with resting CBF in dACC. Higher resting CBF in hippocampus associated with better memory |
| Clark (2015) | Cerebrovascular resistance (CVRi) and MAP; cognition | ASL CVRi; verbal fluency | 58 older adults | Divided into 'young-old': 65-80 years and 'very-old': 85+ years | Very-old participants had elevated CVRi and reduced CBF compared to young-old in regions implicated in AD. Significant age-related interactions between elevated CVRi in the thalamus to reduced performance in verbal fluency |
| Lucas (2012) | Cerebral perfusion; cognition | TCD (MCAv) and NIRS, Stroop | 13 younger (24±5years), 9 older (62±3years) healthy participants | Acute exercise effects: cycling 30% and 70% of HR max | Stroop performance was slower for older than younger group. Cognition improved (similarly for younger and older groups) whilst exercising. Higher MCAv correlated with faster response times at rest, became uncoupled during exercise. Prefrontal NIRS showed smaller increases between intensities in older group |
| Brown (2010) | Resting CBF (MCAv, CVCi and CVRi); cognition | TCD; Global cognitive score (sorting test, colour-word inference, verbal fluency) | 42 healthy women aged 50-90 years | Younger: 50-64 years and older: 65-90 years. Active: $\dot{V}O_2$ max>90% age predicted score; sedentary: $\dot{V}O_2$ max<90% age predicted score | $\dot{V}O_2$ max was a predictor of resting CVCi (positive correlation) and MAP (negative correlation). MAP and CVCi were predictors of cognition. Cognition negatively correlated with age and positively correlated with fitness |
| Gauthier (2015) | Aortic arch elasticity and CVR (RespirActTM); cognition | MRI BOLD CVR; modified Stroop | 13 younger (24±3years), 54 older (63±5y) healthy participants | Older and younger groups defined. No fitness groups. Correlations across sample whilst controlling for age and gender | Older group showed a decline in executive function (slower performance in Stroop), higher aortic pulse wave velocity and lower $\dot{V}O_2$ max than younger. $\dot{V}O_2$ max negatively correlated with BOLD CVR in frontal regions though negatively correlated with CVR in periventricular watershed regions |

| | | | | | |
|---|---|---|---|---|---|
| Albinet (2014) | Cerebral oxygenation; cognition | fNIRS; Control counting task (CNT) and random number generation (RNG) task | 34 older women aged 60-77 years. | High-fit versus low-fit defined by median split of scores (n=17 per group) | High-fit showed significantly better performance on RNG compared to low-fit. High-fit showed significant increases in $HbO_2$ responses during RNG task. Increases in $HbO_2$ found to mediate relationship between V̇O2 max and executive performance |
| Guiney (2015) | Resting CBF and CVR; cognition | TCD (hypercapnia and hypocapnia); inhibition and attention switching task | 55 healthy young adults | NZPARQ self-report questionnaire and V̇O2 max. Correlations across sample | Exercise frequency predicted CBF regulation and inhibitory control. Mediation analysis indicated more frequent exercise may result in improved inhibitory control through improved CBF regulation |
| Wolters (2017) | Resting CBF (perfusion) | PC angiograph (Verbal fluency, Stroop, pegboard) | 4,759 mean age 61 years | 2005-2012, population study, 123 developed dementia | Hypoperfusion associated with accelerated cognitive decline and risk for dementia |

Abbreviations: ASL, arterial spin labelling; BOLD, blood-oxygen-level dependent signal; CBF, cerebral blood flow; CVCi, cerebrovascular conductance; CVRi, cerebrovascular resistance; CVR, Cerebral blood flow responsiveness to carbon dioxide (CVR); MRI, magnetic resonance imaging; fNIRS, functional near-infrared spectroscopy; $HbO_2$, deoxygenated haemoglobin; MCAv, middle cerebral artery blood velocity; NZPARQ, New Zealand Physical Activity Readiness Questionnaire; PA, physical activity; PC angiography, phase contrast angiography; RoI, regions of interest; TCD, transcranial Doppler; V̇O2 max, maximal oxygen consumption.

# 3. CEREBROVASCULAR REACTIVITY TO CARBON DIOXIDE USING TRANSCRANIAL DOPPLER ULTRASOUND

## 3.1. Abstract

**Background:** Cerebrovascular reactivity to carbon dioxide (CVR) is calculated from alterations in arterial carbon dioxide content ($PCO_2$) and is a common functional test to assess brain vascular health. However, studies measuring CVR have used different $CO_2$-stimulus durations to induce the response, and different durations of data extraction, from different time points of the stimulus duration, for its analysis that may alter the CVR outcome measure and explain conflicting findings reported to date. Therefore, the purpose of this study was to examine whether these methodological differences alter the CVR outcome measure.

**Methods:** Eighteen healthy volunteers (24 ± 5 years) completed a familiarisation trial and an experimental trial, which required them to inhale four stimulus durations (1, 2, 4 and 5-min) of 5% $CO_2$ (in air) via the open-circuit Douglas bag method, in a randomised order. A 5-min resting baseline preceded each $CO_2$-stimulus duration. CVR data were derived from transcranial Doppler (TCD) measures of middle cerebral artery blood velocity (MCAv), with concurrent ventilatory sensitivity to the $CO_2$-stimulus (VE-$CO_2$) determined via measures of respiratory rate and volume. Repeated measures ANOVAs compared CVR and VE-$CO_2$ measures between stimulus durations, steady-state time-points (1-min into stimulus versus end of stimulus) and extract durations (30 versus 60 s).

**Results:** An effect of stimulus duration was observed ($p = 0.002$, $\eta^2 = 0.140$), with 1-min ($p = 0.01$) and 2-min ($p < 0.001$) being different from the 4-min, and 2-min being different to the 5-min ($p = 0.02$) duration. VE-$CO_2$ sensitivity increased ~3-fold from 1-min to 4- and 5-min

durations ($p < 0.001$, $\eta^2 = 0.485$). CVR measures calculated from different steady-state time-points within each stimulus duration were different ($p < 0.001$, $\eta^2 = 0.454$); specifically, for the 4-min ($p = 0.001$) and 5-min ($p < 0.001$), but not the 2-min stimulus duration ($p = 0.23$). These findings demonstrate that methodological differences alter the CVR measure. Therefore, caution is needed when comparing studies that use different approaches.

## 3.2. Introduction

Regulation of cerebral blood flow (CBF) in response to a physiological stressor is a common functional test used to assess brain vascular health. Effective regulation of blood flow to and within the brain is vital for optimal brain function momentarily and across the lifespan. CBF declines by approximately 50% across healthy adulthood (Brown et al., 2010; Barnes et al, 2013; Bailey et al., 2013), which unfortunately coincides with a decline in the most potent regulatory mechanism of CBF - its responsiveness to changes in arterial carbon dioxide pressure ($PCO_2$; termed cerebrovascular reactivity to carbon dioxide (CVR)). Elevation in $CO_2$ in the bloodstream is known as hypercapnia. This response demonstrates how well pH balance is maintained and is a crucial homeostatic function to ensure biological processes operate in optimal environments and protect against disease. Reduced CBF and CVR are exacerbated in clinical diseases such as hypertension (Immink et al., 2004), stroke (Markus, 2001) and dementia (den Abeelen et al., 2014). Furthermore, CVR is associated with all-cause cardiovascular mortality (Portegies et al., 2014).

It is well recognized that regular exercise has a positive effect on brain function (Voss et al., 2011), and CVR appears as a sensitive measure to determine this. Generally, a greater CVR is

associated with higher aerobic fitness (Bailey et al., 2013; Barnes et al., 2013) and improves following 12 weeks of exercise training (Murrell et al., 2013). However, conflicting observations have been reported in a group of Masters athletes with life-long history of aerobic exercise training (Thomas et al., 2013). These athletes demonstrated a blunted CVR, contrary to the expected higher response associated with aerobic fitness that is more commonly reported.

A possible explanation for inconsistent findings is that methodological inconsistencies are present throughout the scientific literature in this area. For example, the open-circuit inhalation of $CO_2$ has been administered for a stimulus duration of 1.5 (Vernieri et al., 2009), 3 (Murrell et al., 2013), 4 (Guiney et al., 2015; Kastrup et al., 2001) or 5 minutes (Lavallee et al., 2009), as well as with different $CO_2$-stimulus concentrations (5% or 7%; see Fierstra et al., 2013 for a full review). Further, CVR can be derived from varying proportions of the overall stimulus duration (e.g., 15, 30 or 60 seconds of data extracted from any stimulus duration). In addition, there is variation in how long participants have been breathing $CO_2$ before data is extracted for calculation (i.e., how the time-point of the steady-state period is defined) as well as an unclear methodological approach to determine how to identify the steady-state period.

For example, List and colleagues used measures of change in middle cerebral artery blood velocity (MCAv) in response to $CO_2$ to investigate the effects of transcranial direct current stimulation on cerebral autoregulation in ageing and cerebrovascular diseases (List et al., 2015). The methodology describes how researchers ‘recorded over the MCA during a 60-s period of normal room air breathing and of Carbogen-inhalation (5% $CO_2$) when MCA flow

velocity became stable'. Similarly, Vernieri and colleagues used a '90-s $CO_2$ inhalation period – always including for each subject the 30-s plateau period' (Vernieri et al., 2009). However, in both these studies, determining how MCAv has become stable or reached a plateau period is unclear. Typically, on visual inspection of MCAv, the point at which steady state has been reached is open to interpretation as the trace fluctuates in nature. Further, the MCAv profile will vary between individuals such that some may take longer than 2 minutes (e.g., more than five minutes has been reported) to reach steady state (Regan et al., 2013). Thus, 2 minutes may not be long enough for some people to reach this state. Furthermore, given the known interaction between ventilatory and cerebrovascular responses that occur during hypercapnia at rest (Ogoh et al., 2008), the change in CBF between 1 or 2 minutes of stimulus to that of 4 or 5 minutes will likely be different given the circulatory delay associated with the delivery of the inspired $CO_2$ to the central chemoreceptors. These methodological inconsistencies may account for the different ageing and fitness results seen between the studies described previously (Thomas et al., 2013; Bailey et al. 2013; Barnes et al., 2013).

### 3.2.1. Study Aims and Hypotheses

To maximise the utility of CVR as a measure of brain vascular health it is important to identify whether methodological nuances affect the CVR outcome measure. Therefore, the overall aim of this study was to compare different methods of calculating the CVR outcome measure within the same individuals using the open-circuit technique where individuals inhaled a fixed fractional concentration of 5% $CO_2$ from a pre-mixed Douglas bag. Approaches similar to these are the most commonly used techniques because they use low specification and relatively inexpensive equipment, and are used in clinical settings to determine health status and disease risk (e.g., den Abeelen et al., 2014; Portegies et al., 2014).

VE-$CO_2$ and individual variability were also examined to determine whether these factors may have influenced the CVR measure (described in more detail in Sections 2.3.2 and 2.3.4). The study had two main aims examining variations in data collection and analysis. Specifically, aim (1): Mean CVR outcome measures and VE-$CO_2$ values were compared by calculating the final 30 seconds of four different stimulus durations of breathing 5% $CO_2$ (1, 2, 4 and 5 minutes); Aim (2): Mean CVR calculations derived from different methods of data extraction (including the time-point of the steady-state period used to determine data extraction and the duration of data extraction) within the same stimulus duration were compared. It was hypothesised that: 1) the stimulus duration of 1, 2, 4 or 5 minutes would not give the same calculated CVR outcome; 2) the time-point of data extraction would alter the CVR outcome (i.e., the CVR calculated by extracting data from 90 s into stimulus duration would be different from those calculated from data extracted at the end of the stimulus duration for durations longer than 2 minutes), and 3) CVR outcome measures calculated from 30 s of extracted data will be the same as CVR outcome measures calculated from 60 seconds of extracted data.

## 3.3. Materials and Methods

Ethical approval was obtained for all experimental protocols and procedures by the University of Birmingham Ethics Committee and conformed to the Declaration of Helsinki (project code: ERN_14-0555). All testing took place in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent to participate was obtained (Appendix A3.1 and A3.2).

### 3.3.1. Study Design and Protocol

Eighteen healthy volunteers (9 male and 9 female; mean age 24 ±5 years) participated in the study and were included in the analysis. Participants were required to make two visits. During the first visit participants provided informed consent and completed a general health questionnaire to check for contraindications (Appendix A3.3). Participants were not taking any medication and had no history of cardiovascular, cerebrovascular or respiratory disease. Following successful screening participants then completed a familiarisation session of the open-circuit gas challenge protocol. They were asked to lie in supine position, relax and breathe as naturally as possible. First, participants lay supine for ~20 minutes while they were instrumented with equipment (detailed below, Appendix A3.4). Once instrumented, baseline data were collected for 5 minutes. They then breathed 1 minute of a 5% $CO_2$ (in air) stimulus, followed by 5 minutes of room air, followed by 5 minutes of the 5% $CO_2$ stimulus. After satisfactory completion of familiarisation trials (i.e., no adverse reactions to breathing the $CO_2$ stimulus were experienced by the participant and adequate Doppler signals were identified by the PhD candidate), participants were invited back for the second visit that was a full experimental testing session. For this second visit, four different stimulus durations of the same 5% $CO_2$ stimulus were administered (1, 2, 4 and 5 minutes) and each included a 5-minute baseline recovery period (Figure 3.1). These stimulus durations were performed in a randomised order between participants to avoid order effects. For both the familiarisation and experimental visits, participants were asked to avoid vigorous exercise and alcohol 24 hours prior to study participation, caffeine for 12 hours and heavy meals for 4 hours.

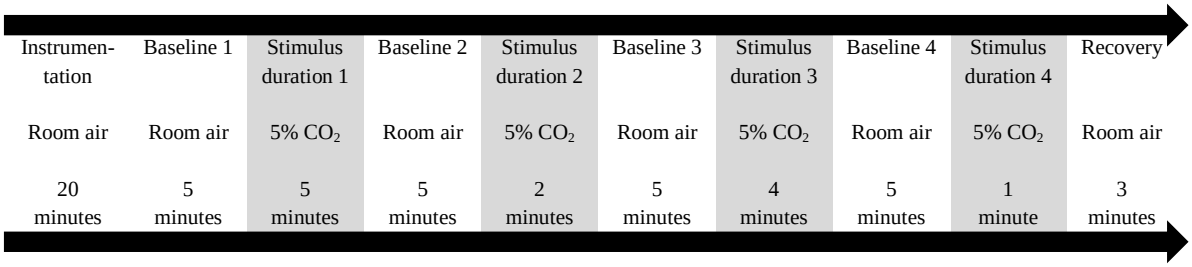



Figure 3.1. Example of gas challenge protocol for the second visit. Shaded boxes show the stimulus durations (1, 2, 4 and 5 minutes of 5% $CO_2$; random order of administration between participants). White boxes show baseline periods where participants were breathing room air.

### 3.3.2. Measures and Equipment

Beat-by-beat middle cerebral blood flow velocity (MCAv) and blood pressure (BP) along with breath-by-breath ventilatory (VE) rate and volume (for measures of VE-$CO_2$, measured in $L \cdot min^{-1} \cdot mm\ Hg^{-1}$) and end-tidal $CO_2$ partial pressure were continuously measured during the gas challenge protocol. MCAv was assessed using transcranial Doppler (TCD) (Multi Dop X, DWL, Compumedics Ltd Germany) with a 2-MHz probe placed over each temporal window to measure bilateral MCAv. Probes were prepared with ultrasound gel and held in place with a headset. Search and identification procedures were performed in accordance with established guidelines (Willie et al., 2011).

BP was measured using a finger cuff placed on the middle finger of the left hand (Portapres, Finapres, Medical System BV, Netherlands). Respiratory rate and volume were measured using a heated pneumotachograph (3813 Series, Hans Rudolph Inc, Kansas, USA) attached to a facemask, while fractional changes in inspired and expired $O_2$ and $CO_2$ were measured via a sample line attached to the facemask and a fast responding gas analyser (ML206, ADInstruments Ltd, New Zealand). Measures were recorded at 1k Hz via an analogue-to-digital converter (Powerlab, ADInstruments) and displayed in real time and stored for offline

analysis using commercially available software (LabChart v7.3.5, ADInstruments). Calibration of equipment was performed before each testing session.

### 3.3.3. Data Analysis

The analysis was split into two parts. For Aim 1, data from the last 30 seconds of each of the four $CO_2$ stimulus durations (1, 2, 4 and 5 minutes) and 60 seconds of baseline data before each stimulus duration were extracted (Figure 3.2). For Aim 2, data were extracted from two different steady-state time-points (i.e., after 60 seconds of stimulus duration and at the end of stimulus duration; Figure 3.3), for two different durations (60 and 30 seconds) from three stimulus durations (2, 4 and 5 minutes). The 1-minute stimulus duration is not included in this analysis as there is not enough time for MCAv to reach steady state (i.e., plateau). Finally, 3-minute stimulus duration CVR measures were calculated from the final 30 seconds of data 3 minutes into the stimulus duration for both the 4- and 5-minute stimulus durations.

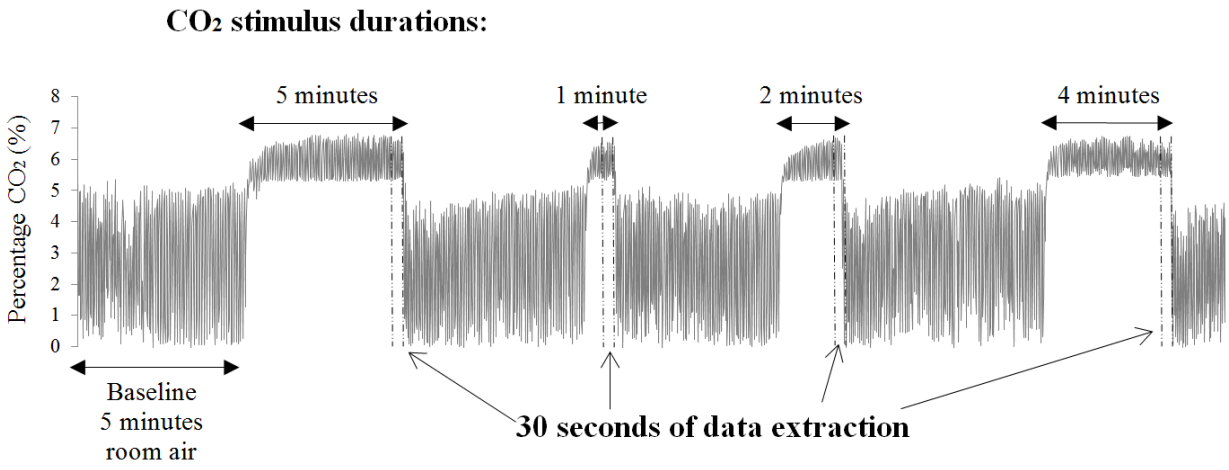



Figure 3.2. A representative $CO_2$ trace of gas delivery to a participant taken from LabChart. Shows where for Aim 1 30 seconds of data were extracted from the end of four different $CO_2$-stimulus durations (1, 2, 4 and 5 minutes).

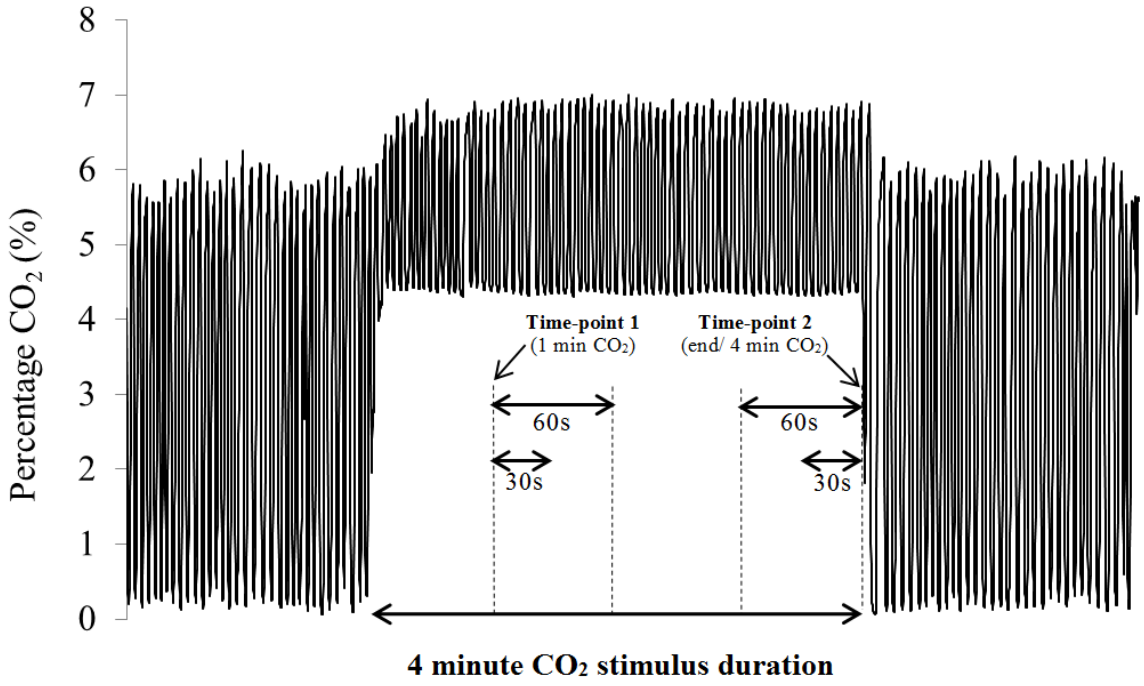



Figure 3.3. Percentage $CO_2$ trace from LabChart showing where data were extracted from two different steady-state time-points. Time-point 1: after 60 seconds of stimulus duration and Time-point 2: end of stimulus duration, for two different durations (60 and 30 seconds).

Mean right and left MCAv and CVR measures were compared for hemispatial effects. Mean baseline MCAv was calculated preceding each gas stimulus and analysed separately to see if they were different. Mean absolute and relative changes (percentage increase from baseline to hypercapnia) in MCAv and CVR measures were calculated. Ventilatory sensitivity was also calculated and compared across stimulus durations. The following equations were used to calculate absolute and relative CVR (Equation 3.1 and 3.2), and VE-$CO_2$ (Equation 3.3) (Brown et al., 2010 and Barnes et al., 2013).

**Absolute CVR:**

$$\frac{\text{hypercapnic (5\% } CO_2\text{) MCAv} - \text{resting MCAv}}{\text{hypercapnic (5\% } CO_2\text{) } P_{ET}CO_2 - \text{resting } P_{ET}CO_2}$$

Equation 3.1

**Relative CVR:**

$$\frac{100\ ((\text{hypercapnic (5\% } CO_2\text{) MCAv} - \text{resting MCAv}) / \text{resting MCAv})}{\text{hypercapnic (5\% } CO_2\text{) } P_{ET}CO_2 - \text{resting } P_{ET}CO_2}$$

Equation 3.2

**VE-$CO_2$:**

$$\frac{\text{hypercapnic (5\% } CO_2\text{) VE} - \text{resting VE}}{\text{hypercapnic (5\% } CO_2\text{) } P_{ET}CO_2 - \text{resting } P_{ET}CO_2}$$

Equation 3.3

After preliminary analysis and for completeness, a further analysis was performed on the 4- and 5-minute stimulus durations to determine whether CVR calculated 3-minutes into the stimulus duration differed to CVR values calculated from the 2-minute stimulus duration.

### 3.3.4. Statistical Analysis

Statistical analysis was performed in SPSS software (IBM SPSS version 22.0, Chicago, IL). Measures were compared using repeated analysis of variance (ANOVAs) with the main factors being stimulus duration (1, 2, 4 and 5 minutes), steady-state time-point (1 and 2), and duration of data extraction (60 and 30 seconds). Data are presented as means and standard deviations. Statistical significance was set at $p = 0.050$ and $\eta^2$ is used as the effect size throughout.

## 3.4. Results

### 3.4.1. Baseline Measures

A 2x4 within-participants ANOVA with two factors: hemisphere side (2 levels: left and right) and baseline number (4 levels: baseline preceding 1, 2, 4 and 5 minute stimulus durations), revealed there was no significant effect for mean MCAv between baseline measures ($F(3,48) = 1.73$, $p = 0.17$, $\eta^2 = 0.098$), nor was there any effect between hemispheres ($F(1,16) = 0.41$, $p = 0.53$, $\eta^2 = 0.025$).

### 3.4.2. Aim 1: Does the $CO_2$ Stimulus Duration Alter the Cerebral Blood Flow Responsiveness to Carbon Dioxide (CVR) Measure?

#### 3.4.2.1. Cerebral Blood Flow Responsiveness to Carbon Dioxide (CVR)

Relative and absolute CVR measures were analysed using a 2x4 within participants ANOVAs with two factors: side (2 levels: right and left), and duration of stimulus (4 levels: 1, 2, 4 and 5 minutes of $CO_2$ stimulus where data were extracted from the last 30 seconds of stimulus duration; see Figure 3.2). For both relative and absolute CVR measures, there was a significant main effect of stimulus duration (relative: $F(3,48) = 3.49$, $p = 0.037$, $\eta^2 = 0.161$; absolute: $F(3,48) = 3.10$, $p = 0.048$, $\eta^2 = 0.162$), whereas neither stimulus duration displayed a significant effect for hemisphere (relative: $F(1,16) = 1.99$, $p = 0.18$, $\eta^2 = 0.111$; absolute: $F(1,16) = 1.86$, $p = 0.19$, $\eta^2 = 0.104$). Therefore, the remaining analysis was performed on average CVR measures of bilateral MCAv (averaged right and left side). Relative and absolute CVR measures and VE-$CO_2$ values are presented in Table 3.1 with significant $p$ values in bold. Post-hoc differences (Bonferroni) are shown in Figure 3.4 and Figure 3.5.

Table 3.1. Summary table showing mean and standard deviation (SD) for average (right and left MCAv) relative and absolute CVR measures and VE-$CO_2$ values for each stimulus duration. Repeated-measures ANOVAs revealed significant differences between stimulus durations.

| | | **$CO_2$ stimulus duration** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **1 minute** | **2 minute** | **4 minute** | **5 minute** | **n** | ***p*** | ***$\eta^2$*** |
| **Relative CVR** (% change in MCAv / mm Hg change in $PETCO_2$) | mean | 2.93 | 3.20 | 2.50 | 2.63 | 18 | **0.036** | 0.153 |
| | SD | 1.05 | 1.25 | 0.93 | 1.00 | | | |
| **Absolute CVR** ($cm \cdot s^{-1} \cdot mm\ Hg^{-1}$) | mean | 1.93 | 2.13 | 1.70 | 1.75 | 18 | **0.036** | 0.153 |
| | SD | 0.70 | 0.94 | 0.70 | 0.67 | | | |
| **VE-$CO_2$** ($L \cdot min^{-1} \cdot mm\ Hg^{-1}$) | mean | 0.32 | 0.70 | 0.90 | 0.95 | 18 | **0.000** | 0.485 |
| | SD | 0.22 | 0.25 | 0.36 | 0.43 | | | |

Abbreviations: $CO_2$, carbon dioxide; CVR, cerebrovascular reactivity; MCAv, middle cerebral artery blood velocity; $P_{ET}CO_2$, end-tidal carbon dioxide; VE-$CO_2$, ventilatory sensitivity to $CO_2$.

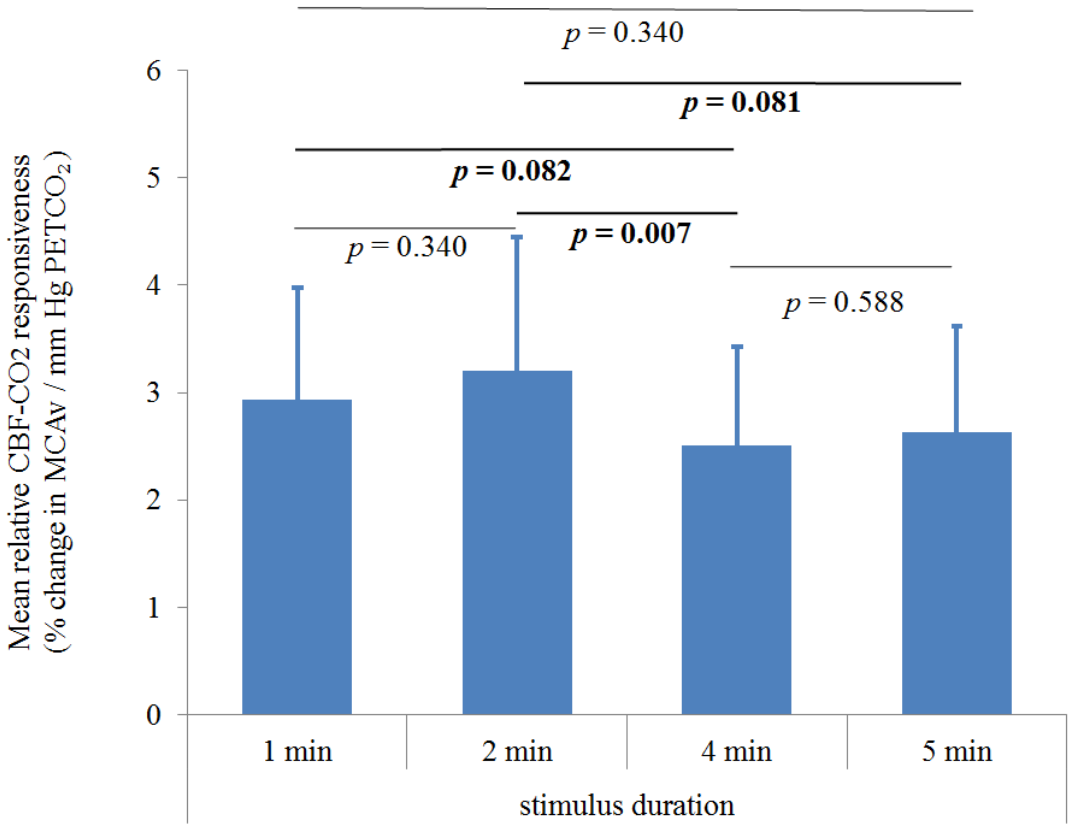



Figure 3.4. Mean (± SD) relative CVR calculated for each stimulus duration. Significant post-hoc effects and trends towards significance of stimulus duration are shown in bold font. Abbreviations: $CO_2$, carbon dioxide; CVR, cerebrovascular reactivity; MCAv, middle cerebral artery blood velocity; $P_{ET}CO_2$, end-tidal carbon dioxide. Adjustment for multiple comparisons was done via Bonferroni.

### 3.4.2.2. Ventilatory Sensitivity to Carbon Dioxide ($VE\text{-}CO_2$)

Ventilatory sensitivity to $CO_2$ ($VE\text{-}CO_2$; average from the 30-second time window selected, see Section 3.3.3 for calculation) increased ~3-fold from the 1 minute to the 4- and 5-minute stimulus durations ($p < 0.001, \eta^2 = 0.485$) (Table 3.1; Figure 3.5).

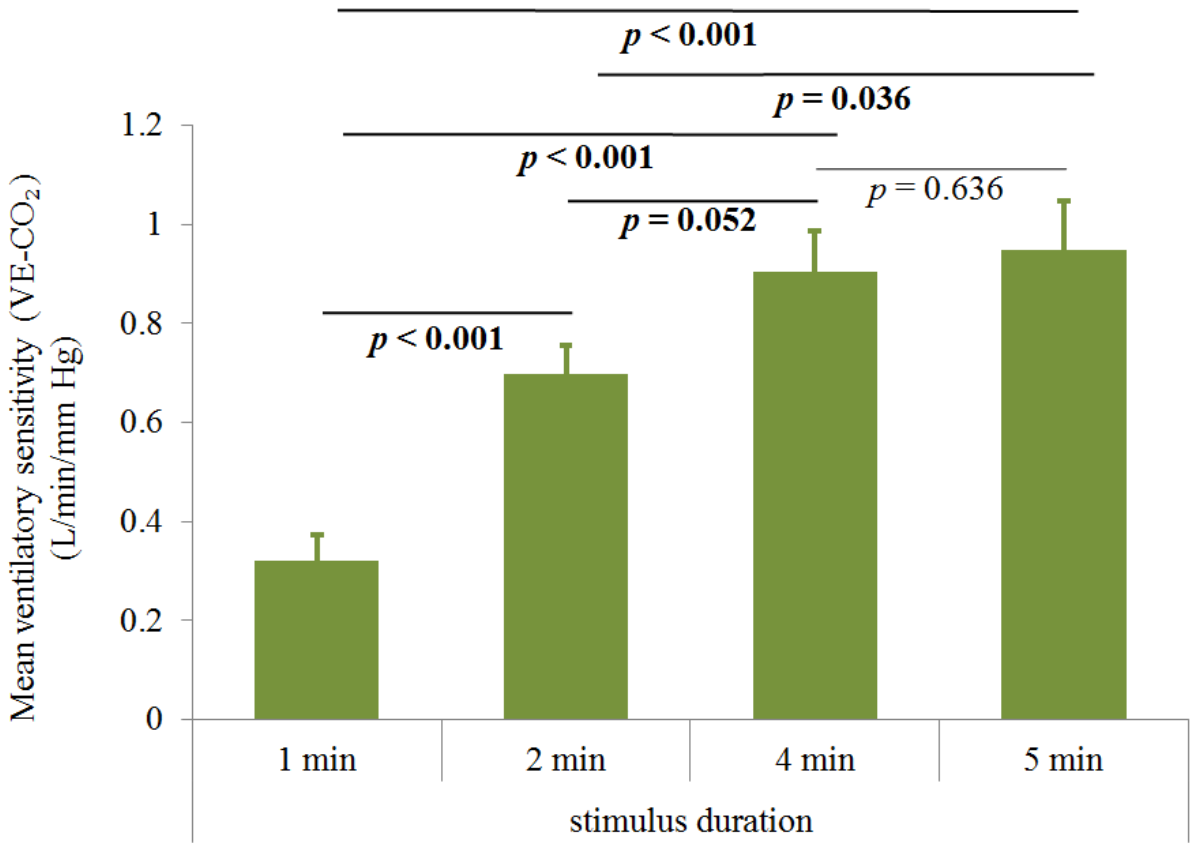



Figure 3.5. Mean (± SD) $VE\text{-}CO_2$ values calculated for each stimulus duration. Significant post-hoc effects and trends towards significance of stimulus duration are shown in bold font. Abbreviations: $CO_2$, carbon dioxide; $VE\text{-}CO_2$, ventilatory sensitivity to $CO_2$. Adjustment for multiple comparisons was done via Bonferroni.

### 3.4.2.3.Within-Individual Variability

We observed visual differences in the beat-to-beat MCAv-response profile between durations. For example, a steady-state profile typified the 4 and 5 minute tests, whereas the 1 minute test tended to peak in the final seconds of the stimulus duration. Further, there was variation

within individuals across the four stimulus durations; CoV ranging from 7 – 46% between individuals (Figure 3.6).

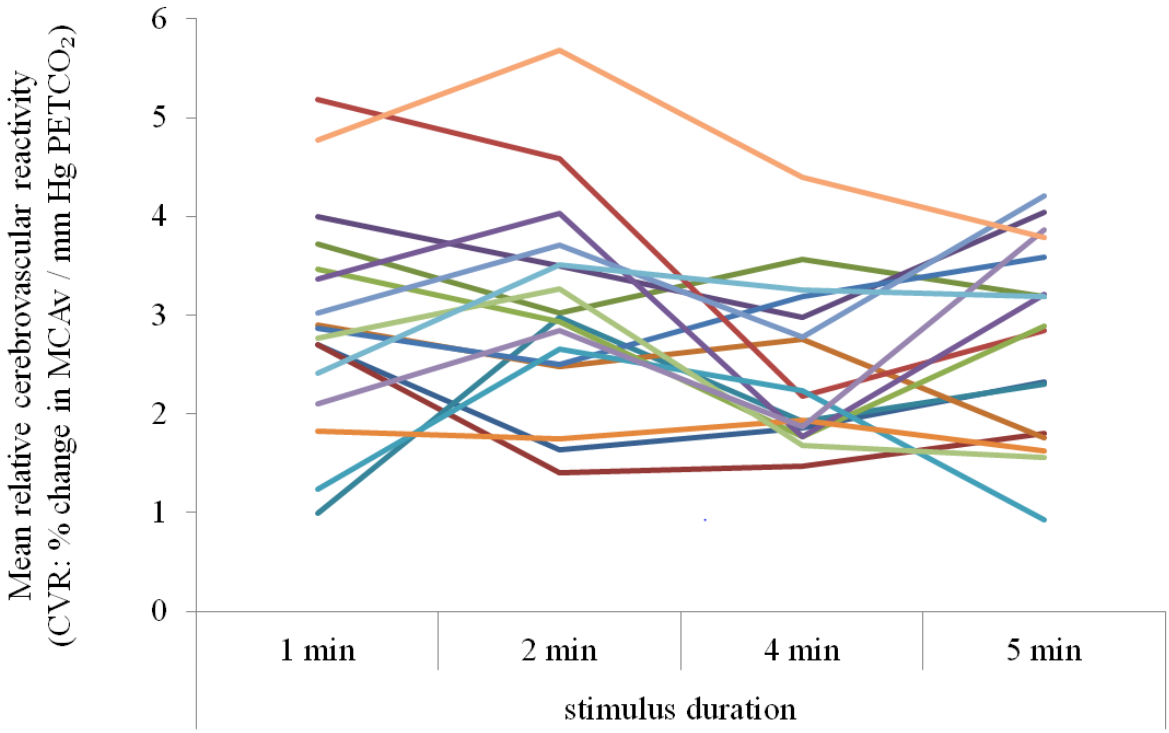



Figure 3.6. Within-individual variability in relative CVR measure (from mean right MCAv) between $CO_2$-stimulus durations. Covariance within participants ranged from 7-46%.

### 3.4.3. Aim 2: Does the Time-Point of Steady State or the Duration of Data Extraction Alter the CVR Outcome Measure?

The purpose of this next analysis was to investigate whether the time-point of steady state used for data extraction (time-point 1 or 2; Figure 3.3) and the duration of data extracted (30 or 60 s) altered the CVR outcome measure. Time-point of steady state and duration of data extraction were examined for the 2-, 4- and 5-minute $CO_2$-stimulus durations for both CVR and VE-$CO_2$ measures. One important note is that for the 2-minute stimulus duration, because of the shorter length, data is extracted from almost identical time-points regardless of the approach used (i.e., data is extracted following 1 minute of the stimulus up to the end of the

stimulus for time-point 1, and data is extracted 1 minute preceding the end of the stimulus backwards to 1 minute into the stimulus for time-point 2 and would be almost identical since the stimulus duration was exactly 2-minutes). Mean MCAv traces and mean $P_{ET}CO_2$ traces for all four stimulus durations (1, 2, 4 and 5 minutes) for one participant are shown in Figure 3.10.

#### 3.4.3.1. Cerebral Blood Flow Responsiveness to Carbon Dioxide (CVR)

Average (right and left MCAv) CVR measures were analysed using a 2x3x2 within participants ANOVA with three factors: steady-state time-point (2 levels: time-point 1 and 2), stimulus duration (3 levels: 2, 4 and 5 minutes of $CO_2$ stimulus), and data extraction duration (2 levels: 30 and 60 seconds). There was a significant effect of stimulus duration (similar to Aim 1): $F(2,68) = 4.78$, $p = 0.02$, $\eta^2 = 0.123$, and steady-state time-point: $F(1,34) = 28.24$, $p < 0.001$, $\eta^2 = 0.454$. The effect of data extraction duration was not significant: $F(1,34) = 1.12$, $p = 0.300$, $\eta^2 = 0.032$. Therefore, a 2x3 ANOVA was performed with two factors: steady-state time-point and stimulus duration, for average CVR measures calculated from 30 and 60 seconds of extracted data.

Repeated measures ANOVA revealed a significant main effect of time-point: $F(1,17) = 14.373$, $p = 0.001$, $\eta^2 = 0.458$, and a trend towards stimulus duration: $F(2,34) = 28.24$, $p = 0.064$, $\eta^2 = 0.149$. Further, a significant time-point by stimulus duration interaction was observed ($p = 0.008$, $\eta^2 = 0.232$). Paired t-tests were used to examine effects of time-point for each of the different stimulus durations separately (Figure 3.7). As expected, given the similar time-points for the 2-minute stimulus duration, CVR measures were not significantly different

($p$ = 0.343). However, there was a significant effect of time-point for the 4-minute ($p$ = 0.012) and 5-minute ($p$ = 0.002) stimulus durations. In summary, this analysis shows that the time-point of steady state used to determine data extraction does alter the CVR measure (Figure 3.7 and Table 3.2).

#### 3.4.3.2. Ventilatory Sensitivity to Carbon Dioxide (VE-$CO_2$)

VE-$CO_2$ values were analysed using a 2x3x2 within participants ANOVA with three factors: steady-state time-point (2 levels: time-point 1 and 2), stimulus duration (3 levels: 2, 4 and 5 minutes of $CO_2$ stimulus), and data extraction duration (2 levels: 30 and 60 seconds). There was a significant effect of time-point: $F(1,17) = 54.76$, $p < 0.001$, $\eta^2 = 0.763$, while effects of extract duration and stimulus duration were not significant ($F(1,17) = 2.52$, $p = 0.131$, $\eta^2 = 0.129$ and $F(2,16) = 0.746$, $p = 0.490$, $\eta^2 = 0.085$ respectively). However, there were significant interactions between time-point and stimulus duration: $F(2,34) = 15.90$, $p < 0.001$, $\eta^2 = 0.483$ and time-point and data extraction duration: $F(2,34) = 14.61$, $p = 0.001$, $\eta^2 = 0.462$. Therefore, four separate ANOVAs were performed for each method of data extraction; 30 and 60 seconds of data extracted from time-point 1 and time-point 2 (Table 3.3; Figure 3.8).

When data were extracted from time-point 2, VE-$CO_2$ sensitivity increased from the 2 minute to the 4 and 5 minute test durations (similar to in Aim 1). In contrast, when data were extracted from time-point 1 there is less variability within the VE-$CO_2$ sensitivity measure between the stimulus durations (i.e., data is extracted from roughly the same time-point despite the stimulus duration).

Table 3.2. Mean and standard deviation (SD) for relative CVR measures calculated using data extracted from different time-points (time-point 1 and 2) from 3 stimulus durations (2, 4 and 5 minutes).

| **CVR** (% change in MCAv / mm Hg change in PETCO2) | | **$CO_2$ stimulus duration** | | | | | |
|---|---|---|---|---|---|---|---|
| **Time-point of data extraction** | | 2 minute | 4 minute | 5 minute | F | *p* | $\eta^2$ |
| Time-point 1 | mean | 3.26 | 2.81 | 3.02 | | | |
| | SD | 1.27 | 0.70 | 0.80 | 1.58 | 0.221 | 0.085 |
| Time-point 2 | mean | 3.22 | 2.50 | 2.62 | | | |
| | SD | 1.24 | 0.92 | 0.97 | 4.61 | **0.024*** | 0.213 |

Abbreviations: CVR, cerebrovascular reactivity (CVR); MCAv, middle cerebral artery blood velocity.

Table 3.3. Mean and standard deviations (SD) for VE-$CO_2$ values calculated using different methods of data extraction (steady state time-point and data extraction duration) from 3 stimulus durations (2, 4 and 5 minutes). Separate within subject ANOVAs were performed for each steady state time-point (with stimulus duration and duration of data extraction as factors) and each stimulus duration (with steady state time-point and duration of data extraction as factors).

| **VE-$CO_2$** (L/min/mm Hg) | | **$CO_2$ stimulus duration** | | | | | |
|---|---|---|---|---|---|---|---|
| **Method of data extraction** | | 2 minute | 4 minute | 5 minute | F | *p* | $\eta^2$ |
| Time-point 1 | mean | 0.64 | 0.60 | 0.54 | | | |
| | SD | 0.21 | 0.23 | 0.23 | 1.25 | 0.299 | 0.069 |
| Time-point 2 | mean | 0.63 | 0.89 | 0.91 | | | |
| | SD | 0.20 | 0.31 | 0.40 | 5.46 | **0.009**** | 0.243 |

Abbreviations: $CO_2$, carbon dioxide; VE-$CO_2$, ventilatory sensitivity to $CO_2$.

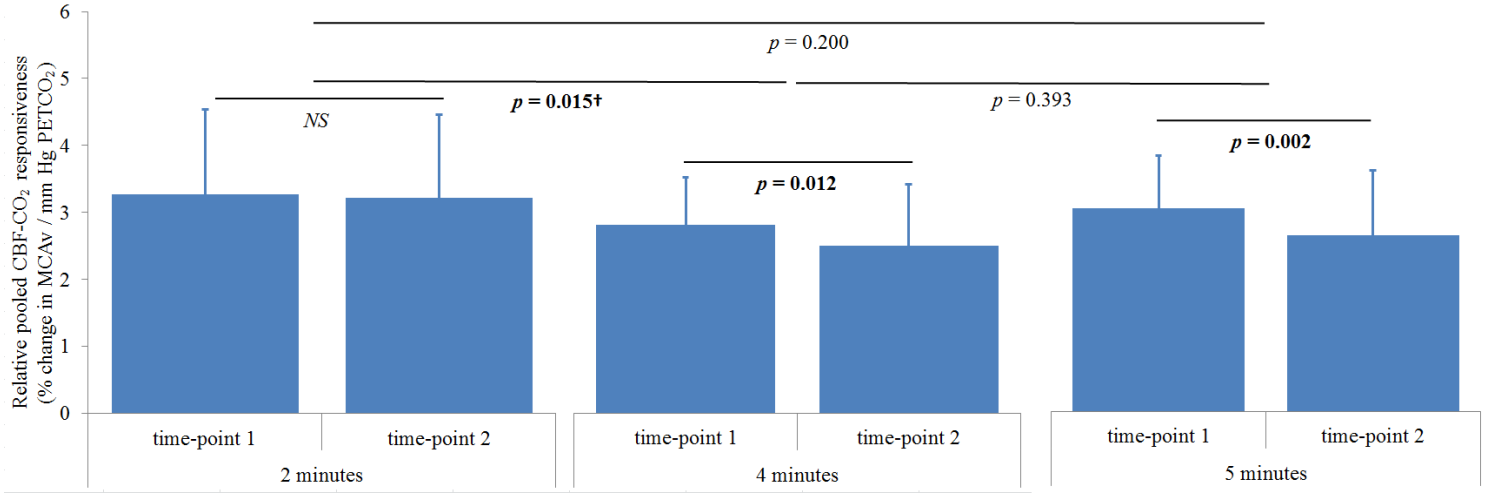



Figure 3.7. Mean ± SD relative CVR measures (averaged right and left MCAv). Graphs shows measures calculated from two steady-state time-points from three stimulus durations (2, 4 and 5 minutes). Paired t-tests revealed significant effect of time-point for the 4-minute and 5-minute stimulus durations. Abbreviations: $CO_2$, carbon dioxide; CVR, cerebrovascular reactivity; MCAv, middle cerebral artery blood velocity; $P_{ET}CO_2$, end-tidal carbon dioxide.

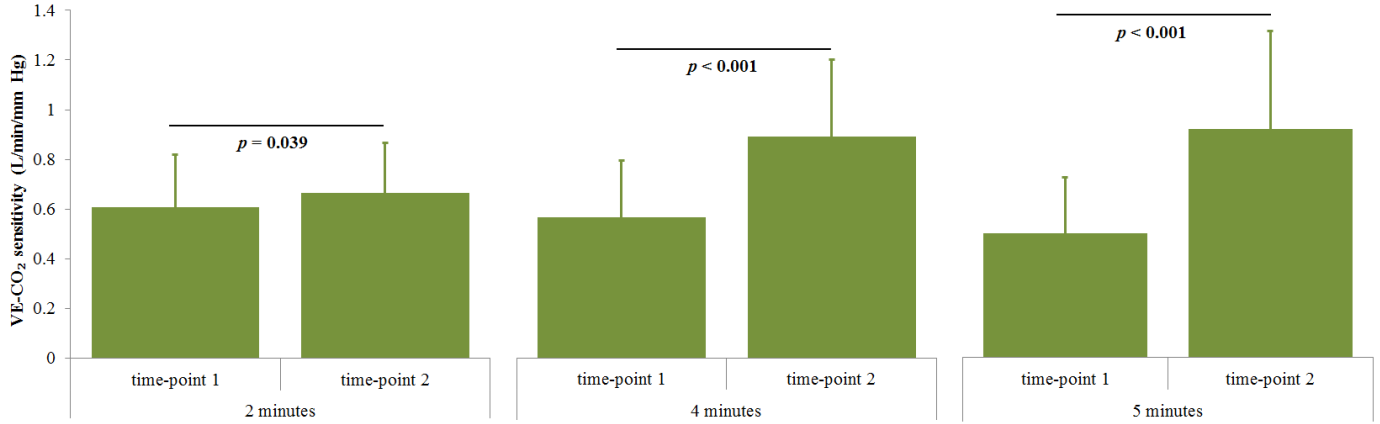



Figure 3.8. Mean ± SD VE-$CO_2$ values. Graphs shows measures calculated from: two steady state time-points from three stimulus durations (2, 4 and 5 minutes). Paired t-tests revealed significant effect of time-point for the 2- minute, 4-minute and 5-minute stimulus durations. Abbreviations: $CO_2$, carbon dioxide; VE-$CO_2$, ventilatory sensitivity to $CO_2$.

### 3.4.3.3. Three Minute Stimulus Duration CVR Measures

In addition, CVR measures were calculated from the final 30 seconds of data 3 minutes into the stimulus duration for both the 4 and 5 minute stimulus durations. Paired t-tests revealed no differences between relative CVR measures calculated from the mean right MCAv ($t$ = 0.474; $p$ = 0.643), a trend for a difference between relative CVR measures calculated from the left MCAv ($t$ = 1.965; $p$ = 0.070) and no differences for measures calculated from average (right and left) MCAv ($t$ = 1.686; $p$ = 0.504) when comparing between the 4 minute and 5 minute stimulus durations. Further, a repeated-measures ANOVA comparing average CVR measures obtained from 3 minutes into the 4- and 5-minute stimulus duration with the CVR measures obtained from the 2-minute stimulus duration, were also not significantly different ($p$ = 0.184, $\eta^2$ = 0.121) (Figure 3.9).

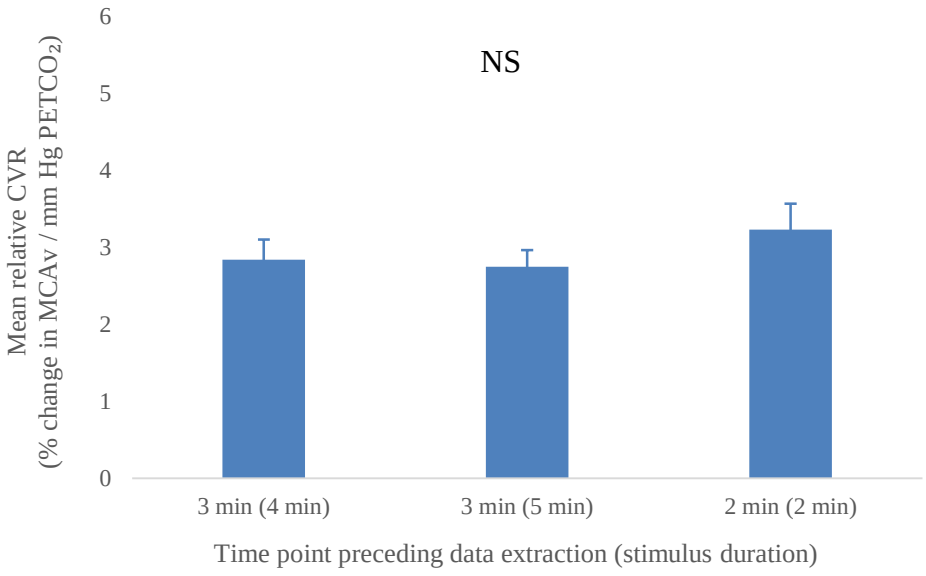



Figure 3.9. Mean relative average (right and left MCAv) CVR calculated from data extracted 3 minutes into the 4-minute and 5-minute stimulus durations and at the end of the 2-minute stimulus duration.

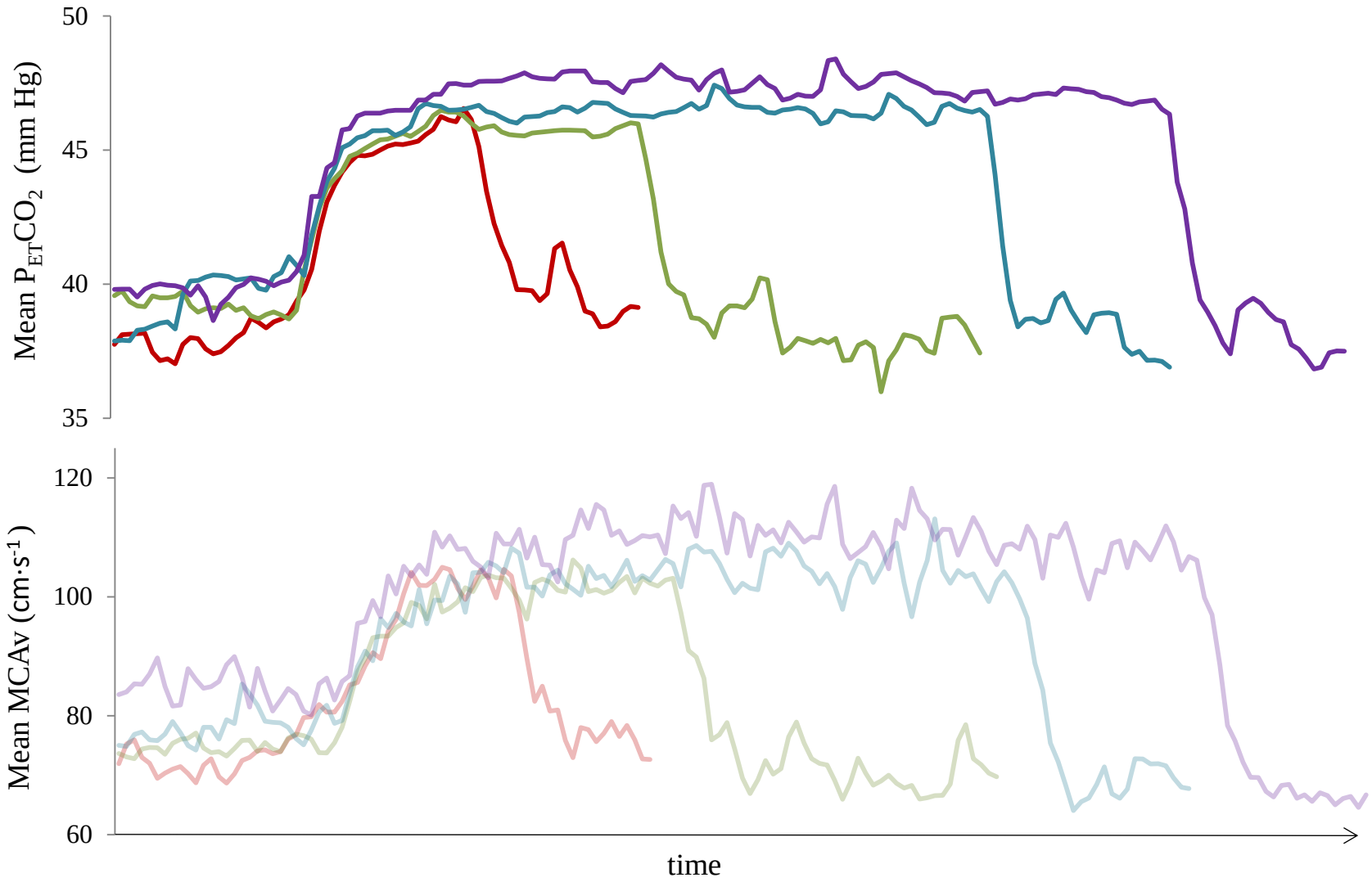



Figure 3.10. Mean MCAv (lighter lines; lower four traces) and mean $P_{ET}CO_2$ (darker lines; upper four traces) for all four stimulus durations (1, 2, 4 and 5 minutes) for one participant.

## 3.5. Discussion

The purpose of this study was to compare CVR measures and VE-$CO_2$ values calculated from: 1) different $CO_2$ stimulus durations, and 2) different durations of data extraction and different time-points of steady state to determine data extraction. The main findings were: 1) CVR outcome measures calculated after 60 seconds of stimulus (time-point 1) were higher than measures obtained at the end of the stimulus duration (time-point 2); 2) there was less variability in the CVR outcome measure when it was derived from 60 seconds after stimulus onset (time-point 1), indicating a more reliable point to determine CVR; 3) within-participant variation increased when the stimulus duration was longer than 2 minutes, likely due to increased effects of the ventilatory response. Collectively, these findings demonstrate that methodological differences of stimulus duration and time-point used to determine the steady-state, do indeed alter the CVR outcome measure and VE-$CO_2$ values.

CVR is a commonly used method of assessing brain health and has been applied in both healthy and clinical populations. Therefore, it is important that the methodologies used are consistent across research and clinical centres. Based on the findings of the present study, CVR outcome measures calculated 60 seconds after stimulus onset (time-point 1) with stimulus duration of between 2 and 3 minutes will be less affected by individual variability and therefore more reliable for both within (i.e., repeated measures) and between (cross-sectional) study comparison.

In aim 1, we found a significant effect of the $CO_2$ stimulus duration on the CVR outcome measure, as well as marked variability within the same individuals across the different stimulations (see Figure 3.6). On average, the CVR measure increased between the 1-

minute and the 2-minute stimulus duration, and was lowest when taken from the 4- and 5-minute stimulus durations. This observation was also clear in the second part of the analysis for aim 2, where CVR measures were higher for the 2-minute stimulus duration and lower for the 4- and 5-minute stimulus durations. These differences are likely driven by the change in VE-$CO_2$ that increased approximately 3-fold as the stimulus duration increased. Indeed, the difference in VE-$CO_2$ values between 1 and 2 minutes were significant, becoming progressively less different as the stimulus duration increased to 4 and 5 minutes. These values support those previously shown in the literature (Lucas et al., 2011; Murrell et al., 2013; Brothers et al., 2014) and were again similar in the second part of the analysis.

In aim 2, we found significant effects of steady-state time-point on CVR measures, though only when calculated from the 4- and 5-minute stimulus durations. The lack in variability from the 2-minute duration was expected since the data are extracted from the same point for both steady-state time-point approaches (i.e., for time-point 1, data are extracted 1 minute into gas delivery and for time-point 2, data are extracted during the final minute of gas delivery which is also 1 minute into gas delivery given that the stimulus duration is 2 minutes). In contrast, there was no effect of data extraction duration for each stimulus duration on the CVR measure (30 vs. 60 seconds; $p = 0.300$).

When considering the 2-minute stimulus duration to calculate CVR, MCAv was just reaching steady-state, or was still increasing to reach steady-state in some people (Regan et al., 2013), and calculated CVR measures were higher. Consequently, measures calculated by extracting data from this period may be less susceptible to increased ventilation effects, particularly with responders who are slow to reach steady-state and particularly if 60

seconds of data are extracted. However, this may lead to an overestimation of the CVR measure. To address this, CVR measures from the final 30 seconds of data 3 minutes into the stimulus duration for both the 4-and 5-minute stimulus durations were compared with the CVR measure calculated using the 2-minute stimulus duration. This comparison revealed no significant difference between these CVR measures. Given this, perhaps a 2- or 3-minute stimulus duration is the most suitable to use to obtain an accurate CVR measure, since they are least affected by intra-individual variation in vascular and respiratory responses, including the time to reach steady state. Further, a shorter duration will also not unnecessarily overstress participants.

The duration of data extraction (60 or 30 seconds) does not seem to affect CVR measures based on this dataset. However, a consistent approach should be used that avoids any interference resulting from the on-kinetics of the stimulus and the gradual increase to steady state that occurs within the first 2 minutes. Extracting a relative small proportion of the dataset is the commonly used approach in research centres using Doppler ultrasound to calculate the CVR measure. In contrast, research centres using magnetic resonance imaging (MRI) to measure CVR will extract data from the entire time course as well as the preceding baseline to calculate the CVR measure using a linear regression. Further, in protocols using a number of different levels of hypercapnia, data will be extracted from the entire protocol (Driver et al., 2016), rather than a relatively small segment. These approaches that utilise more of the dataset may avoid the possible sensitivity issues observed here. This leads to question whether CVR outcome measures (in addition to resting CBF measures) are different between different imaging modalities (i.e., TCD and MRI), a question investigated in Chapters 4 and 5.

### 3.5.1. Limitations and Methodology Considerations

Limitations within this study include that it is difficult to test natural variation that may appear in the same individual across time. This is because it is impossible to test exact reliability between stimulus durations, as we cannot give a subject the different stimulus durations at the same time (i.e., the 2-minute stimulus duration cannot be given at the same time as the 4- or 5-minute stimulus duration). This study came as close as possible in achieving this by comparing four different stimulus durations within an hour whilst allowing adequate recoveries between them. The stimulus durations were also administered in a randomised order to account for potential order effects. Further research is needed to compare these measures between days. Individual variation in $CO_2$ responses may be explained by genetic factors as well as sex, fitness level and other circumstances (Secher, 2015), indicating that the CVR measure is far more complicated than often presumed. Nevertheless, the CVR outcome measure is often collected from a single clinical visit, where the individual has not had time for their physiological measures to reach a resting baseline, and then used to predict current health status and disease risk.

Another limitation with this study is that we are unable to consider possible effects of vessel diameter change in response to $CO_2$ and how these may contribute to changes in blood flow velocity, which is currently debated in the literature (e.g., Brothers and Zhang, 2016; Hoiland and Ainslie, 2016). However, our findings can direct us towards an approach that avoids as much variability as possible and lead us to consider other factors. For example, future research may aim to establish where the peak CVR is likely to occur and the extent to which individual responses range from the lowest to highest CVR measure. Our findings demonstrated that some individual's CVR measures vary considerably depending on the stimulus duration used, whereas others would remain

relatively stable across the different durations tested here (as shown in Figure 3.6). We could also consider implementing the ventilation response into the calculation in some way. For example, determining the point where CVR is at its peak and the ventilation response is at its lowest. Perhaps this approach would give a more accurate representation of CVR measures that is less influenced by within subject variability and the ventilatory response.

A familiarization trial was performed with each participant to ensure they were comfortable with the 5% $CO_2$ gases and experienced no adverse effects. This trial also ensured that the PhD candidate could obtain sufficient MCA signals that could be replicated for the second visit. Mean MCAv values were also compared between the four baselines. On visual inspection there is notable variability between the baseline velocities with some participants showing more variability than others (i.e., CoV ranging from 1 – 8%). This may be a natural variability that occurs across time in response to many environmental influences and warrants further investigation, particularly if day-to-day variation within the same individual may also influence the CVR measure. In contrast, there is less variability between the baseline mean $P_{ET}CO_2$ values (i.e., CoV ranging from 0 – 5%; mean CoV = 2.4%) (Appendices Table A3.2).

### 3.5.2. Perspectives and Significance

To maximise the utility of CVR as a measure of brain vascular health it is important to identify where methodological nuances affect CVR outcome measures and consider where these inconsistencies between studies alter the measure, and thus, their collective interpretation of how CVR depicts brain vascular health in various populations. It may then be necessary to move towards a standardised, optimised method to measure CVR. This is

imperative to investigate given that study findings are often compared and some studies have been used to predict risk of cardiovascular mortality (Markus and Cullinane, 2001; Portegies et al., 2014).

In addition to the open-circuit technique, re-breathing and stepped end-tidal clamping are other approaches that are used to measure CVR with transcranial Doppler (TCD), though investigating these are beyond the scope of this study, but are likely to introduce more variability for between study comparisons of the CVR outcome measure. Further, magnetic resonance imaging (MRI) also provides a measure of CVR. Though in contrast to TCD, responsiveness measures are derived from changes in the blood-oxygen-level dependent (BOLD) signal (Bhogal et al., 2016; Thomas et al., 2013; Zhou et al., 2015). As well as variations within one methodological approach, the choice of measurement technique differs across studies (TCD or MRI).

Recommended areas that focus on methodological inconsistencies to be explored include: 1) investigating how the respiratory and vascular responses interact; 2) individual variability in average, maximum and minimum CVR measures, maximum change in CVR measure and time in which CVR response takes to peak, and 3) the response to $CO_2$ stimulus being applied or discontinued (i.e., the on and off-kinetics), as time to reach steady-state or return to baseline may also be an important indicator of cerebrovascular health. Further, these areas of investigation are likely to be influenced by ageing and fitness.

Given conflicting findings on CVR, where fitness has been associated with both higher CVR (Bailey et al., 2013; Barnes et al., 2013) and lower CVR (DuBose et al., 2016;

Thomas et al., 2013), and the findings shown by this study demonstrating possible causes of this variability, future research is required to elucidate other causes of variability on the CVR measure.

In conclusion, the present study showed that the stimulus duration does alter the CVR measure, and so does the method of data extraction (i.e., choice of steady-state time-point); though these effects are different between stimulus durations. Given these findings, whilst also considering that slow responders may take longer to reach steady-state (this study recruited only young and healthy participants), we recommend using a 3-minute stimulus duration where data is extracted from the end of the stimulus duration (taking a 30-second average). Our findings strongly indicate that a more consistent approach in collecting data and calculating the CVR measure is required. This should be employed across studies exploring brain health, particularly where results between studies are compared. In order to achieve this, a better understanding of what is indeed the best method of calculating this response is also required. Gold-standard approaches are needed so that findings between studies can be compared and clinical use of CVR measures are more robust.

# 4. RESTING CEREBRAL BLOOD FLOW AND PERFUSION USING TRANSCRANIAL DOPPLER AND MAGNETIC RESONANCE IMAGING

## 4.1. Abstract

**Background:** Resting cerebral blood flow (CBF) and perfusion measures are used to quantify brain health and to better understand neurological conditions (e.g., dementia and stroke). Yet, different imaging modalities are used to obtain these measures, and different measurements can be described as representing resting CBF. This study examined whether different approaches alter the resting CBF outcome measure between age and fitness groups. In the same cohort, we examined: (1) imaging modality (transcranial Doppler (TCD) approach measuring middle cerebral artery blood velocity (MCAv) and cerebrovascular conductance (CVCi) vs. magnetic resonance imaging (MRI) approach using arterial spin labelling (ASL)), and 2) typical analysis approach (resting MCAv and CVCi (TCD) vs. quantitative estimate of cerebral perfusion and blood flow transit times (MRI ASL)) for determining resting CBF.

**Methods:** Thirty-five healthy volunteers participated (20 young: 24 ± 7 years; 15 old; 66 ± 7 years). Participants completed two experimental sessions (TCD/MRI) on separate days (randomised and counter-balanced). Resting CBF outcome measures were calculated for TCD and MRI data using typical analysis approaches (e.g., Brown et al., 2010; Parkes 2004, respectively). Between group ANOVAs examined effects of ageing and aerobic fitness within each imaging modality. CBF measures were correlated (Pearsons) against age and fitness status, and between analysis approaches.

**Results**: Differences were observed between younger and older participants for resting CBF measures derived using both TCD (MCAv: 69 vs 55 $cm \cdot s^{-1}$; $p < 0.01$; CVCi: 0.89 vs 0.67 $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$; $p < 0.01$) and MRI (transit times: 0.67 vs 0.73 s; $p < 0.01$). On average, the younger group had higher grey matter cerebral perfusion (MRI: 69 vs 61 $mL \cdot 100g^{-1} \cdot min^{-1}$; $p = 0.13$). Older fit participants had higher TCD CVCi compared to their unfit counterparts (0.77 vs 0.48 $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$; $p = 0.02$), and fitness was positively associated with both MCAv ($r = 0.52$) and CVCi ($r = 0.73$) measures ($p < 0.05$). Unexpectedly, the younger fit group had *slower* MCAv (TCD) and transit times (MRI ASL) compared to their unfit counterparts (61 vs 75 $cm \cdot s^{-1}$, $p = 0.02$ and 0.70 vs 0.65 s, $p < 0.01$, respectively), and fitness was negatively correlated with TCD MCAv ($r = -0.49$, $p < 0.05$) and positively correlated with MRI transit times ($r = 0.61$, $p < 0.05$). No significant difference was observed between fitness groups for the other resting CBF measures. Across the whole cohort, MRI transit times correlated with TCD MCAv and CVCi ($r = -0.60$ and $r = -0.46$ respectively; both $p < 0.01$).

**Conclusions:** Findings indicate that TCD and MRI modalities can provide complementary resting CBF measures (e.g., TCD velocity and MRI transit times), with similar differences across the whole cohort and between subgroups (age/fitness) observed across modalities. However, having accounted for differences in transit times between cohorts, tissue perfusion measures obtained with MRI ASL (the most common measure used for MRI), did not show significant age or fitness effects between groups. Thus, to enable comparison of studies across modalities that assess resting CBF the metrics of CBF measured must be comparable (e.g., flow velocity (TCD) vs blood transit time (MRI)).

## 4.2. Introduction

Resting cerebral blood flow (CBF) is a common measure used to determine brain health and has been shown to predict clinical outcomes (as reviewed in Chapter 2). Studies measuring resting CBF have used different methodological approaches to determine this outcome measure, including the choice of imaging modality to collect the data as well as the analysis approach or metric used to determine the resting CBF. Neuroimaging modalities include transcranial Doppler (TCD) ultrasound and magnetic resonance imaging (MRI). Arterial spin labelling (ASL) MRI measures are commonly used to assess cerebral perfusion into all grey matter tissue (global flow) or grey matter tissue in specific regions of interest (RoIs), and can also be used to estimate blood transit times through the cerebral vasculature. TCD-based measures include middle cerebral artery blood flow velocity (MCAv) and/or cerebrovascular conductance (CVCi) (calculated by dividing MCAv by mean arterial blood pressure (MAP)), and cerebrovascular resistance (CVRi) (the inverse of CVCi, calculated by dividing MAP by MCAv)); and are often used to assess resting CBF. Given the physiological and theoretical differences between MRI and TCD approaches (i.e., phenomena being measured and approach to measurement, discussed in detail in Chapter 2, Section 2.3), it is important to consider whether the results obtained reveal similar differences between groups irrespective of modality acquisition and analysis approach or metric used. The purpose of this study was to examine whether these methodological differences alter the outcome measure of resting CBF between groups with expected differences (i.e., younger/older and fit/ unfit).

### 4.2.1. Measuring Resting Cerebral Blood Flow (CBF)

Resting CBF has been shown to decline in healthy ageing and these effects are offset by greater physical fitness (Ainslie et al., 2008; Bailey et al., 2013; Barnes et al., 2013;

Bastos-Leite et al., 2008; Zimmerman et al., 2014). This finding is consistent across studies using different neuroimaging modalities, including Doppler ultrasound (Ainslie et al., 2008; Bailey et al., 2013; Barnes et al., 2013) and MRI (Bastos-Leite et al., 2008; Zimmerman et al., 2014) (previous findings described in more detail in Chapter 2, Section 2.3.2). Further, measures of resting CBF are impaired in cerebrovascular disease (Detre et al., 1998) and neurocognitive conditions, including: stroke (Markus et al., 2001) dementia (TCD studies: Meel-van den Abeelen et al., 2014; Wolters et al., 2017; MRI studies: Alsop et al., 1996, 2000; Zhang et al., 2017) and traumatic brain injury (Kim et al., 2010). From a mechanistic perspective, cerebral hypoperfusion may lead to cognitive decline during sedentary ageing and the development of neurocognitive conditions via reduced substrate delivery (i.e., oxygen and glucose) to the brain (see Chapter 6 for investigation into cognitive performance measures and effects of age and fitness).

The MCA is the largest branch of the internal carotid artery (ICA) and supplies the frontal, temporal and parietal lobes of the brain (described in more detail in Chapter 2, Section 2.3). This makes it an ideal blood vessel to investigate blood flow to the brain. Blood pressure is also important to consider when investigating resting CBF due to links between elevated blood pressure, hypertension and cognitive impairment (Elias et al., 2012). Concurrent recordings of mean arterial blood pressure (MAP) taken from the arm or index finger enable researchers to calculate cerebrovascular conductance indices (CVCi), calculated as MCAv/MAP. This metric is an alternative measure of resting CBF and has the additional benefit of accounting for changes in CBF driven by blood pressure (Harper et al., 1966). Studies have shown that TCD measures of MCAv-derived and CVCi-derived resting CBF decline with age and are offset by physical fitness (e.g., Ainslie et al., 2008

and Brown et al., 2010). Further, these measures have been associated with measures of cognitive performance (see Chapter 6).

Another imaging method often used to provide a measure of resting CBF is MRI. One of the most common MRI sequences used is ASL, which provides quantitative measures of resting cerebral perfusion and blood flow transit times. Cerebral perfusion refers to the rate at which blood is delivered to the capillary bed and is commonly expressed in millilitres of blood per 100 grams of tissue per minute ($mL \cdot 100g^{-1} \cdot min^{-1}$; Liu and Brown 2007). Transit times refer to the time taken for blood to travel to various regions of the brain from the neck where the blood has been 'tagged' (see Chapter 2, Section 2.3.3.1 for more detail).

Similarly to resting CBF measures reported in the TCD literature, ASL-derived cerebral perfusion and transit time measures have been shown to differ between older and younger populations for all grey matter (Bastos-Leite et al., 2008) and frontal and parietal RoIs (Zimmerman et al., 2014) (discussed in Chapter 2, Section 2.3.3.1). Further, cerebral perfusion, as measured with ASL MRI, has shown to be impaired in several clinical conditions including dementia (Alsop et al., 2000; Zhang et al., 2017) and traumatic brain injury (TBI) (Kim et al., 2010) (discussed in more detail in Chapter 2, Section 2.3.3.1).

One important consideration with ASL measures is that cortical atrophy due to healthy ageing will affect measures of cerebral perfusion and transit times due to an overall reduction in tissue. If transit times are not accounted for then incorrect estimates of perfusion will be obtained as the images are not acquired at the correct time relative to the 'tag' inversion pulse. Therefore, it is important to consider transit times when calculating resting perfusion measures so that ageing effects on brain volume (i.e., cortical atrophy due

to healthy ageing) are accounted for. Another consideration when investigating resting CBF measures and perfusion using TCD and MRI modalities is that they are targeting different aspects of the vascular tree. TCD is targeting a specific point (e.g., the centre of the MCA), whereas ASL is measuring perfusion in the tissue consisting many micro-vessels.

In summary, previous studies have use different neuroimaging modalities that target different aspects of the vascular tree, combined with different analysis approaches and metrics to report outcome measures of resting brain blood flow. Therefore, it is important to consider whether these differences alter interpretation of the outcome measure and differences between groups. This can be achieved by comparing these measures in the same individuals and selecting groups to compare that have expected differences (e.g., age and fitness). Further, no previous studies have directly compared TCD and MRI (ASL) measures of resting CBF in the same cohort.

### 4.2.2. Study Aims and Hypotheses

The overall aims of this chapter were to: (1) investigate differences between age (younger versus older) and fitness (fit versus unfit) groups on resting CBF outcome measures, and (2) determine whether differences in neuroimaging modality (i.e., TCD versus MRI) and associated analysis approaches and metrics would influence the resting CBF outcome measure and its interpretation across all participants. In addition, associations between different measures of resting CBF were examined (i.e., resting MCAv, CVCi and CVRi (TCD) and cerebral perfusion and transit times in the whole of grey matter and RoIs (MRI)).

There were two main research questions addressed in this chapter:

1. *Ageing and fitness effects on resting CBF outcome measures:* Do TCD and MRI approaches show the same differences between populations where differences in resting CBF outcome measures are expected? (i.e., younger/older and fit/unfit).
2. *Imaging modality and metric used to obtain the resting CBF outcome measure:* Do resting CBF outcome measures obtained from data collected using different brain imaging modalities (TCD versus MRI) directly correlate with one another?

It was hypothesised that: (1) TCD and MRI assessment of resting CBF will provide a similar pattern between younger versus older and fit versus unfit participants, where younger participants will have higher resting CBF measures than older participants, and fit participants will have higher resting CBF than unfit participants, and (2) resting CBF measures obtained using the TCD modality will correlate with resting CBF measures obtained using the MRI modality across the whole group. Thus, indicating that although different imaging and analysis approaches are being used to obtain the resting CBF measures, they are conceptually considered to be similar.

## 4.3. Materials and Methods

Ethical approval was obtained for all experimental protocols and procedures by the University of Birmingham Ethics Committee and conformed to the Declaration of Helsinki (project code: ERN_14-1423). Participants completed five visits on separate days to either the School of Sport, Exercise and Rehabilitation Sciences (four visits) or the Birmingham University Imaging Centre (one visit) at the University of Birmingham. Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent was obtained (see Appendix A4.1 and A4.2).

### 4.3.1. Participants

Thirty-five healthy volunteers in two age groups participated: 20 younger participants, mean age 24 ± 7 years and 15 older participants, mean age 66 ± 7 years. These groups were further divided into fit and unfit groups, as determined by performance on a maximum oxygen consumption ($\dot{V}O_2$ max) fitness test (see below for details). The partitioning of fitness for each age group was as follows. For younger participants, a $\dot{V}O2$ max greater than or less than 45 $mL \cdot kg^{-1} \cdot min^{-1}$ placed them in the fit or unfit group, respectively. For the older participants, a $\dot{V}O_2$ max greater or less than 25 $mL \cdot kg^{-1} \cdot min^{-1}$ placed them in the fit or unfit group, respectively. Partitioning values were decided by the PhD candidate after referring to several sources with varying normative data, though ranges described in the general population show that male 20 to 30 year olds with a $\dot{V}O2$ max of 33 $mL \cdot kg^{-1} \cdot min^{-1}$ and below would be described as ‘very poor’, ~45 $mL \cdot kg^{-1} \cdot min^{-1}$ would be described as ‘good’, and 52 $mL \cdot kg^{-1} \cdot min^{-1}$ and above would be described as ‘superior’. In contrast, male 60+ year olds with a $\dot{V}O2$ max of 21 $mL \cdot kg^{-1} \cdot min^{-1}$ and below would be described as ‘very poor’, ~35 $mL \cdot kg^{-1} \cdot min^{-1}$ would be described as ‘good’, and 44 $mL \cdot kg^{-1} \cdot min^{-1}$ and above would be described as ‘superior’ (Heyward 1998).

### 4.3.2. Experimental Visits

#### 4.3.2.1. Overview

The first visit for the participants included general health screening, MRI safety screening (see Appendix A4.3), fitness questionnaires (see Appendix A4.4), quality of life assessments and an electrocardiogram (if over 50 years of age). The second visit included the aerobic fitness test on a treadmill or stationary bike. The third visit included a cognitive performance assessment (data presented in Chapter 6). The fourth visit was a familiaristaion of the CBF measures using TCD and included a gas challenge protocol. The final two visits involved collecting CBF measures, one using TCD and the other using MRI. The order of these final two visits was randomised and counterbalanced between participants. Across the entire study, participants completed measures of resting cerebral blood flow (CBF) (investigated in this Chapter), cerebrovascular reactivity (CVR) (data presented in Chapter 5), and measures of cognitive performance and quality of life (data presented in Chapter 6). For all visits, participants were asked to avoid vigorous exercise and alcohol 24 hours prior to study participation, caffeine for 12 hours and heavy meals for 4 hours prior.

#### 4.3.2.2. Electrocardiogram (ECG) and general health screening

All participants underwent a pre-exercise evaluation prior to the exercise testing and completed a general health questionnaire and the New Zealand Physical Activity and Readiness Questionnaire Short Form (NZPARQ-SF). Participants over 50 years of age also completed a resting 12-lead electrocardiogram (ECG) assessment and a resting blood pressure measurement, which was reviewed by a cardiologist. Participants who revealed a contraindication to non-medically supervised exercise testing in the general health

questionnaire (e.g., family history of heart attack), had high resting blood pressure (systolic >160, diastolic >90), or showed ECG abnormalities (e.g., S-T suppression, multiple ectopic beats in a row (i.e., >3)) were excluded from the aerobic fitness testing and the rest of the study (and referred on to their GP). All participants included in the study were not taking any medication and had no history of cardiovascular, cerebrovascular or respiratory disease.

#### 4.3.2.3. Aerobic fitness assessment

Measures of aerobic fitness included maximal oxygen consumption ($\dot{V}O_2$ max) and measures scored from the NZPARQ. After screening and inclusion into the study, all participants completed a maximal aerobic fitness test to determine $\dot{V}O_2$ max. Participants were able to choose to either cycle on an electromagnetically braked cycle ergometer or run on a treadmill. For the cycling protocol, participants were asked to cycle at a rate of 70 rpm (rotations per minute) or above. The initial workload began at 35 Watts and then depending on the participant's sex, body mass and habitual physical activity levels, workload increased by between 20 and 35 Watt increments every three minutes. This continued until the participant reached volitional exhaustion, or heart rate reached 100% of the participant's estimated maximum heart rate (i.e., 220 $b \cdot min^{-1}$ (beats per minute) minus the participant's age), or the participant was unable to maintain over 50 rpm on the bike (in line with ACSM Guidelines for Exercise Testing and Prescription, 2013). For the running protocol, participants' heart rate and pace were monitored during the warm-up to determine pacing during the test. The initial pace began at a speed where heart rate was approximately 65% of their predicted maximum, and then depending on the participant's sex, body mass and habitual physical activity levels, speed increased by 0.5 to 1.0 $km \cdot hour^{-1}$ every two minutes for the first four stages, and then at a 1% incline per minute.

This continued until the participant reached volitional exhaustion or heart rate reached 100% of the participant's estimated maximum heart rate. Respiratory gases and gas volume were collected for measurement of the rate of $\dot{V}O_2$. $\dot{V}O_2$ max was then calculated from a 30-second average around the peak $\dot{V}O_2$ (i.e., highest value) and divided by body weight (kilograms).

In addition to the $\dot{V}O_2$ max test, participants' physical activity levels were assessed using the NZPARQ (McLean and Tobias, 2004). Three measures of activity were scored from the NZPARQ: 1) last 7-days frequency of activity (0-7 days), self-rated activity category (1-5; 1 being the least active to 5 being the most active), and activity category (1-3; assessed by adding together participant responses to frequency of mild, moderate and intense activity; category 1 being the least active and category 3 being the most active). This questionnaire was completed by all participants during the initial screening visit.

#### 4.3.2.4. Resting cerebral blood flow and perfusion outcome measures

Participants completed a familiarisation of the $CO_2$ gas challenge (including measures of resting CBF and CVR measured with TCD; see Chapter 5 for CVR measures), and were required to tolerate the gases before continuing the rest of the study. Following familiarisation of the breathing protocol, participants completed two experimental sessions (TCD/MRI) on separate days (randomised and counter-balanced). During resting CBF measures for the TCD experimental session, participants wore a nose-peg and breathed through a mouthpiece that was connected to a Douglas bag circuit that allowed switching from room air to premixed concentrations of $CO_2$ in air, though were only breathing room air for resting measures. For the MRI session, participants wore a nose-peg though had the

mouthpiece resting on their chest, which was placed in the mouth after the resting measures presented in this Chapter were done (for CVR measures, see Chapter 5).

All resting measures were recorded from the period during which participants were breathing room air prior to any gas challenge and following at least 20 minutes of supine rest (see schematic Figure 4.1). For the TCD session, the supine rest period included locating the right and left MCA and complementary physiological measures (described below). For the MRI visit the supine period consisted of setting up breathing equipment and standard set-up scans required for planning the experimental scans. Resting CBF measures were recorded prior to the gas challenge using a flow-sensitive alternating inversion recovery (FAIR) pulsed ASL (PASL) sequence (see Section 4.3.3, Figure 4.2).

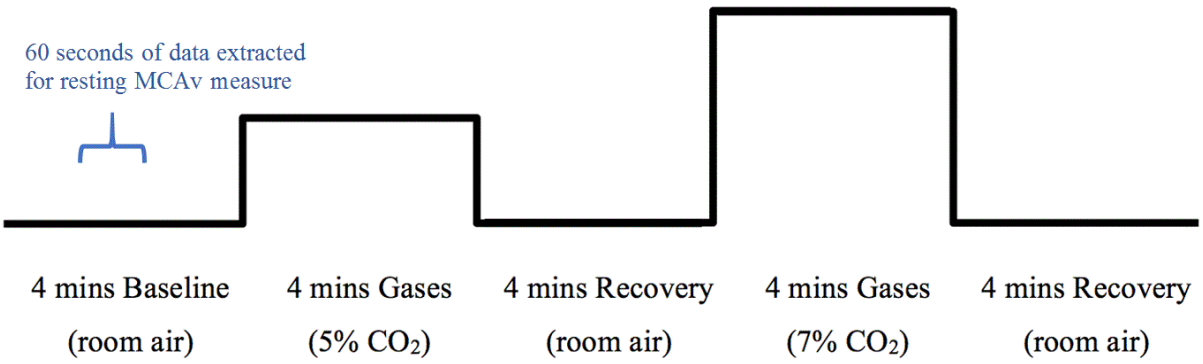



Figure 4.1. Gas challenge protocol schematic for the TCD visit. Resting CBF measures were taken during the initial four-minute baseline. Data for CVR measures (see Chapter 5) were taken separately during the 4-minute 5% $CO_2$ and 4-minute 7% $CO_2$ stimulus duration and the preceding baseline. The protocol lasted approximately 20 minutes.

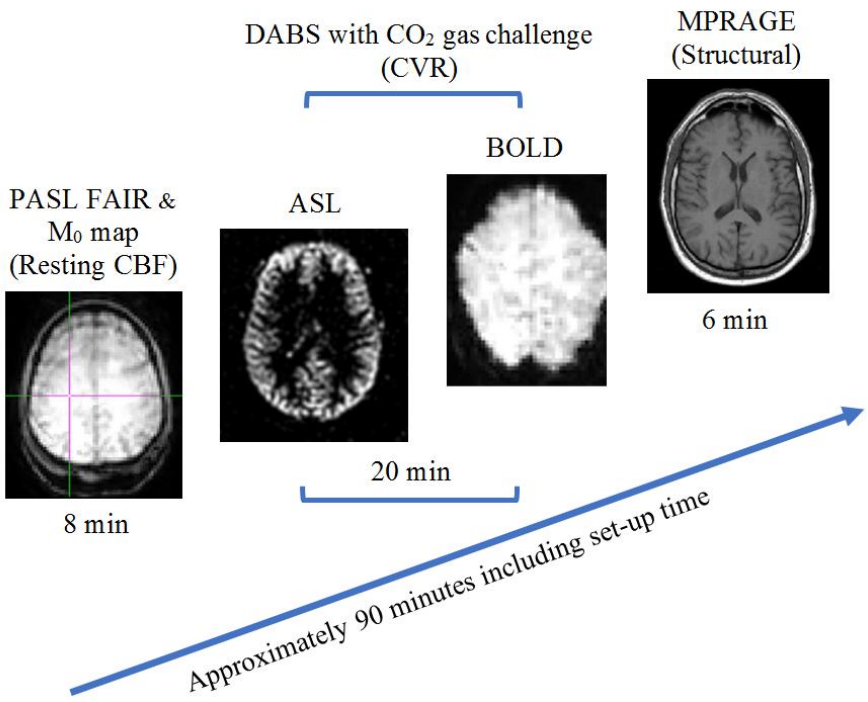



Figure 4.2. MRI protocol schematic. The eight-minute PASL FAIR resting scans combined with the $M_0$ scan (1 minute) were used to determine measures of resting CBF. The DABS sequence allowed simultaneous acquisition of ASL and BOLD scans, during which the same gas challenge protocol illustrated in Figure 4.1was administered to allow measures of CVR to be acquired (see Chapter 5). A $T_1$-weighted anatomical scan (MPRAGE) was also acquired to allow individual subject identification of grey and white matter. The whole MRI protocol, including set-up time, lasted approximately 90 minutes.

### 4.3.3. Data Acquisition

Thirty-five datasets were obtained using the TCD modality and thirty-three datasets were obtained from the MRI modality. One participant chose not to complete the MRI visit and one MRI dataset was lost due to technical issues.

*TCD session:* Bilateral blood flow velocity in the right and left middle cerebral artery (MCAv) was measured using TCD (Doppler Box, DWL, Compumedics Ltd, Germany), with a 2-MHz probe placed over each temporal window on the right and left side of the head. Probes were prepared with ultrasound gel and held in place with a headset. Search and identification procedures were done in accordance with established guidelines (Willie et al.,

2011). Beat-by-beat blood pressure (BP) was measured using photoplethysmography via a finger cuff placed on the middle finger of the left hand (Portapres, Finapres, Medical System BV, The Netherlands). A 3-lead electrocardiogram (ECG) was used to continuously measure heart rhythm and electrical activity.

*MRI session:* All MRI data were acquired on a 3-T Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) (see Figure 4.2 for protocol). A whole-body transmit coil and 32 channel head receiver coil were used for all data acquisition. Prior to scanning sessions, a customised MR sequence code was uploaded to the MRI scanner to allow the MRI sequence acquisitions acquired in this study (i.e., the scanner was 'patched'). The MRI scanning protocol included two ASL sequences to answer different questions, one for resting CBF and one for CVR. This chapter focuses on the MRI acquisitions required to produce resting CBF measures from the MRI data. The acquisition protocol used to obtain CVR measures from MRI data is described in Chapter 5.

To allow quantification of resting perfusion using MRI, images were acquired using a FAIR PASL sequence with two-dimensional echo-planar imaging (2D-EPI) readout. The imaging parameters were: echo time (TE): 9 ms; repetition time (TR): 8 s; inversion times (TIs): 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 s voxel size: 3.25 mm in plane; slice thickness: 5 mm; slices: 12; field of view (FOV): 212 x 212 mm; label duration: 1 s; no background suppression or vascular crushing; sensitivity encoding in parallel imaging (SENSE factor): 2.5. Four volumes of data were acquired for TIs of 0.4 $\rightarrow$ 1.4 s whilst 10 volumes of data acquired for TIs of 1.6 $\rightarrow$ 1.8 s. A base equilibrium $M_0$ scan was acquired with the same parameters but without the inversion pulses required for ASL sequence. Slices were positioned to cover as much of the cortex as possible and the area imaged was identical to that used in Chapter 5.

A whole head T1-weighted anatomical image (magnetisation prepared rapid gradient echo; MPRAGE) with 1 $mm^3$ resolution was also acquired to allow definition and segmentation of the grey and white matter so that resting CBF and CVR measures in these tissues could be assessed separately.

### 4.3.4. Data Analysis

*TCD data:* Mean resting MCAv ($cm \cdot s^{-1}$) data were extracted from a 60-s duration during the initial baseline. CVCi was calculated by dividing the mean MCAv by the mean MAP during the same 60-s duration. CVRi, the inverse of CVCi, was calculated by dividing the mean MAP by the mean MCAv data extracted from the same 60-s duration.

*MRI data:* Data were checked for movement between TIs. The data were then segmented into different TIs and averaged over repeats for each TI. The FMRIB Software Library (FSL) Brain Extraction Tool (BET) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) was used to create a whole brain image without the skull and scalp and grey matter mask from the anatomical image (Smith et al., 2002). This allowed for grey matter to be defined as well as the whole brain to be used for normalisation of individual participants to standard space (see later). A region of interest (RoI) in a single slice was checked to ensure the signal followed the expected pattern (gradual increase in signal strength and then beginning to decrease; inverted-u shape, reflecting increase and loss of signal with increasing inversion time). Resting CBF data were corregistered to the participant's whole brain image from the MPRAGE data. The extracted brain image from the MPRAGE data was then normalised to Montreal Neurological Institute (MNI) standard brain (MNI152_T1_2mm_brain) using the FSL Linear Regression Tool (FLIRT) (Jenkinson et al., 2001; 2002; Greve and Fischi,

2009). Corregistered resting CBF data were then moved from native space into MNI space with FLIRT using the transform derived for the anatomical data.

Resting cerebral perfusion ($mL \cdot 100g^{-1} \cdot min^{-1}$) and transit time (seconds) measures were calculated using the FSL Bayesian Inference for Arterial Spin Labeling MRI (BASIL) toolset (Chappell et al., 2009) to assess the delivery rate for blood to reach all grey matter brain tissue followed by several brain tissue RoIs. RoI masks were defined from the conjunction of the Harvard atlas (in FSL) and the normalised individual subject's grey matter mask. RoIs used were: cingulate gyrus, frontal lobe, motor lobe, occipital lobe and parietal lobe. To determine resting cerebral perfusion and transit time the inversion of a kinetic model of label inflow (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL) was found using FSL BASIL toolbox, along with a separate calculation of the equilibrium magnetisation of arterial blood using the $M_0$ scan (Chappell et al., 2009). ASL data acquired at multiple times post-inversion of the arterial blood were fitted to the kinetic curve model so that perfusion estimation errors associated with variable transit times (i.e., bolus arrival times) across subject groups could be avoided, since the arrival time becomes a parameter of the model where the value is determined from the data. The model fitting was performed with a least squares technique, providing parameter estimates of cerebral perfusion and transit time over the imaged region. This was carried out using the functions 'oxford_asl' and 'asl_calib' in from the BASIL toolbox (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/ BASIL). The parameters input to the models based on measured literature values and the ASL data acquisition were: bolus duration: 1.0 s; bolus arrival time (BAT): 0.8 s; tissue relaxation time ($T_1$): 1.3 s (FSL default setting; Chappell et al., 2009); blood relaxation time ($T_1b$): 1.65 s (Lu et al., 2004); timing between slices:19 ms; label efficiency (alpha): 0.98 (Wong et al., 1998); $T_{1CSF}$ (reference tissue):

3.3 s (at 3T); $T_{2CSF}$ (reference tissue): 0.3 s; $T_{2blood}$: 0.15 s; and echo time (TE): 9 ms (unreferenced relaxation times are set to standard FSL values).

Finally, mean cerebral perfusion and transit time group maps were created for all participants and the younger and older groups separately by averaging the participant's individual mean perfusion and transit time maps (see Appendix A4.5 and A4.6). In addition, the mean cerebral perfusion and transit times of the groups were determined for the whole of the imaged grey matter and the different RoIs.

### 4.3.5. Statistical Analysis

This analysis includes typical approaches to calculate resting measures of CBF obtained from MRI (cerebral perfusion and transit times) (Chen et al., 2011; Parkes 2004) and TCD (MCAv and CVCi) (Brown et al., 2010), and measures of resting MAP and HR. Means were calculated for younger and older groups and fit and unfit sub-groups (younger fit and younger unfit; older fit and older unfit). Between group ANOVAs were used to examine effects of ageing and aerobic fitness. Outcome measures were also correlated against age for all participants together, and against fitness separately for the younger and older groups (see Table 4.2 and Table 4.3). Resting CBF measures using different imaging modalities were compared using Pearson's *r* correlations (Table 4.4).

## 4.4. Results

Thirty-five participants completed the resting TCD measures. Thirty-three participants completed all the resting CBF measures. Mean participant characteristics are reported in

Table 4.1 including: age, fitness ($\dot{V}O_2$ max), heart rate (HR) and MAP (Figure 4.3). There were more male than female participants.

Table 4.1. Characteristics of participants who completed the resting CBF measures using TCD and MRI brain imaging modalities.

| Group (n) | Younger (20) | Older (15) | *ANOVA* *p* value | Younger-fit (8) | Younger-unfit (12) | *ANOVA* *p* value | Older-fit (9) | Older-unfit (6) | *ANOVA* *p* value |
|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 24.5 (6.9) | 66.5** (6.7) | <0.001 | 27.6 (8.1) | 22.4 (5.3) | 0.097$^{t}$ | 64.2 (6.8) | 69.8 (5.4) | 0.114 |
| $\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$) | 43.8 (10.7) | 33.0* (15.9) | 0.026 | 54.5 (5.0) | 36.0** (5.5) | <0.001 | 42.9 (13.9) | 19.7** (3.3) | 0.001 |
| Heart rate ($b \cdot min^{-1}$) | 64 (8) | 57 (8) | 0.124 | 55 (7) | 70* (15) | 0.018 | 55 (7) | 61 (8) | 0.181 |
| MAP (mm Hg) | 80 (11) | 83 (11) | 0.445 | 77 (12) | 82 (9) | 0.297 | 80 (9) | 89 (12) | 0.120 |
| Sex (male: female) | 12:8 | 10:5 | | 7:1 | 5:7 | | 8:1 | 2:4 | |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years), while the criterion for being fit was defined as $\geq 45$ $mL \cdot kg^{-1} \cdot min^{-1}$ and $\geq 25$ $mL \cdot kg^{-1} \cdot min^{-1}$ for the younger and older groups respectively. Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$. Abbreviations: $\dot{V}O_2$ max, maximum oxygen consumption; MAP, mean arterial blood pressure.

### 4.4.1. Age, Fitness and Resting Cerebral Blood Flow (CBF) Outcome Measures

Between-group outcome measures are shown in Table 4.2, Figure 4.3 and Figure 4.4. Correlations between age and fitness are shown in Table 4.3, Figure 4.5 and Figure 4.6.

*MAP and HR data:* No significant differences were observed between younger and older groups for MAP ($p = 0.445$) or HR ($p = 0.124$) (Table 4.2, Figure 4.3). In line with existing literature, MAP and HR were on average lower in the fit groups compared to their unfit counterparts for both the older (HR: 55 vs. 61 $b \cdot min^{-1}$, MAP: 79 vs. 89 mm Hg; Figure 4.3), and younger group (HR: 55 vs. 70 $b \cdot min^{-1}$, MAP: 77 vs. 82 mm Hg; Figure 4.3)

though differences only reached statistical significance for HR in the younger group ($p$ = 0.018).

*TCD data:* Significant group differences were observed between younger and older participants for measures of MCAv ($p$ = 0.008) and CVCi ($p$ = 0.005), where averaged MCAv and CVCi values were ~30% higher in the younger group than the older group (Figure 4.4). As expected, MCAv and CVCi were higher (31% and 60%, respectively) in the older fit group compared to the unfit group, though was only significant for the CVCi measure ($p$ = 0.016; MCAv: $p$ = 0.099). In contrast, the younger fit group had on average lower MCAv and CVCi (23% and 7%, respectively) compared to their unfit counterparts, though was only significantly so for the MCAv measure (MCAv: $p$ = 0.024 and CVCi: $p$ = 0.170; Figure 4.4).

Correlational analysis showed that as age increased MCAv significantly decreased (at a rate of 3.9 $cm \cdot s^{-1}$ every 10 years), and CVCi significantly decreased (at a rate of 0.06 $cm \cdot s^{-1} \cdot$ mm Hg every 10 years) (Figure 4.5, C and D). Correlations between resting CBF TCD measures and fitness ($\dot{V}O_2$ max) are shown in Figure 4.6 and Table 4.3 separating younger and older groups. In the younger group, fitness negatively correlated with MCAv ($r$ = -0.484; $p < 0.05$), where increased fitness was associated with slower resting CBF. In contrast, the older group showed that fitness positively correlated with MCAv ($r$ = 0.520; $p < 0.05$) and CVCi ($r$ = 0.732; $p < 0.01$), where increased fitness was associated with faster resting CBF.

*MRI data:* Significant group differences were observed between younger and older participants for measures of grey matter transit time, where transit times were on average

7% faster in the younger group ($p$ = 0.001). Grey matter cerebral perfusion was 13% higher in the younger group than the older group, though this difference did not reach statistical significance ($p$ = 0.129). No differences were observed between the older fit and unfit groups for measures of transit times or grey matter cerebral perfusion (Figure 4.4). Unexpectedly, the younger fit group had significantly slower (8%) grey matter transit time compared to their unfit counterparts ($p < 0.001$; Figure 4.4).

Correlational analysis showed that grey matter transit times significantly increased with age ($r$ = 0.606; Figure 4.5B, Table 4.3). In addition, analysis of different RoIs demonstrated that this reduction in transit time was widespread, occurring in the frontal lobe, motor lobe, parietal lobe and occipital lobe (see Table 4.4). Age also correlated (negatively) with grey matter cerebral perfusion, though this did not reach statistical significance (Figure 4.5A, Table 4.3). Correlations between resting CBF measures and fitness are shown in Figure 4.6, separating younger and older groups. In the younger group, fitness significantly correlated with MRI grey matter transit time ($r$ = 0.785; $p < 0.01$), where increased fitness was associated with slower resting CBF. In the older group, no fitness effects were observed for resting MRI measures. Perfusion measures showed no fitness effects in the younger or older group.

Table 4.2. Means ± standard deviations for resting CBF measures obtained from MRI data (grey matter cerebral perfusion and transit times) and TCD data (MCAv and CVCi). Measures are shown for all participants followed by groups separated into: younger and older; younger fit and unfit; and older fit and unfit.

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | | Younger | Older | Younger fit | Younger unfit | Older fit | Older unfit |
| MRI: GM cerebral perfusion ($mL \cdot 100g^{-1} \cdot min^{-1}$) | 65.9 ± 15.0 | 69.3 ± 12.9 | 61.2 ± 16.8 | 72.2 ± 8.55 | 67.2 ± 15.3 | 61.6 ± 16.3 | 60.8 ± 19.1 |
| MRI: GM transit time (s) | 0.70 ± 0.46 | 0.67 ± 0.34 | **0.73 ± 0.44**** | 0.70 ± 0.02 | **0.65 ± 0.27**** | 0.72 ± 0.04 | 0.73 ± 0.05 |
| TCD: MCA velocity ($cm \cdot s^{-1}$) | 63.1 ± 16.2 | 69.2 ± 13.6 | **54.9 ± 16.2*** | 61.1 ± 7.14 | **74.7 ± 14.4*** | 60.6 ± 14.1 | **46.4 ± 16.6$^{t}$** |
| TCD: CVCi ($cm \cdot s^{-1} \cdot$ mm $Hg^{-1}$) | 0.80 ± 0.23 | 0.89 ± 0.19 | **0.67 ± 0.23**** | 0.81 ± 0.17 | 0.94 ± 0.20 | 0.77 ± 0.18 | **0.48 ± 0.19*** |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Significant age/fitness effects (different from younger group/ fit group): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 4.3. Correlation (Pearson's *r*) of resting CBF measures with age, and fitness (separately for the younger and older group).

| | All participants<br>Age | Younger<br>Fitness: $\dot{V}O_2$ max | Older<br>Fitness: $\dot{V}O_2$ max |
|---|---|---|---|
| Measure | *r* | *r* | *r* |
| MRI: GM cerebral perfusion ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.231 | 0.099 | 0.086 |
| MRI: GM transit time (s) | **0.606**** | **0.785**** | -0.126 |
| TCD: MCA velocity ($cm \cdot s^{-1}$) | **-0.535**** | **-0.494*** | **0.520*** |
| TCD: CVCi ($cm \cdot s^{-1} \cdot$mm $Hg^{-1}$) | **-0.586**** | -0.250 | **0.732**** |

Values represent Pearson's *r* correlations. Significant (2-tailed) age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Abbreviations: CBF, cerebral blood flow; MRI, magnetic resonance imaging; TCD, transcranial Doppler; GM, grey matter; MCAv, middle cerebral artery blood velocity; CVCi, cerebrovascular conductance; MAP, mean arterial blood pressure; $\dot{V}O_2$ max, maximum rate of oxygen consumption.

Table 4.4. Correlations of measures of resting CBF with age and other measures of resting CBF, calculated using TCD (MCAv and CVCi) and MRI ASL (grey matter cerebral perfusion and transit times; including RoIs of the cingulate gyrus and frontal, motor, occipital and parietal lobes) brain imaging.

| | | TCD data | | | ASL data | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | age | MCAv | CVCi | CVRi | GM CP | GM TT | Cingulate CP | Frontal CP | Motor l CP | Occipital CP | Parietal CP | Cingulate TT | Frontal TT | Motor TT | Occipital TT | Parietal TT |
| age | _ | | | | | | | | | | | | | | | |
| $\dot{V}O_2$ max | -0.42* | | | | | | | | | | | | | | | |
| MCAv ($cm \cdot s^{-1}$) | -0.54** | _ | | | | | | | | | | | | | | |
| CVCi ($cm \cdot s^{-1} \cdot mm\ Hg^{-1}$) | -0.59** | 0.71** | _ | | | | | | | | | | | | | |
| CVRi ($mm\ Hg^{-1} \cdot cm \cdot s^{-1}$) | 0.57** | -0.74** | -0.91** | _ | | | | | | | | | | | | |
| GM CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.23 | 0.12 | 0.21 | -0.34 | _ | | | | | | | | | | | |
| GM TT (s) | 0.61** | -0.60** | -0.46** | 0.43* | -0.18 | _ | | | | | | | | | | |
| Cingulate gyrus CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | 0.12 | -0.06 | 0.01 | -0.06 | 0.84** | 0.13 | _ | | | | | | | | | |
| Frontal CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.33 | 0.18 | 0.32 | -0.43* | 0.85** | -0.31 | 0.52** | _ | | | | | | | | |
| Motor CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.19 | 0.12 | 0.25 | -0.37* | 0.94** | -0.12 | 0.76** | 0.86** | _ | | | | | | | |
| Occipital CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.41* | 0.30 | 0.33 | -0.42* | 0.86** | -0.47** | 0.63** | 0.75** | 0.74** | _ | | | | | | |
| Parietal lobe CP ($mL \cdot 100g^{-1} \cdot min^{-1}$) | -0.33 | 0.19 | 0.25 | -0.36* | 0.96** | -0.30 | 0.80** | 0.79** | 0.87** | 0.89** | _ | | | | | |
| Cingulate gyrus TT (s) | 0.32 | -0.35* | -0.34 | 0.25 | -0.15 | 0.86** | -0.06 | -0.27 | -0.16 | -0.41* | -0.23 | _ | | | | |
| Frontal TT (s) | 0.64** | -0.65** | -0.45** | 0.42* | -0.03 | 0.92** | 0.14 | -0.15 | 0.03 | -0.37* | -0.16 | 0.70** | _ | | | |
| Motor TT (s) | 0.62** | -0.52** | -0.42* | 0.38* | -0.01 | 0.84** | 0.26 | -0.19 | 0.03 | -0.33 | -0.14 | 0.71** | 0.81** | _ | | |
| Occipital TT (s) | 0.34* | -0.29* | -0.17 | 0.12 | 0.33 | 0.51** | 0.41* | 0.07 | 0.32 | 0.27 | 0.26 | 0.33 | 0.50** | 0.47** | _ | |
| Parietal TT (s) | 0.63** | -0.51** | -0.32 | 0.31$^{t}$ | 0.16 | 0.80** | 0.25 | -0.19 | 0.73 | -0.26 | -0.10 | 0.65** | 0.76** | 0.90** | 0.61** | _ |

Values represent Pearson's *r* correlations. Significance (2-tailed): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$Shows a trend towards significance: $0.05 < p \leq 0.1$. Correlations in the orange block show resting CBF measures from TCD versus MRI data modality. Red numbers show correlations between typical approaches from each modality. Green numbers show correlations between measures that are derived from taking the measurement from anatomically close areas though from different modalities (TCD: MCA and MRI: motor lobe and frontal lobe). Abbreviations: ASL, arterial spin labelling; CP, cerebral perfusion; CVCi, cerebrovascular conductance; MRI, magnetic resonance imaging; GM, grey matter; MAP, mean arterial blood pressure; MCAv; middle cerebral artery blood velocity; RoI, region of interest; TCD, transcranial Doppler; TT, transit time; $\dot{V}O_2$ max, maximum oxygen consumption.

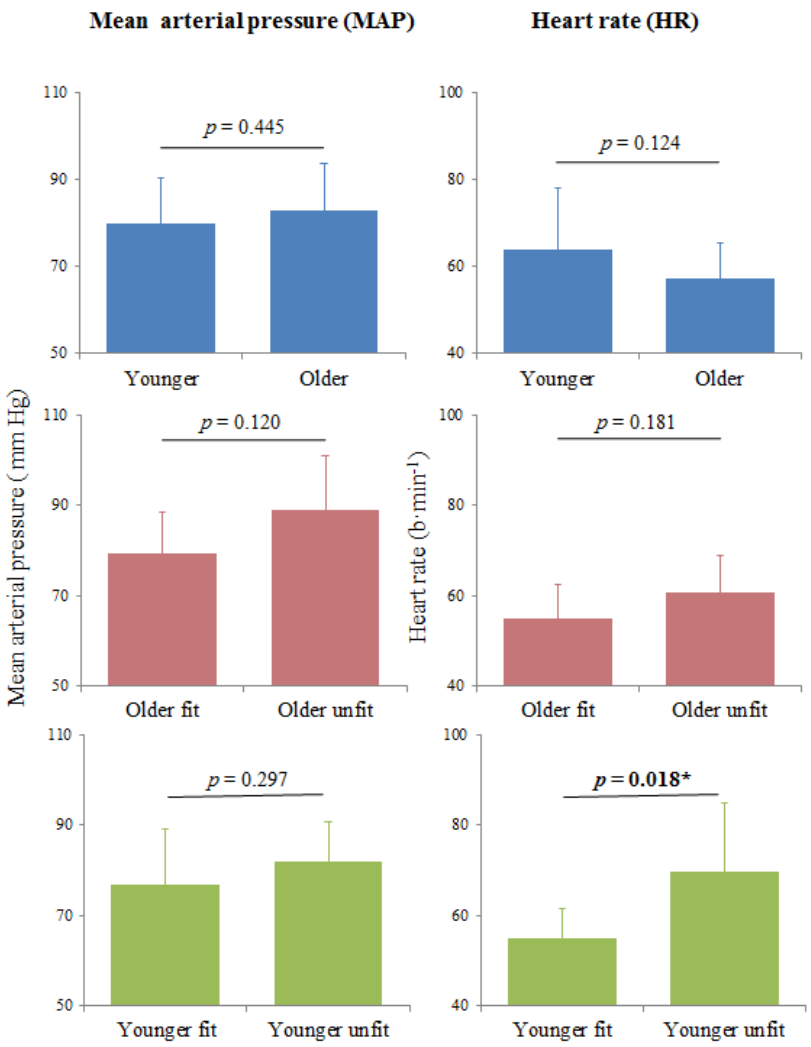



Figure 4.3. Graphs show resting mean arterial blood pressure (MAP) and heart rate (HR). Top row compare means for the younger and older groups, middle row compare means for older fit and unfit sub-groups. Bottom row compare means for younger fit and unfit sub-groups. Error bars show standard deviation. Significance: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

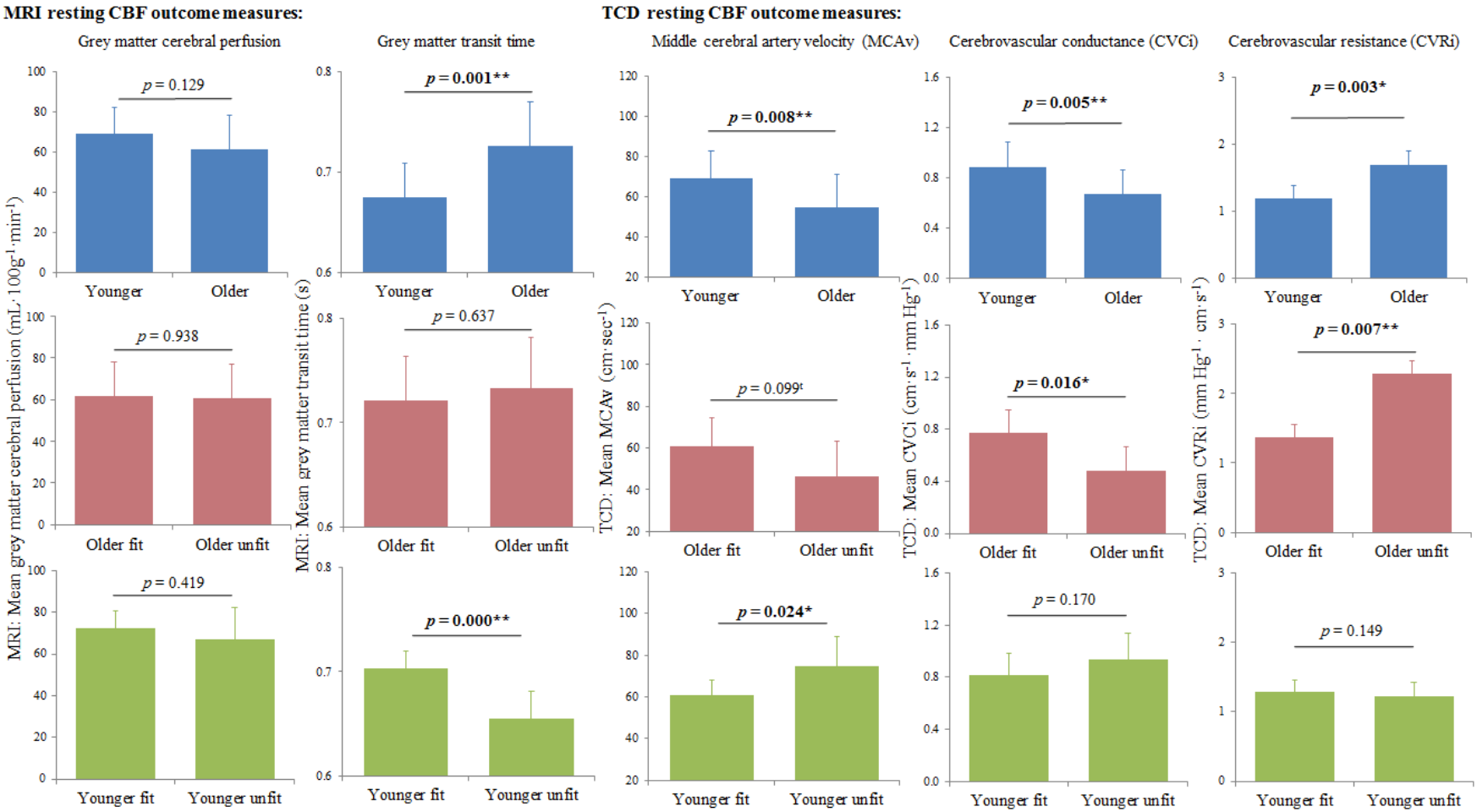



Figure 4.4. Graphs show resting CBF measures obtained from MRI data (grey matter cerebral perfusion and transit times) and TCD data (MCAv and CVCi). Top row of graphs compare means for the younger and older groups respectively, middle row compare means for older fit and unfit sub-groups. Bottom row compare means for younger fit and unfit sub-groups. Abbreviations: ASL, arterial spin labelling; CVCi, cerebrovascular conductance (MCAv/ MAP); CVRi, cerebrovascular resistance (MAP/ MCAv); MRI, magnetic resonance imaging; MAP, mean arterial blood pressure; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler; TT, transit time; $\dot{V}O_2$ max, maximum rate of oxygen consumption. Error bars show standard deviation. Significance: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$Shows a trend towards significance: $0.05 < p \leq 0.1$.

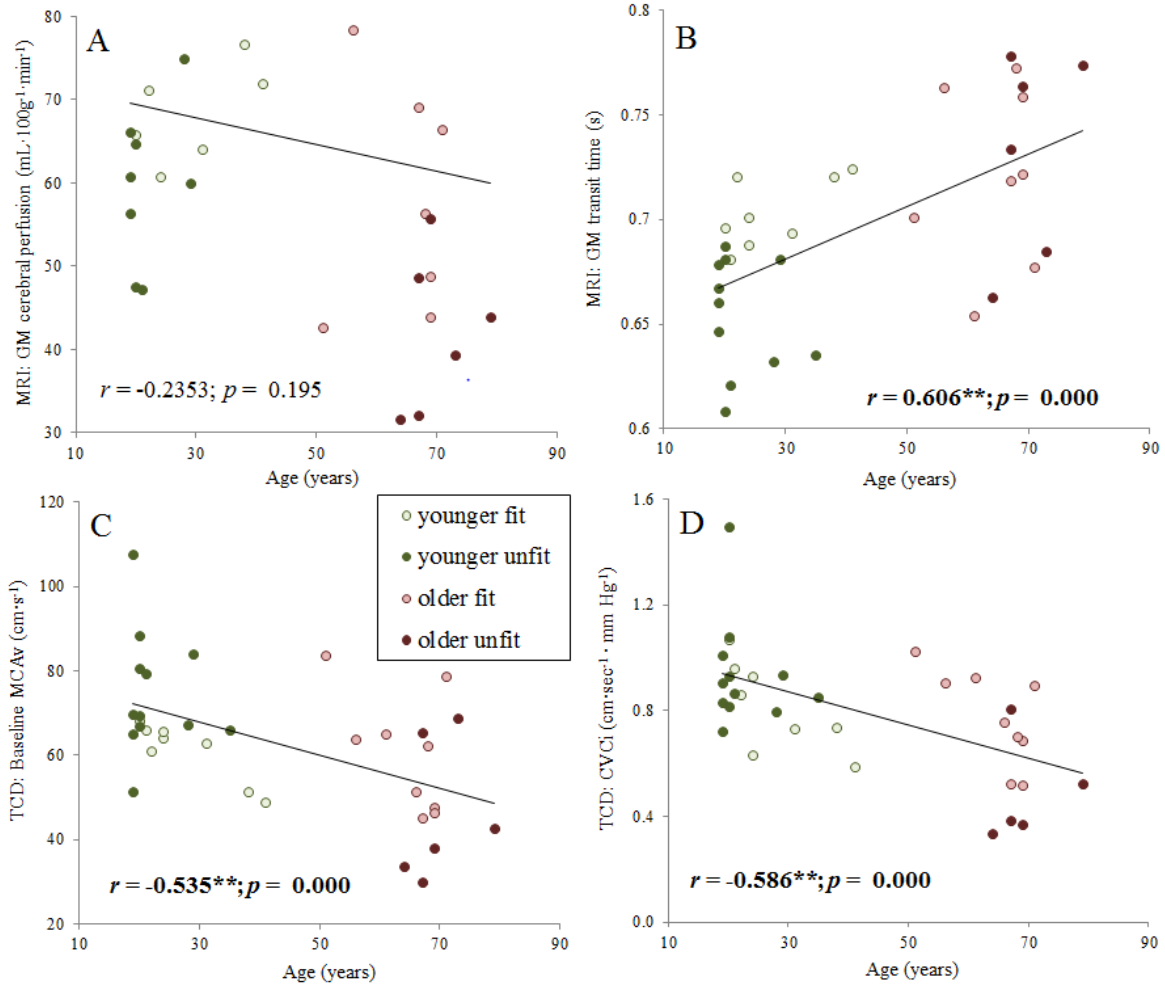



Figure 4.5. Correlations between age (years) and resting CBF measures. A. Grey matter (GM) cerebral perfusion (MRI), B. GM transit time (MRI), C. MCAv (TCD), and D. CVCi (TCD). Black lines denote lines of best fit with Pearson's *r* and associated *p* values reported. Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MAP, mean arterial blood pressure; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler.

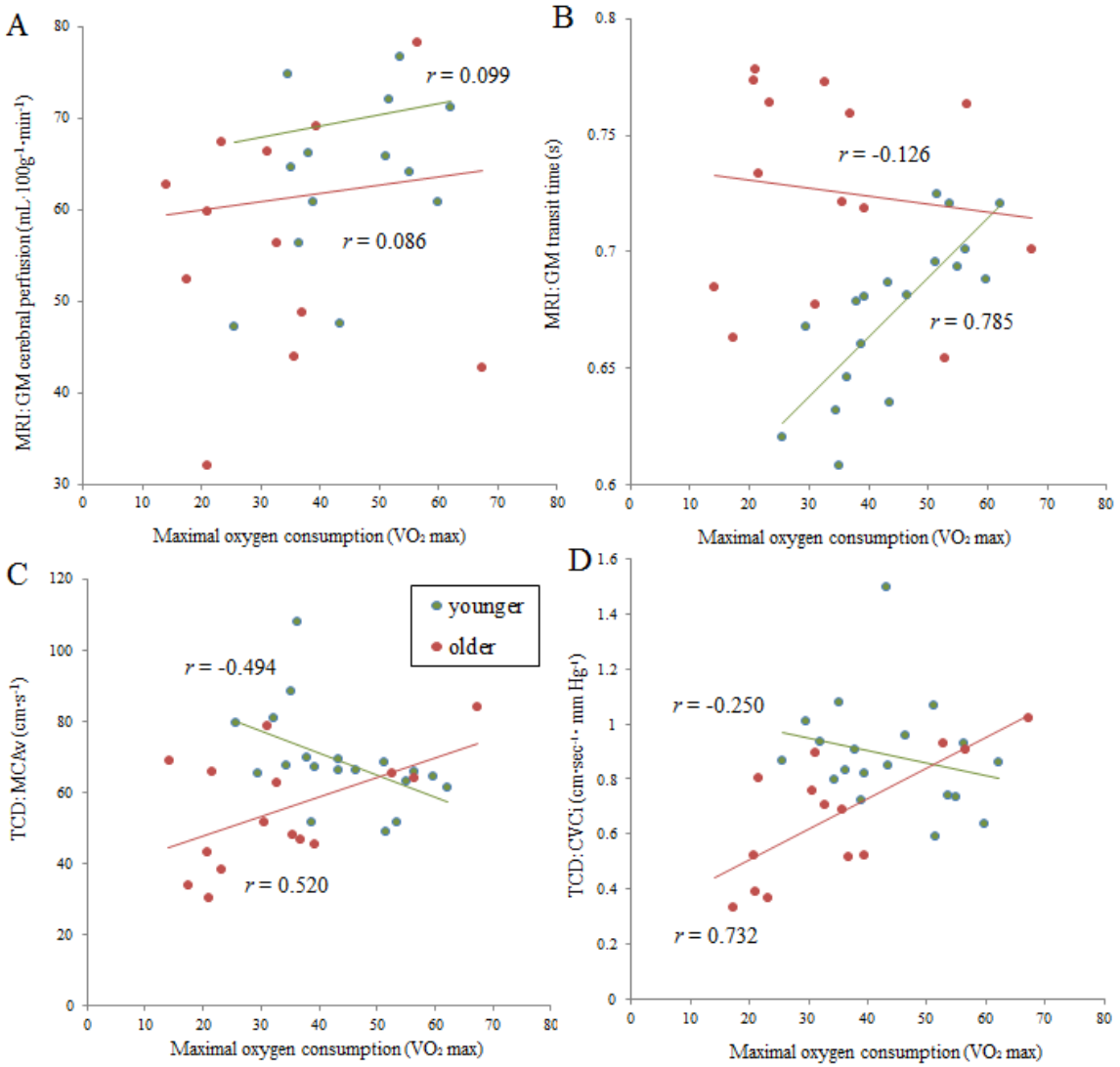



Figure 4.6. Correlations for younger and older groups between fitness ($\dot{\mathbf{V}}\mathbf{O_2}$ max) and resting CBF measures. A. GM cerebral perfusion (MRI), B. GM transit time (MRI), C. MCAv (TCD), and D. CVCi (TCD). Lines of best fit are shown with Pearson's *r* values presented. Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MAP, mean arterial blood pressure; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler. Black lines denote lines of best fit with Pearson's *r* and associated *p* values reported.

### 4.4.2. MRI Resting CBF Measures for Specific RoIs

The effects of ageing on MRI resting CBF measures are shown in Figure 4.7A, including whole grey matter cerebral perfusion and transit times (also presented in Table 4.2) and

specified RoIs. The effects of fitness are shown in Figure 4.7B and Figure 4.7C for whole grey matter and RoIs, for older and younger groups respectively. Whilst a significant difference in transit times was observed between the younger and older groups over all grey matter, no differences were observed for cerebral perfusion. However, this may be due to differences being region specific. Therefore, region specific effects were investigated in the specific RoIs.

*Cerebral perfusion in RoIs:* Significant group differences were observed between younger and older participants for measures of cerebral perfusion in the occipital lobe ($p = 0.01$) and the parietal lobe ($p = 0.05$). Specifically, perfusion in the occipital lobe was 37% higher and in the parietal lobe was 24% higher in the younger group compared to the older group. Perfusion was also higher in the younger group in the cingulate gyrus (1%), frontal lobe (21%) and motor lobe (10%), though these did not reach significance (Figure 4.7A left panel). Similar to the whole grey matter cerebral perfusion observations, cerebral perfusion in the RoIs were similar between fitness groups for both younger and older participants (Figure 4.7B and Figure 4.7C left panels).

*Transit times for RoIs:* Significant group differences were observed between younger and older participants for measures of transit times in the frontal lobe ($p = 0.01$), motor lobe ($p < 0.01$), and the parietal lobe ($p < 0.01$). Specifically, blood flow was 7%, 9% and 10% faster in the frontal, motor and parietal lobes, respectively, in the younger compared to the older group (Figure 4.7A right panel), while the on average faster times for the other RoIs did not reach significance. Transit times for the specified RoIs were similar between the fitness groups in the older participants (Figure 4.7B right), whereas in the younger participants, blood flow was significantly faster in the unfit group than the fit group for all

considered RoIs (cingulate gyrus ($p$ = 0.01), frontal lobe ($p$ = 0.01), motor lobe ($p$ = 0.02), occipital lobe ($p$ = 0.00) and parietal lobe ($p$ = 0.02)) (Figure 4.7C right). This latter observation was consistent with the MCAv observations for these younger participants.

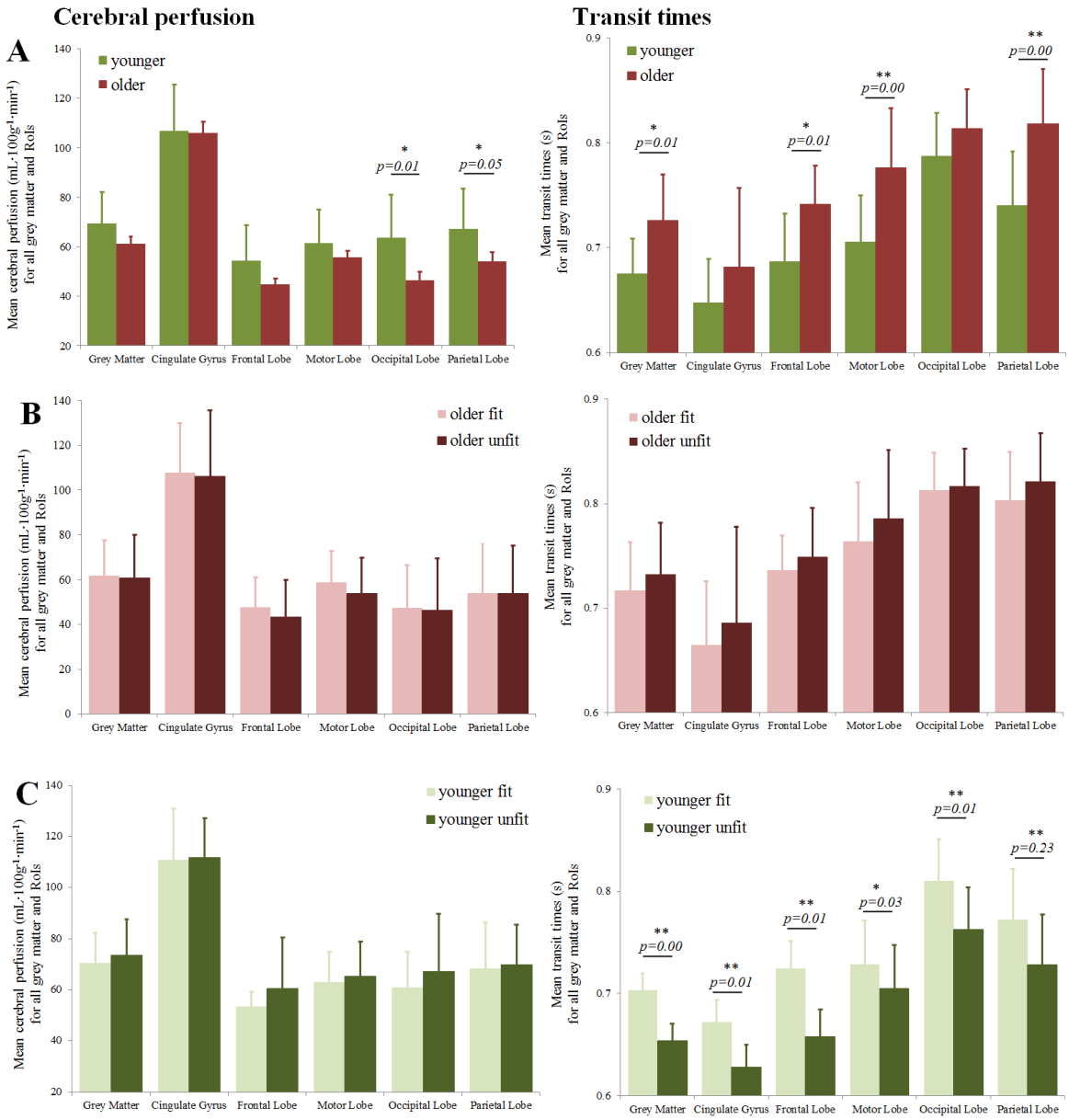



Figure 4.7. MRI resting CBF outcome measures of cerebral perfusion (left panel) and transit times (right panel) for all grey matter and specified regions of interest (RoIs). Metrics were separated into: A. Younger and older groups, B. Older fit and unfit groups, and C. Younger fit and unfit groups. Error bars denote standard deviations. Significance values: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

### 4.4.3. Correlations Between Different Approaches of Measuring Resting Cerebral Blood Flow (CBF) in Regions of Interest

Correlations between different approaches of measuring resting CBF are summarised in Table 4.4.

*ASL cerebral perfusion and TCD:* MRI CBF perfusion measures for all grey matter and RoIs showed no correlation with TCD resting measures calculated using MCAv and CVCi (Table 4.4). However, TCD CVRi measures negatively correlated with MRI CBF perfusion measures in the following RoIs: frontal lobe ($p = 0.02$), motor lobe ($p = 0.04$), occipital lobe ($p = 0.02$), and parietal lobe ($p = 0.04$), where higher cerebral perfusion was associated with decreased resistance.

*CBF transit times and TCD:* MRI CBF transit time measures for all grey matter significantly correlated with all TCD resting measures (MCAv: $p < 0.01$; CVCi: $p = 0.01$, and CVRi: $p = 0.01$). In addition, resting MCAv significantly correlated with transit times in all RoIs (all $p < 0.05$, see Table 4.4 and Figure 4.8), indicating this relation was not region specific. Resting CVCi significantly correlated with transit times in the frontal ($p = 0.01$) and motor ($p = 0.02$) lobes, and showed a trend towards significance for the cingulate gyrus ($p = 0.06$) and parietal lobe ($p = 0.07$). Finally, there was no correlation between CVCi and occipital lobe transit time ($p > 0.1$).

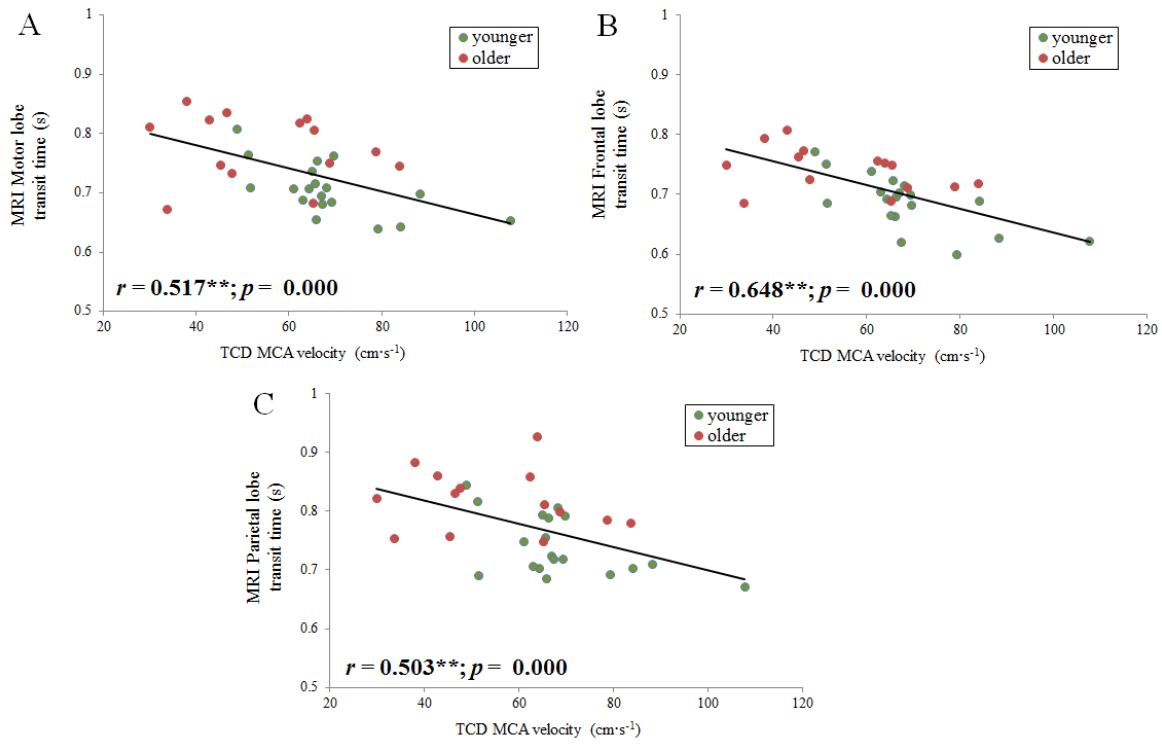



Figure 4.8. Correlation (Pearson's *r*) between resting CBF measures for TCD MCA velocity (MCAv) and MRI transit times to the motor (A), frontal (B) and parietal (C) lobe.

### 4.4.4. Sub-Set Analysis on Eight Participants Pair-Match for Age and Sex

Due to the unexpected significantly higher CBF transit times observed in the younger fit group compared to their unfit counterparts (i.e., the unfit group had significantly faster blood flow), a separate analysis was performed on a sub-set of the participants who were pair-matched for age and sex (6 males and 2 females). After pair-matching the younger group for age and sex they still showed that transit times were faster in the unfit group compared to the fit group; specifically showing significance in the whole of the grey matter ($p$ = 0.046), the cingulate gyrus ($p$ = 0.050), and the occipital lobe ($p$ = 0.048). These observations were therefore consistent with that observed for the whole younger group (Figure 4.7C). No between group differences were observed in TCD measures for this subset (MCAv: $p$ = 0.263 and CVCi: $p$ = 0.879).

## 4.5. Discussion

The aims of this chapter were to examine how measures of resting CBF differed between healthy age groups and how they were affected by aerobic fitness. This was done by examining group differences in mean outcome measures in younger and older, fit and unfit individuals, and by correlating outcome measures with age and fitness. Further, this chapter investigated whether differences in neuroimaging modality (i.e., MRI and TCD) would influence the resting CBF outcome measure and its interpretation. In addition, associations between different measures of resting CBF were examined (i.e., MRI: cerebral perfusion and transit times in all grey matter and RoIs; and TCD: resting MCAv, CVCi and CVRi).

*Group differences in resting CBF measures*

Outcome measures from the MRI modality revealed that resting CBF perfusion was lower in the older group compared with the younger group in the whole grey-matter and all RoIs, though group differences were only significant in the occipital and parietal lobes. Transit times were longer in the older group compared with the younger group in the whole grey-matter and all RoIs (significant for whole grey-matter, frontal lobe, motor lobe and parietal lobe, the cingulate gyrus and occipital lobe showed a trend towards significance). Outcome measures from the TCD modality revealed that resting MCAv and CVCi were significantly lower in the older group compared to the younger group. Fitness effects were observed in the younger group, though these were not in line with the existing literature. Specifically, the younger fit group showed significantly slower MCAv determined by TCD, and longer (i.e., slower) transit times determined by MRI. In contrast, findings for the older group were in line with existing literature (Barnes et al., 2013; Brown et al.,

2010), where the fit group had higher resting CVCi and MCAv, although the latter measure did not reach statistical significance (0.099; see Figure 4.4).

*Correlations between resting CBF measures from different modalities*

The correlational analysis across the entire cohort revealed that as age increased both MCAv and CVCi metrics decreased. Specifically, MCAv decreased at a rate of 3.9 $cm \cdot s^{-1}$ every 10 years, while CVCi decreased at a rate of 0.06 $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$ every 10 years (Figure 4.6C and D). This is in line (though not as high) with findings from Ainslie and colleagues (2008), where they reported that MCAv decreased at a rate of 0.76 $cm \cdot s^{-1}$ every year (i.e., 7.6 $cm \cdot s^{-1}$ every 10 years). The difference in the magnitude of change may be related to the much larger sample size used in that large cohort study, which had 154 trained and 153 untrained male only participants compared. In addition, both males and females were included in the current study, which may provide another explanation for lower rate of change across the age range that was compared here.

Although age effects were not observed in all grey matter, significant group differences in CBF perfusion were observed between younger and older participants in RoIs including the occipital lobe (37% higher in the younger group) and the parietal lobe (24% higher in the younger group). Age differences were also observed in transit times and were clearest in the frontal and motor regions as well as the parietal lobe. This is likely due to these areas being associated with higher executive functions including attention, planned movements and spatial reasoning (Frank et al., 1998; Ridderinkhof et al., 2004). Performance in these domains is known to decline across the lifespan and to be impaired in clinical conditions such as dementia. This decline in cognitive performance co-occurs with a decline in resting CBF measures (discussed in Chapter 2; see Chapter 6 for

investigation into cognitive performance measures and effects of age and fitness). One point worth noting is that the findings of regional differences in these particular regions could also be explained by these areas having generally higher perfusion (Zhou et al., 2015), which might mean we have more sensitivity to detect differences in these regions due to a higher signal-to-noise ratio.

The results showing higher MCAv in the younger unfit compared to fit group, and supported by the differences in CBF transit times (though not perfusion measures), are not in line with existing literature (Ainslie et al., 2008; Barnes et al., 2013). One potential explanation for this finding is due to the unbalanced numbers of males and females in these groups. Specifically, there were more males than females in the fit group, whereas more females than males were in the unfit group. Given that previous studies have reported higher resting CBF measures in females compared with males (Marinoni et al., 1998; Parkes et al., 2004), this may explain the inconsistent findings here, though this cannot be reliably tested due to the small sample sizes of these subgroups. Further, resting CBF is known to decline with age, and the average age of the young fit group was higher than the young unfit group. Although this difference was not significant, there was a trend towards significance. To try and interrogate these issues, further analysis was performed, specifically looking at the TCD resting MCAv, and MRI transit time data where participants were pair-matched for age and sex. Despite this procedure, the same pattern was observed where the younger unfit group had faster transit times than the younger fit group, though no differences were observed in the TCD data. This observation needs to be considered with caution given the very low numbers in each group and further investigation is required to test for reproducibility of these observations in a larger cohort where participants are closely pair matched for age and sex between fitness groups.

Significant correlations were observed between resting CBF measures calculated using MRI and TCD approaches when measures used the speed of blood flow as an index of resting CBF (i.e., velocity ($cm \cdot s^{-1}$) with TCD vs. transit times (s) with MRI). Indeed, the two imaging approaches were able to differentiate the younger from the older and the fit from the unfit groups similarly, including the unexpected finding of increased resting CBF (MCAv and transit times for TCD and MRI, respectively) in the younger unfit group compared to the fit group (see Figure 4.4). The age-related findings complement those currently in the literature, where resting CBF naturally declines across the lifespan (e.g., Bailey et al., 2013; Ainslie et al., 2008; and Brown, 2010). However, there were no associations between MRI measures of cerebral perfusion and TCD measures of CVCi or MCAv. Further, though MRI ASL measured cerebral perfusion declined across the lifespan, the correlation with age was not significant, and no differences between fitness groups were observed for either the younger or the older group (in contrast to MCAv and transit time measures). These findings show that the modality used to determine the resting CBF measure can affect interpretation of the measure and its association with age or fitness. However, there were some associations between approaches and some consistent patterns between age and fitness groups (e.g., the interpretations from MRI grey matter transit times and TCD MCAv were in agreement). Given that MRI transit time and TCD MCAv are physiologically similar metrics of flow (both measure the speed of blood flow through the vasculature), whereas perfusion is measuring the rate at which blood is delivered to the capillary bed (i.e., tissue), it makes sense that these measures may not necessarily strongly correlate.

### 4.5.1. Study Limitations

As mentioned previously, this study recruited more males than females, particularly in the fit groups and this may have affected the results. The scientific literature reports mixed findings on the effects of sex on resting CBF; specifically, studies using the TCD modality have shown both lower resting CBF in females compared to males (Marinoni et al., 1998) or vice versa (Purkayastha and Sorond, 2012), and studies using MRI measures have shown higher resting CBF in males compared to females (Kassner et al., 2010) or vice versa (Parkes et al., 2004). One confounding factor was that the fit younger group was on average older than the unfit group (28 vs. 22 years), meaning that the fitness effects within this group may be less likely to be detected due to the natural decline in resting CBF that occurs during ageing (Ainslie et al., 2008; Bailey et al., 2013). Future research in this area would benefit from better pair-matching of participants for both sex and age in order to differentiate the fitness effect on resting CBF.

To try and address this issue a subgroup of the cohort were pair-matched for age and sex and their data compared. However, this did not alter the pattern of results, only removed the significance. Higher MCAv and transit times were still observed in the younger unfit group compared to their fit and age-matched counterparts. Therefore, individual variability may be causing these unexpected findings that would be less likely to be an influence in a study with a larger sample size. Examples of individual variability factors include: level of cognitive activity participants engage in (i.e., education, speaking multiple languages, playing a musical instrument, amongst many others) and activities that combine several functional skills including physical, cognitive and social/team working (e.g., dancing). These factors may further drive improvements in the cerebrovascular measures investigated in this chapter and have additive effects in a dose-dependent manner.

### 4.5.2. Summary and Conclusion

In line with existing research, these data show that younger individuals have elevated resting CBF compared to older individuals. However, in this study, the younger fit group had lower resting CBF compared to their unfit counterparts. This result is surprising given that fitness is typically associated with elevated CBF (i.e., elevated MCAv and lower transit times) and although may be explained by a small sample size, warrants further investigation into both intra- and inter-individual variability. Further, MRI CBF measures can give more information about regional differences, where prefrontal areas of the brain are likely to be more sensitive to changes resulting from ageing and/ or diseases such as dementia (Gunning-Dixon et al., 2009; Alexopoulos et al., 2012).

In conclusion, TCD and MRI imaging modalities provide complementary resting CBF measures when assessing similar metrics of flow (e.g., TCD velocity and MRI transit times), where similar differences across the whole cohort and within subgroups were observed across modalities. This indicates that findings between studies using different modalities to assess resting CBF can be compared when healthy participants are being investigated and the metrics of CBF measured are compatible (e.g., flow velocity vs. blood transit time). However, measures of MRI cerebral perfusion and associations with transit times and velocity (MCAv, CVCi and CVRi) were less clear, likely due to the difference in metric being used (rate of blood flow through the vessel/ vasculature vs. tissue perfusion in the capillary bed); thus, further research is needed investigating differences within and between modalities and associations with different populations (i.e., younger, older, disease, etc). Further, although beyond the scope of this study, it is important to consider the significance of the chemoreflex in the control of CBF (see Ogoh et al., 2013) and its effects on various measures of resting CBF obtained from different imaging modalities

investigated here. The findings in this study cannot be generalised to other imaging modalities for resting CBF or to disease populations. Therefore, further investigation is warranted to determine whether TCD and MRI resting CBF measures complement other resting CBF measures obtained using different imaging modalities (e.g., NIRS), and also to determine whether TCD and MRI resting CBF measures remain complementary in specific disease states.

# 5. CEREBROVASCULAR REACTIVITY TO CARBON DIOXIDE USING TRANSCRANIAL DOPPLER AND MAGNETIC RESONANCE IMAGING

## 5.1. Abstract

**Background:** Cerebrovascular reactivity (CVR) to carbon dioxide ($CO_2$) has emerged as a key marker of brain health. However, recent findings report conflicting interpretations of CVR in healthy ageing, fitness and neurodegenerative conditions (e.g., dementia and stroke). This may be related to methodological differences and different typical disciplinary analysis approaches (i.e., imaging modality, analysis approach and/or $CO_2$ stimulus concentration). The purpose of this study was to examine whether these methodological and analysis differences alter the CVR outcome measure across a cohort. Within the same cohort, the following were examined: (1) imaging modality (magnetic resonance imaging (MRI) versus transcranial Doppler (TCD)); 2) typical analysis approach (linear regression (MRI) versus 30-s mean steady state (TCD)), and 3) $CO_2$ stimulus concentrations (5% vs. 7% $CO_2$), for determination of CVR.

**Methods:** Thirty-five healthy volunteers participated (20 young: 24 ± 7 years; 15 old: 66 ± 7 years). Participants completed two experimental sessions (TCD/MRI) on separate days (randomised and counter-balanced). For both sessions, CVR data were obtained using two concentrations of $CO_2$ via the same Douglas bag open circuit (4-min cycles of: room air, 5% $CO_2$ (in air), room air, 7% $CO_2$ (in air)). CVR outcome measures were calculated for both MRI and TCD data using typical analysis approaches for each modality (e.g., Driver et al., 2010 and Vernieri et al., 2009 respectively), and then analysis approaches were applied to the other imaging modality for comparison. Differences in CVR outcome measures between stimulus concentrations (5% versus 7% $CO_2$) were also investigated.

**Results**: There were no correlations (Pearson's *r*) between TCD and MRI modalities for CVR measures calculated using typical analysis approaches from either stimulus concentrations (5% $CO_2$: $r = 0.033$, $p = 0.866$; 7% $CO_2$: $r = 0.303$, $p = 0.117$). Within each modality, there were strong correlations between CVR measures calculated via either analysis approach ($r > 0.737$, $p < 0.001$). Regardless of the modality used to obtain CVR data (TCD or MRI), CVR measures calculated using the TCD analysis approach (30-s steady state) were higher than CVR measures calculated using the MRI analysis approach (linear regression). Stimulus concentration altered CVR measures for the TCD ($p > 0.001$) but not the MRI modality.

**Conclusions:** The imaging modality used to calculate CVR, as well as the $CO_2$ concentration for TCD measures, had significant effects on reported CVR outcome measures. Therefore, caution is needed when comparing studies that use different methodological approaches. Observed discrepancies between CVR measures across imaging modalities require further investigation to elucidate their cause. To improve the utility of CVR as an outcome measure, methodological guidelines are required that address modality and analysis approaches separately.

## 5.2. Introduction

Cerebrovascular reactivity (CVR) is a vascular response to alterations in arterial carbon dioxide ($P_{ET}CO_2$) content and is a common test to assess brain health, described in more detail in Chapter 2, Section 2.3.2.3. Traditionally, higher CVR is associated with higher aerobic fitness (Bailey et al., 2013; Barnes et al., 2013), while a lower CVR is associated with natural ageing and brain-related conditions including dementia (Wolters et al., 2017; Meel-van den Abeelen et al., 2014) and stroke (Markus and Cullinane, 2001). However,

recent findings challenge some of these observations. For example, blunted CVR was reported in a group of Masters athletes relative to sedentary age-matched controls (Thomas et al., 2013). Further, in another study of older adults, higher cardiorespiratory fitness was associated with lower CVR (DuBose et al., 2016).

Thomas and colleagues suggested that this blunted responsiveness was due to repeated exposure to elevated $CO_2$ during exercise; however, another explanation for this contradictory finding, and findings from DuBose and colleagues, may be due to the methodology used to measure CVR, which was different to the method used by Bailey and colleagues and different again to that used by Barnes and colleagues (i.e., $CO_2$ stimulus concentration and duration, steady-state time point; discussed in Chapter 2 and investigated in Chapter 3). In addition, another key difference of the Thomas et al., (2013) and DuBose et al., (2016) studies relates to the imaging technique used to determine CVR. These researchers used magnetic resonance imaging (MRI) to assess CVR, while the other studies discussed used transcranial Doppler (TCD) ultrasound (Markus and Cullinane, 2001; Bailey et al., 2013; Barnes et al., 2013; Meel-van den Abeelen et al., 2014; Wolters et al., 2017). This highlights an important question within the research field of brain health assessment; namely, does the method by which CVR is assessed affect this outcome measure?

Therefore, inconsistent findings in the literature regarding the directionality of the association between CVR and fitness, and indeed whether there is an association at all, may be related to different neuroimaging acquisition and analysis methods used to determine CVR. Firstly, studies use different imaging modalities to collect data (i.e., TCD or MRI). Secondly, these disciplines typical use different analysis approaches to calculate

the CVR measure (30/60 s data extraction in TCD, and linear regression in MRI). Finally, studies within both neuroimaging disciplines use different stimulus concentrations of carbon dioxide (4%, 5% or 7% $CO_2$), and different stimulus durations during the experimental procedure that may also alter the reported CVR outcome measure (as explored in Chapter 3).

### 5.2.1. Measuring Cerebrovascular Responsiveness

TCD and MRI are examples of two neuroimaging modalities that can be used to measure and record brain blood flow responses to hypercapnia during a gas challenge involving the administration of elevated levels of $CO_2$, and thus, give a measure of CVR. However, the way these techniques measure brain blood flow is quite different, despite both being referred to as CVR. Further, hypoxia has also been used to induce this response and other research groups have focused on the significance of the chemoreflex for the control of cerebral blood flow (Ogoh et al., 2013). TCD measures the echoes of ultrasound waves moving transcranially and typically involves placing two probes on either side of the head to allow bilateral recording of middle cerebral artery blood flow velocity (MCAv). A key assumption of this measure is that MCAv represents cerebral blood flow (CBF). In contrast to this approach, one typical approach within the MRI field uses the blood-oxygen level dependent (BOLD) signal to measure the haemodynamic response to a $CO_2$ stimulus. The BOLD signal is reduced in the presence of increased deoxyhaemoglobin because of the difference in the magnetic susceptibility of deoxy and oxyhaemoglobin. The BOLD signal is influenced by a complex balance of physiological factors combined to calculate an arbitrary CVR outcome measure (specifically, blood flow, blood volume and tissue oxygenation). It therefore provides an indirect indication of changes in blood flow, and the BOLD signal is used extensively to examine brain vascular response to an external stimuli

such as $CO_2$, assuming no change in neuronal activity (Febo 2011; Buxton 2013). However, studies have shown that $CO_2$ is in fact not neuronally neutral and that not only does $CO_2$ change CBF, it alters other neural frequencies (Hall et al., 2011).

While research disciplines have recently begun to collaborate and merge together (e.g., see Chapter 2, section 2.5 for detail), traditionally different disciplines or research fields (e.g., neuroscience and physiology) use different modalities (i.e., Doppler ultrasound, near-infrared spectroscopy (NIRS), MRI) and analysis approaches to measure brain blood flow. One similarity across modalities is that the vascular response may be measured either at rest or during functioning, such as physical activity, a cognitive task, or through manipulation of inhaled gases to obtain CVR. However, the analysis approaches used to derive a specific brain vascular outcome measure also differ between modalities. For example, the typical TCD approach of calculating CVR involves extracting either 30 or 60 s of data from the end of the gas stimulus duration and then plotting the change in MCA velocity relative to 30 s of a resting period, against the change in $P_{ET}CO_2$ taken from these same time points. Further, TCD approaches also use a resting index of cerebrovascular conductance (CVCi) to calculate CVCi-derived CVR, where the MCAv measure is divided by a measure of mean arterial blood pressure (MAP), thereby avoiding potential influences on changes in arterial blood pressure on cerebral blood velocity during the hypercapnic challenge (e.g., Low et al., 2008). By comparison to TCD, the typical MRI approach of calculating CVR involves a linear regression of the BOLD signal change against the $P_{ET}CO_2$ changes over the entire gas challenge protocol, including the baseline period of data collection (Driver et al., 2010), which can be performed for each voxel of the brain or an average signal over the whole brain. An alternative MRI method to investigate CVR is arterial spin labelling (ASL). ASL is a direct measure of CBF in the

capillaries, often termed perfusion. However, few studies have used ASL to assess CVR and instead use BOLD (Ekstrom et al., 2010; Bhogal et al., 2014, 2015, 2016; Duffin et al., 2017) due to the inherently low SNR of ASL data (Buxton 2013).

This leads us to consider whether the choice of neuroimaging modality or analysis approach used will alter the CVR measure. Further, no research has been done to compare whether these approaches differentiate between age and fitness groups similarly.

### 5.2.2. Study Aims and Hypotheses

The overall aims of this study were to: (1) investigate differences between age (younger versus older) and fitness (fit versus unfit) groups on the CVR outcome measure, and (2) compare the most robust and clinically transferrable CVR test (identified in previous studies) using TCD and MRI. Based on common CVR protocols previously used, and the findings from Chapter 3 (indicating that CVR measures calculated from 3-minutes into the stimulus duration would show the least variability, whilst allowing individuals enough time to reach steady-state), a 4-minute (to allow for error), two-stepped 5 and 7% $CO_2$ gas challenge protocol was employed for both MRI and TCD acquisition approaches. The 5% and 7% $CO_2$ stimuli were both used to investigate whether the different stimulus concentrations used in previous work discussed above, alter the CVR measure.

There were two main research questions addressed in this chapter:

1. *Ageing and fitness effects on the CVR outcome measures:* Do different methodological approaches show similar patterns in populations where differences in the CVR outcome measure are expected? (i.e., younger/older and fit/unfit).

2. *Imaging modality and analysis approaches used to obtain the CVR outcome measure:* Do CVR outcome measures obtained from data collected using different brain imaging modalities (TCD vs. MRI) directly correlate with one another? Further, do typical analysis approaches normally used in different disciplines give similar CVR measures? (i.e., TCD approach: 30/60 seconds of extracted data vs. MRI approach: linear regressions).

It was hypothesised that: (1) MRI and TCD assessment of CVR will provide a similar pattern across the entire cohort as well as separation between younger/older and fit/unfit participants, and (2) the assessment of the CVR measure will be different between the typical analysis approach used, though group differences (younger/ older and fit/unfit) will remain the same.

### 5.3. Materials and Methods

The imaging modalities used for this chapter are the same as those described in Chapter 4 (i.e., TCD and MRI). However, the data acquisition and analysis methods employed specifically for obtaining the outcome measures of CVR are described in more detail in this chapter (see below). CVR measures were calculated using data obtained from TCD and MRI BOLD signal acquisition methods and their accompanying typical analysis approaches. Further, both TCD and MRI analysis approaches were used for data obtained using both modalities (i.e., typical TCD analysis approaches were used with MRI data and typical MRI analysis approaches were used with TCD data).

### 5.3.1. Participants

Thirty-five healthy participants (20 younger and 15 older) completed the CVR $CO_2$ gas challenge and fitness test. One participant chose not to go onto complete the MRI visit. One participant chose not to complete the 7% $CO_2$ gas challenge during the TCD visit and 3 participants chose not to complete the 7% $CO_2$ gas challenge during the MRI visit or ended the 7% $CO_2$ gas challenge early. CVR datasets for two participants from the MRI visit could not be used due to technical issues and missing data. This resulted in 28 participants (16 younger and 12 older) with complete datasets for TCD and MRI CVR measures from both the 5% and 7% $CO_2$ stimulus durations. All participants completed the same experimental visits described in Chapter 4 (see Section 4.3.2), with the gas challenge to obtain CVR measures included during both the TCD and MRI visits (Figure 4.1 and 4.2).

### 5.3.2. Pilot Testing

The final protocol was developed after several pilot scans to test the sequences and refine the protocol as necessary, as well as to develop an easy to use method for delivering gases into the scanner room, which ultimately could be implemented by other researchers in the field. Initially the practical set-up and optimisation of the MRI scanning protocols was performed to achieve the desired measures. The set-up of the breathing equipment was tested on eight pilot participants with both TCD and MRI modalities to check that: 1) reliable data could be collected; 2) the equipment was all MRI compatible; 3) the equipment could fit into the MRI head coil with a variety of head sizes, and 4) measures of end-tidal $CO_2$ could be synchronised to MRI acquired data via post-processing analysis. The final experimental set up developed through piloting the equipment both inside and outside (for TCD visits) the MRI scanner and was influenced partly by experience from

using the equipment outside of the MRI scanner at the School of Sport, Exercise and Rehabilitation Sciences and from using a similar methodological approach to that previously employed by others (Tancredi et al., 2014, 2015; Moreton et al., 2016).

Some practical issues were encountered that affected the quality of the data, which required solutions to be developed in order to be overcome and be consistently applied thereafter. The size of the mouth piece bore affected the participant's breathing ability and the data, where a larger mouth piece improved data quality and participant comfort (large Hans Rudolph T shaped valve and silicone rubber mouthpiece; Figure 5.1). Tape was used across the participant's nose to prevent the nose peg from slipping. A researcher was present in the MRI scanning room to visually and verbally check the set-up before the CVR acquisition commenced. On occasion, leaking of breath around the mouthpiece was observed in the end-tidal traces, so clear instructions were given to participants explaining the procedures prior to scanning (i.e., requesting they kept a tight seal around the mouth piece throughout) and participants practiced putting in place and removing the mouthpiece before going into the MRI scanner. The finalised breathing equipment set-up is shown in Figure 5.1, and was used for both TCD and MRI data acquisitions. All the equipment was MRI safety checked by the Birmingham University Imaging Centre safety officer.

### 5.3.3. Familiarisation of Cerebrovascular Reactivity Gas Challenges

Participants completed a full familiarisation session of the gas challenge protocol (Chapter 4, Figure 4.1) whilst MCAv measures were recorded using TCD. Participants were asked to lie in supine position, relax and breathe as naturally as possible. A 20-minute resting period was followed by a 4-minute resting data collection period. They then breathed 4 minutes of 5% $CO_2$ stimulus, followed by 4 minutes of room air, followed by 4 minutes of

7% $CO_2$ stimulus, followed by 4 minutes of room air. After satisfactory completion of familiarisation trials (i.e., no adverse reactions to breathing the $CO_2$ stimulus were experienced by the participant and adequate Doppler signals were identified), participants were invited back for the two full testing sessions, one where MCAv was measured using TCD and one where BOLD and ASL were measured using MRI to give different indirect measures of brain blood flow. The order of these two testing session visits was randomised between participants to avoid order effects.

### 5.3.4. Gas Challenge set up for Cerebrovascular Reactivity Outcome Measures

To ensure mouth breathing only, participants wore a nose peg whilst breathing through a mouthpiece connected to the gas delivery apparatus shown in Figure 5.1. A large 3-way valve (2700 series: NRBV; Hans Ruldolph Inc., Kansos City, MO, USA) was connected to the mouthpiece, an inhalation tube that was connected to one of two open-circuit Douglas bags (containing 5% $CO_2$ or 7% $CO_2$ gas in dry air) or left open to room air, and an exhalation tube that lead to room air. Within this circuit for the TCD session only, was a heated pneumotachograph (3813 Series, Hans Rudolph Inc., Kansas, USA); however, due to the non-MRI compatibility of any available spirometer, ventilation volume and rate was not recorded during the MRI session. Switching the Douglas bag valves from ambient room air to the 5 and 7% $CO_2$ mixture was done by a researcher sitting beside the participant for both TCD and MRI sessions.

During both visits, fractional changes in inspired and expired oxygen ($O_2$) and carbon dioxide ($CO_2$) were measured via a sample line inserted into the mouth piece, as shown in Figure 5.1. The sample line connected to a fast responding gas analyser (ML206, ADInstruments Ltd, New Zealand), with the flow rate at maximum (200 $mL \cdot min^{-1}$). $O_2$

and $CO_2$ data were acquired continuously at a sample rate of 1kHz via an analogue-to-digital converter (PowerLab 16/35, ADInstruments) interfaced with a computer, displayed in real time and stored for offline analysis using commercially available software (LabChart v7.3.5, ADInstruments). Beat-by-beat blood pressure (BP) was measured using photoplethysmography via a finger cuff placed on the middle finger of the left hand (Portapres, Finapres, Medical System BV, the Netherlands) during the TCD visit. A 3-lead electrocardiogram (ECG) was used to continuously measure heart rhythm and electrical activity. During the MRI visit, cardiac and respiratory cycles were simultaneously recorded using the vector cardiogram (VCG; Philips Medical Systems, The Netherlands) and respiratory belt, respectively.

Calibration of the breathing equipment through PowerLab was performed before each testing session for airflow and gas concentration. A 3-L calibration syringe was used for volume measurement and certified reference gases used for gas concentration (5.00% $CO_2$, 15.01% $O_2$) (BOC Gases, Surrey, UK).

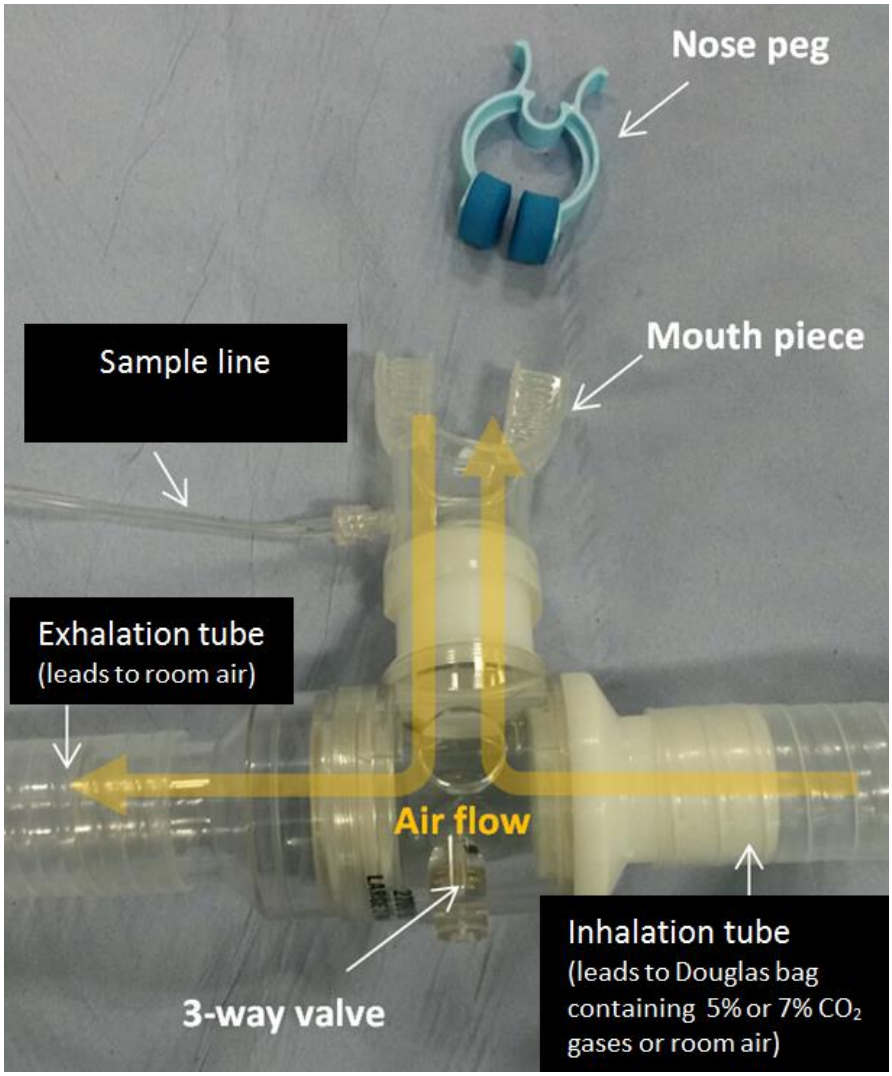



Figure 5.1. Breathing equipment set-up used for gas challenge protocol. Participants wore a nose peg and breathed through the mouth piece. The mouthpiece connected to a 3-way valve with inhalation and exhalation tubes. The inhalation tube connected to room air or one of two open-circuit Douglas bags (containing 5% $CO_2$ or 7% $CO_2$ gas in dry air).

### 5.3.5. Data Acquisition

*MRI data:* All MRI data were acquired on a 3-T Philips Achieva MRI scanner (Philips Medical Systems, Best, Netherlands) using the same hardware and software as described in Chapter 4 (see Chapter 4, Figure 4.2 for protocol). To allow measurement of CVR using MRI, a flow sensitive alternating inversion recovery (FAIR) double acquisition background suppression (DABS) sequence (Mullinger et al., 2013, 2017) was used for simultaneous acquisition of ASL and BOLD data. The same gas challenge paradigm used

in the familiarisation and TCD visits (Figure 4.1) was used with the BOLD and ASL signals recorded simultaneously (total scan duration of 18 min).

Importantly, in the DABS sequence, ASL data are collected with a short echo time (TE) following two background suppression pulses, ensuring that the signal from the static tissue in the label and control ASL images is close to suppressed in order to reduce physiological noise and any BOLD contamination in the CBF response. To maximize BOLD contrast, the BOLD data are acquired at the end of each TR period to allow partial recovery of the static signal, with an echo time approximately matched to the T2* of grey matter. Imaging parameters used in this study were: TR = 5.2 s (label-control pair), with background suppression pulses at TBGS1/TBGS2: 339 ms/899 ms, label delay = 1400 ms, TE = 9 ms (ASL)/40 ms (BOLD), voxel size: 3.25 × 3.25 × 5 mm, 12 slices of ASL and BOLD data, FOV: 212 × 212 mm, SENSE factor: 2.3, volumes: 205 (label-control pairs).

As mentioned in Chapter 4, Section 4.3.3, a whole head T1-weighted anatomical image (magnetisation prepared rapid gradient echo; MPRAGE) with 1 $mm^3$ resolution was also acquired to allow normalisation of the individual subject to the standard brain and definition, and segmentation of the grey and white matter so that CVR measures in these tissues could be assessed separately.

*TCD session:* Bilateral blood flow velocity in the right and left middle cerebral artery (MCAv) was measured using TCD (Doppler Box, DWL, Compumedics Ltd, Germany), with a 2-MHz probe placed over each temporal window on the right and left side of the head. Probes were prepared with ultrasound gel and held in place with a headset. Search and identification procedures were done in accordance with established guidelines (Willie et al., 2011).

### 5.3.6. Data Analysis

For both TCD and MRI visits, collected gas fractions were converted to partial pressure measures of end tidal $CO_2$ and $O_2$ using the measured barometric pressure (Fortin Barometer, Russell Scientific Instruments Ltd, Norfolk, UK) of that day ((percentage $CO_2$/100)*(760-barometric pressure)).

#### 5.3.6.1. Calculating CVR from MRI data

*Pre-processing*

The DABS data were separated into the BOLD and ASL data for subsequent analysis. The BOLD data were physiologically corrected for cardiac and respiratory effects using RETROICOR (Glover et al., 2000). All data were then motion corrected using Oxford University Functional Magnetic Resonance Imaging of the Brain (FMRIB) Linear Regression Tool (FLIRT) (Jenkinson et al., 2002) in the FMRIB Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Using in-house Matlab programmes, separately the ASL data (perfusion (CBF)-weighted images acquired at TE = 9 ms) and BOLD data (BOLD-weighted images acquired at TE = 40 ms) were then linearly interpolated (Interp function, Matlab, Mathworks USA) to an effective TR of 2.6 s. Label-control pairs of ASL data were subtracted (using simple subtraction) to create perfusion weighted (CBF) images. BOLD-weighted image pairs were averaged to produce mean BOLD-weighted data. Further pre-processing was carried out in FSL. BOLD data were normalised to the standard Montreal Neurological Institute (MNI) template using the anatomical-to-standard space transform derived in the resting CBF data analysis (Chapter 4, Section 4.3.4). After inspection of CBF data it was decided that further analysis could not be performed due to the inherently low signal-to-noise producing spurious variability during baseline and CVR

gas challenges. Therefore, the post-processing stages outlined below were only carried out on the BOLD data to give an MRI measure of CVR.

*Post-processing*

The CVR outcome measure was calculated using several different methodological approaches that are commonly used and are described in more detail below. This was done to determine whether they gave similar results and showed similar differences between groups. Approaches included calculating a linear regression and a general linear model (GLM) analysis across the entire gas challenge and across the 5% $CO_2$ and 7% $CO_2$ gas challenges separately.

*Linear regression:* Firstly, the mean BOLD signal for each volume over the individual subject grey matter tissue mask (i.e., excluding veins) was calculated. The mask to exclude veins was created by plotting a histogram of the percentage BOLD signal change to the entire gas challenge across the grey matter and where this was greater than 15% was regarded as a vein so excluded. To account for the delay in the $P_{ET}CO_2$ readings relative to the BOLD readings (due to the sample line length, enabling readings to be taken from the facemask on the participant to the MRI control room), the mean % BOLD signal intensity was temporally aligned with the $P_{ET}CO_2$ (mm Hg) trace using an iterative process to maximise the correlation of the BOLD and $P_{ET}CO_2$ trace, with an example shown in Figure 5.2C. The BOLD signal was converted to a percentage change relative to baseline and plotted against the corresponding $P_{ET}CO_2$ values to give a plot such as that shown in Figure 5.2D. The data were then modelled using a linear regression, with Equation 5.1 used to obtain the line of best fit over the whole data acquisition (including periods of breathing room air, 5% and 7% $CO_2$).

$$y = mx + c$$

Equation 5.1

where m is the gradient and c is the intercept of the line with the y axis at x=0 and y and x are variables (in this case y is the BOLD data and x is the $P_{ET}CO_2$ trace). The gradient (m) of the line of best fit was the first BOLD CVR outcome measure.

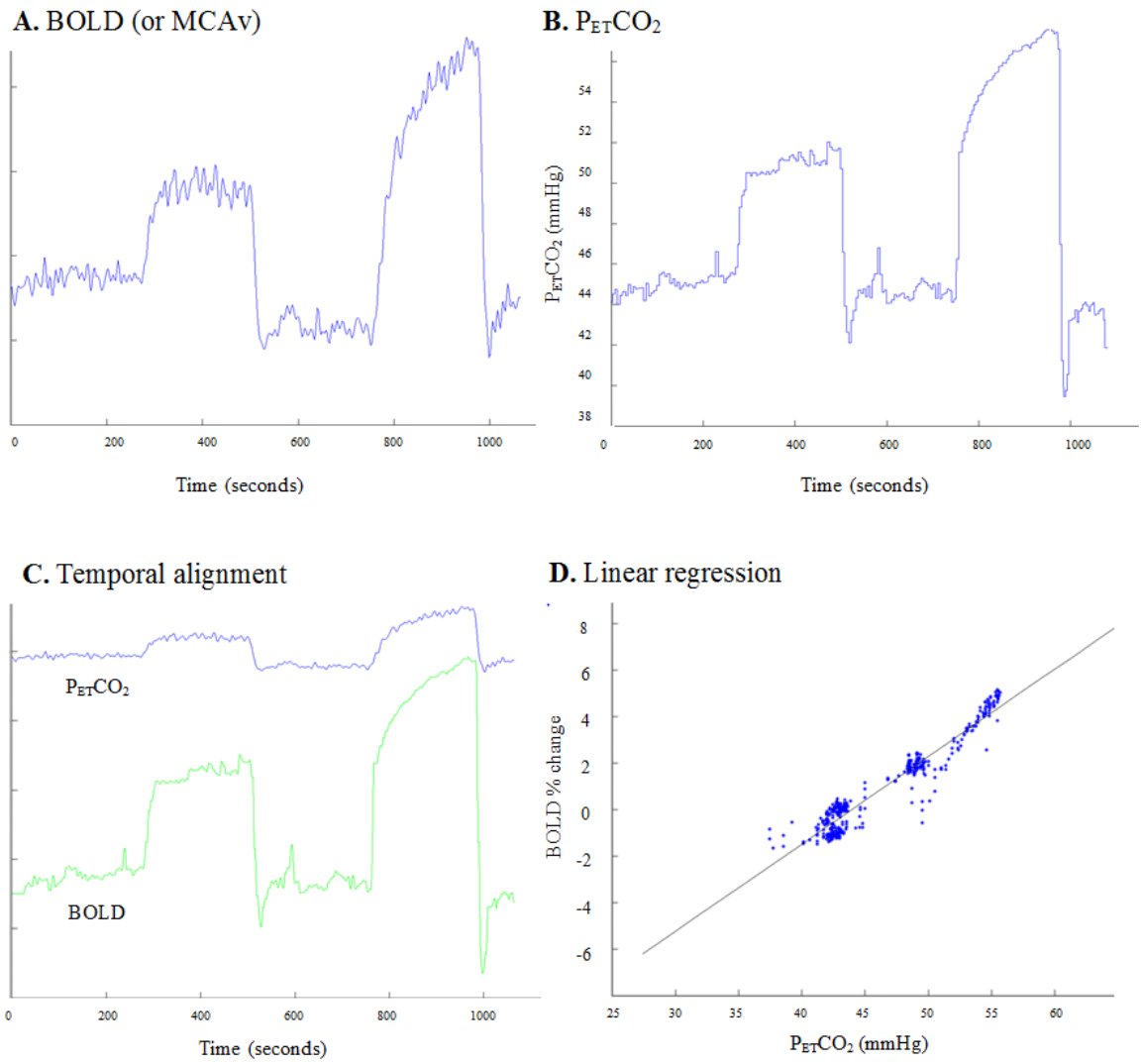



Figure 5.2. Obtaining the CVR measure using a linear regression analysis approach. The BOLD (or MCAv) data (A) and the $P_{ET}CO_2$ data (B) were temporally aligned (C). Percentage change in the BOLD signal relative to baseline was then plotted against the corresponding $P_{ET}CO_2$ values (D). The gradient of the line of best fit was used as the CVR measure.

*GLM analysis:* The GLM uses a mass univariate approach by applying the same statistical analysis on every single voxel. For these data, this process involved looking at the relationship between the dependent variable: Y (the BOLD signal) and the eight dependent variables (i.e., regressors): $X_1$, $X_{2...}$ $X_8$ ($P_{ET}CO_2$, linear drift and six movement parameters) (see Equation 5.2).

$$\boldsymbol{Y = Constant + X_1 ß_1 + X_2 ß_2 + X_3 ß_3 + X_4 ß_4 + X_5 ß_5 + X_6 ß_6 + X_7 ß_7 + X_8 ß_8 + \varepsilon}$$

Equation 5.2

where in this case: X1 is the $P_{ET}CO_2$ regressor, X2 the linear drift regressor, $X_3$-$X_8$ the motion regressors, the constant is the mean BOLD signal over time and ε the noise term. Y is the measured BOLD signal time-course in a given voxel.

The beta weights are regression coefficients that are used when both the variables are standardised. For the purpose of calculating CVR, it is clear from the similarity of Equations 5.1 and 5.2 that the beta weight $ß_1$ (related to the $P_{ET}CO_2$ regressor) at each voxel provided a metric of CVR in units of % BOLD signal change/ mm Hg $P_{ET}CO_2$ change at each voxel (e.g., Thomas et al., 2013). These BOLD-derived CVR measures using the GLM take account of any BOLD signal changes linked to motion or drift due to the nuisance regressors modelled (Equation 5.2), and therefore should provide a more accurate estimate of CVR than the linear regression method. The GLM-derived CVR measures were masked to only include grey matter tissue (excluding veins, see linear regression analysis for details). The mean CVR over all grey matter was then calculated and, in addition, CVR measures in different RoIs were also assessed. The RoI masks described in Chapter 4, Section 4.3.4 were used (akin to Thomas et al., 2013). Both the linear regression and GLM-derived BOLD CVR measures were compared with Doppler-

derived measures of CVR. Finally, to further understand regional variation, mean CVR maps from the GLM analysis were created over all participants and the younger and older groups separately by averaging the participants individual mean GLM-derived CVR for each RoI (see Appendix A5.1).

#### 5.3.6.2. Calculating CVR using TCD

CVR was calculated using Equations 5.3 and 5.4 and the same methods described in Chapter 3. Given that the findings of Chapter 3 indicated that 3 minutes into the gas challenge produced the least variability in relative CVR, this 3-minute time-point was used for analysis in this Chapter. Further relative CVR is used as the main TCD analysis approach (see Chapter 3; Results, and Discussion, Sections 3.5 and 3.5). TCD analysis approaches included extracting 60 seconds of stable resting data, and 30 seconds of data from both the 5% $CO_2$ and 7% $CO_2$ stimulus durations, preceding 3 minutes of the stimulus duration (i.e., starting from 2.5 minutes into the stimulus duration).

**Absolute CVR:**

$$\frac{\text{hypercapnic (5\% or 7\% } CO_2\text{) MCAv} - \text{resting MCAv}}{\text{hypercapnic (5\% or 7\% } CO_2\text{) } P_{ET}CO_2 - \text{resting } P_{ET}CO_2}$$

Equation 5.3

**Relative CVR:**

$$\frac{100\ ((\text{hypercapnic (5\% or 7\% } CO_2\text{) MCAv} - \text{resting MCAv}) / \text{resting MCAv})}{\text{hypercapnic (5\% or 7\% } CO_2\text{) } P_{ET}CO_2 - \text{resting } P_{ET}CO_2}$$

Equation 5.4

TCD CVR measures using cerebrovascular conductance (CVCi-derived CVR) were also calculated using mean arterial blood pressure (MAP) (Equation 5.5), where CVCi replaces MCAv in Equation 5.3 and 5.4 (Brown et al., 2010 and Barnes et al., 2013).

$$CVCi = \frac{MCAv}{MAP}$$

Equation 5.5

#### 5.3.6.3. Calculating CVR using different gas concentrations and analysis approaches

Above, the CVR measures calculated for both MRI and TCD data are described using typical analysis approaches (e.g., Thomas et al., 2013, Driver et al., 2010 and Vernieri et al., 2009). Whilst the MRI-derived CVR calculations described above were over the entire data, using 5% and 7% $CO_2$ challenges, previous work has indicated that there may be a non-linearity in responses between gas challenges both in MRI response (Bhogal et al., 2014) and TCD responses (Battisti-Charbonney et al., 2011). Therefore to allow for this possibility, additional analyses of the MRI BOLD data were performed.

*Calculating CVR using different gas concentrations:* MRI analysis approaches included calculating a linear regression using BOLD signal intensity and $P_{ET}CO_2$ data from: i) the entire gas challenge (as described above); ii) the first baseline period and the 5% $CO_2$ stimulus duration, and iii) the second baseline period and the 7% $CO_2$ stimulus duration. The GLM approach was also adapted to use the same periods and interrogate 5% and 7% $CO_2$ responses separately for the whole of grey matter and the ROIs described above. This then would serve to match the TCD measures on the 5% and 7% $CO_2$ data, which were done separately using the typical CVR analysis described above (see Section 5.3.6.2). Figure 5.3 provides an overview of the typical measures made with each imaging modality.

*Calculating CVR using different analysis approaches:* To allow comparison between CVR measures from different imaging modalities it was important to match analyses as closely as possible. Therefore, BOLD data (in this case the mean over all grey matter tissue) were analysed using the TCD approach (outlined in Section 5.3.6.2) by extracting 30 seconds of data from 3 minutes into the stimulus duration to calculate the mean BOLD response in this time window, and 60 seconds of BOLD data from the resting baseline. The average BOLD signal then replaced the MCAv in Equation 5.4 to calculate CVR. An important note is that the TCD MCAv and $P_{ET}CO_2$ data were not temporally aligned as they were for the MRI BOLD and $P_{ET}CO_2$ data when using the linear regression approach described above (Section 5.3.6.1). This was because this is not typical for TCD methodological approaches and the aim was to keep the approaches used in this study as close to typical approaches as possible. Therefore, in addition, the TCD modality data were used to form a linear regression analysis, akin to that described in Section 5.3.6.1, linear regression subsection. Linear regressions were performed for: i) the entire gas challenge, ii) the first baseline and the 5% $CO_2$ stimulus duration, and iii) the second baseline and the 7% $CO_2$ stimulus duration – matching the BOLD MRI linear regression method.

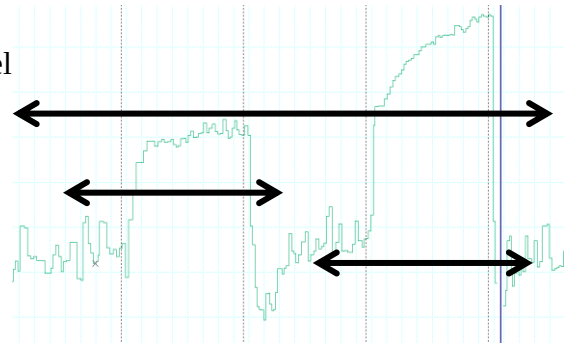
*Regional comparisons*: Although MRI RoI approaches cannot be directly compared to TCD approaches, an analysis investigating CVR measures calculated between modalities from anatomically similar locations was also performed (i.e., TCD MCAv measures were compared with MRI motor, parietal and frontal lobe measures).

**A: Typical MRI approaches for calculating CVR:**

Linear regressions and General Linear Model

CVR measures calculated from:

1. Entire gas challenge (18 minutes)
2. Baseline and 5% $CO_2$ (8 minutes)
3. Baseline and 7% $CO_2$ (8 minutes)

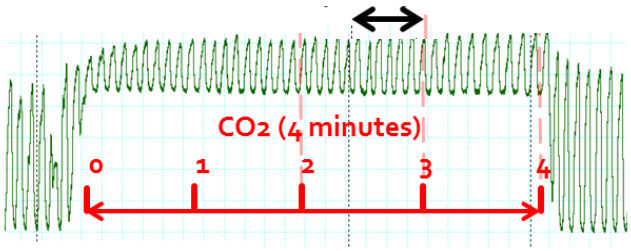


Calculations were made, first for all grey matter, followed by several regions of interest

**B: Typical TCD approaches for calculating CVR:**

60 s of resting data and 30 s of data extracted preceding 3 minutes of the stimulus duration from:

1. 5% $CO_2$ stimulus
2. 7% $CO_2$ stimulus




Calculations were performed using: MCAv and CVCi (MCAv/MAP).

Figure 5.3. Typical MRI (A) and TCD (B) analysis approaches for calculating the CVR outcome measure.

### 5.3.7. Statistical Analysis

Group means were compared between younger and older groups, younger fit and unfit groups, and older fit and unfit groups using ANOVAs. Differences between analysis approaches were compared where possible using ANOVAs and t-tests. Stimulus concentrations (5% versus 7% $CO_2$) were also compared using t-tests. Where analysis

approaches were measured using different units, correlational analyses were performed to investigate correlations between approaches.

Change in $P_{ET}CO_2$ values from baseline to both stimulus concentrations were compared between TCD and MRI visits using paired t-tests to ensure they were not significantly different. Further, maximum $P_{ET}CO_2$ values were checked to see if they went over 55 mm Hg, as this would introduce a further potential confound on the CVR outcome measure where linearity of changes between CBF and $P_{ET}CO_2$ would no longer be expected (Bhogal et al., 2014; Battisti-Charbonney et al., 2011; discussed in detail in Chapter 2).

## 5.4. Results

The following results only includes participants who completed both the 5% $CO_2$ and 7% $CO_2$ stimulus concentrations during both TCD and MRI modality visits (n = 28; 16 younger and 12 older). Mean participant characteristics are shown in Table **5.1**, including: age, fitness ($\dot{V}O_2$ max), resting heart rate and MAP measures. ANOVAs were performed to test for differences between age and fitness groups. Tests of homogeneity of variances showed no significant effects apart from V̇O2 max measures between the older fit and unfit group, where the fit group had a significantly higher variance (SD = 14.06 versus 3.67; p = 0.003). The ratio of males to females is also reported.

Table 5.1. Characteristics of participants who completed the CVR challenge for both 5% and 7% $CO_2$ stimulus concentrations, using both TCD and MRI brain imaging modalities.

| Group (*n*) | Younger (16) | Older (12) | *ANOVA* *p* value | Younger-fit (8) | Younger-unfit (8) | *ANOVA* *p* value | Older-fit (7) | Older-unfit (5) | *ANOVA* *p* value |
|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 25 (7) | 66** (8) | 0.000 | 28 (8) | 23 (6) | 0.183 | 64 (8) | 70 (6) | 0.127 |
| V̇O2 max ($mL \cdot kg^{-1} \cdot min^{-1}$) | 45.8 (11.0) | 34.2* (16.8) | 0.040 | 54.5 (5.0) | 35.9** (6.2) | 0.000 | 44.7 (14.1) | 19.4** (3.7) | 0.003 |
| Heart rate ($b \cdot min^{-1}$) | 63 (14) | 56 (7) | 0.149 | 55 (7) | 70* (16) | 0.026 | 54 (6) | 59 (8) | 0.233 |
| MAP (mm Hg) | 79 (11) | 84 (12) | 0.240 | 77 (12) | 81 (10) | 0.482 | 80 (9) | $92^{t}$ (12) | 0.095 |
| Sex (male: female) | 11:5 | 7:5 | | 7:1 | 4:4 | | 6:1 | 1:4 | |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years), while the criterion for being fit was defined as $\geq 45$ $mL \cdot kg^{-1} \cdot min^{-1}$ and $\geq 25$ $mL \cdot kg^{-1} \cdot min^{-1}$ for the younger and older groups respectively. Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$. Abbreviations: V̇O2 max, maximum oxygen consumption; MAP, mean arterial blood pressure.

$P_{ET}CO_2$ values were assessed using paired t-tests to ensure there were no significant differences between visits for change in $P_{ET}CO_2$ values between the two visits. There were no significant differences in $P_{ET}CO_2$ change from baseline to the 5% ($p = 0.153$) or the 7% $CO_2$ stimulus ($p = 0.280$) between the TCD and MRI visits (see Appendix A5.2 for values). To determine whether there were significant differences between groups in movement during the MRI protocol that may have influenced the CVR outcome measure, paired t-tests were performed on the total translational movement ($x^2 + y^2 + z^2$) from the movement parameters. No significant differences were observed between younger and older groups in mean movement ($p = 0.254$), standard deviation of movement ($p = 0.413$) or maximum movement ($p = 0.252$), neither were differences in movement observed between fitness groups (younger fit and unfit groups: $p = 0.298$, $p = 0.344$ and $p = 0.947$, respectively; older fit and unfit groups: $p = 0.579$, $p = 0.928$ and $p = 0.848$, respectively) (see Appendix A5.3 for values).

### 5.4.1. Aim 1: Effects of Ageing and Fitness on the Cerebrovascular Responsiveness Outcome Measure

The following section looks at differences in CVR outcome measures in different age and fitness groups. This section will also look at CVR outcome measures obtained from specific regions of interest from the MRI modality. The between group (i.e., age and fitness) effects on all CVR outcome measures are summarised in Table 5.2, and Table 5.3 shows correlations between all CVR measures, as well as between CVR measures and age and fitness subgroups. Figure 5.4 shows differences between younger and older groups, whilst Figures 5.5 and 5.6 show differences between fit and unfit groups in the younger and older cohorts respectively.

*Age:* No significant differences were observed between age groups on any of the CVR measures derived from the BOLD data, though they did all show the same pattern where CVR was higher in the younger group than the older group (Figure 5.4A). In contrast, CVR measures derived from the TCD-MCAv data showed the opposite pattern where CVR was lower in the younger group than the older group (Figure 5.4B), though this was only significant for absolute measures from the 5% $CO_2$ stimulus concentration ($p$ = 0.008). No age group differences were observed in TCD-CVCi CVR measures (Figure 5.4C). As the BOLD data with TCD relative CVR analysis approach showed the same pattern as the BOLD data with linear regression approach, this indicates that differences in the CVR measure are more related to acquisition method (i.e., imaging modality) than the analysis approach. However, the CVR measure was sensitive to the analysis approach as significant effects were not always observed.

*Fitness:* Figure 5.5 shows some effects of fitness in the younger group though only when CVR was calculated from the BOLD data using 7% $CO_2$ stimulus and a linear regression analysis approach ($p$ = 0.028), or using 7% $CO_2$ stimulus and the TCD relative analysis approach ($p$ = 0.013). No other significant fitness group differences were observed, though all CVR measures were lower in the younger fit group than the unfit group (Figure 5.6A and 5.6B). Figure 5.6 shows no significant effects of fitness on CVR in the older group regardless of imaging modality, analysis approach or stimulus concentration.

*Regions of interest:* The CVR outcome measures calculated from separate RoIs within the MRI modality showed no significant age or fitness effects (all $p > 0.10$). However, in the older group CVR significantly decreased as age increased, for CVR measures calculated using the 5% $CO_2$ stimulus (all $p < 0.05$) from all RoIs: frontal lobe, motor lobe and parietal lobe. There were no correlations between the same measures and age from the 7% $CO_2$ stimulus (all $p > 0.1$).

Table 5.2. Means and standard deviations for CVR measures obtained from MRI BOLD data and TCD MCAv data using typical analysis approaches from a 5% $CO_2$ and 7% $CO_2$ stimulus duration. Analysis approaches include, for MRI: linear regressions and general linear model (GLM) for all grey matter (GM); and for TCD: averaged right and left relative and absolute CVR calculated using MCAv and CVCi from 30-seconds of data. CVR measures are shown for all participants followed by groups: younger/ older; younger fit/ unfit; and older fit/ unfit.

| | | | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|---|---|
| Modality | Analysis approach (units) | Stimulus | (28) | Younger (16) | Older (12) | Younger fit | Younger unfit | Older fit | Older unfit |
| | age | | 42.8 ± 22.1 | 25.1 ± 7.4 | **66.4 ± 7.5**** | 27.6 ± 8.1 | 22.6 ± 6.0 | 63.6 ± 7.7 | 70.4 ± 5.8 |
| | V̇O2 max | | 40.6 ± 14.8 | 45.8 ± 11.0 | **34.2 ± 16.8*** | 54.5 ± 5.0 | 35.9 ± 6.2 | 44.7 ± 14.1 | 19.4 ± 3.7 |
| TCD | LR MCAv 5% | 5% $CO_2$ | 2.20 ± 0.72 | 2.22 ± 0.51 | 2.17 ± 0.96 | 2.13 ± 0.32 | 2.32 ± 0.79 | 2.36 ± 0.83 | **1.92 ± 1.16*** |
| | LR MCAv 7% | 7% $CO_2$ | 2.11 ± 1.00 | 1.93 ± 0.87 | 2.35 ± 1.15 | 1.76 ± 0.79 | 2.10 ± 1.00 | 2.67 ± 1.00 | **1.90 ± 1.31*** |
| | LR CVCi 5% | 5% $CO_2$ | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.02 | 0.02 ± 0.01 |
| | LR CVCi 7% | 7% $CO_2$ | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.01 |
| | MCAv relative 5% | 5% $CO_2$ | 3.79 ± 0.85 | 3.44 ± 0.77 | **4.26 ± 0.73**** | 3.52 ± 0.81 | 3.36 ± 0.76 | 4.27 ± 0.61 | 4.26 ± 0.95 |
| | MCAv relative 7% | 7% $CO_2$ | 4.74 ± 1.09 | 4.55 ± 0.83 | 5.00 ± 1.36 | 4.85 ± 0.84 | 4.24 ± 0.76 | 5.14 ± 0.75 | 4.81 ± 2.04 |
| | MCAv absolute 5% | 5% $CO_2$ | 2.39 ± 0.78 | 2.31 ± 0.75 | 2.49 ± 0.84 | 2.15 ± 0.56 | 2.48 ± 090 | 2.73 ± 0.66 | 2.15 ± 1.01 |
| | MCAv absolute 7% | 7% $CO_2$ | 2.83 ± 0.90 | 2.86 ± 0.68 | 2.79 ± 1.17 | 2.72 ± 0.41 | 3.0 ± 0.89 | 3.05 ± 0.95 | 2.43 ± 1.46 |
| | CVCi relative 5% | 5% $CO_2$ | 2.58 ± 0.99 | 1.96 ± 1.51 | 2.56 ± 0.85 | 2.57 ± 1.25 | 2.65 ± 1.08 | 2.69 ± 0.65 | 2.38 ± 1.13 |
| | CVCi relative 7% | 7% $CO_2$ | 3.25 ± 0.88 | 2.98 ± 1.22 | 3.30 ± 0.87 | 3.50 ± 0.87 | 2.80 ± 0.86 | 3.46 ± 0.93 | 3.08 ± 0.83 |
| | CVCi absolute 5% | 5% $CO_2$ | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| | CVCi absolute 7% | 7% $CO_2$ | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| MRI | LR BOLD 5% | 5% $CO_2$ | 0.25 ± 0.10 | 0.28 ± 0.11 | 0.22 ± 0.09 | 0.24 ± 0.70 | 0.32 ± 0.13 | 0.21 ± 0.10 | 0.22 ± 0.07 |
| | LR BOLD 7% | 7% $CO_2$ | 0.29 ± 0.80 | 0.31 ± 0.08 | **0.25 ± 0.07**$^{t}$ | 0.27 ± 0.08 | **0.35 ± 0.05*** | 0.24 ± 0.08 | 0.27 ± 0.06 |
| | GLM GM 5% | 5% $CO_2$ | 0.30 ± 0.10 | 0.31 ± 0.12 | 0.27 ± 0.10 | 0.29 ± 0.13 | 0.33 ± 0.12 | 0.29 ± 0.07 | 0.30 ± 0.09 |
| | GLM GM 7% | 7% $CO_2$ | 0.28 ± 0.09 | 0.29 ± 0.09 | 0.27 ± 0.10 | 0.26 ± 0.07 | 0.32 ± 0.10 | 0.28 ± 0.11 | 0.25 ± 0.10 |
| | 30-s 5% | 5% $CO_2$ | 0.27 ± 0.12 | 0.30 ± 0.12 | 0.23 ± 0.11 | 0.28 ± 0.09 | 0.31 ± 0.15 | 0.23 ± 0.13 | 0.23 ± 0.09 |
| | 30-s 7% | 7% $CO_2$ | 0.31 ± 0.09 | 0.33 ± 0.10 | **0.27 ± 0.08** $^{t}$ | 0.28 ± 0.09 | **0.31 ± 0.15*** | 0.23 ± 0.09 | 0.29 ± 0.05 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years).

* Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Abbreviations: MRI, magnetic resonance imaging; LR, linear regression; TCD, transcranial Doppler, GM, grey matter; MCAv, middle cerebral artery blood velocity; CVCi, cerebrovascular conductance; MAP, mean arterial blood pressure, V̇O2 max, maximal rate of oxygen consumption.

Table 5.3. Correlations (Pearson's *r*) with CVR outcome measures and age, followed by fitness for the younger group and older group separately.

| | | All participants (n=28) Age | Younger (n=16) Fitness: V̇O2 max | Old (n=12) Fitness: V̇O2 max |
|---|---|---|---|---|
| Modality | Analysis approach | *r* | *r* | *r* |
| | V̇O2 max | **-0.453*** | | |
| TCD | LR MCAv 5% | -0.080 | -0.197 | 0.276 |
| | LR MCAv 7% | 0.098 | -0.285 | 0.413 |
| | LR CVCi 5% | -0.123 | -0.039 | **0.700*** |
| | LR CVCi 7% | -0.329 | -0.280 | **0.525**$^{t}$ |
| | MCAv relative 5% | **0.503**** | 0.072 | -0.140 |
| | MCAv relative 7% | 0.187 | 0.192 | 0.088 |
| | MCAv absolute 5% | 0.043 | -0.217 | 0.285 |
| | MCAv absolute 7% | -0.133 | -0.268 | 0.329 |
| | CVCi relative 5% | -0.066 | -0.082 | 0.229 |
| | CVCi relative 7% | 0.064 | 0.130 | 0.552 |
| | CVCi absolute 5% | -0.070 | 0.148 | 0.117 |
| | CVCi absolute 7% | -0.136 | 0.169 | 0.434 |
| MRI | LR BOLD 5% | **-0.406*** | **-0.534*** | 0.386 |
| | LR BOLD 7% | **-0.408*** | **-0.454**$^{t}$ | 0.038 |
| | GLM GM 5% | -0.149 | -0.083 | 0.193 |
| | GLM GM 7% | -0.278 | -0.271 | 0.276 |
| | 30-s 5% | **-0.383*** | -0.305 | 0.397 |
| | 30-s 7% | **-0.418*** | **-0.582*** | -0.133 |

Values represent Pearson's *r* correlations. Significance (2-tailed) Significant age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

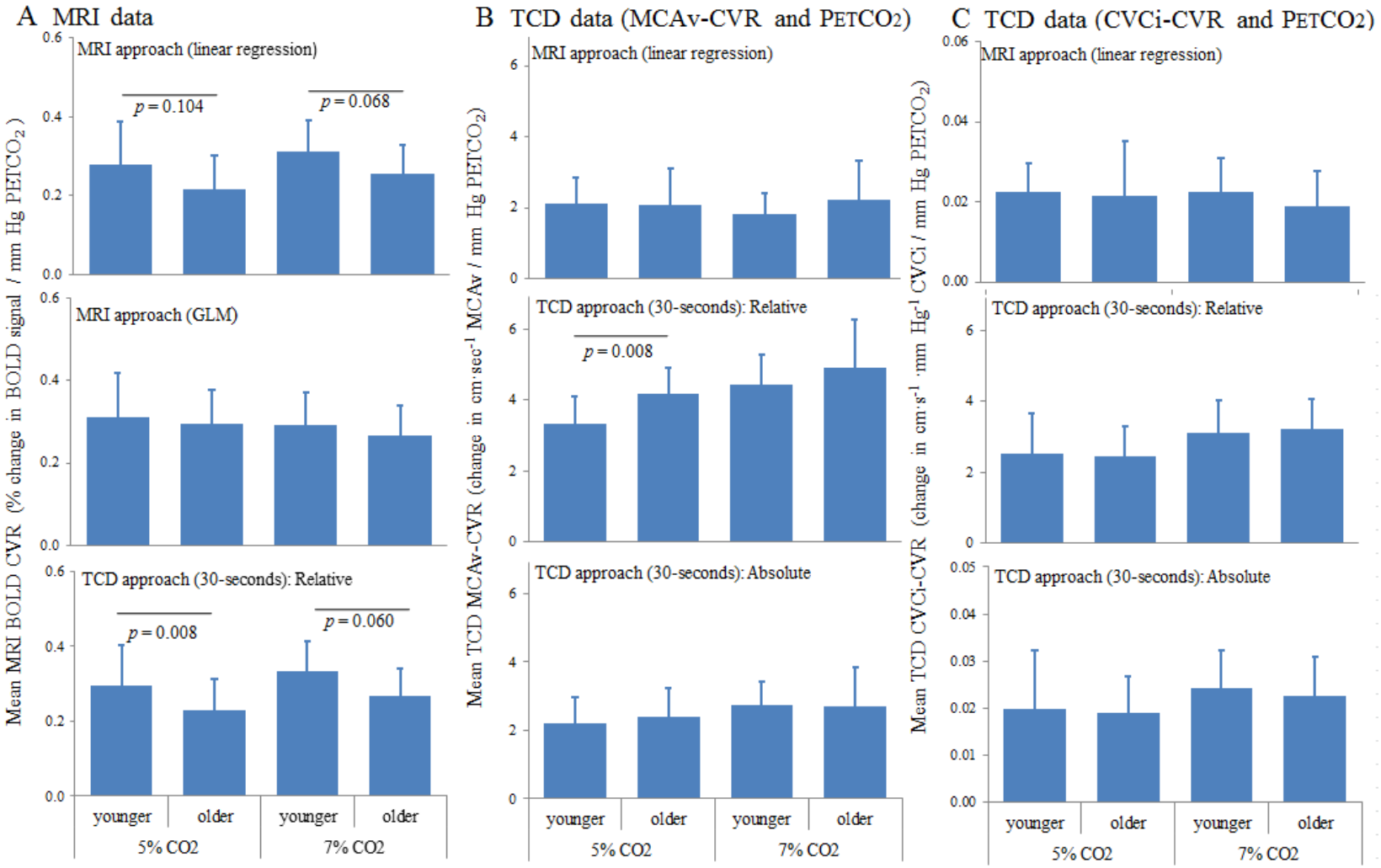



Figure 5.4. Graphs showing CVR outcome measures for younger and older groups. Measures were calculated using different neuroimaging modalities (A. MRI vs. B and C TCD data). Graphs show different analysis approaches and different stimulus concentration (5 versus 7% $CO_2$). Significant effects of age are shown above relevant bars.

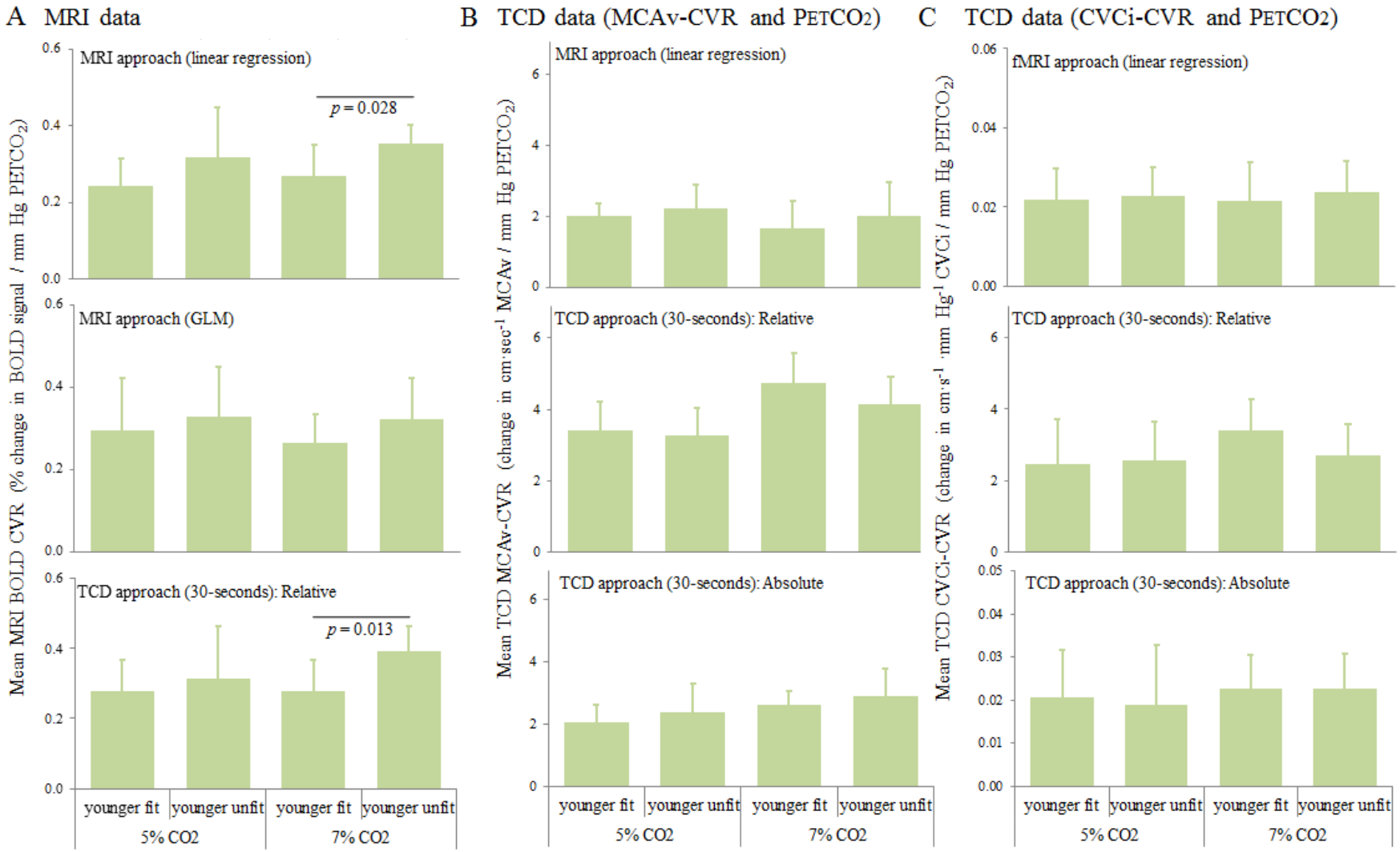



Figure 5.5. Graphs showing CVR outcome measures for younger fit and unfit groups. Measures were calculated using different neuroimaging modalities (A. MRI vs. B and C TCD data). Graphs show different analysis approaches and different stimulus concentration (5 vs. 7% $CO_2$). Significant effects of fitness are shown above relevant bars.

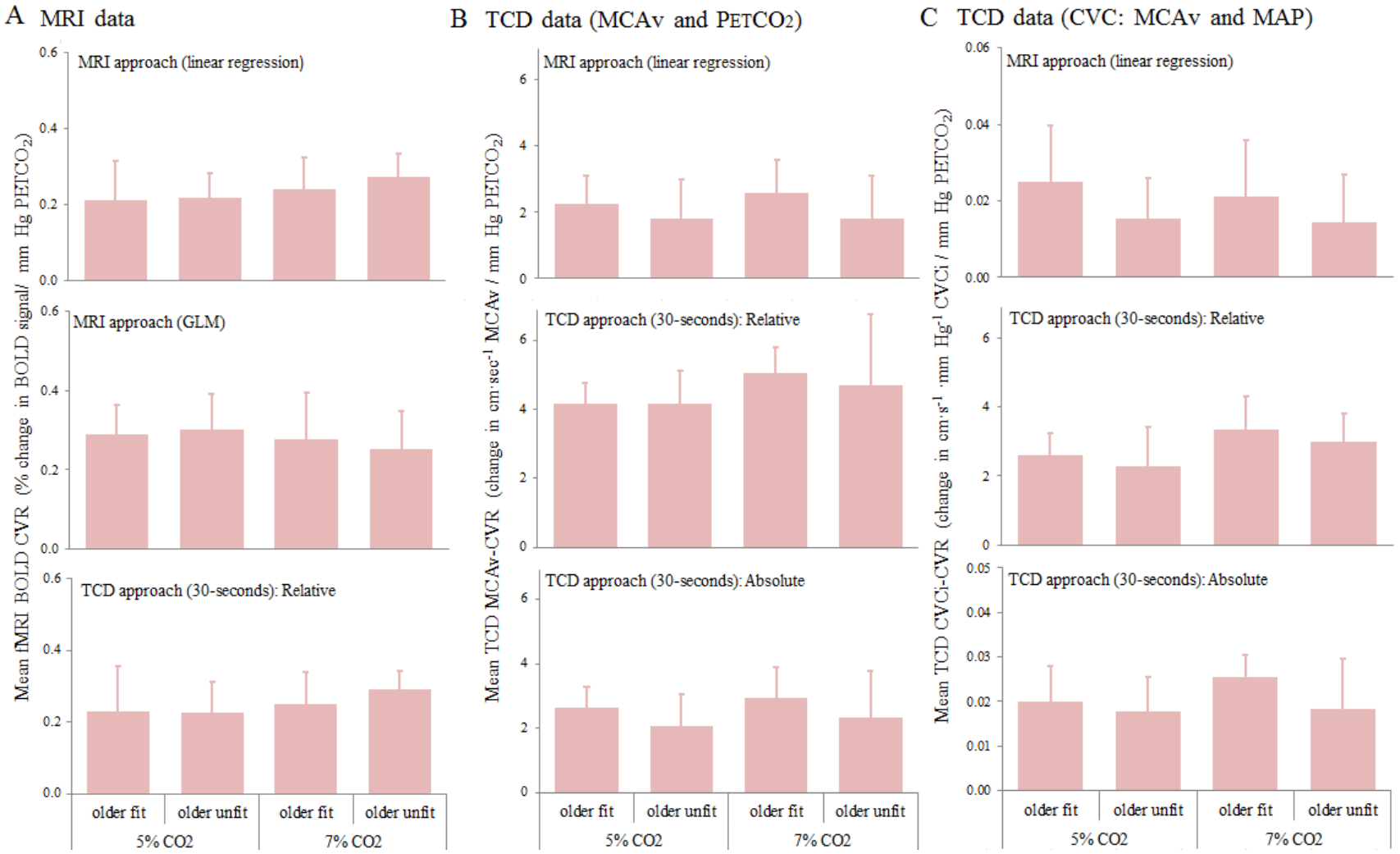



Figure 5.6. Graphs showing CVR outcome measures for older fit and older unfit groups. Measures were calculated using different neuroimaging modalities (A. MRI vs. B and C TCD data). Graphs show different analysis approaches and different stimulus concentration (5 versus 7% $CO_2$). No significant fitness effects were observed.

### 5.4.2. Aim 2: Effects of Imaging Modality and Analysis Approaches on the Cerebrovascular Responsiveness Outcome Measure

Given that the differences in CVR observed between groups were inconsistent depending on the imaging modality used (e.g., MRI approaches showed higher CVR in the younger group whereas TCD approaches showed higher CVR in the older group), it is important to see if differences are observed across the whole group due to the imaging modality, stimulus concentration and analysis approach. Therefore, this section looked at differences between the imaging modality and analysis approach for all the participants as one group.

*Effect of imaging modality with typical analysis approach*

As expected MRI BOLD-derived CVR measures calculated using the linear regression significantly correlated with MRI BOLD-derived CVR measures calculated using a general linear model (GLM) as shown in Figure 5.7.

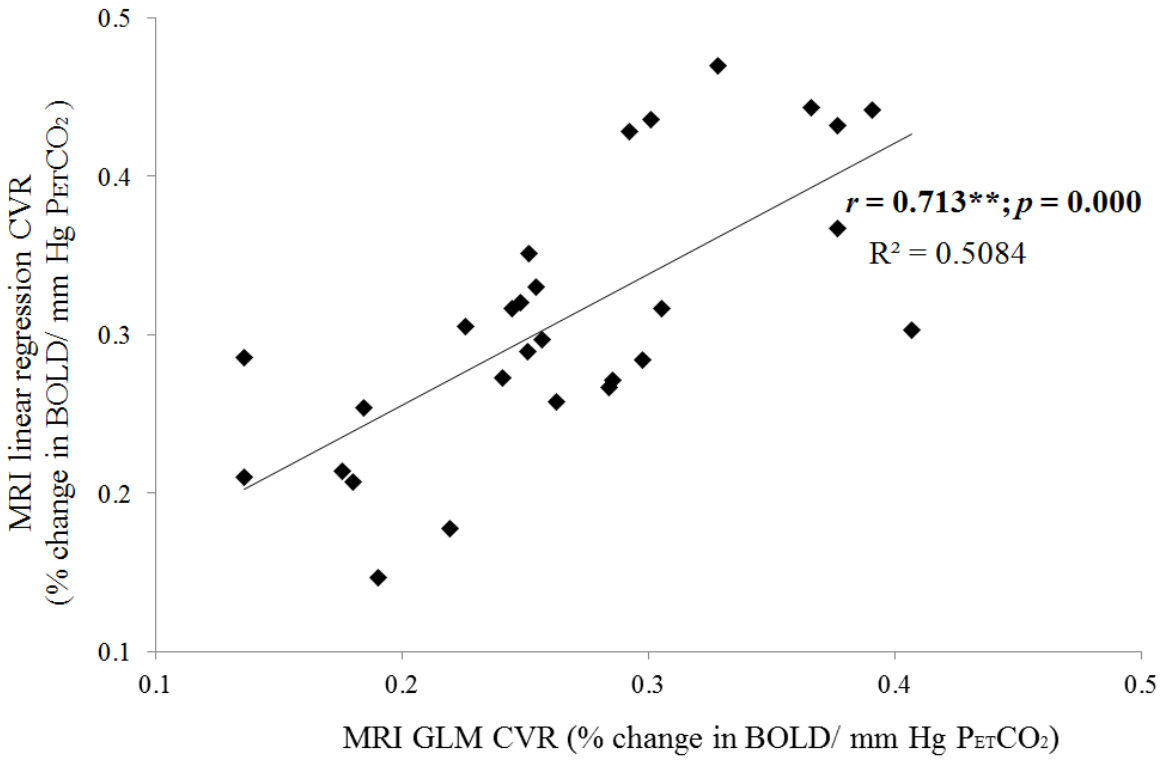



Figure 5.7. Correlation between MRI linear regression CVR and MRI GLM CVR analyses of BOLD data.

*Effects of imaging modality and analysis approach:*

For all participants, MRI GLM-derived CVR showed no correlation with TCD CVR measures calculated from the 5% $CO_2$ stimulus or 7% $CO_2$ stimulus using respective typical analysis approaches (relative TCD: 30-s steady state versus MRI: GLM, see Figure 5.8A). However, MRI GLM CVR measures did correlate with absolute TCD CVR measures calculated from the 7% $CO_2$ (Figure 5.8B).

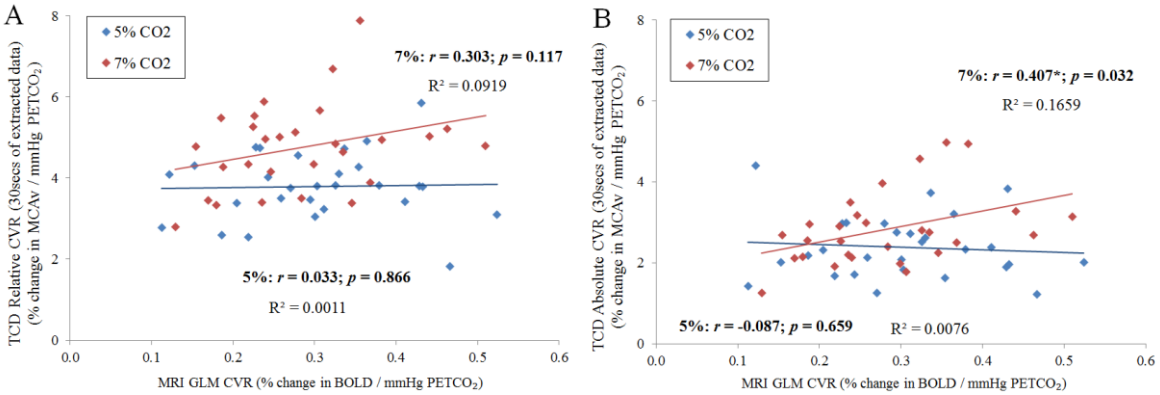



Figure 5.8. Correlation (Pearson's *r*) of the CVR outcome measure between different modalities (MRI versus TCD using their typical analysis approaches: MRI GLM (BOLD) using the entire gas challenge and TCD using a 30-s steady state for the 5 and 7% $CO_2$ gas challenges separately. A: shows relative TCD CVR values, and B: shows absolute TCD CVR values. Solid lines show lines of best fit.

CVR measures obtained from the same modality but calculated using different analysis approaches correlated significantly in most cases ($p < 0.05$, Figure 5.9), except for the MRI data obtained during the 5% $CO_2$ stimulus concentration (Figure 5.9). Specifically, MRI-derived CVR measures calculated using the MRI GLM approach did not correlate with CVR measures calculated using the TCD 30-s steady-state approach ($r = 0.168$; $p = 0.393$) (Figure 5.9B). However, with the 7% $CO_2$ challenges these CVR measures correlated better ($r = 0.472$; $p = 0.011$).

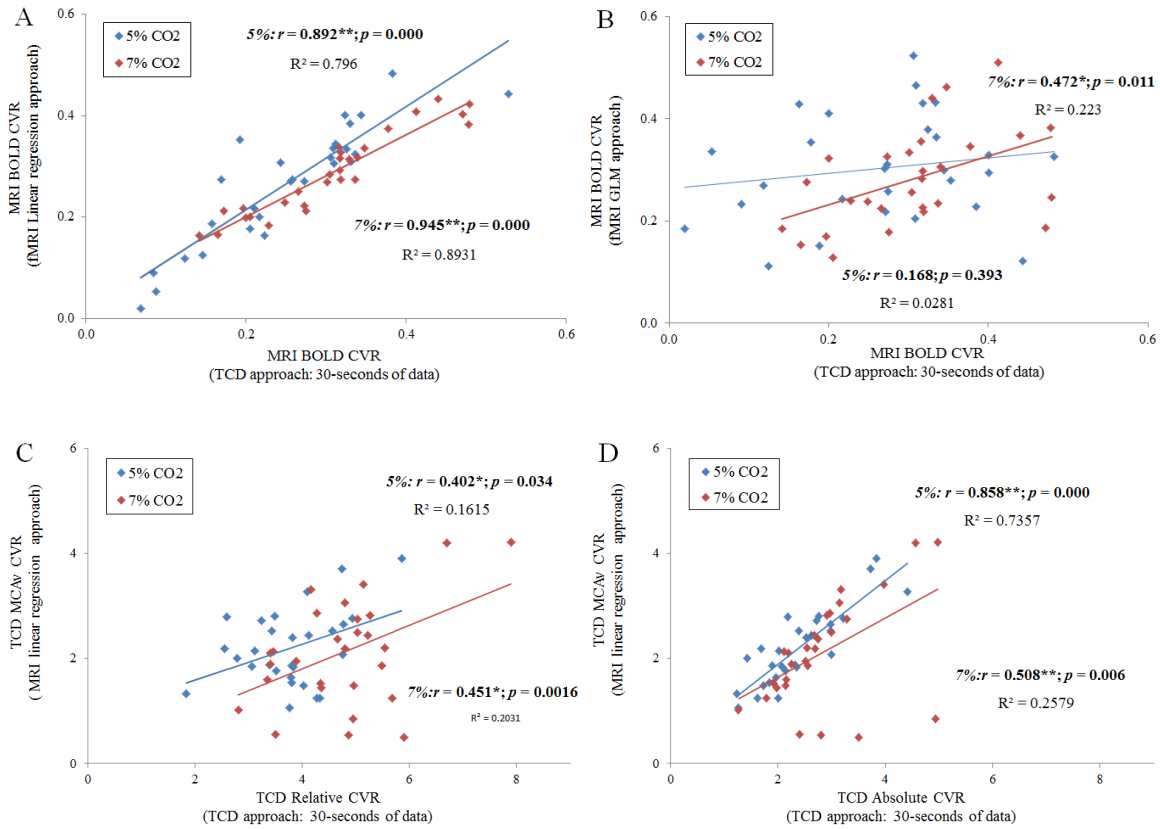



Figure 5.9. Correlations of the CVR outcome measure obtained from MRI and TCD modalities analysed using different analysis approaches. A and B show MRI BOLD data: A. MRI linear regression and TCD 30-s steady-state data analysis approaches; B. MRI GLM and TCD 30-s steady-state data analysis approaches. C and D show TCD MCAv data: C. MRI linear regression and TCD 30-s steady-state data (relative) analysis approaches. D. MRI linear regression and TCD 30-s steady-state data (absolute) analysis approaches. Lines of best fit are shown along with R values and associated *p* values (Pearson's *r*).* $p \leq 0.05$; ** $p \leq 0.01$.$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

A repeated-measures ANOVA revealed significant effects of analysis approach on MRI CVR measures (linear regression vs. GLM vs. 30-s steady state) ($p$ = 0.010) and TCD CVR measures (linear regression vs. 30-s relative steady state vs. 30-s absolute steady state) ($p < 0.05$; Figure 5.10).

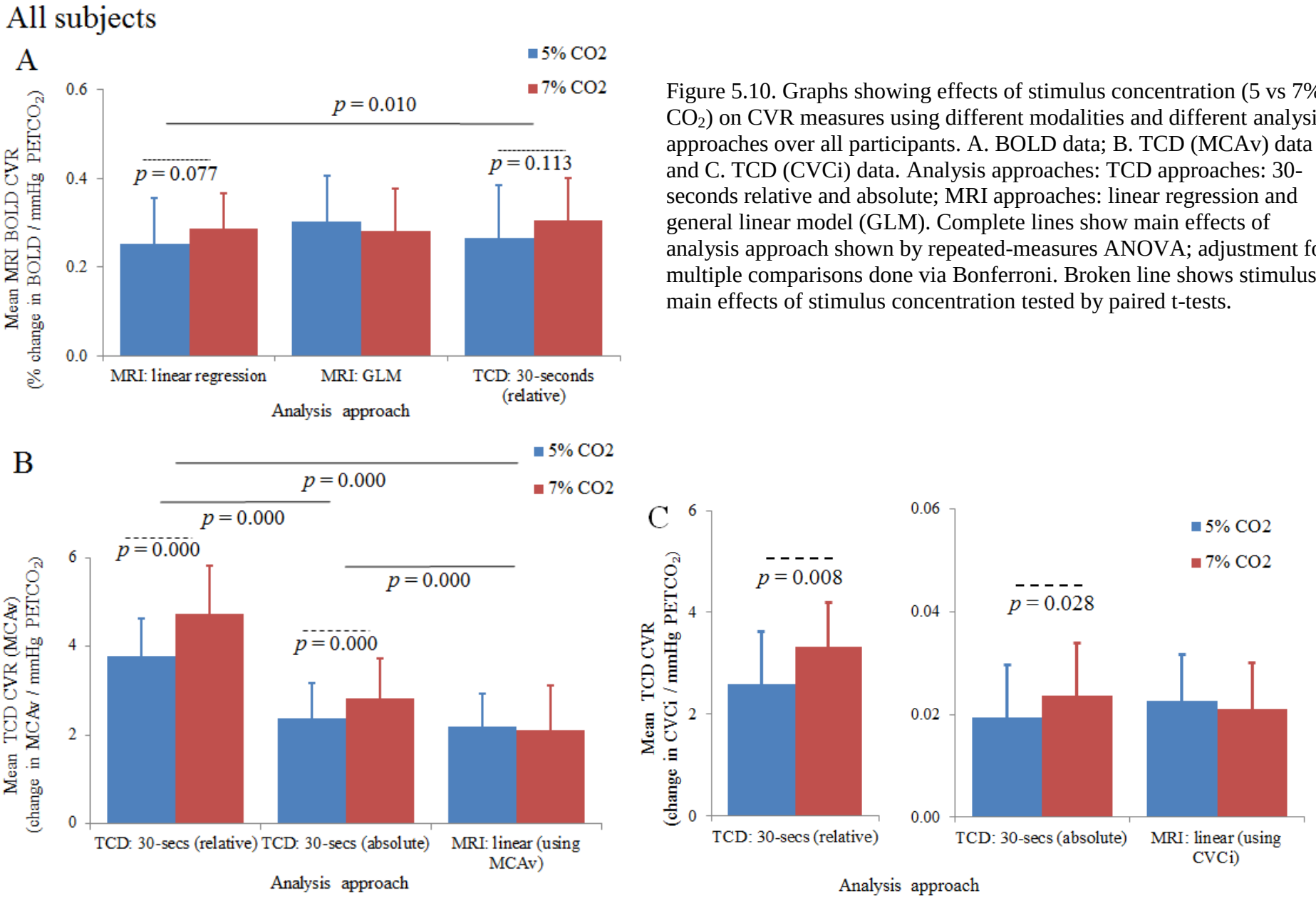



Figure 5.10. Graphs showing effects of stimulus concentration (5 vs 7% $CO_2$) on CVR measures using different modalities and different analysis approaches over all participants. A. BOLD data; B. TCD (MCAv) data and C. TCD (CVCi) data. Analysis approaches: TCD approaches: 30-seconds relative and absolute; MRI approaches: linear regression and general linear model (GLM). Complete lines show main effects of analysis approach shown by repeated-measures ANOVA; adjustment for multiple comparisons done via Bonferroni. Broken line shows stimulus main effects of stimulus concentration tested by paired t-tests.

*Effects of stimulus concentration:*

CVR measures correlated significantly between stimulus concentrations in most cases when using measures (BOLD and MCAv) with typical analysis approaches from the corresponding modality (all $p < 0.05$; Table 5.4), except the relative CVCi-CVR measure ($r = 0.214$). However, they did not correlate when a TCD approach (30-s steady state) was used to calculate CVR from MRI BOLD data ($r = 0.305$). Although there were significant correlations between CVR measures from different stimulus concentrations, the magnitude of the CVR outcome value was significantly different for the TCD modality whereas the stimulus concentration had less effect on the CVR measure for the MRI modality (Table 5.4, Figure 5.4).

Table 5.4. Correlations and paired sample t test between 5% and 7% $CO_2$ CVR measures from MRI and TCD data modalities calculated using typical analysis approaches.

| Modality | Analysis approach | n | Correlation (Pearson's *r*) | Paired sample *t*-test (*t*) |
|---|---|---|---|---|
| TCD | MRI: linear regression (MCAv) | 28 | 0.579** | 0.596 |
| | MRI: linear regression (CVCi) | 28 | 0.596** | 0.955 |
| | TCD: 30-s steady state (MCAv relative) | 28 | 0.502* | -5.08** |
| | TCD: 30-s steady state (MCAv absolute) | 28 | 0.828** | -4.573** |
| | TCD: 30-s steady state (CVCi relative) | 28 | 0.214 | -2.88* |
| | TCD: 30-s steady state (CVCi absolute) | 28 | 0.546** | -2.33* |
| MRI | MRI: linear regression | 28 | 0.442* | $-1.84^{t}$ |
| | MRI: GLM | 28 | 0.517** | 1.15 |
| | TCD: 30-s steady state | 28 | 0.305 | -1.64 |

Abbreviations: CVCi, cerebrovascular conductance; GLM, general linear model; MCAv, middle cerebral artery blood velocity; TCD, transcranial Doppler.
* $p \leq 0.05$; ** $p \leq 0.01$.$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

When comparing CVR outcome measures obtained from the 5% and 7% $CO_2$ stimulus concentrations, paired t-tests showed no difference for measures calculated from the MRI data modality (all $p > 0.05$). However, when looking at data from the TCD modality, all measures calculated using typical TCD analysis approaches showed significant differences between stimulus concentrations ($p < 0.05$) (Figure 5.10, Table 5.4).

Correlations between all data acquisition and analysis approaches on the CVR measures in the same participants are shown in Table 5.5. Generally, CVR measures were more likely to correlate if the same imaging modality had been used to acquire the data, regardless of the analysis approach used (Figure 10; Table 5.5, white blocks). Where CVR measures were correlated between different imaging modalities they were less likely to correlate (Table 5.5, orange block). As shown in Table 5.4 and Table 5.5, most CVR measures calculated using a 5% or 7% $CO_2$ stimulus concentration correlated if the same imaging modality and analysis approach was used (Table 5.5, blue values). As shown in Figure 5.9, there were no correlations between CVRs calculated using TCD or MRI modalities with typical analysis approaches (Table 5.5, red values).

Table 5.5. Correlations between age, fitness, and the measures of CVR calculated using TCD and MRI brain imaging modalities and analysis approaches.

| | | TCD data (MCAv) | | | | | | | | | | | | MRI data (BOLD) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MRI approaches | | | | TCD approaches | | | | | | | | MRI approaches | | | | TCD approaches | |
| | age | LR MCAv 5% | LR MCAv 7% | LR CVCi 5% | LR CVCi 7% | MCAv relative 5% | MCAv relative 7% | MCAv absolute 5% | MCAv absolute 7% | CVCi relative 5% | CVCi relative 7% | CVCi absolute 5% | CVCi absolute 7% | LR BOLD 5% | LR BOLD 7% | GLM GM 5% | GLM GM 7% | 30-seconds 5% | 30- seconds 7% |
| age | _ | | | | | | | | | | | | | | | | | | |
| V̇O2 max | -0.45* | | | | | | | | | | | | | | | | | | |
| LR MCAv 5% | -0.08 | _ | | | | | | | | | | | | | | | | | |
| LR MCAv 7% | 0.10 | 0.58** | _ | | | | | | | | | | | | | | | | |
| LR CVCi 5% | -0.12 | 0.56** | 0.30 | _ | | | | | | | | | | | | | | | |
| LR CVCi 7% | -0.33 | 0.71** | 0.53** | 0.59** | _ | | | | | | | | | | | | | | |
| MCAv relative 5% | 0.50** | 0.40* | 0.19 | 0.32 | 0.16 | _ | | | | | | | | | | | | | |
| MCAv relative 7% | 0.19 | 0.54** | 0.45* | 0.38* | 0.37t | 0.50** | _ | | | | | | | | | | | | |
| MCAv absolute 5% | 0.04 | 0.86** | 0.35 | 0.57** | 0.63** | 0.65** | 0.43* | _ | | | | | | | | | | | |
| MCAv absolute 7% | -0.13 | 0.91** | 0.51** | 0.53** | 0.73** | 0.35 | 0.68** | 0.83** | _ | | | | | | | | | | |
| CVCi relative 5% | -0.66 | 0.63** | 0.43* | 0.60** | 0.54** | 0.58** | 0.36 | 0.63** | 0.46* | _ | | | | | | | | | |
| CVCi relative 7% | 0.06 | 0.26 | 0.25 | 0.21 | 0.19 | -0.02 | 0.37 | -0.01 | 0.20 | 0.21 | _ | | | | | | | | |
| CVCi absolute 5% | -0.07 | 0.27 | 0.35 | 0.38* | 0.24 | 0.31 | 0.16 | 0.23 | 0.11 | 0.74** | -0.02 | _ | | | | | | | |
| CVCi absolute 7% | -0.14 | 0.49** | 0.49** | 0.48** | 0.44** | 0.01 | 0.45* | 0.22 | 0.45* | 0.37 | 0.48* | 0.55** | _ | | | | | | |
| LR BOLD 5% | -0.41* | 0.20 | -0.25 | 0.30 | 0.29 | 0.01 | -0.12 | 0.32 | 0.28 | 0.02 | -0.17 | -0.32 | -0.24 | _ | | | | | |
| LR BOLD 7% | -0.41* | 0.21 | 0.18 | -0.34 | 0.25 | 0.15 | -0.07 | 0.10 | 0.18 | -0.07 | -0.18 | 0.03 | -0.19 | 0.44* | _ | | | | |
| GLM GM 5% | -0.15 | 0.01 | 0.32 | -0.01 | -0.03 | 0.03 | 0.05 | -0.09 | 0.01 | 0.17 | -0.18 | 0.06 | -0.14 | 0.22 | 0.47* | _ | | | |
| GLM GM 7% | -0.28 | 0.27 | 0.22 | 0.16 | 0.29 | 0.16 | 0.30 | 0.28 | 0.41* | 0.16 | -0.09 | 0.02 | -0.03 | 0.40* | 0.56** | 0.52** | _ | | |
| 30-secs 5% | -0.38* | 0.16 | -0.25 | 0.34 | 0.24 | 0.09 | -0.20 | 0.30 | 0.16 | 0.32 | -0.30 | -0.17 | -0.15 | 0.89** | 0.28 | 0.17 | 0.30 | _ | |
| 30-secs 7% | -0.42* | 0.17 | 0.04 | -0.00 | 0.20 | -0.17 | -0.16 | 0.13 | 0.15 | 0.12 | -0.32 | 0.07 | -0.19 | 0.47* | 0.95** | 0.28 | 0.47* | 0.31 | _ |

Values represent Pearson's *r* correlations. Significance (2-tailed): * $p \leq 0.05$; ** $p \leq 0.01$. t Shows a trend towards significance: $0.05 < p \leq 0.1$. Correlations in the orange block show CVR measures from TCD versus MRI data modality. Red numbers show correlations between TCD and MRI measures using typical analysis approaches. Blue numbers show correlations between 5 and 7% $CO_2$ from the same modality and approach. Abbreviations: LR; linear regression (pre-processing), MCAv; middle cerebral artery blood velocity, CVCi, cerebrovascular conductance; BOLD, blood-oxygen-level dependent signal, GLM, general linear model (post-processing), GM, grey matter

## 5.5. Discussion

The overall aim of this chapter was to determine whether differences in neuroimaging modality (i.e., MRI/ TCD) would influence the CVR outcome measure and its interpretation. To do this, associations between different measures of CVR within and between imaging modalities and different analysis approaches were examined (i.e., MRI: linear regressions and GLM for all GM and RoIs; and TCD: 30-s of steady-state data extracted for relative and absolute CVR measures and CVCi derived CVR) for groups of individuals where differences in CVR were expected. Specifically, group differences in CVR outcome measures for younger versus older and fit versus unfit individuals were compared and outcome measures were correlated with age and fitness. Collectively, these findings demonstrated that the imaging modality used (TCD versus MRI) as well as the stimulus concentration (5% versus 7% $CO_2$) will significantly affect the calculated CVR outcome measure. The implications of these findings is that studies using different imaging modalities or different stimulus concentrations to determine the CVR measure cannot be easily compared to one another.

These findings showed directional discrepancies between age groups when using different neuroimaging modalities (Figure 5.4). MRI-derived CVR measures were elevated in the younger group compared to the older group, in line with other research studies (Bailey et al., 2013; Guiney et al., 2015). However, TCD CVR measures were lower in the younger group compared to the older group. It may be that large-scale studies (for example, Bailey and colleagues study included 81 participants) are more likely to report higher mean CVR measures at the population level, while studies such as the present with smaller samples sizes are more susceptible to individual variability. Therefore, study sample size is another factor that should be considered when comparing findings across studies.

Participants with higher aerobic fitness showed a blunted response in CVR when measured with MRI (similar to Thomas et al., 2013). However, this was in the younger group and not the older group as found from Thomas and colleagues study looking at Masters Athletes (Thomas et al., 2013). The differences between fitness (V̇O2 max) between fit and unfit groups in the current study were not as large as reported in other studies. For example, participants in Bailey and colleagues (2013) study had a mean V̇O2 max score of 62 and 33 $mL \cdot kg^{-1} \cdot min^{-1}$ for the younger 'trained' and 'sedentary' groups, respectively (compared to 55 and 36 $mL \cdot kg^{-1} \cdot min^{-1}$ in the present study) and 40 and 24 $mL \cdot kg^{-1} \cdot min^{-1}$ for the older 'trained' and 'sedentary' groups respectively (compared to 45 and 20 $mL \cdot kg^{-1} \cdot min^{-1}$ in the present study). Although the older group were more similarly defined, there were fewer of them (12 vs. 42 older participants in total). An important difference in this study is that 'fit' and 'sedentary' individuals were defined by reported lifetime physical activity levels (trained >150 minutes recreational aerobic activity per week versus sedentary, no activity) and V̇O2 max was calculated after group assignment. Further, sex differences may explain the unexpected CVR results given that females typically have higher resting CBF (see Chapter 4), and the present study recruited more males than females, and more of the fit group were male and the unfit group were female. However, this area of research has also reported conflicting findings where either males or females have been reported to have greater CVR depending on the modality used. For example, in an MRI study using BOLD, a 22% greater response to $CO_2$ was reported in males compared to females (Kassner et al., 2010).

No significant correlations between CVR outcome measures obtained using TCD and MRI modalities with their typical analysis approaches for all of the stimulus concentrations were observed (Figure 5.8), though the correlation between the modalities was stronger for

the 7% $CO_2$ ($r$ = 0.303) than the 5% $CO_2$ ($r$ = 0.033) stimulus. This higher correlation may be explained by an increased signal-to-noise ratio within the TCD modality when the 7% $CO_2$ concentration is used, and may also be because the typical MRI analysis approach used the entire gas challenge whereas the typical TCD approach calculates CVR from one gas concentration.

An important point to consider is that discrepancies between modalities may be explained by the technology targeting different areas of the vascular bed; i.e., TCD is imaging a precise location within the centre of the blood vessel (in this case the MCA) whereas MRI BOLD is imaging changes (global or regional) further down the vascular tree within micro-vessels. Larger vessels such as the MCA may also be more affected by changes in blood pressure compared to the cerebral capillaries. Given the known pressure effect of $CO_2$ on MCAv (Battisti-Charbonney et al., 2011), the differences seen between 5 and 7% with TCD but not MRI are likely explained by the targeted vasculature used to determine CVR. Further, in this study, the CVR measures correlated better with the 7% $CO_2$ challenges. This could be because MRI signal changes are longer and will also drive GLM BOLD fits.

Comparing typical analysis approaches within the same modality showed that typical conventional approaches were closely matched to the alternative modality (i.e., using 30-s steady state extracted data from the BOLD data and calculating CVR using a linear regression with the TCD data). Although significant differences were observed between calculated CVR when comparing analysis approaches and stimulus concentrations within the same modality (Figure 5.4), the measures did significantly correlate in most cases for a given imaging modality (Table 5.3 and Figure 5.10). Correlations were highest in the TCD

modality where TCD analysis approaches were used, indicating that most of the variability is removed when using this approach.

It was also considered whether more variability in the MRI modality data could be explained by movement parameters. Analysis of motion parameter measures between age and fitness groups was performed to determine whether they were significantly different, and could have influenced the CVR measure calculated by MRI GLM. On visual observation, participants displayed more movement during the 7% than the 5% $CO_2$ stimulus concentration. However, no statistically significant differences in movement parameters between different age or fitness groups were observed, indicating that the increased variability in the MRI CVR measures is caused by other factors. Although movement was not recorded with TCD, due to the way TCD probes are secured to the head it could be argued that the TCD modality is less affected by movement artefacts. Certainly a more multifactorial approach in determining CVR that considers other physiological changes that occur in response to a stimulus may be beneficial and lead to less conflicting findings.

Another explanation for conflicting findings in the literature and confusion around the CVR measure, as well as different methodological approaches, may be explained by a publication bias where studies that find no association between fitness (as determined by V̇O2 max) and CVR fail to be reported. Reasons for this could be that the lack of findings is explained by limitations within the study such as small sample sizes, rather than addressing the issue of the different methodological approaches being used leading to different interpretations of the CVR measure. Therefore, further investigation into different

methodological approaches is required to examine variability observed in the CVR outcome measure.

### 5.5.1. Limitations

In this study the sample sizes became quite small after the age groups had further been divided into sub-groups of fit and unfit (8 younger fit and 8 unfit; 7 older fit and 5 unfit) compared with previously published studies (Ainslie et al., 2008; Bailey et al., 2013). The reason for this is that this study required a large amount of data to be collected on each participant due to the modality comparison limiting the total number of participants that could be recruited and data acquired from in the time frame available. Another consideration is the use of maximal oxygen consumption as a measure of fitness and how motivation may influence this measure. Particularly in individuals who exercise less, they may be less motivated or less physiologically aware of their bodies, so despite receiving the same verbal encouragement, may be less likely to reach their V̇O2 max. One way to address this issue is to use other methods of assessing fitness such as questionnaires (see Chapter 6, Section 6.2.2, where the New Zealand physical activity readiness questionnaire (NZPARQ) is discussed in more detail). However, the issue with these was that the age groups differed in the method of interpreting the questionnaire (i.e., 7-day activity frequency, self-rated activity rating, or activity category), such that fitness effects were not consistent across different methods of calculating fitness. Another approach may be to assess participant motivation at performing well in the fitness test with a questionnaire, similar to the Situational Motivational Scale (SIMS) (Guay et al., 2000), though this would need to be modified slightly to fit the context of the fitness test.

### 5.5.2. Perspectives and Significance

In addition to setting out gold-standard guidelines to determine CVR, a clearer understanding around what the different methodological approaches are revealing about cerebrovascular health is required, and more descriptive terminology needed to describe what, where and how CVR is being measured. The currently used term 'CVR' for 'cerebrovascular reactivity' (also described as 'CBF-$CO_2$ responsiveness') to describe this outcome measure fails to recognise its complexity and what it means in the context of health and disease. It may be that certain blood vessels (i.e., macro versus micro) and locations within the vascular tree will be more relevant for certain disease pathologies and stages of disease progression. This will therefore determine the most appropriate modality to use (i.e., MRI BOLD or TCD MCAv) on an individual basis.

### 5.5.3. Conclusion and Recommendations

The findings in this chapter support recent literature reporting conflicting interpretations of CVR in healthy ageing, fitness and neurodegenerative conditions, making the fidelity of this biomarker problematic. This study demonstrated that discrepancies arise from the range of image acquisition and analysis approaches used to determine CVR, making direct comparison questionable. A validated 'gold standard' test across imaging modalities is needed. Internationally, few research groups can directly compare CVR across imaging modalities, due to limited access and/or expertise. Our findings showed no significant correlation in the standard CVR measures across modalities (TCD MCAv-derived measures and MRI BOLD-derived measures), raising the question of why this arises given the two techniques are theorised to provide similar measures of brain vascular function. We hypothesize this is due to over-simplistic standard literature methods employed, which may combine with differences in standard analysis strategies across

techniques (e.g., GLM using all data collected in MRI-BOLD analysis versus using a small subset of the data collected (30-60s once a steady state is reached compared with a short baseline measure) and calculating a fractional change in these data in TCD analysis). This hypothesis needs to be tested with advanced imaging and analysis techniques on larger cohorts with intermediatory measures taken such as CBF measures using MRI phase contrast (PC) angiography analysis (Stoquart-ElSankari et al., 2009; Verbree et al., 2014) and Duplex Doppler ultrasound (Willie et al., 2014), where vessel diameter and movement can be accounted for more reliably.

# 6. COGNITIVE PERFORMANCE AND QUALITY OF LIFE

## 6.1. Abstract

**Background**: The incidence of dementia and age-related neurodegenerative disease is increasing in line with an ageing and increasingly sedentary population. Recent studies have shown that physical activity benefits the brain and can protect against factors associated with neurodegeneration. Dementia and natural ageing are associated with impaired performance on cognitive tasks that rely on the prefrontal cortex (e.g., attention switching task (AST) and mental arithmetic). Further, neurodegenerative conditions drastically impair quality of life (QoL) and well-being. This study investigated the effects of age and aerobic fitness on brain health outcome measures including tasks assessing cognitive performance and questionnaires assessing quality of life (QoL).

**Methods:** During separate visits, thirty-six healthy volunteers (nineteen younger: 25±7 yrs, seventeen older: 67±7 yrs) completed an aerobic fitness test, cognitive tasks (three pen and paper tasks, five tablet-based tasks using Cambridge Cognition CANTAB software, and a mental arithmetic task), two quality of life questionnaires and the PASAT mental arithmetic task. ANOVAs and correlations were used to examine the effects of age and fitness. The cognitive tasks included: attention switching, paired associated learning, working memory, mental arithmetic, digit-decoding and verbal fluency.

**Results:** Cognitive performance was lower in the older group compared to the younger group in attention switching (by 18%), learning (by 65%) and spatial working memory (by 56%), whereas QoL was higher. In the older group, attention switching, and mental arithmetic were significantly higher in the fit group versus the unfit group ($p<0.05$). Aerobic fitness significantly correlated with overall QoL and psychological subscale outcome measures in both age groups (Pearson's $r$ ranging from 0.410 to 0.527).

**Conclusions**: Age-related decline in attention switching was offset by physical fitness. Higher physical fitness also improved mental arithmetic in older participants. Further, physical fitness may significantly improve well-being and quality of life across ages. Taken together, findings lend further support to the evidence that physical activity may be protective against neurodegenerative disease.

## 6.2. Introduction

The incidence of dementia and age-related neurocognitive disorders is increasing in line with an ageing and increasingly sedentary population (Deary et al., 2009). Therefore, effective prevention and treatment strategies are desperately needed, as well as reliable tools to measure brain health. Brain health outcome measures include several different disciplinary approaches that are associated with neurocognitive disorders. These include, though are not limited to: measures of cerebrovascular health (e.g., resting cerebral blood flow (CBF), and functional cerebrovascular reactivity (CVR) to carbon dioxide); reviewed in Chapter 2 and investigated in Chapters 3-5), measures of cognitive function determined by performance in tasks that target specific domains (e.g., attention, mental arithmetic, memory, learning, verbal fluency and decoding), and measures of quality of life and well-being, often determined via questionnaire responses (including overall health scores as well scores in sub-domains focusing on psychological, physical, emotional, social and environmental aspects of a person's well-being). However, studies seldom combine these measures to assess brain health to determine possible associations between cognitive function and general well-being (i.e., QoL), in addition to ageing and fitness.

Cognition encompasses a range of higher executive brain functions that, when working optimally, are considered to enable humans to perform at their best in professional and personal endeavours. Examples of these higher cognitive abilities include: the ability to switch attention between tasks; retain information in short-term memory; make effective use of strategy, and fast and effective learning. Performance in these tasks is known to naturally decline during healthy ageing and impairment is associated with neurological and psychiatric conditions including dementia, stroke and depression (see Voss et al., 2011). Thus, tasks like these can be used as important outcome measures to reveal possible therapeutic effects of targeted interventions and have also been used to pre-screen individuals for early Alzheimer's disease in clinical trials (Barnett et al., 2016; Nathan et al., 2017).

Aerobic fitness has been associated with improved cognition in several cross-sectional and intervention studies in humans and animals (Kramer et al., 1999; Vaughan et al., 2014; Barcelos et al., 2015). Further, interventions involving physical *and* cognitive activity (e.g., motor tasks requiring sustained attention and concentration) have been investigated in humans and revealed improvements in cognition (Vaughan et al., 2014; Barcelos et al., 2015). In Vaughan and colleague's randomised controlled trial they investigated the effects of a 16-week multimodal exercise programme that included cardiovascular, strength conditioning and motor fitness training. This study was performed in older women and they found improvements in cognition, including: working memory; inhibition; shifting; verbal fluency, and reaction times. This was in addition to improvements in physical function measured by a six-minute walk test and timed up and go. Similarly, Barcelos and colleagues (2015) examined a combination of stressors by observing the effects of physical exercise (cycling) whilst undertaking cognitive tasks of

varying loads (virtual bike tour and video gaming). They found that everyday function improved in both conditions, though those in the high cognitive demand group performed better than those in the low cognitive demand condition; providing support to further 'stressing' the system by using multiple approaches and the additive benefits that can occur in a dose-dependent manner. Such findings are consistent with observations from cross-sectional studies (Eskes et al., 2010), where the diversity of cognitively stimulating activities was an independent predictor of cognitive function in older (female) adults, and that there was an additive effect on neuropsychological performance with the combination of fitness, cerebrovascular reserve and cognitive stimulation. Research should further explore the underlying mechanisms driving these adaptations with additional cognitive loading, as well as focusing on specific brain areas affected through neurodegenerative disease.

The beneficial role of exercise for brain health in clinical populations and older adults has been demonstrated in numerous studies. For example, physically active dementia patients may deteriorate at a slower rate than their sedentary counterparts (Buchner et al., 2007; Zschucke et al., 2013). Grey matter hippocampal volume and performance on memory tasks has also been positively correlated with physical exercise in healthy adult human populations and those at risk for dementia (Erickson et al., 2011; Killgore et al., 2013). While such findings indicate that exercise improves markers of brain health (e.g., brain volume and memory performance), many tend to use self-report questionnaires to assess levels of exercise. These may be less reliable than quantitative measures such as a maximal oxygen consumption ($\dot{V}O_2$ max) test.

Quality of life (QoL) and well-being outcome measures also reveal important information about the subjective experience of individuals in the face of debilitating conditions. The measurement of health-related quality of life for people with dementia (DEMQOL) is a quality of life questionnaire carefully adapted specifically for dementia patients (Smith et al., 2007). The questionnaire considers language and mental capacity barriers elicited by the disease, and has a reported reliability coefficient of 0.82 (Whitaker et al., 2014). A randomised controlled trial using this measure has shown improvements in well-being in dementia patients in care homes following non-pharmacological interventions that included exercise (Ballard et al., 2016). In populations like these, performance in cognitive tasks may no longer be considered a primary outcome measure since cognitive impairment is so severe and rapidly deteriorating. Alternatively, the subjective well-being of these patients is arguably far more important, where tests of cognition are likely to miss important factors (Banerjee et al., 2006).

Similar questionnaires have also been used in the general population, including the World Health Organisation Quality of Life (WHOQOL) questionnaire (WHOQOL group, 1998) and RAND-36 (Hays et al., 1993), which have been shown to have excellent psychometric properties of reliability and perform well in tests of validity (Skevington et al., 2004; Vlander Zee et al., 1996). The literature reports mixed findings on the effects of healthy ageing on such QoL measures, where some studies report QoL decreases with ageing (Demura et al., 2003) and this is further exacerbated by sex where depression increases more in older-old (>74 years) females compared to males. In contrast, other studies have reported that older respondents display lower levels of negative affect than their young-old counterparts (Mroczek et al., 1998), indicating there is an improvement in well-being among older people. In a clinical context, ageing is an accompanying factor alongside

clinical deterioration that will be associated with reduced QoL revealed by the DEMQOL, though has been shown to be offset by physical activity (Ballard et al., 2016). Further, in healthy populations, physical activity levels have been associated with improved well-being in younger (Hinkley et al., 2014) and older (meta-analysis of 36 studies) healthy individuals (Netz et al., 2005).

In summary, a wealth of evidence has emerged in support of the effects of physical activity and improved cardiorespiratory fitness on brain health outcome measures relating to cognition and QoL. Interventions involving physical activity have been shown to improve such measures in healthy and clinical populations (Gillison et al., 2009), though the underlying mechanisms are poorly understood (Lucas et al., 2015). Further, little research has been done investigating a combination of brain health outcome measures including cognitive function and QoL in the same individuals; or considered how they may differ between significantly different age groups (some of the findings in the studies discussed compare 'young-old' with 'old-old' rather than two samples with a larger age-gap) with significantly different levels of fitness determined using both quantitative and qualitative measures (i.e., with V̇O2 max test and a physical activity questionnaire).

### 6.2.1. Study Aims and Hypotheses

Therefore, the overall aims of this cross-sectional study were to examine whether differences in brain health measures, including several measures of cognitive function and QoL, will be observed in healthy younger and older individuals with varying levels of physical fitness (determined by V̇O2 max and the New Zealand physical activity readiness questionnaire (NZPARQ)). These outcome measures included: cognitive function (attention, memory, learning and mental arithmetic) and QoL. Further, this study aimed to

provide evidence that physical activity can be a first-call consideration that is beneficial and neuroprotective to individuals across the lifespan, and may help protect against neurodegenerative conditions.

It was hypothesised that the brain health outcome measures would differ between younger and older groups, such that measures of cognitive performance would decline with age in line with existing research, and measures of QoL would be different between age groups. However, the directionality of QoL measures hypothesis was 2-tailed due to the discrepancies in the existing scientific evidence. In addition, it was hypothesised that differences between fitness groups would be observed in our brain health outcome measures, such that both cognitive and QoL outcome measures would improve with increased aerobic fitness (as determined by V̇O2 max and/ or NZPARQ).

### 6.3. Materials and Methods

Thirty-six healthy volunteers in two age groups participated, 19 younger participants (age, 25 ± 7 years) and 17 older participants (age, 67 ± 7 years). The age groups were further divided into fit and unfit groups, determined by performance on a maximal aerobic (i.e., V̇O2 max) fitness test (younger group V̇O2 max: 9 fit >45 $mL \cdot kg^{-1} \cdot min^{-1}$ vs. 10 unfit <45 $mL \cdot kg^{-1} \cdot min^{-1}$; older group V̇O2 max: 10 fit >25 $mL \cdot kg^{-1} \cdot min^{-1}$ vs. 7 unfit <25 $mL \cdot kg^{-1} \cdot min^{-1}$). Ethical approval was obtained for all experimental protocols and procedures by the University of Birmingham Ethics Committee and the study conformed to the Declaration of Helsinki (ethics code: ERN_14-1423). Participant visits took place at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham.

Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent to participation was obtained.

In summary (described in more detail below), during separate visits, participants completed: 1) an electrocardiogram (ECG) and general health screening; 2) aerobic fitness assessment (including self-reported frequencies and a maximal oxygen uptake assessment ($\dot{V}O2$ max)); 3) familiarisation in cerebrovascular measures; 4) collection of cerebrovascular measures (data presented in Chapters 4 and 5); 5) cognitive outcome measures (including 3 pen and paper tasks and 5 tablet-based tasks), 6) a mental arithmetic task, and 7) two QoL questionnaires. Prior to all testing visits, participants were asked to abstain from heavy physical exercise and alcohol for 24 hours. For all visits, participants were asked to avoid vigorous exercise and alcohol 24 hours prior to study participation, caffeine for 12 hours and heavy meals for 4 hours prior.

### 6.2.1. Electrocardiogram (ECG) and General Health Screening

All participants underwent a pre-exercise evaluation prior to the exercise testing and completed a general health questionnaire. Participants over fifty years of age also completed a resting 12-lead electrocardiogram (ECG) assessment and a resting blood pressure measurement, which was reviewed by a cardiologist. Participants who revealed a contraindication to non-medically supervised exercise testing in the general health questionnaire (e.g., family history of heart attack), had high resting blood pressure (systolic >160, diastolic >90), or showed ECG abnormalities (e.g., S-T suppression, multiple ectopic beats in a row (i.e., >3)) were excluded from the aerobic fitness testing (and referred on to their GP).

### 6.2.2. Aerobic Fitness Assessment

Measures of aerobic fitness included maximal oxygen consumption (V̇O2 max) and measures scored from the New Zealand Physical Activity and Readiness Questionnaire (NZPARQ). This particular questionnaire was chosen as previous research identified associations between the NZPARQ and CBF-$CO_2$ responsiveness measures (Guiney et al., 2014). After screening and inclusion into the study all participants completed a maximal aerobic fitness test to determine V̇O2 max (procedure described in Chapter 4, section 4.3.3).

Three measures of activity were scored from the NZPARQ completed by participants, these were: 1) last 7-days frequency of activity (0-7 days); 2) self-rated activity category (1-5; 1 being the least active to 5 being the most active), and 3) activity category (1-3; assessed by adding together participant responses to frequency of mild, moderate and intense activity; category 1 being the least active and category 3 being the most active).

### 6.2.3. Cognitive Performance Outcome Measures

Firstly, participants completed three pen and paper tasks administered by the PhD candidate. These included the Hopkins verbal learning task (HVLT; Brandt 1991), the verbal fluency task and a digit decoding task. These tests were selected due to previously established associations between performance scores and age-related conditions including dementia (Hogervorst 2014). The HVLT was scored by counting the total number of recalled items across the three trials with a total possible score of 36. Verbal fluency was scored by counting the number of animal names participants could say out loud in 60

seconds. Digit-coding was scored by counting how many numbers participants could successfully draw the corresponding code for in 90 seconds.

Secondly, participants completed a mental arithmetic task under stressful conditions using the paced auditory serial addition task (PASAT), where the total number of correct responses was calculated. Stress was induced by a combination of factors that were initiated in the same way across participants. These included: the paced serial addition increasing in speed during the duration of the task; the sound of a buzzer indicating an incorrect answer or too much hesitation; the PhD candidate explaining that they were competing against other participants in the study and their results would be displayed in tables; participants being required to watch themselves on a TV screen throughout whilst being recorded, and the PhD candidate explaining to them that their body language would be assessed by two independent body language experts. Throughout the task heart rate, blood pressure and ECG were continuously measured. A 4-minute resting baseline preceded the 4-minute mental arithmetic task, which was then followed by a 4-minute recovery period.

Finally, participants completed five tablet-based cognitive tasks on the CANTAB® Cognitive assessment software (Cambridge Cognition, UK). Tasks were administered in the same order across participants in a quiet laboratory room where they were not disturbed. Completion of the tasks took approximately 30-40 minutes. The tests included: reaction time (RTI); attention switching task (AST), paired associates learning (PAL), spatial working memory (SWM), and the emotion recognition task (ERT) (Figure 6.1).

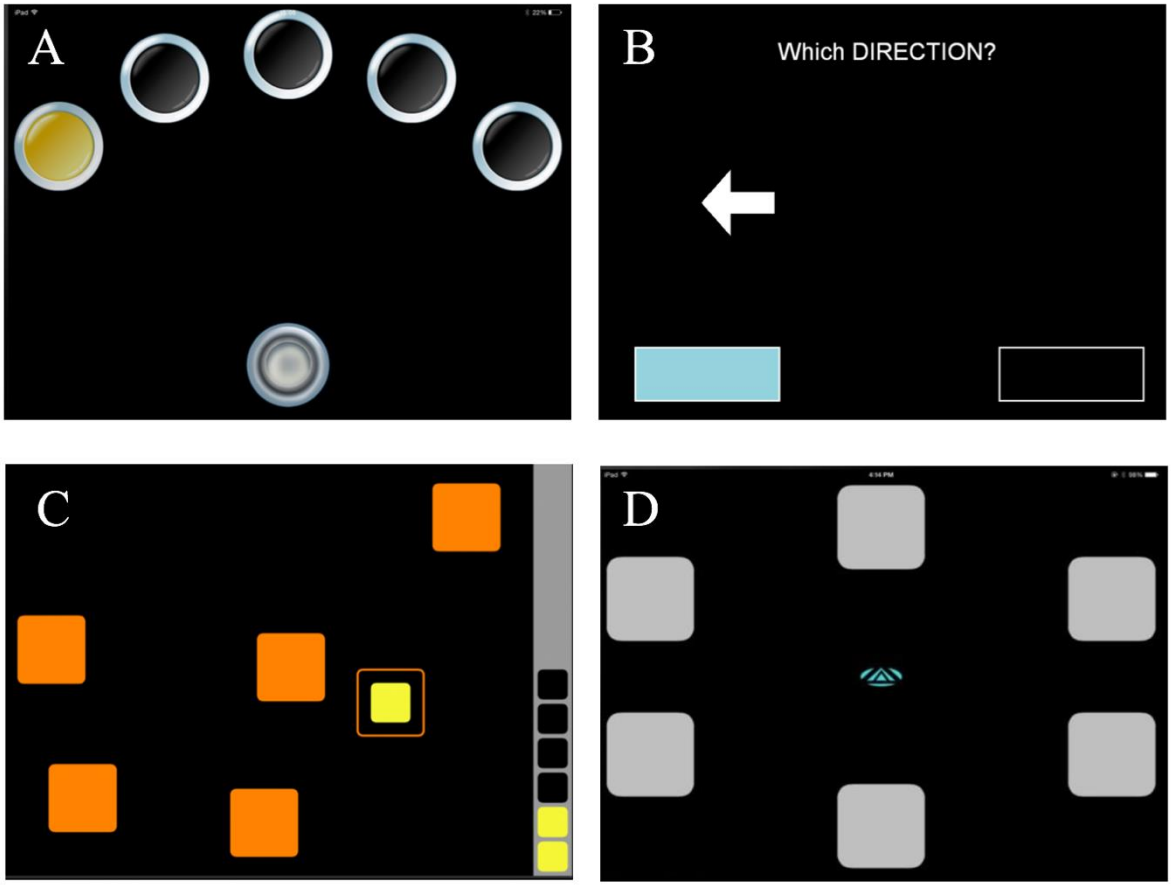



Figure 6.1. Examples of each of the CANTAB tasks. A. Reaction time (RTI); B. Attention switching task (AST); C. Spatial working memory task (SWM), and D. Paired associates learning (PAL) task.

The RTI, AST, SWM and PAL have been associated with ageing and neurodegenerative disease in several studies (Barnett 2016; Nathan 2017) and form part of the CANTAB Connect cognitive assessment for Alzheimer's clinical trials. The main outcome measures for the CANTAB tasks involved assessing the number of correct and incorrect responses and reaction latencies (in ms) in several conditions.

During the RTI, participants were required to respond as quickly as possible by moving the finger from a starting position, to a circle that would appear at the top of the screen, and then returning to the starting position. Outcome measures included the number of correct

responses and reaction times (i.e., latency) during several conditions including: mean simple reaction time (time taken from the onset of the circle to the participant pressing on the circle) and mean simple movement time (time taken from when the participant's finger left the starting position to touching the circle) for trials where the stimulus would appear in one location only and in trials where the stimulus could appear in one of five locations (five-choice reaction/movement time).

The AST involved responding as quickly as possible to stimuli on the screen whilst ignoring conflicting information. Conditions included: congruent, incongruent, non-attention switching, attention switching, and responding to either the side or direction of stimuli whilst ignoring irrelevant stimulus information (i.e., a distractor). As with the RTI, outcome measures included number of correct responses and reaction times/latency. The main outcome measures from the AST included in the analysis for this study were: total correct (TC); total incorrect (TI); mean reaction latency during congruent trials (RLC); mean reaction latency during incongruent trails (RLI); congruent errors (CE); incongruent errors (IE); congruency cost (CC); omission errors (OE); commission errors (CE); mean reaction latency (RL); mean reaction latency from non-switching trials (RLN), and mean reaction latency from switching trials (RLS).

Outcome measures relating to attention switching between side and direction included: total side errors (SE); mean side reaction latency (SRL); total direction errors (DE), and mean direction reaction latency (DRL). The CANTAB software also calculated congruency cost and switching cost using algorithms. The congruency cost algorithm calculated the difference between the mean reaction time of a response (from stimulus appearance to button press) on the trials that were congruent versus the trials that were

incongruent. This was calculated by subtracting the mean congruent latency from the mean incongruent latency. Values close to zero indicate less variation in reaction times across congruent and incongruent trials. A positive value indicates that the participant is faster on congruent trials and a negative score indicates that the participant is faster on incongruent trials. The switching cost algorithm calculated the difference between the mean reaction time (from stimulus appearance to button press) during assessed blocks where the rule is switching, versus assessed blocks in which the rule remains constant. This was calculated by subtracting the mean latency of response during non-switching block from the mean latency of response during switching blocks. Values close to zero indicated less variation in latencies across non-switch and switch trials. A positive score indicated that the participant responded more quickly in non-switching blocks.

The SWM task began with a number of coloured squares that represented boxes. The participant needed to find a yellow token in each of a number of boxes and use them to fill up the empty column on the right-hand side of the screen by selecting the boxes and using a process of elimination. The number of boxes increased to make the task more difficult until the participant failed a level. The colour and position of the boxes used were changed from trial to trial to discourage the use of stereotyped search strategies. The main outcome measures for the SWM were working memory defined by the number of errors, and higher executive function defined by use of strategy. Use of strategy refers to where participants used distinct boxes to begin a new search, rather than returning to a previously opened box where they had already seen the contents.

The PAL task involved boxes that were displayed on the screen and opened by the participants in a randomised order. One or more of them contained a pattern. The patterns

were then displayed in the middle of the screen, one at a time and the participant had to select the box in which the pattern was originally located. If the participant made an error, the boxes were all opened in sequence to remind the participant of the locations of the patterns. The outcome measures for the PAL task were the total adjusted errors calculated by the number of times the participant chose the incorrect box for a stimulus on assessment problems, but with an adjustment for the estimated number of errors they would have made on any problems, attempts and recalls they did not reach due to failing the test. Other measures included in this analysis were: the first attempt memory score; the total number of patterns reached, and the total number of errors before adjustment.

### 6.2.4. Quality of Life Outcome Measures

Quality of life was assessed using two questionnaires; the World Health Organisation Quality of Life (WHOQOL) questionnaire and the Research and Development 36-item short form survey instrument (RAND36). Participants completed the questionnaires in the same order in a quiet room with no interruptions. Prior to completing the questionnaires, the PhD candidate explained that all answers were anonymous, confidential, and that they did not have to answer anything they were not comfortable with. All participants completed both questionnaires in their entirety. Outcome measures included overall health scores and sub-scores focusing on specific domains (e.g., physical, psychological, social, etc.) for each of the questionnaires.

### 6.2.5. Statistical Analysis

T-tests were used to compare participant characteristics between groups. All mean outcome measures were calculated for the whole group, younger/ older groups, younger

fit/ unfit groups, and older fit and unfit groups and compared using one-way between subjects ANOVAs. Outcome measures were also correlated (Pearson's *r*) against age and across the whole group, while fitness was correlated against the outcome measures separately for the younger group and the older group. All data were analysed using SPSS statistical software (SPSS version 17.0, SPSS Inc., Chicago, IL). Statistical significance was established at a level of 0.05, and data are expressed as means (± standard deviation). Data were tested for normal distribution using Shapiro Wilks W Tests.

## 6.4. Results

Thirty-five participants completed the QoL questionnaire. Thirty-three participants completed the cognitive tasks, though three had to be repeated on a separate session due to software errors. Two participants did not complete the cognitive tasks due to availability. Thirty-one participants completed the mental arithmetic task (see Table 6.1 for participant characteristics).

### 6.4.1. Participant Characteristics and Fitness Assessment

Participant characteristics are summarised in Table 6.1. By design, there was a significant difference in age between the younger and older group, while fitness (determined by V̇O2 max) and resting blood pressure were higher and lower, respectively, in the younger compared to the older group. There was also a strong negative correlation between V̇O2 max and age (Figure 6.2), indicating that V̇O2 max declined with age. There were no differences between BMI, resting heart rate or NZPARQ scores between the younger and older groups.

In the younger participants, V̇O2 max was higher, resting heart rate lower, and NZPARQ self-rated activity rating and activity category higher in the younger fit group compared to the unfit group. There were no differences between age, BMI, blood pressure or NZPARQ 7-day activity frequency between the fit and unfit younger groups. In the older participants, V̇O2 max was higher, resting heart rate lower, and NZPARQ 7-day activity frequency higher in the older fit group compared to the unfit group. There were no differences between age, BMI, blood pressure, NZPARQ self-rated activity rating or activity category between fit and unfit older groups.

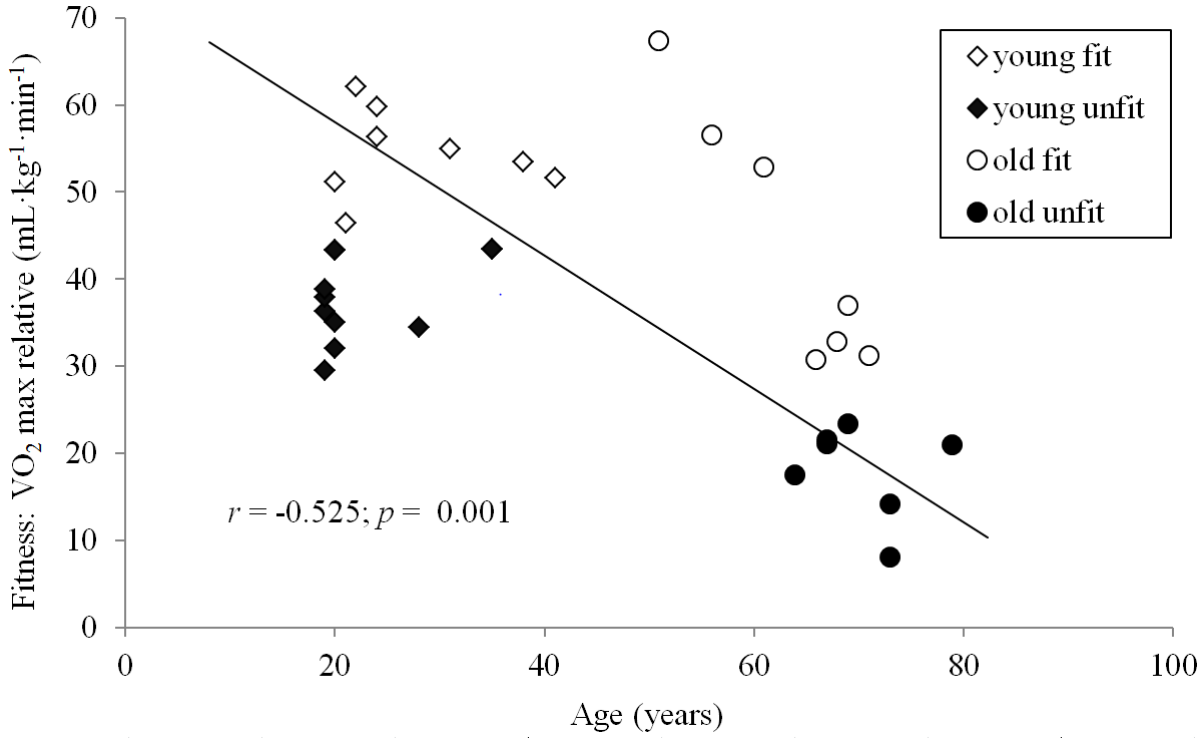



Figure 6.2. Correlation between age and physical fitness (V̇O2 max). Data illustrate that physical fitness declines with age.

Table 6.1. Characteristics of study participants.

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | *n* = 35 | Younger *n*= 19 | Older *n*= 16 | Younger fit *n*= 9 | Younger unfit *n* = 10 | Older fit *n* = 9 | Older unfit *n* = 7 |
| Age | 43.9 ± 22.4 | 24.6 ± 7.0 | **66.9 ± 6.7**** | 27.7 ± 7.6 | 21.9 ± 5.3 | 64.2 ± 6.8 | 70.3 ± 5.1 |
| Sex ratio (male: female) | 22:13 | 12:7 | 10:6 | 8:1 | 4:6 | 8:1 | 2:5 |
| Body mass index ($kg \cdot m^2$) | 23.6 ± 2.7 | 22.7 ± 2.3 | 24.8 ± 2.8 | 23.4 ± 2.3 | 22.0 ± 2.2 | 23.7 ± 1.3 | 25.7 ± 3.5 |
| V̇O2 max relative ($mL \cdot kg^{-1} \cdot min^{-}$ | 38.7 ±14.3 | 44.5 ± 9.8 | **32.1 ± 15.9*** | 53.8 ± 5.5 | **37.0 ± 4.5**†† | 42.3 ± 13.4 | **19.0 ± 6.3**†† |
| Resting systolic BP (mm Hg) | 120 ± 16 | 111 ± 12 | **130 ± 14**** | 115.0 ± 8.8 | 107.0 ± 13.9 | 128.6 ± 10.3 | 132.8 ± 17.9 |
| Resting diastolic BP (mm Hg) | 71 ± 9 | 67 ± 9 | **76 ± 7*** | 69 ± 10 | 66 ± 7 | 78 ± 4 | 73 ± 8 |
| Resting heart rate ($b \cdot min^{-1}$) | 59 ± 10 | 64 ± 10 | 56 ± 6 | 55 ± 8 | **68 ± 9**† | 52 ± 6 | **61 ± 3** † |
| PARQ_7 days activity freq. | 5.0 ± 2.0 | 5.1 ± 2.0 | 4.9 ± 2.0 | 6 ± 2 | 4.2 ± 2.1 | 6.0 ± 1.4 | **3.4 ± 1.8**† |
| PARQ_Self activity rating (1-5) | 4.6 ± 0.9 | 4.5 ± 1.0 | 4.8 ± 0.6 | 5.0 ± 0.0 | **1.2 ± 0.4**† | 5.0 ± 0.0 | 4.4 ± 0.9 |
| PARQ_Activity category (1-3) | 2.5 ± 0.7 | 2.7 ± 0.7 | 2.5 ± 0.7 | 3.0 ± 0.0 | **2.3 ± 0.9**† | 2.6 ± 0.5 | 1.8 ± 0.8 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (60-80 years). Fitness groups were defined as: younger fit (relative V̇O2 max > 45 $mL \cdot kg^{-1} \cdot min^{-1}$) and unfit (relative V̇O2 max < 45 $mL \cdot kg^{-1} \cdot min^{-1}$), older fit (relative V̇O2 max > 25 $mL \cdot kg^{-1} \cdot min^{-1}$) and unfit (relative V̇O2 max < 25 $mL \cdot kg^{-1} \cdot min^{-1}$). Abbreviations: V̇O2 max, maximal oxygen consumption; NZPARQ, New Zealand Physical Activity Readiness Questionnaire.

* Significant AGE effect (different from younger group): * $p \leq 0.01$; ** $p \leq 0.001$.

† Significant FITNESS effect (different from fit group): † $p \leq 0.01$; †† $p \leq 0.001$.

### 6.4.2. Cognition Outcome Measures

#### 6.4.2.1. Hopkins Verbal Learning Task, Verbal Fluency, Digit-Coding and Mental Arithmetic

No significant age or fitness effects were observed using the HVLT ($p$ = 0.878 and $p$ = 0.785, respectively), verbal fluency ($p$ = 0.766 and $p$ = 0.801, respectively) or digit-coding tasks ($p$ = 0.702 and $p$ = 0.316, respectively). In the mental arithmetic task, ANOVA post-hoc analysis revealed a main effect of fitness on the number of correct responses in the older group ($p$ < 0.004), but not the younger group ($p$ = 0.113; Figure 6.3).

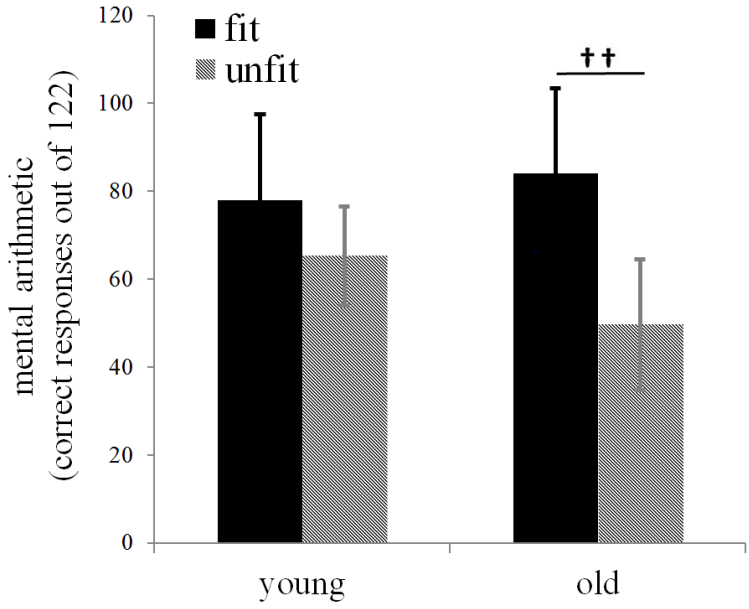



Figure 6.3. Number of correct responses in a mental arithmetic task for younger (18-40) and older (50-80 y) participants. Groups separated into fit (defined as >45 or >25 mL·kg$^{-1}$·min$^{-1}$, for younger and older, respectively) and unfit (<45 or <25 mL·kg$^{-1}$·min$^{-1}$, for younger and older, respectively) groups. † Significant FITNESS effect (different from fit group): † $p \leq 0.01$; †† $p \leq 0.001$.

#### 6.4.2.2. Reaction Time (RTI)

The following results were found from the CANTAB reaction time task (see Tables 6.2 and 6.3). Simple reaction time (mean duration between the onset of the stimulus and the time at which the participant released the button where the stimulus could appear in one

location only) was not different between age ($p$ = 0.364) or fitness (younger group: $p$ = 0.455 and older group: $p$ = 0.519) groups. However, there was a significant effect of age ($p$ < 0.01) but not fitness (younger group: $p$ = 0.819 and older group: $p$ = 0.522) on the simple movement time (mean time taken to touch the stimulus after the button has been released). Similarly, there was a significant effect of age ($p$ < 0.001) but not fitness (younger group: $p$ = 0.841 and older group: $p$ = 0.694) on the five-choice movement time (when the stimulus could appear in one of five locations), while five-choice reaction time was not different between age and fitness groups (age: $p$ = 0.387; fitness: younger, $p$ = 0.157 and older, $p$ = 0.811). In addition, correlational analysis demonstrated strong associations between age and RTI performance (see Table 6.3), where age was significantly positively associated with reaction time for both the simple movement ($r$ = 0.454) and 5-choice movement ($r$ = 0.577) RTs. No associations were observed for simple RT ($r$ = 0.188) or 5-choice RT ($r$ = 0.201). No fitness effects were observed for any of the RTI measures.

#### 6.4.2.3. Attention Switching Task (AST)

AST outcome measures are summarised in Tables 6.4 and 6.5. Consistent with the RTI findings noted above, reaction times for the non-switching and switching trials, and congruent and incongruent trials of the AST were all slower in the older group (all $p$ < 0.05, see Figure 6.4, Panel A). Overall, the number and type (e.g., side, congruency) of errors were higher in the younger group compared to the older group, although statistical significance was only reached for the difference in error rate for the non-switching block trials ($p$ = 0.027, Table 6.3). The exception to this age-effect pattern was the larger number of omission errors in the older group ($p$ = 0.018 vs. younger). Switching cost and congruency cost measures were impaired with age (both p<0.05), with the younger group

showing less variation in responses between congruent and incongruent trials, and switching and non-switching trials. In addition, correlational analysis demonstrated moderate-to-strong associations between age and AST performance (see Table 6.5), where age was negatively associated with number of errors obtained (*r*: -0.235 to -0.443), and positively associated with longer reaction times (*r*: 0.314 to 0.601).

In the older group, the higher fitness group had faster reaction times for all AST outcome measures; although the threshold for significance was not reached on 3 of the 9 measures (see Table 6.4 and Figure 6.4, Panel B). In contrast, fitness status did not affect AST reaction time performance in the younger group. In addition, fitness status did not alter the number of correct or incorrect responses, nor the congruency/switching costs in either age group (all $p > 0.05$, see Table 6.4).

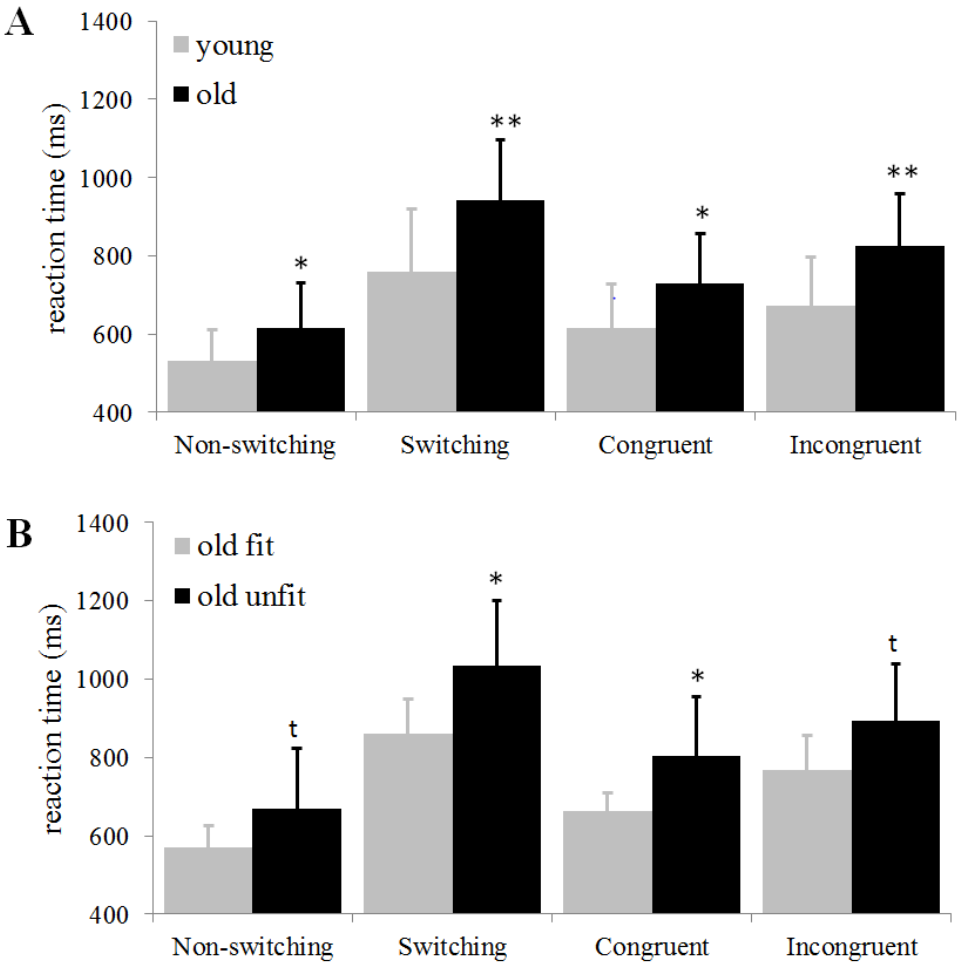



Figure 6.4. Attention switching task (AST) outcome measures showing mean (±SD) reaction time (ms) during non-switching and switching trials, and congruent and incongruent trials. A. shows younger and older groups. B. shows older fit and unfit groups. Significant age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.2. Mean ± standard deviation in performance scores on reaction time cognitive task (RTI) outcome measures. Scores are shown for all participants followed by groups: younger/ older groups; younger fit/ unfit, and older fit/ unfit.

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | | Younger | Older | Younger fit | Younger unfit | Older fit | Older unfit |
| Simple RT (ms) | 353.9 ± 39.7 | 348.0 ± 40.5 | 360.8 ± 39.0 | 367.8 ± 45.4 | 336.8 ± 18.2 | 375.8 ± 36.8 | 343.8 ± 36.6 |
| Simple Movement RT (ms) | 197.1 ± 59.7 | 172.1 ± 41.3 | **227.0 ± 65.7*** | 162.4 ± 37.1 | 189.9 ± 47.9 | 221.2 ± 62.2 | 233.9 ± 73.9 |
| 5 choice RT (ms) | 401.8 ± 42.6 | 395.9 ± 46.7 | 408.9 ± 36.2 | 415.5 ± 55.6 | 382.5 ± 19.3 | 406.7 ± 42.5 | 411.4 ± 30.7 |
| 5 choice Movement RT (ms) | 218.0 ± 65.6 | 183.5 ± 33.8 | **259.4 ± 71.4**** | 191.2 ± 29.8 | 188.0 ± 33.0 | 252.3 ± 67.6 | 267.6 ± 80.0 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Higher values are slower reaction times (ms).
* Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$.
$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.3. Correlations (Pearson's *r*) with reaction time cognitive task (RTI) outcome measures and age, followed by fitness (V̇O2 max) for the younger group, followed by fitness for the older group.

| | All participants<br>Age | Younger (n=19)<br>Fitness: V̇O2 max | Older (n=16)<br>Fitness: V̇O2 max |
|---|---|---|---|
| Measure | *r* | *r* | *r* |
| Simple RT | 0.188 | -0.217 | -0.197 |
| Simple Movement RT | **0.454**** | 0.067 | 0.196 |
| 5-choice RT | 0.201 | -0.216 | 0.110 |
| 5-choice Movement RT | **0.577**** | -0.291 | 0.087 |

Values represent Pearson's *r* correlations. * Significant age/fitness effects:
* $p \leq 0.05$; ** $p \leq 0.01$.$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.4. Mean ± standard deviation in performance scores on the attention switching cognitive task (AST) outcome measures. Scores are shown for all participants followed by groups: younger/ older groups; younger fit/ unfit; and older fit/ older unfit.

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | *n* = 33 | Younger *n*= 19 | Older *n*= 16 | Younger fit *n*= 9 | Younger unfit *n* = | Older fit *n* = 9 | Older unfit *n* = 7 |
| Total correct | 152.9 ± 7.3 | 151.8 ± 7.9 | 154.3 ± 6.5 | 151.8 ± 8.3 | 151.8 ± 7.9 | 154.0 ± 7.9 | 154.6 ± 5.3 |
| Total incorrect | 6.2 ± 6.9 | 7.9 ± 7.6 | 4.2 ± 5.4 | 7.8 ± 7.7 | 8.1 ± 8.0 | 5.0 ± 6.9 | 3.3 ± 3.0 |
| Switching block errors | 4.4 ± 5.5 | 5.2 ± 5.6 | 3.3 ± 5.4 | 5.1 ± 5.3 | 5.3 ± 6.3 | 4.4 ± 7.2 | 2.1 ± 2.2 |
| Non-switching block errors | 1.9 ± 2.4 | 2.7 ± 2.9 | **0.9 ± 1.1*** | 2.7 ± 2.9 | 2.8 ± 3.1 | 0.6 ± 0.9 | 1.1 ± 1.2 |
| Side block errors | 0.5 ± 1.3 | 0.8 ± 1.6 | **0.1 ± 0.3$^{t}$** | 0.9 ± 2.0 | 0.8 ± 1.3 | 0.1 ± 0.4 | 0.0 ± 0.0 |
| Direction block errors | 1.4 ± 1.9 | 1.9 ± 2.3 | 0.8 ± 1.1 | 1.8 ± 2.5 | 2.0 ± 2.3 | 0.5 ± 0.9 | 1.1 ± 1.2 |
| Congruent errors | 0.9 ± 1.6 | 1.4 ± 2.1 | **0.4 ± 0.5$^{t}$** | 1.4 ± 1.7 | 1.3 ± 2.6 | 0.5 ± 0.5 | 0.3 ± 0.5 |
| Incongruent errors | 5.3 ± 5.9 | 6.6 ± 6.3 | 3.8 ± 5.2 | 6.3 ± 6.5 | 6.8 ± 6.4 | 4.5 ± 6.7 | 3.0 ± 3.1 |
| Side errors | 2.5 ± 3.4 | 3.3 ± 4.3 | 1.4 ± 1.7 | 3.1 ± 4.4 | 3.6 ± 4.3 | 1.3 ± 1.7 | 1.6 ± 1.9 |
| Direction errors | 3.8 ± 4.2 | 4.6 ± 4.3 | 2.8 ± 4.1 | 4.7 ± 4.3 | 4.6 ± 4.5 | 3.8 ± 5.4 | 1.7 ± 1.6 |
| Omission errors | 0.8 ± 1.5 | 0.3 ± 0.6 | **1.5 ± 1.9*** | 0.4 ± 0.7 | 0.1 ± 0.3 | 0.9 ± 1.2 | 2.1 ± 2.5 |
| Commission errors | 0.0 ± 0.2 | 0.0 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.4 | 0.0 ± 0.0 |
| Mean reaction time (RT)(ms) | 704.7 ± 136.6 | 644.2 ± 116.1 | **777.3 ± 126.1**** | 650.0 ± 147.6 | 638.3 ± 82.5 | 715.0 ± 63.0 | **848.6 ± 146.2*** |
| Switching RT (ms) | 842.1 ± 181.5 | 758.2 ±161.3 | **942.7 ± 154.3**** | 760.7 ± 212.0 | 755.7 ± 101.5 | 862.2 ± 88.2 | **1034.7 ± 167.3*** |
| Non-switching RT (ms) | 569.1 ± 104.7 | 530.5 ± 79.5 | **615.4 ± 114.7*** | 539.8 ± 89.3 | 521.2 ± 72.2 | 569.5 ± 56.0 | **667.8 ± 144.9$^{t}$** |
| Side blocks RT (ms) | 513.1 ± 137.3 | 477.5 ± 134.4 | **555.8 ± 132.5$^{t}$** | 493.7 ± 165.8 | 461.4 ± 101.4 | 500.3 ± 44.2 | **619.2 ± 173.0$^{t}$** |
| Direction block RT (ms) | 625.3 ± 102.0 | 583.8 ± 79.5 | **675.1 ± 105.9*** | 586.6 ± 96.9 | 581.0 ± 63.3 | 638.8 ± 74.7 | 716.6 ± 126.1 |
| Congruent reaction RT (ms) | 666.9 ± 130.8 | 615.1 ± 113.3 | **729.2 ± 126.0*** | 623.5 ± 142.1 | 606.7 ± 83.3 | 663.7 ± 44.7 | **803.9 ± 150.0$^{*}$** |
| Incongruent RT (ms) | 742.7 ± 147.0 | 673.3 ± 122.7 | **826.1 ± 132.2**** | 676.5 ± 154.9 | 670.1 ± 89.4 | 766.7 ± 90.4 | **894.0 ± 145.4$^{t}$** |
| Side RT (ms) | 657.5 ± 154.9 | 592.9 ± 146.2 | **735.0 ± 130.7*** | 606.4 ± 182.0 | 579.4 ± 109.2 | 677.6 ± 74.8 | **800.6 ± 154.7$^{t}$** |
| Direction RT (ms) | 751.8 ± 125.7 | 695.3 ± 96.4 | **819.6 ± 125.7**** | 693.5 ± 125.2 | 697.2 ± 63.9 | 752.3 ± 56.3 | **896.4 ± 142.4*** |
| Congruency cost | 75.8 ± 51.9 | 58.2 ± 43.4 | **96.9 ± 54.7*** | 52.9 ± 35.7 | 63.4 ± 51.6 | 102.9 ± 66.7 | 90.1 ± 41.2 |
| Switching cost | 273.0 ± 110.3 | 227.7 ± 102.8 | **327.3 ± 95.7*** | 220.9 ± 135.5 | 234.5.1 ± 63.2 | 292.7 ± 75.6 | 367.0 ± 106.1 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Higher AST RT outcome measures indicate slower RTs.* Significant age/fitness effect (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$. $^{t}$Shows a trend towards significance: $0.05 < p \leq 0.1$

Table 6.5. Correlations between attention switching task (AST) outcome measures and age, followed by fitness (V̇O2 max) for the younger group and fitness for the older group.

| | All<br>Age: years | Younger (n=19)<br>Fitness: V̇O2 max | Older (n=16)<br>Fitness: V̇O2 max |
|---|---|---|---|
| Measure | *r* | *r* | *r* |
| Total correct | 0.236 | 0.147 | 0.048 |
| Total incorrect | **-0.346*** | -0.134 | -0.041 |
| Switching block errors | -0.235 | -0.117 | -0.101 |
| Non-switching block errors | **-0.443**** | -0.123 | 0.307 |
| Side block errors | -0.340 | -0.088 | -0.111 |
| Direction block errors | **-0.337**$^{t}$ | -0.092 | 0.333 |
| Congruent errors | **-0.365*** | -0.110 | 0.357 |
| Incongruent errors | **-0.301**$^{t}$ | -0.127 | -0.077 |
| Side errors | **-0.340**$^{t}$ | -0.070 | -0.027 |
| Direction errors | -0.284 | -0.173 | -0.043 |
| Omission errors | **0.420*** | -0.228 | -0.007 |
| Commission errors | **0.219** | all 0 | -0.341 |
| Mean reaction time | **0.565**** | -0.305 | -0.208 |
| Switching RT (ms) | **0.585**** | -0.330 | -0.205 |
| Non-switching RT (ms) | **0.476**** | -0.228 | -0.175 |
| Side blocks RT (ms) | **0.314**$^{t}$ | -0.225 | -0.158 |
| Direction block RT (ms) | **0.552**** | -0.058 | -0.178 |
| Congruent reaction RT | **0.507**** | -0.235 | -0.163 |
| Incongruent RT (ms) | **0.601**** | -0.357 | -0.237 |
| Side RT (ms) | **0.517**** | -0.285 | -0.248 |
| Direction RT (ms) | **0.591**** | -0.297 | -0.151 |
| Congruency cost | **0.425*** | -0.343 | -0.191 |
| Switching cost | **0.511**** | -0.331 | -0.089 |

Values represent Pearson's *r* correlations. * Significant age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$.$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$. For all AST RT outcome measures, higher scores mean slower RTs.

#### 6.4.2.4. Spatial Working Memory (SWM)

SWM outcome measures are summarised in Tables 6.6 and 6.7. There were marked age effects on measures of working memory (number of errors; $p < 0.01$) and higher executive function (use of strategy; $p < 0.01$), as illustrated by ~3-fold difference in performance scores between the younger and older groups where the younger group performed better on both of these measures (see Table 6.6). In addition, correlational analysis demonstrated moderate-to-strong associations between age and SWM performance (see Table 6.7).

Fitness effects were less clear, with no significant differences between fitness groups for either the younger or older group ($p > 0.05$); although fit participants in both age groups had on average better performance for error rate and the use of strategy (see Figure 6.5). In support of this fitness effect, correlational analysis showed some associations between fitness and performance on some of the SWM outcome measures, although none reached the 0.05 threshold for significance in the older group (see Table 6.7).

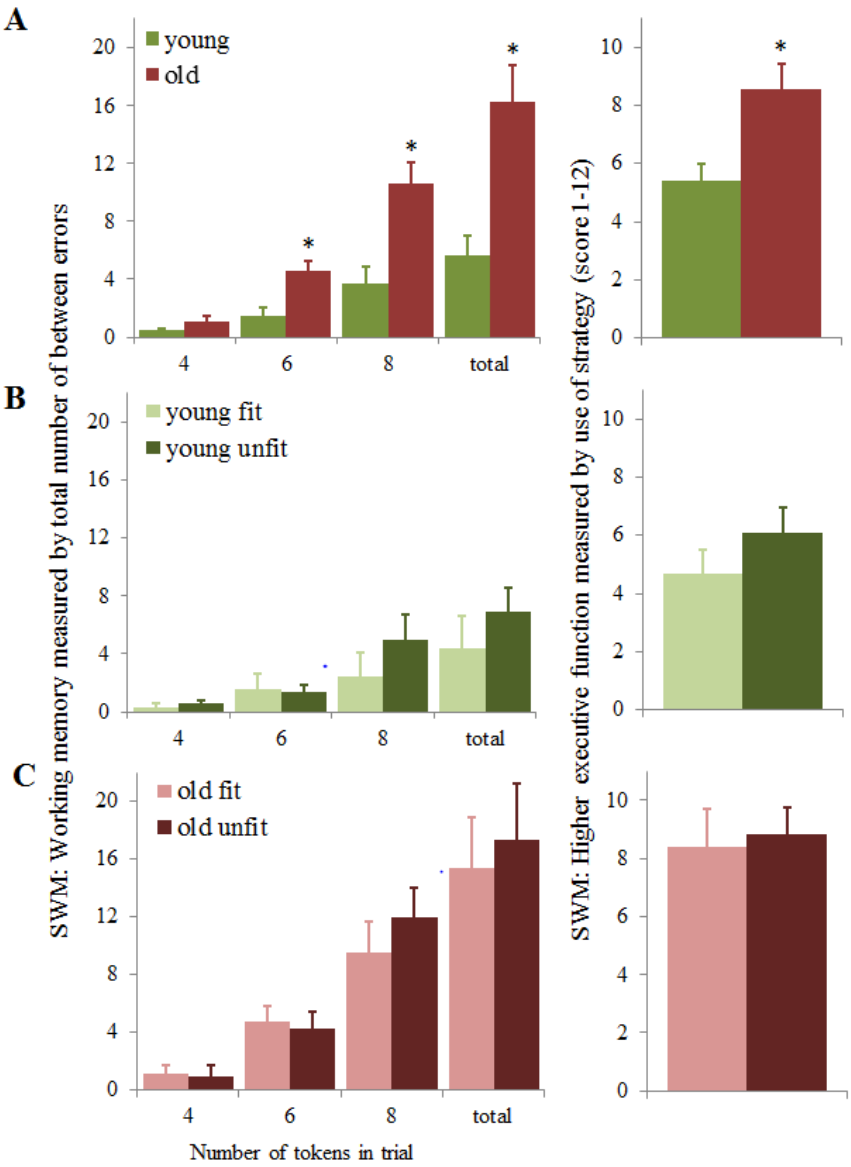



Figure 6.5. Spatial working memory task (SWM) outcome measures of number of errors in the total number of trials and trials with 4, 6 and 8 tokens. Use of strategy score is also shown (high scores indicate poor use of the best strategy). (A) shows younger/ older groups, (B) shows younger fit/ unfit groups, and (C) shows older fit/ unfit groups.

Table 6.6. Mean ± standard deviation in performance scores on spatial working memory (SWM) cognitive task outcome measures. Scores are shown for all participants followed by groups: younger/ older groups; younger fit/ unfit; and older fit/ unfit. Higher values indicate poorer performance (i.e., more errors or failure to use strategy and memory recall).

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | *n* = 33 | Younger | Older | Younger fit | Younger unfit | Older fit | Older unfit |
| | | *n*= 19 | *n*= 16 | *n*= 9 | *n* = 10 | *n* = 9 | *n* = 7 |
| Between errors (all trials) | 9.9 ± 9.2 | 5.6 ± 6.1 | **15.1 ± 9.7**** | 4.3 ± 7.0 | 6.9 ± 5.1 | 15.4 ± 9.9 | 14.9 ± 10.3 |
| Between errors (4 boxes) | 0.7 ± 1.3 | 0.4 ± 0.7 | 1.1 ± 1.7 | 0.3 ± 0.7 | 0.6 ± 0.7 | 1.1 ± 1.6 | 1.0 ± 1.8 |
| Between errors (6 boxes) | 2.8 ± 3.0 | 1.4 ± 2.4 | **4.5 ± 2.9**** | 1.6 ± 3.1 | 1.3 ± 1.5 | 4.8 ± 3.0 | 4.3 ± 2.9 |
| Between errors (8 boxes) | 6.7 ± 6.2 | 3.7 ± 5.0 | **10.6 ± 5.5**** | 2.4 ± 4.9 | 5.0 ± 5.1 | 9.5± 6.1 | 12.0 ± 4.9 |
| Within errors (all trials) | 0.3 ± 0.6 | 0.2 ± 0.5 | 0.4 ± 0.6 | 0.0 ± 0.0 | **0.4 ± 0.7$^{t}$** | 0.4 ± 0.5 | 0.3 ± 0.8 |
| Within errors (4 boxes) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Within errors (6 boxes) | 0.1 ± 0.3 | 0.1 ± 0.3 | 0.7 ± 0.3 | 0.0 ± 0.0 | 0.2 ± 0.4 | 0.1 ± 0.4 | 0.0 ± 0.0 |
| Within errors (8 boxes) | 0.2 ± 0.5 | 0.1 ± 0.3 | 0.3 ± 0.6 | 0.0 ± 0.0 | 0.2 ± 0.4 | 0.3 ± 0.5 | 0.3 ± 0.8 |
| Total errors (all trials) | 10.4 ± 9.2 | 5.8 ± 6.3 | **16.4 ± 9.2**** | 4.3 ± 7.0 | 7.2 ± 5.5 | 15.6 ± 10.1 | 17.3 ± 8.7 |
| Total errors (4 boxes) | 0.7 ± 1.3 | 0.4 ± 0.7 | 1.1 ± 1.7 | 0.3 ± 0.7 | 0.6 ± 0.7 | 1.1 ± 1.6 | 1.0 ± 1.8 |
| Total errors (6 boxes) | 2.9 ± 3.0 | 1.6 ± 2.5 | **4.5 ± 2.9**** | 1.6 ± 3.1 | 1.6 ± 1.7 | 4.7 ± 3.0 | 4.3 ± 2.9 |
| Total errors (8 boxes) | 6.8 ± 6.3 | 3.8 ± 5.1 | **10.7 ± 5.6**** | 2.4 ± 4.9 | 5.1 ± 5.2 | 9.8 ± 6.3 | 12.0 ± 4.9 |
| Use of strategy | 6.8 ± 3.2 | 5.4 ± 2.5 | **8.6 ± 3.1**** | 4.7 ± 2.4 | 6.1 ± 2.6 | 8.4 ± 3.8 | 8.8 ± 2.2 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (60-80 years). For all SWM outcome measures, higher scores mean poorer performance.* Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$.$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.7. Correlations between spatial working memory (SWM) outcome measures and age, followed by fitness (V̇O2 max) for the younger group and fitness for the older group.

| | All<br>Age: years | Younger (n=19)<br>Fitness: V̇O2 max | Older (n=16)<br>Fitness: V̇O2 max |
|---|---|---|---|
| Measure | *r* | *r* | *r* |
| Between errors (all trials) | **0.456**** | **-0.332[t]** | -0.267 |
| Between errors (4 boxes) | 0.227 | **-0.508*** | -0.189 |
| Between errors (6 boxes) | **0.472**** | **-0.452*** | **-0.394[t]** |
| Between errors (8 boxes) | **0.519**** | -0.100 | -0.169 |
| Within errors (all trials) | 0.097 | -0.129 | -0.150 |
| Within errors (4 boxes) | Technical fault | 0.309 | Technical fault |
| Within errors (6 boxes) | -0.114 | 0.328 | -0.200 |
| Within errors (8 boxes) | 0.184 | 0.182 | -0.069 |
| Total errors (all trials) | **0.532**** | -0.289 | -0.274 |
| Total errors (4 boxes) | 0.227 | **-0.508*** | -0.189 |
| Total errors (6 boxes) | **0.449**** | **-0.396[t]** | **-0.394[t]** |
| Total errors (8 boxes) | **0.517**** | -0.078 | -0.184 |
| Use of strategy | **0.504**** | -0.147 | -0.262 |

Values represent Pearson's *r* correlations.
* Significantly age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$.
[t] Shows a trend towards significance: $0.05 < p \leq 0.1$
For all SWM outcome measures, higher scores mean poorer performance.

### 6.4.2.5. Paired Associates Learning (PAL)

Between groups analysis of outcome measures from the PAL are summarised in Table 6.8 and correlations are summarised in Table 6.9. Significant ageing effects were observed where the younger group achieved a significantly higher first memory score ($p < 0.01$) and made fewer errors compared to the older group ($p < 0.05$). No significant fitness effects were observed in either of the age groups. However, the younger group did show a trend towards more errors in the fit group compared to the unfit group.

There were marked age effects on the measure of first memory score ($p < 0.01$), and the younger group made 63% fewer errors than the older group ($p < 0.01$). In addition, correlational analysis demonstrated moderate-to-strong associations between age and PAL

performance (see Table 6.9), where a greater age was associated with lower performance on the first attempt memory score and a higher number of errors.

Fitness effects were less clear, with no significant differences between fitness groups for either the younger or older group ($p > 0.05$); although the younger fit group showed a trend towards significance where the unfit group made fewer errors (see Table 6.8). In contrast, the older fit group made fewer errors than the unfit group and performed better, although none reached the 0.05 threshold for significance (see Table 6.8).

Table 6.8. Mean ± standard deviation in performance scores on paired associates learning (PAL) cognitive task outcome measures. Scores are shown for all participants followed by groups: younger/ older groups; younger fit/ unfit; and older fit/ unfit. Higher values indicate poorer performance (i.e., more errors).

| | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|
| | | Younger | Older | Younger fit | Younger unfit | Older fit | Older unfit |
| 1st attempt memory score | 14.3 ± 3.4 | 15.8 ± 2.3 | **12.6 ± 3.7**** | 15.4 ± 2.5 | 16.2 ± 2.2 | 12.0 ± 4.0 | 13.3 ± 3.5 |
| Total patterns reached | 7.9 ± 0.5 | 7.7 ± 0.7 | 8.0 ± 0.0 | 8.0 ± 0.0 | 8.0 ± 0.0 | 7.8 ± 0.7 | 7.7 ± 0.8 |
| Total errors | 9.3 ± 6.6 | 6.9 ± 5.1 | **11.9 ± 7.3*** | 9.1 ± 5.7 | **5.0 ± 3.7$^{t}$** | 13.1 ± 8.4 | 10.4 ± 6.0 |
| Total errors adjusted | 11.0 ± 9.6 | 6.9 ± 5.1 | **15.6 ± 11.4*** | 9.1 ± 5.7 | **5.0 ± 3.7$^{t}$** | 16.6 ± 11.3 | 14.4 ± 12.4 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Higher scores mean better performance for 1st attempt memory score and total patterns reached. Higher scores mean poorer performance for total errors and total errors adjusted.

* Significantly age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$.

$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.9. Correlations (Pearson's *r*) with paired associates learning (PAL) outcome measures and age, followed by fitness (V̇O2 max) for the younger group, followed by fitness for the older group.

| | All participants<br>Age | Younger (n=19)<br>Fitness: V̇O2 max | Older (n=16)<br>Fitness: V̇O2 max |
|---|---|---|---|
| Measure | *r* | *r* | *r* |
| 1st attempt memory | **-0.498**** | 0.100 | 0.158 |
| Total patterns reached | **-0.311$^{t}$** | | 0.219 |
| Total errors | **0.445*** | 0.001 | -0.221 |
| Total errors adjusted | **0.530**** | 0.001 | -0.323 |

Values represent Pearson's *r* correlations.

* Significantly age/fitness effects: * $p \leq 0.05$; ** $p \leq 0.01$.

$^{t}$ Shows a trend towards significance: $0.05 < p \leq 0.1$.

### 6.4.3. Quality of Life Outcome Measures

Quality of life measures are summarised in Tables 6.10 and 6.11. Data from the WHOQOL questionnaire revealed significant age effects in measures of overall health ($p$ = 0.016) and the social subscale ($p$ = 0.025). From the RAND36, significant age effects were observed for: general health ($p$ = 0.010), limitations due to emotional well-being subscale ($p$ = 0.013), energy and fatigue ($p$ = 0.020), emotional well-being ($p$ = 0.004) and social functioning ($p$ = 0.032). Older participants scored higher (more desirable scores) on all measures compared to their younger counterparts (Table 6.10).

In the younger group, significant fitness effects were found in the RAND36 limitations due to emotional well-being subscale ($p$ = 0.004) and a trend was observed in the WHOQOL psychological health subscale ($p$ = 0.077). In the older group, significant fitness effects were observed for the RAND36 physical limitations subscale ($p$ = 0.007) and a trend was observed for the WHOQOL social health subscale ($p$ = 0.078; Table 6.10). A number of significant correlations between QoL outcome measures and fitness were observed and these are summarised in Table 6.11 and Figure 6.6.

Table 6.10. Mean ± standard deviation in quality of life outcome measures from two quality of life (QoL) questionnaires (WHOQOL and RAND-36). Scores are shown for all participants followed by groups: younger/ older groups; younger fit/ unfit; and older fit/ unfit.

| | | All participants | By age groups | | By age and fitness groups | | | |
|---|---|---|---|---|---|---|---|---|
| | Measure | | Younger | Older | Younger fit | Younger unfit | Older fit | Older unfit |
| WHOQOL | Overall health score | 8.5 ± 1.0 | 8.1 ± 1.1 | **8.9 ± 0.8*** | 8.4 ± 0.9 | 7.9 ± 1.2 | 9.0 ± 0.8 | 8.9 ± 0.9 |
| | Subscale: Physical health | 28.5 ± 2.6 | 27.9 ± 3.2 | 29.1 ± 1.6 | 28.1 ± 2.6 | 27.8 ± 3.7 | 29.1 ±1.1 | 29.0 ± 2.2 |
| | Subscale: Psychological | 24.8 ± 2.9 | 24.1 ± 2.7 | 25.5 ± 2.9 | 25.4 ± 2.0 | **23.1 ± 2.9**[t] | 26.3 ± 1.9 | 24.3 ± 3.8 |
| | Subscale: Social health | 11.4 ± 1.9 | 10.7 ± 2.1 | **12.2 ± 1.5*** | 11.5 ± 1.3 | 10.1 ± 2.5 | 12.7 ± 1.1 | **11.4 ± 1.7**[t] |
| | Subscale: Environmental | 32.1 ± 4.0 | 31.0 ± 4.8 | 33.2 ± 2.7 | 32.6 ± 4.7 | 29.7 ± 4.6 | 32.8 ± 2.7 | 33.7 ± 2.8 |
| RAND-36 | Overall general health | 75.6 ± 14.9 | 69.4 ± 15.4 | **82.1 ± 11.5*** | 75.6 ± 3.3 | 64.5 ± 17.9 | 85.0 ± 10.3 | 77.9 ± 12.5 |
| | Subscale: Physical function | 93.3 ± 16.8 | 92.8 ± 22.3 | 93.8 ± 8.2 | 99.4 ± 1.8 | 87.5 ± 29.5 | 98.0 ± 3.5 | **87.9 ± 9.5**** |
| | Subscale: Limit. physical | 93.6 ± 18.8 | 88.9 ± 24.6 | 98.5 ± 6.1 | 93.8 ± 11.6 | 85.0 ± 31.6 | 100.0 ± 0.0 | 96.4 ± 9.4 |
| | Subscale: Limit. emotional | 83.8 ± 33.7 | 70.4 ± 42.6 | **98.0 ± 8.1*** | 100.0 ± 0.0 | **46.7 ± 45.0**** | 100.0 ±0.0 | 95.2 ± 12.6 |
| | Subscale: Energy/ fatigue | 68.4 ± 15.9 | 62.4 ± 15.5 | **74.7 ± 14.2*** | 67.5 ± 17.0 | 58.3 ± 13.9 | 78.0 ± 10.6 | 70.0 ± 18.0 |
| | Subscale: Emotional | 77.7 ± 15.6 | 70.7 ± 17.5 | **85.2 ± 8.6**** | 68.5 ± 18.8 | 71.7 ± 17.3 | 86.0 ± 8.1 | 84.0 ± 9.8 |
| | Subscale: Social | 90.7 ± 17.2 | 84.7 ± 21.7 | **97.1 ± 7.0*** | 89.1 ± 22.6 | 81.2 ± 21.4 | 98.8 ± 4.0 | 94.6 ± 9.8 |
| | Subscale: Pain | 88.2 ± 10.5 | 88.9 ± 8.8 | 87.5 ± 12.2 | 86.9 ± 9.5 | 90.5 ± 8.2 | 89.0 ± 13.7 | 85.4 ± 10.5 |

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Higher scores indicate better QoL.

* Significantly age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$.

[t] Shows a trend towards significance: $0.05 < p \leq 0.1$.

Table 6.11. Correlations between quality of life (QoL) outcome measures and age, followed by fitness (V̇O2 max) for the younger group, followed by fitness for the older group.

| | | All participants (n = Age (years) | Younger (n=18) Fitness (V̇O2 max) | Older (n=17) Fitness (V̇O2 max) |
|---|---|---|---|---|
| Questionnaire | Measure | Pearson's *r* | | |
| WHOQOL | Overall health score | **0.455**** | **0.410$^{*}$** | -0.234 |
| | Subscale: Physical health | 0.251 | **0.319$^{t}$** | -0.013 |
| | Subscale: Psychological health | 0.236 | **0.527*** | **0.494*** |
| | Subscale: Social health | **0.375*** | **0.525*** | **0.368$^{t}$** |
| | Subscale: Environmental | 0.313 | 0.353 | **-0.366$^{t}$** |
| RAND36 | Overall general health score | **0.424*** | **0.482*** | **0.331$^{t}$** |
| | Subscale: Physical function | 0.040 | 0.090 | **0.638**** |
| | Subscale: Limitations-physical | 0.266 | 0.112 | 0.181 |
| | Subscale: Limit. emotional | **0.468**** | **0.580**** | 0.170 |
| | Subscale: Energy/ fatigue | **0.404*** | **0.311$^{t}$** | 0.063 |
| | Subscale: Emotional wellbeing | **0.458**** | -0.098 | 0.028 |
| | Subscale: Social functioning | **0.397*** | 0.303 | 0.161 |
| | Subscale: Pain | -0.009 | -0.019 | 0.022 |

Values represent Pearson's *r* correlations. Age groups were defined as: younger (18-40 years) and older (50-80 years). Fitness groups were defined as: younger-fit (relative V̇O2 max > 45 $mL \cdot kg^{-1} \cdot min^{-1}$) and unfit (relative V̇O2 max < 45 $mL \cdot kg^{-1} \cdot min^{-1}$); older fit (relative V̇O2 max > 25 $mL \cdot kg^{-1} \cdot min^{-1}$) and unfit (relative V̇O2 max < 25 $mL \cdot kg^{-1} \cdot min^{-1}$).
Abbreviations: WHOQOL: World Health Organisation Quality of Life questionnaire; RAND36: Research and Development 36-item short form survey instrument.
***** Significant correlation with age/fitness: * $p \leq 0.05$; ** $p \leq 0.01$.
**$^{t}$** Shows a trend towards significance: $0.05 < p \leq 0.1$.

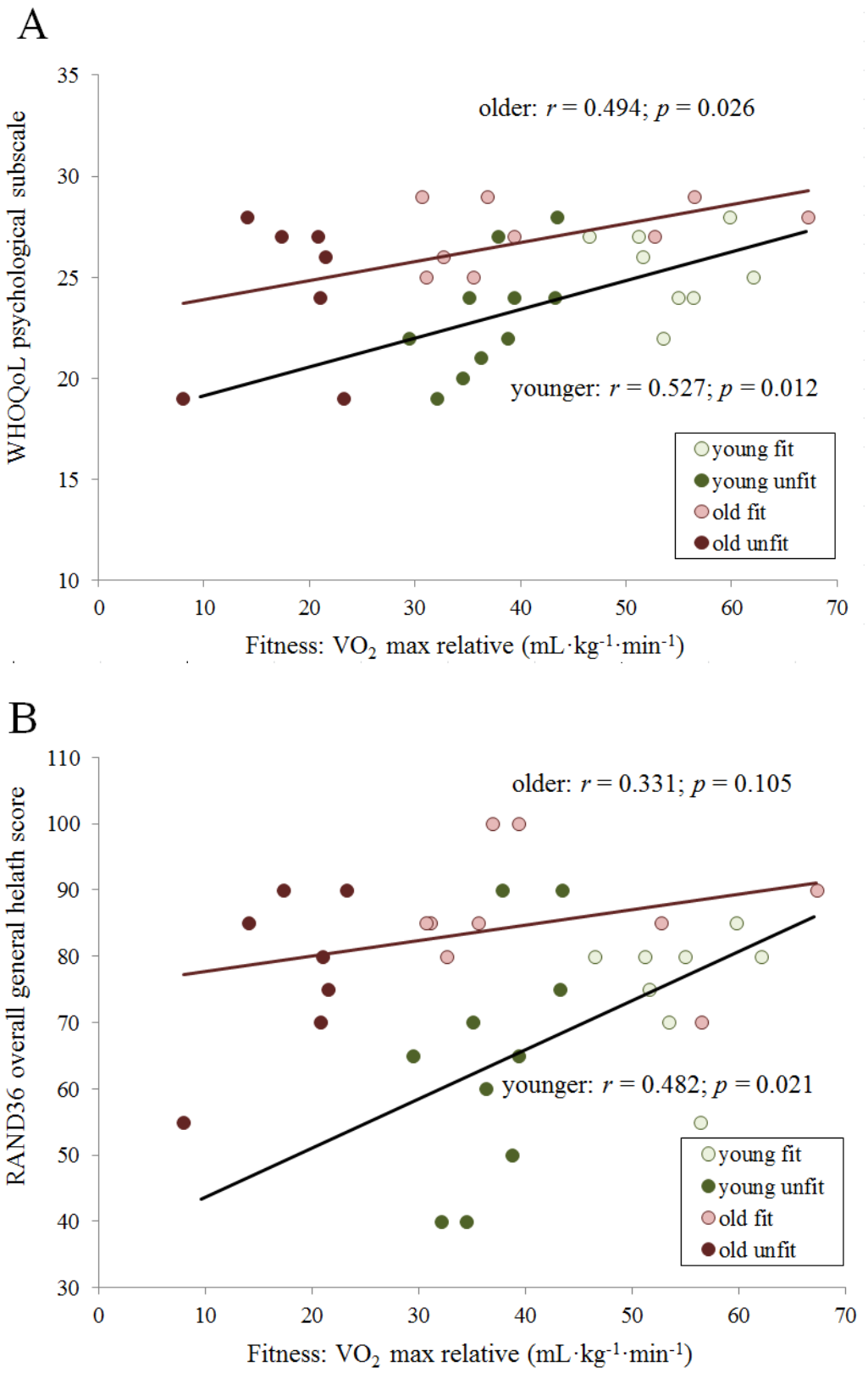



Figure 6.6. Correlations between fitness (V̇O2 max) and quality of life (A. WHOQOL: psychological subscale and B. RAND36: overall general health scale) in younger and older groups.

## 6.5. Discussion

The aims of this study were to compare outcome measures of cognitive function and QoL between younger and older groups with varying levels of aerobic fitness. Significant ageing and fitness effects were observed in several of the investigated outcome measures. Overall, our findings demonstrate that higher aerobic fitness is associated with a range of brain health outcome measures, including performance in attention switching and mental arithmetic, and quality of life. Therefore, physical activity may protect against the natural decline associated with healthy ageing, in addition to declines associated with sedentary behaviour. Further, aerobic fitness may protect against neurodegenerative conditions by modifying factors associated with disease pathology (i.e., cognitive function and QoL). However, these findings should be taken with caution given that the study design is cross-sectional.

Maximal oxygen consumption was significantly higher in fit compared to unfit groups for both the young participants and the older participants. The NZPARQ 7-day activity frequency was better at distinguishing between fit and unfit groups for the older participants, whereas the self-rated activity category and researcher calculated activity category were better at differentiating the younger fit and unfit groups. These differences in self-reported activity ratings between age groups should be considered in research where fitness is determined by using similar methods. Certainly, issues around the use of self-reported activity ratings have been discussed extensively in the literature (see Hays et al., 1993; Ainsworth 1994; Skevington et al., 2004; Banerjee et al., 2006; Smith et al., 2007).

As expected and consistent with the literature (Deary et al., 2009; Shiroma and Lee, 2010), significant age effects were observed for outcome measures of cognitive function, where performance in cognitive tasks decreased with age. The older group reported higher scores in outcome measures of QoL, in line with existing research associating older age with less anxiety, lower stress and a decreased risk of depression (Mroczek et al., 1998).

The results from the AST cognitive task showed that the younger participants were less affected by the task that required attention switching and that their response times were less likely to slow down due to trying to ignore conflicting irrelevant stimuli. While fitness showed no effect on the younger cohort, in the older cohort the fit group responded more quickly to the task, indicating that fitness improves performance in ignoring irrelevant stimuli (that declines with healthy ageing). The findings from the QoL questionnaires showed significant positive associations between fitness and QoL in both age groups. Benefits were seen particularly in subscales relating to psychological health, providing further evidence that physical activity is good for our emotional health, as well as our cognitive health (e.g., ability to attend relevant stimuli and ignore distractors) and physical health.

In line with existing literature, clear ageing effects were observed in the memory and learning tasks (SWM and PAL), where the older group showed a significant increase in reaction time, scored higher in the strategy score (indicating less use of higher executive function) and made more errors than the younger group. This decline in performance has also been shown to increase in mild cognitive impairment and dementia (Saunders & Summers 2011). Although no significant fitness effects were observed, the results did show a similar pattern where poor fitness mimicked the effects of ageing (slower reaction

times and poor use of strategy) in the unfit group compared to the fit group, in both the younger and older cohorts.

The tablet-based cognitive measures were better at differentiating between age and fitness groups, likely due to the greater sensitivity and ability to detect subtle differences, as well as a larger array of outcome measures to assess (i.e., number of correct responses as well as time to respond in several different conditions). Further development of cognitive measures with increased sensitivity and sophistication will greatly improve our understanding of brain function in health and disease, particularly if measures are designed that are more translatable to everyday life, can be individually tailored, and designed to target the specific symptoms of disease pathology through associated brain regions.

### 6.5.1. Study Limitations and Methodology Considerations

This study recruited a relatively small sample size and our participants were not perfectly pair-matched for age and sex. Age effects were clearer than fitness effects due to the larger sample size and many significant effects of age on cognition were observed. In contrast, the fitness effects tended to reach borderline significance (due to the smaller sample size after the age groups were further divided in sub-groups 'fit' and 'unfit'). Based on these findings, it is recommended that a sample size of 40 participants in each subgroup be recruited in similar studies investigating ageing and fitness using similar cognitive measures. Further, all participants were healthy volunteers so findings cannot be generalised to clinical populations. However, our findings do support existing research where interventions involving physical activity have been used in clinical populations, and found improvements in brain health outcome measures of cognition similar to those used in this study. Further, our participants only completed the various measures on one

occasion, so any practice or learning effects that may be a confounding factor in intervention studies (Cacciamani et al., 2017) have been avoided in this study. Despite this, it is also important to consider that participants may have previously been exposed to similar tasks prior to taking part in this study, though it is unlikely that this would have affected the results.

Caution should also be taken in generalising results from cognitive tasks to everyday life and consider transferability of specific cognitive function to other tasks and situations. Similar cognitive tasks to those used as outcome measures in this study are often used in brain training tools (e.g., Luminosity, Lumo Labs Inc., https://www.lumosity.com). Certainly, there is heated debate around whether 'brain-training' (using tasks similar to those used in this study as outcome measures) improves cognitive function (Simons et al., 2016). Further, cognitive training and investigation has been extended to the affective domain by adding emotional material to traditionally dry cognitive tasks (Schweizer et al., 2011). Interestingly in our study, better performance was observed in cognition in association with objective measures of aerobic fitness, without any repeated exposure to these tasks (i.e., learning effects), providing further evidence that physical activity is beneficial for cognitive health.

It is always necessary to consider issues around cause and effect in cross-sectional studies that rely on associations between measures. It cannot be concluded that increased physical activity will lead to increased objective fitness measures that will lead to benefits in every other aspect of physical and psychological health. Although the findings from this study do indicate that individuals are more likely to experience benefits across a range of health measures if they have higher aerobic fitness. Conducting intervention studies that improve

physical activity and fitness and then using outcome measures assessing cognition would demonstrate the cause and effect relation.

### 6.5.2. Conclusion and Recommendations

These data support existing literature showing that physical activity can benefit the brain and improve cognitive health. More research is needed that investigates the underlying mechanisms that drive these improvements as well as links between different types of brain health measures (i.e., cerebrovascular measures). Longitudinal studies that incorporate more holistic approaches would allow the investigation of mechanisms and demonstrate the cause and effect nature of physical activity interventions. In line with better understanding of how to adequately measure brain health we will be better equipped to tackle neurocognitive disorders and develop individualised treatments.

# 7. CONCLUSION

This thesis aimed to investigate brain health measures of cerebrovascular health, cognitive performance and quality of life, whilst also examining methodological differences in the obtainment of some of these measures (i.e., resting cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) measures derived using transcranial Doppler (TCD) ultrasound and magnetic resonance imaging (MRI)). Further, the effects of ageing and fitness on these outcome measures were investigated.

One key finding from this thesis was that different methodological approaches used to calculate CVR, including stimulus duration, stimulus concentration, imaging modality and analysis approach will alter this widely used outcome measure. These alterations can be explained by physiological differences underpinning the process by which these measures are derived. With regard to stimulus duration and concentration, these factors affect the balance between the contribution of the ventilatory and vascular response to carbon dioxide ($CO_2$) during data collection processes for CVR, which is further influenced by the choice of steady-state location.

In terms of imaging modalities, TCD and MRI are measuring distinctly different physiological phenomena to determine resting CBF and CVR values that likely explains some of the discrepancies shown in the results of Chapters 4 and 5, including different effects of ageing and fitness on the CVR measure. Specifically, MRI arterial spin labelling (ASL) calculates regional perfusion and transit times in the tissue for resting CBF measures, and regional changes in blood-oxygen-level dependent (BOLD) signal intensity (driven by a delicate balance of CBF velocity, volume and tissue oxygenation) for CVR measures. In contrast, TCD measures of CBF and CVR are calculated by targeting the

middle cerebral artery blood velocity (MCAv), essentially a conduit vessel that supplies a large proportion of cerebral tissue. These differences may explain the contradictory ageing and fitness effects observed with CVR measures, though resting CBF measures appear to be complementary between imaging modalities. These findings may help explain why there is conflicting information in the literature about ageing and fitness effects on CVR. Further research is warranted to investigate the discrepancies between modalities, specifically looking at how vessel diameter and structure change may influence these measures and observed differences between groups.

Measures of resting CBF and cognition (including reaction time, attention switching and spatial working memory) were observed to decline across the lifespan; though attention switching and mental arithmetic improved with cardiorespiratory fitness. Further, quality of life measures significantly correlated with fitness in both younger and older groups. Taken together, these findings show that physical activity has many benefits for the brain, as has been shown previously in the literature, and discussed throughout this thesis. Future research is required to investigate how various measures of brain health are associated with one another in carefully matched groups (for age, sex and fitness).

This research highlights existing pitfalls within the scientific literature around inconsistent methodological approaches used to determine measures, indicating that guidelines are needed and perhaps a gold-standard approach to be developed (or specific gold-standard approaches depending on the vessel of interest, clinical condition and/or population being investigated). This would make it easier for findings between research centres to be directly compared, assuming the studies were using the same methods in similar populations. Further, standardised approaches specifically for resting CBF and CVR

measures may be useful in recognising brain vulnerability to neurodegeneration and cognitive decline, which can be detected earlier than currently used approaches, and if measured in the same individual over time, may eventually coincide with later biomarkers of neurodegeneration, including structural imaging and cognitive decline.

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# APPENDICES