Cerebrovascular Reactivity, Metabolism and Cognitive Function in Healthy Aging

Doctor of Philosophy
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Abstract

Normal cognitive aging is characterized by a general slowing of mental processing, including reduced memory and attentional control abilities, while some cognitive processes remain intact. Pathological cognitive aging is indicated when one or more cognitive abilities decline at a greater rate than average; this decline can signify the presence of dementia. Discovering the underlying biological foundations behind successful cognitive aging remains an active area of research. A better understanding is needed to improve cognitive function and, at the same time, to lessen the impact of cognitive impairment, particularly at a time when the world population is aging rapidly.

The functioning of the cerebral blood vessels and the adequate supply of oxygen over the lifespan is vital for optimum performance of neural structures. Chronic hypoperfusion is believed to impact cognitive aging in numerous ways. Reduced cerebral blood flow has been linked to poor cognitive performance in studies of stroke, cerebral vessel occlusion, and arteriosclerosis. Investigations of neurodegenerative disorders such as Alzheimer’s disease have uncovered connections between the severity of cognitive impairment, brain perfusion, and blood vessel integrity.

Extensive research associates impaired cognition with defective vascular mechanisms in pathological models, yet investigation of physiological mechanisms behind healthy brain aging is incomplete. It is necessary to establish effective
therapeutic options for those affected by vascular-based cognitive impairments. This requires further investigation.

This thesis reports on the findings of two empirical studies investigating the contribution of specific vascular and metabolic mechanisms to cognitive performance in healthy aging. A systematic review of research using magnetic resonance imaging (MRI) to measure cerebrovascular reactivity (CVR) and its contribution to cognitive function provides the rationale for CVR investigations.

Participants were 59 healthy adults aged 21-44 years or 55-75 years. Cerebrovascular function was assessed using several MRI methods, including phase contrast, arterial spin labeling (ASL) and T2-relaxation-under-spin-tagging (TRUST), and cognitive performance was measured using a validated computerized assessment battery. Findings indicated significant differences between younger and older adults on attention and memory function, as well as differing contributions of blood flow and vascular reactivity to specific cognitive domains.

Attention task performance was related to blood flow in older adults, yet no such relationship was seen in younger adults. An imbalance between oxygen supply, demand and consumption was observed in the older group. Older brains extracted more oxygen from the deteriorating supply to maintain oxygen metabolism at a normal rate. CVR was not implicated in cognitive performance in younger adults; however, there were significant correlations between CVR in the hippocampus and memory performance in older adults. This novel finding was supported by a
significant relationship observed between CVR in the hippocampus and subjective memory concerns.

Identification of individuals at risk for vascular impairment would allow health professionals to provide more vascular-focused therapeutic options, with greater potential for prevention and/or treatment of cognitive decline.
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I would also like to acknowledge and thank the participants who donated their time to volunteer for the study, Professor Denny Myers for her assistance with statistical analysis, and the Barbara Dicker Foundation for the research grant.
Declaration

This thesis contains no material which has been accepted for the award to the candidate of any other degree or diploma, except where due reference is made in the text. To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in text. Where the work is based on joint research or publications, this thesis discloses the relative contributions of the respective authors.

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Contribution to Jointly Authored Papers

The candidate would like to acknowledge the help of all-co-authors who contributed to the papers included in this thesis. All co-authors have given approval for the relevant papers to be included as part of this dissertation. As reflected by the candidate’s position as first author on all included papers (excluding papers in the appendices), the candidate confirms that she has made the major contribution to all papers forming this dissertation.

All papers were primarily written by the candidate. The author indication forms are provided in the appendices.
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List of Conference Presentations and Posters

Clinical Trials in Alzheimer's disease (CTAD), San Diego, 2013; Poster

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Chapter 1

Introduction and Overview

There is an urgent need to understand physiological mechanisms underlying the cognitive decline which occurs in aging. Understanding how vascular and metabolic factors impact cognition is essential to help guide prevention, diagnosis and treatments for cognitive losses in older age. Similarly, untangling the secrets underlying successful cognitive aging is a worthy pursuit in today's rapidly aging population. It is known that common disorders of an aging cardiovascular system, such as high blood pressure, high cholesterol and diabetes, can contribute to the onset of deteriorating cognitive functions (Jurado et al., 2017) and increase the risk of developing dementia (Kivipelto et al., 2001). These risk factors have a negative impact on cerebral blood flow.

There is increasing evidence that the functioning of the blood vessels supplying the brain is a significant determinant of cognitive health over the lifespan (Kalaria, 2010; DeCarli et al., 2001; Gorelick et al., 2011). Vascular risk factors can trigger a variety of pathophysiological processes leading to neurodegeneration, possibly initiating the onset of cognitive impairment and dementia (Nelson et al., 2016, Duron and Hanon, 2008). Even normal, healthy aging is associated with some degree of cognitive slowing, due to multiple natural processes, including decreased production of neurotransmitters, neural pruning and cortical atrophy (Bishop et al., 2010, Peters, 2006). Yet some people are more affected by cognitive impairment than others. The gradual slowing of cognitive processes appears to occur concurrently with a slowly
deteriorating oxygen supply and consumption, dysregulation of blood flow and disruptions to neurovascular coupling via an aging cerebral vascular system. Alzheimer’s disease (AD), the most common cause of dementia (McKhann et al., 2011), shows a characteristic pattern of accumulation of amyloid-β plaques and neurofibrillary tangles within the brain, often coupled with alterations in vascular function (Sabayan et al., 2012). Impaired response of the cerebral blood vessels to changes in gas tension of the blood is one mechanism related to the cognitive deterioration which is the hallmark of this disease (Silvestrini et al., 2006). However, it is not fully understood how impaired cerebrovascular function contributes to cognitive impairment in the milder cognitive changes seen in normal healthy aging.

The goal of the current thesis is to examine the contributions of specific vascular factors to cognition across the adult lifespan. It is understood that poor cardiovascular health is linked to cognitive decline and dementia, and evidence suggests a role of cerebrovascular functioning in the development and progression of these impairments (Duron and Hanon, 2008, Nelson et al., 2016). Identification of suboptimal cerebrovascular functioning could help to distinguish those who would benefit from vascular-specific therapeutic approaches and could potentially lead to detection of early dysfunction of the vascular system in people at risk. This knowledge could help advance identification and treatment of vascular dysfunctions, slowing the progression of age- and vascular-related cognitive impairments. There is a need for research in this area would enable better understanding of the cerebrovascular effects of interventions designed to prevent or treat age-related cognitive impairments.
1.1 Population aging

Rapid global aging is becoming a critical concern worldwide. Advances in medical science are enabling people to live longer than ever before, with the number and proportion of older persons growing exponentially every year in most nations. In 2006 there were approximately 500 million people aged 65 years and older, and it is estimated by the U.S National Institute on Aging (2007) that by 2030 there will be 1 billion people in this demographic, outnumbering children under 5 years old for the first time in history. It is also expected that the number of individuals living to 85 years and older will become the fastest growing demographic in many countries and regions. The implications of these changes are multifaceted; virtually all sectors of society will be affected, including economies, employment and retirement, and provision of health services. Although many people are living healthier lives, this increase in population aging is also leading to a rise in non-communicable age-related disease and impairment, such as Alzheimer's disease and hypertension, overtaking the predominance of infectious and parasitic illnesses for loss of health and life (Prince et al., 2013). Aging is associated with increasing prevalence of both cerebrovascular and cognitive disorders (Popa-Wagner et al., 2015).

1.2 Cognitive aging

While the life expectancy of the population continues to increase, the issue of declining cognition in aging is ubiquitous. Age-related cognitive health is of critical importance in a time where there are increasing numbers of older Australians. Advancing age is the number one risk factor for the development of dementia. As
people live longer and continue to grow older, the prevalence of dementia will continue to increase. Dementia is a chronic and progressive process in which cognitive functions such as memory, thinking, orientation and learning capacity deteriorate beyond what is expected in normal aging. This syndrome is a leading cause of disability and dependence amongst the elderly; however it is not a part of the normal aging process (AIHW, 2012). Dementia results from various causes that primarily affect the brain. Dementia Australia (2018) estimated 425,000+ Australians (1.7% of the population) are living with dementia in 2018; this is projected to increase to more than 536,000 (~2% of the population) by 2025, and over 1,100,000 by 2056. The problem of dementia has a severe financial impact on societies around the world. In 2017 the annual costs of dementia were estimated at $14.67 billion in Australia alone, and this is projected to increase to over $1 trillion over the next 40 years (Brown et al., 2017). AD cases are the most common dementia diagnosis, followed by vascular dementia, which is reported to make up approximately 10% of dementia cases (McKhann et al., 2011).

Age-related cognitive decline is the term given to reflect the normal course of diminishing cognitive abilities over the lifespan. Although there is wide variation, most people will experience some cognitive slowing with increasing age. Large individual differences exist, and some people show more successful cognitive aging than others. It is also established that certain abilities show greater age-associated decline than others (Pipingas et al., 2010). Vocabulary, numerical skills and general knowledge show little reduction over the lifespan, whereas more ‘fluid’ processes such as attention, multi-tasking and memory function may undergo a gradual decline from middle adulthood (Harada et al., 2013). Attention and certain aspects of
memory are two domains known to be markedly affected by the aging process, even in healthy, cognitively-intact individuals. While some degree of age-related decline is expected, there is evidence to suggest that this diminishment of capabilities can be at least partially offset by dietary modifications, physical activity and mental training activities (Baltes et al., 2005), or somewhat compensated for by obtaining a certain level of practical competence and experience over the course of a lifetime, known as cognitive reserve (Muscari, et al., 2010).

Some degree of cognitive decline is normal with advancing age, bolstered by the multiple assaults of neural pruning, mitochondrial dysfunction and reduced cortical volume (Salthouse, 2010), amongst various other pathologies. Due to the heterogeneous rates of cognitive decline seen in longitudinal studies (Christensen, 2001, Morris et al., 1993) not all adults will experience deterioration in mental processing to the same extent. It is often the case that mild cognitive decline precedes the development of AD (Petersen et al., 2001). Often one of the first warning signs of impending AD is the onset of subjective memory concerns. These memory impairments are greater than simply forgetting a persons’ name or misplacing car keys; subjective memory concern refers to increasing instances of confusion and/or forgetting how to do things that one would normally be capable of, or things that one would normally know.

Subjective memory concerns, particularly in verbal episodic memory, are often linked with increased risk of conversion to dementia (Clarnette et al., 2001), although inconsistently indicate current cognitive impairment (Reid and MacLullich, 2006). In fact, it is estimated that approximately 1/3rd of individuals presenting to memory
clinics perform normally on neuropsychological tests, showing no cognitive impairments (Hejl et al., 2002). While many believe that increasing concerns about one’s memory may herald the very first signs of incipient dementia, it is also often the case that memory complainers do not progress to clinically-recognised cognitive impairment. It is theorized that the onset of memory concerns may be induced in party by subtle brain changes, which are as such inadequate to cause impairments severe enough to be detected by formal cognitive assessments (van der Flier et al., 2004).

Research using brain imaging has correlated cognitive change with structural and functional changes and AD pathology, suggesting that these conditions may elicit similar patterns of alteration in the brain, including reduced total volume, as well as specific regional volume losses (the hippocampus and entorhinal areas are often implicated) (Fox et al., 2000, Jack et al., 2004, Apostolova and Thompson, 2008). Moreover, it has also been observed that even cognitively normal individuals who report subjective memory complaints may have smaller hippocampal and medial temporal lobe volumes (van der Flier et al., 2004). Growing evidence also indicates a role of vascular impairments in the initiation and progression of neural dysfunction in aging (Kalaria, 1996).

1.3 Vascular aging

Clinically, cerebrovascular function is widely recognised as having crucial effects on cognition (Bowler, 2002, Popa-Wagner et al., 2015). Chronic pathology of the cerebral vessels is a central cause of dementia, and has been touted as the most
primary of the modifiable risk factors for Alzheimer’s disease (AD) (Iadecola, 2013). Cerebral vessel integrity is of utmost importance to the maintenance of optimum neurological functions across the lifespan. It is not surprising that the functioning of the vasculature supports brain and cognitive processes, given that this highly metabolic organ receives 20% of the total cardiac output despite making up only 2% of the total body mass (Owen and Sunram-Lea, 2011). The brain has little to no capacity to store energy, and thus requires a constant steady stream of blood containing oxygen and glucose to maintain optimum functioning.

Blood vessels undergo a multitude of changes in the normal aging process, even when unaffected by ill-health or disease. It is somewhat rare for individuals to reach older age with no vascular pathology whatsoever (Kalaria, 2010). Vascular aging is mostly associated with hardening and loss of elasticity of the blood vessels. Arteriosclerosis is the term given to this vessel stiffening with age, and is a common pathology in older people which is a leading cause of vascular mortality worldwide (Herrington et al., 2016). This overall stiffening of vessels in the brain affects cerebral blood flow in two main ways. Firstly, the cushioning capacity of the vessels is reduced, leading to increased pulsatile surges of inflowing blood. The low vascular resistance in the brain coupled with torrential flow can cause damage to the delicate microvessels, potentially resulting in micro-bleeds or injury to tissues. Neural tissue is particularly vulnerable to damage in watershed regions of the brain. These regions have a decreased blood supply due to being in the border-zone territories supplied by the major cerebral arteries. Tissues in watershed regions are perfused by more than one blood supply, however given they are the most distal region of the vascular territory, these areas are exposed to greater risk of ischemia during times of
hypoperfusion or hypoxia (Torvik, 1984). Secondly, arterial stiffening renders small blood vessels less responsive to changes in arterial gas tensions, pressure and metabolic needs, potentially resulting in neurovascular uncoupling, pathogenesis of cerebrovascular disease and white matter lesions (Poels et al., 2012).

1.4 Vascular function and cognitive performance

It is well established that poor cardiovascular health is associated with greater risk of developing dementia (Larson et al., 2006, Lopez et al., 2003). The physiological processes that underlie cognitive impairment in dementia and other neurodegenerative disorders have been examined extensively (Kalaria, 1996, Farkas and Luiten, 2001, De la Torre, 2004, Iadecola, 2004), yet cerebrovascular mechanisms that support healthy cognitive aging have not been explored to the same extent. Cardiovascular risk factors such as diabetes, hypertension and arteriosclerosis have been shown to correlate with impaired regulation of cerebral blood flow and vascular reactivity (Zeiher et al., 1993). Reduced cerebrovascular health is also likely to contribute to neurodegeneration (Ruitenberg et al., 2005, Gorelick et al., 2011, Iadecola, 2010). Dysregulation of cerebral blood flow (CBF) caused by neurovascular uncoupling in cardiovascular disorders has been linked to impaired mental processing (Toth et al., 2017, Tarantini et al., 2016, Tarantini et al., 2015). The integrity of small cerebral blood vessels impacts brain health and has been linked to deficits in cognition which can range from subtle impairments to advanced-stage dementia (Iadecola, 2013).
What is unknown is the extent to which cerebrovascular health contributes to cognition across the lifespan and in age-related cognitive decline. If physiological processes underpinning the onset and perpetuation of cognitive decline are found to be modifiable, specifically targeted therapeutic interventions may be useful to slow the progression of cognitive aging. Moreover, there may be potential to use these parameters as preclinical indicators of brain dysfunction in older age.

The current thesis aims to provide new knowledge on the contributions of specific cerebrovascular mechanisms to cognitive performance across a normal healthy lifespan. Increasing age is often associated with loss of elasticity of the blood vessels, both peripherally and in the brain. Age-related increases in blood pressure, coupled with stiffening vasculature, results in reduced cushioning of the pulsatile flow of blood. The brain has a low vascular resistance, rendering the small cerebral capillaries and neural tissues vulnerable to damage from flows of surging blood over time (Pase et al., 2014, DeCarli, 2012). While blood flow regulatory mechanisms are generally maintained over the healthy lifespan, morphological changes in the cerebrovasculature may impact the functioning of the neurovascular unit, compromising cerebral blood flow (CBF), cerebral metabolism of oxygen (CMRO₂), and cerebrovascular reactivity (CVR). These physiological parameters underpin the supply, regulation and consumption of oxygenated blood in the brain. CBF is the delivery of oxygenated blood to the neural tissue; this denotes the energy supply to the brain. CMRO₂ refers to the rate at which the brain tissues utilize oxygen and represents the energy demand. CVR reflects the capacity for the small cerebral blood vessels to respond to vasoactive stimuli, and this index signifies the integrity and health of the brain microvasculature. Impaired CVR is a marker of damaged
microvasculature, and is implicated in multiple brain pathologies, due to the reduced capabilities to regulate blood flow necessary to maintain optimum neural function. Extensive reviews have examined the physiological mechanisms underlying cognitive change in neurodegenerative illnesses and dementia (Kalaria, 1996, Farkas and Luiten, 2001, Iadecola, 2004, De la Torre, 2004), yet the mechanisms that support healthy cognitive aging are less explored. The extent to which these normal age-associated alterations in vascular integrity affect neurocognitive function is not well established in the literature and requires further investigation.

1.5 Research aims

The primary aims of this thesis are:

1) To gain an understanding of region-specific relationships between CVR and cognitive abilities by systematically reviewing current investigations of contributions of MRI-estimated cerebrovascular reactivity (CVR) to cognition;

2) To investigate the relationship of CVR to memory and attention task performance across the lifespan by examining age differences in regional CVR to carbon dioxide;

3) To examine how CBF, CMRO₂ and blood oxygenation contribute to memory and attention in cognitively healthy younger and older adults using a variety of neuroimaging methods.
1.6 Thesis overview

This first chapter is the Introduction and Overview, which has outlined the overall scope of the work, including study aims and structure. The second chapter is a general synopsis of the anatomy and physiology of the cerebral vasculature. Structural changes that occur in the cerebral microvasculature with age are discussed, as are the possible mechanisms behind cerebral dysfunction with age, including changes to endothelial functioning, biochemical production in the endothelium, and arterial stiffening. This chapter will finish with a brief overview of the features of cerebral metabolism and circulation in the aging brain.

Chapter Three will consider cerebrovascular reactivity, the different vascular challenges employed to measure this index and the various tools that are used to non-invasively measure CVR and other blood flow parameters. Evidence of the contributions of CVR to cognition is also discussed.

Chapter Four builds on this by providing a systematic review of all published studies that examined the relationship between cognition and CVR using MRI (Catchlove et al., 2018c). This published paper synthesizes information from 10 studies that assessed CVR using MRI and its relationship to cognitive performance in adults. The purpose of this paper is to provide a rationale for research investigating the role of cerebral vascular reactivity in cognition.

Chapter Five contains the methods used in the current research, including a background of MRI principles and history. It details the screening tools, cognitive
assessment, hypercapnic challenge and specific MRI sequences used for data collection in both the empirical papers included in the chapters that follow.

Following this, two empirical papers are presented. The first published experimental paper, which forms Chapter Six, extending from the recommendations made in Chapter Four, investigates the contributions of cerebrovascular reactivity (CVR) to cognition in normal aging using MRI and CO₂-gas inhalation to elicit increases in cerebral blood flow. This paper was accepted for publication on the 3rd June 2018 in the Journal for Experimental Neuroscience and is currently in press.

Chapter Seven, entitled ‘An investigation of cerebral oxygen utilization, blood flow and cognition in healthy aging’ examines age-related differences in cerebral metabolic rate of oxygen use (CMRO₂), cerebral blood flow (CBF) and cognitive performance in healthy younger and older adults. This paper was published in PLOS One (Catchlove et al., 2018b).

Chapter Eight is a general discussion of the major findings of these investigations in the context of the literature, as well as the clinical implications and limitations of the current work. Future research directions are also discussed, followed by conclusions.
Chapter 2

Cerebrovascular Physiology: Functional Changes that Occur with Normal Aging

It is commonly accepted that aging is accompanied by some degradation of the brain. However, some people appear to age more successfully than others. An important aspect of brain aging is the widespread changes in the blood vessels; these changes will affect brain health by limiting blood supply, which is essential to the functioning of neurons. The integrity of the cerebrovascular system is vital to maintain optimal functioning of the brain and cognition across the lifespan. Extensive research has investigated the changes that occur in the cerebral vasculature with healthy aging, and the mechanisms behind these alterations. Structural, functional and/or chemical dysfunctions in the delivery of oxygen and nutrients to the brain will ultimately bring about declines in cognition and behavior. It is important to elucidate the specific processes that are responsible for accelerated aging in these areas.

The first half of the current chapter will present an overview of the anatomy and physiology of the cerebrovascular system, age-related changes that commonly occur in the structure of the cerebral vasculature from larger vessels down to more in-depth look at the microvasculature, the potential mechanisms that contribute to decline in cerebral microvascular function, and the impact that these changes can have on cognitive performance with aging. The second half of this chapter will provide a more in-depth discussion of changes to cerebrovascular processes with
increasing age, and the effect of these changes on brain perfusion. Core measures of the research studies presented in Chapters 4, 6 and 7 will be introduced, including how they are assessed, and methodological considerations associated with these indices. The aim of this chapter is not to provide an exhaustive review of the literature, rather a more generalized overview of potential alterations that occur in the normally-aging cerebrovascular circulation, and how cognitive functions can be affected by these.

2.1 Basic anatomy and physiology of cerebrovascular system

2.1.1 Vessel wall structure
The vascular walls of cerebral arteries and smaller arterioles are comprised of three layers known as tunics: the tunica adventitia, tunica media and the tunica intima. The tunica adventitia is the outermost section; this tier is made up of collagen fibers and fibroblasts and is in contact with other cell types - pericytes and astrocytic end-feet in the neurovascular unit, and the perivascular nerves that innervate the pial and larger vessels. Tunica media is sandwiched in between the adventitia and the intima, this layer is composed of mostly smooth muscle cells, elastin and collagen fibers. Arterioles contain a large amount of smooth muscle cells, enabling the regulation of blood flow between the larger vessels and the delicate capillaries through vasodilatory and vasoconstrictive functions. The tunica intima is the innermost section, consisting of a single layer of endothelial cells which lines the entire circulatory system, and the internal elastic lamina, as opposed the systemic circulation, which has an external elastic lamina not present in the cerebral circulation (Cipolla, 2009, Lee, 1995). Capillaries consist of a single-cell thick layer of
endothelium only, and this single cell layer enables the exchange of gas and nutrients into the capillary beds. Figure 1 shows the structure of the blood vessel wall.

![Artery structure with tunics](image.png)

*Figure 1 Structure of arterial wall showing the tunics. Reproduced from Martini et al. (2013), Essentials of Anatomy and Physiology, with permissions. Copyright © Pearson Education Limited*

While vessels of the cerebral circulatory system are generally understood to be structurally similar to systemic arteries, some slight differences are apparent, such as fewer pinocytotic vesicles in the endothelial layer, and the presence of tight junctions between capillaries (Kalaria, 1996). In comparison to the systemic
circulation, the cerebral arteries differ in that they are lacking an external elastic lamina, the tunica media appears to contain far fewer elastic fibers than seen in the peripheral vasculature, and the tunica adventitia is very thin in the brain (Cipolla, 2009).

Interestingly, when compared to an artery or arteriole of the same size diameter in the heart, blood pressure in the microvessels is lower in the brain (Faraci and Heistad, 1990). Pressure differences also exist regionally within the brain, as it has been reported that blood pressure varies from the cortex to the brain stem (Mayhan et al., 1986). Researchers attribute this to the large cerebral arteries having greater resistance than those in other organs, most likely due to variations in relative diameter, length and branching pattern of the supplying vessels (Faraci and Heistad, 1990).

### 2.1.2 Arteries and arterioles

The brain is one of the most heavily perfused organs on the body, receiving roughly 20% of the total cardiac output, despite making up only 2% of total body weight (Kalaria, 2010). The cerebrum receives blood from two pairs of major arteries- the left and right internal carotids, which supply blood to the anterior portion, and the left and right vertebral arteries, which join to form the basilar artery at the medulla-pons junction and are responsible for the posterior circulation. From here the vasculature is tree-like in structure; the circle of Willis is formed by large, interconnected arteries at the base of the brain. This formation is comprised of the internal carotids and the basilar system, interconnected by the communicating arteries. Blood carried by
these is reallocated to distinct areas of the brain by the anterior, middle and posterior cerebral arteries.

Blood vessels are innervated by perivascular nerves. Research has provided evidence that vascular function may be affected by age-related losses in innervation (Bleys and Cowen, 2001). While all cerebral arteries can be potentially affected by aging, those that form the circle of Willis are the most commonly investigated. The arteries of the left side of the circle are larger than those of the right, most likely due to the fact that the left cerebral hemisphere is commonly dominant (Lee, 1995). The territories supplied by the major arteries are shown in Figure 2 below.
Figure 2 Lateral, sagittal and inferior views of the cerebral artery territories and major cerebral arteries

The circle-like organization of the main cerebral arteries is such that the brain remains constantly perfused, if any one vessel in the circulatory system becomes occluded blood flow is redistributed to an alternate vessel to maintain adequate pressure throughout the brain. From the base of large internal carotid arteries, the vessels then branch off sequentially into smaller formations, giving rise to the pial arteries, then to the intracerebral arteries that penetrate into the depths of the brain hemispheres, then finally divide into parenchymal arterioles and the vast capillary

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network that supplies the parenchyma (Kandel et al., 2000), see Figure 3. This highly dynamic, multicellular structure has at its core the neurovascular unit (NVU).

*Figure 3* Perivascular nerves surround the pial arteries on the cortex. These then penetrate the depth of the parenchyma within the Virchow-Robin space, becoming less innervated. Parenchymal arterioles in the neuropil of the brain are thus controlled by astrocytes and neurons. From here the arterioles branch into smaller and smaller microvessels to form the neurovascular unit (NVU). Note also the differences in smooth muscle components ranging
from pial arteries through to capillaries. Image reproduced with permission from (Cipolla, 2009). Digital Object Identifier for image
https://doi.org/10.4199/C00005ED1V01Y200912ISP002

2.1.3 Capillaries and the neurovascular unit

The microcirculation of the brain is comprised of a complex interconnected web of capillaries, made up of endothelial cells lacking smooth muscle. It has been estimated that there is one capillary for each neuron in the brain (Cipolla, 2009). Capillaries have the ideal structure to maintain the blood brain barrier (BBB), they are comprised of three main cell types- endothelial cells; these are innervated by sporadically-occurring pericytes; and astrocytes, the end-feet of which attach to the outside of the basement membrane. Because the vasculature lacks smooth muscle at this level, the regulation of the tone and diameter of capillaries is under the control of astrocytes and pericytes. These microglia completely surround the endothelial cells and support and maintain the BBB. Highly active neural areas are more heavily vascularized, with greater capillary density observed in areas of greater synaptic density (Farkas and Luiten, 2001).

The endothelial cells surround the capillary lumen in a single layer. Tight junctions create a virtually impermeable seal between the endothelial surfaces; these junctions are the basis for the integrity of the BBB system. The integrity of this system is of utmost importance in maintaining optimum functioning of the brain. A disproportionately large number of mitochondria are found in cerebral capillaries to
provide for the greater energy needs required by the blood brain barrier transport proteins.

The neurovascular unit (NVU) consists of neurons, blood vessels and astrocytes that interact as a unit to maintain neurological activity. It is comprised of the endothelial cells, pericytes at the level of the capillaries, vascular smooth muscle cells at the level of the arterioles, astrocytes and other microglia, neurons and the extracellular matrix (Winkler et al., 2011, Brown and Thore, 2011). It is the site of gas exchange and the BBB. This structure ultimately regulates the blood vessel response to changes in neuronal and metabolic demand. The NVU transmits change in neuronal activity to the capillaries to regulate blood supply proximally along the vessels via vasodilation and constriction. Maintenance of optimum neurovascular coupling is crucial to brain function. There are several means by which blood supply is regulated. The most important of these are autoregulation and neurovascular coupling (also known as functional hyperemia), which will be discussed in depth in Chapter 3.
Figure 4 Diagram of the blood brain barrier. Reproduced with permission from (RCT, 2017).
2.1.4 Veins and venules

The venous system of the brain consists of the dural sinuses and cerebral veins. The cerebral veins are valve-less, with no muscular tissue and thin walls to allow for drainage. These vessels are divided into two groups: the superficial cortical veins and the central or deep veins (Cipolla, 2009). The cortical draining veins lie superficially on the surface of the brain against the underside of the arachnoid mater, helping to maintain the aperture of the sulci (Kilic and Akakin, 2008). These superficial vessels drain the cortex and the subcortical white matter into the nearest dural sinus, either the superior sagittal sinus (SSS) or the transverse sinus via the straight sinus, depending on the origin of flow. As a general rule the superficial aspects of the cerebral hemispheres duct into the SSS and the posterior-inferior surfaces drain into the transverse sinus (Kilic and Akakin, 2008).

The deep central veins drain blood from the deeper white and gray matter in the interior of the brain surrounding the ventricles, where they conjoin with the cortical veins to empty into the SSS. From here blood flows via the confluence of sinuses to the sigmoid sinuses then empties into the jugular veins of the neck (Cipolla, 2009).
2.2 Structural changes in the cerebral vasculature with age that impact cognition

2.2.1 Larger vessels - structural change with aging

Aging is often accompanied by widespread changes in vasculature, both peripherally and in the brain (Mitchell, 2008). Alterations in the structure and function of the blood vessels occur at all levels of the vessel composition: the endothelium, the smooth muscle cells that control vascular tone, and the extracellular matrix of arteries (Scioli et al., 2014). Most generally, aging effects on the larger cerebral vessels generally involve the thickening and stiffening of the arterial walls. These changes can lead to vascular dysfunction which may result in reduced volumes of blood flow to the brain.
Cerebral blood flow disruption predates a variety of impairments in brain structure and function (Ajmani et al., 2000), with previous studies showing that impaired brain vascularization is a predictor of cognitive decline (Gorelick et al., 2011, DeCarli et al., 2001).

Aging is associated with an increase in arteriosclerosis. While build-up of atherosclerotic plaques adds to the width of the arterial walls, thickening of artery walls occurs with age even when arteriosclerosis is absent (Barodka et al., 2011). Larger vessels become stiffer and less distensible as vascular wall stresses tend to increase in old age (Desjardins, 2015). These biomechanical changes are the result of the thickening of the vessel wall, primarily due to an imbalance in the ratio of vascular elastin to collagen content seen in elderly individuals (Nagasawa et al., 1979, O'Rourke and Hashimoto, 2007, Hashimoto and Ito, 2009). While the amount of elastin present in cerebral walls is generally unchanged (Fonck et al., 2009), collagen content has been shown to markedly increase, and it is this altered ratio between the two that is believed responsible for overall stiffening. Elastin may lose its functionality with increasing age, adding to the loss of compliance of the vessels (Fonck et al., 2009). One study observed that pial vessels in mice undergo age-related alterations whereby the ratio of collagen to elastin increases with age. This change in ratio is understood to contribute to decreased vascular wall distensibility and ultimately suboptimal blood flow (Kang et al., 2015).

The modification to wall thickness also leads to decreased overall vessel diameter, leading to heightened shear wall stress and flow pulsatility (Desjardins, 2015), affecting the cyclic distension of the vessels, i.e., the beat-to-beat variation in vessel
diameter in response to the pulsatile blood pressure changes (Gorelick et al., 2011). Damage to the elastin and increased collagen deposition in the cerebral microvessels can occur as a result of increased flow pulsatility due to less cushioning effect in stiff large arteries (O'Rourke and Safar, 2005, Pase et al., 2014, Desjardins, 2015). These alterations in the components of the vascular wall have been shown to result in an increase of as large as three-fold in the intima-media thickness across the lifespan (Gorelick et al., 2011). Thickening of carotid artery intima-media has been shown to correlate with poorer cognition in both cross-sectional (Morović et al., 2009, Muller et al., 2007) and longitudinal research (Wendell et al., 2009).

Blood pressure and vascular tone in larger cerebral vessels are also affected by build-up of atherosclerotic plaques (Faraci and Heistad, 1990), increases in reactive oxygen species and mitochondrial oxidative stress (Davenport et al., 2012), fibrohyaline thickening of vascular walls, necrosis of smooth muscle cells and thickening of the basement membrane (D’Esposito et al., 2003), all of which lead to an overall decrease in compliance of the vascular system. Vessels throughout the white matter of the brain have been reported as becoming more tortuous with increasing age (Brown and Thore, 2011, Kang et al., 2015) causing increased arterial resistance, though this relationship is not observed in grey matter vessels (Iadecola et al., 2009, Kalaria, 2009). Veins and venules can also deteriorate with age. It is reported that increasing age can be accompanied by excess collagen deposits in the venous walls, which may result in periventricular collagenosis (D’Esposito et al., 2003, Brown and Thore, 2011). This degenerative disorder is
known to occlude venous flow in the brain and is strongly linked to leukoaraiosis (Moody et al., 1995) and cognitive deterioration (Schmidt et al., 2007).

These widespread detrimental changes in the compliance of the larger cerebral vessels result in greater stiffness, which in turn affects blood pressure and blood flow, and ultimately leads to injury downstream in smaller arteries and microvasculature. The brain relies heavily on an uninterrupted flow of oxygen and nutrients to support neurological function; thus, any age-related deterioration of the vasculature supplying it can have serious consequences for overall cognition.

### 2.2.2 Microvasculature- structural changes with age

The cerebral microvascular system is comprised of the arterioles, capillaries and venules that are $<300\mu m$ in diameter (Gates et al., 2009, Serné et al., 2007). Age-related alterations in the structure of the microvessels of the brain can contribute to changes in blood flow, blood pressure, the delivery of matter across the capillary walls and impede the removal of metabolic waste products such as carbon dioxide. Numerous reviews of age-related morphologic microvascular alterations have been published (Kalaria, 1996, Kalaria, 2010, Farkas and Luiten, 2001, Brown and Thore, 2011, Desjardins, 2015). It has been reported that increasing age can affect almost all the elements involved in the neurovascular unit, including the capillary endothelial cells, basement membrane, pericytes and the astrocytic end-feet that innervate the outer surface of the vessels (see Farkas and Luiten (2001), Ballabh et al. (2004) for reviews).
Numerous morphological changes can occur in the microvasculature with age, and it appears as though these structures are also affected by larger vessel impairment upstream (Faraci and Heistad, 1990). Both Kalaria (2010) and Farkas and Luiten (2001) have provided extensive reviews of studies investigating structural age-related microvessel changes. As in the larger vessels, thickening of the basement membrane and extracellular matrix due to increased collagen fibers is a commonly reported major impairment (D’Esposito et al., 2003). Astrocytic end-feet and pericytes have also been shown to increase in number with age (Rodríguez-Arellano et al., 2016).

Microvessels regulate blood flow and maintain neurovascular coupling. Age-related structural and physiological deformations of the vascular bed in the brain can impact cognitive performance by impairing blood flow responses to neural activation (Tarantini et al., 2016, Toth et al., 2017) due to disturbed hemodynamic flow patterns and impaired vasodilatory capacity (De la Torre, 1997).

2.2.2.1 Functional alterations in the microvasculature with age

Changes in the shape of cerebral arterioles and capillaries in elderly individuals were first reported in the early 1900’s (Cerletti, 1910). While the extent to which abnormalities in the size, shape and tortuosity of cerebral blood vessels occur in normal aging has been disputed (De la Torre, 1997), there is extensive evidence to support the notion that alterations to vessel structure occurs in many neural regions over the lifespan (Sonntag et al., 2007, Kalaria, 1996, Iadecola, 2004, Iadecola, 2010, Iadecola et al., 2009).
It is well documented that both the diameter and density of capillaries affect brain perfusion (Meier-Ruge et al., 1980, Bell and Ball, 1981, Kalaria, 2010, D'Esposito et al., 2003, Brown and Thore, 2011). Increasing age is associated with a reduction of arterioles throughout the periphery of the body, and loss of arteriole-to-arteriole connections in the microvasculature (Hutchins et al., 1996). While some research has indicated that the diameter of the capillaries and arterioles actually increases with age (Bell and Ball, 1981) others have found either no change or regionally-dependent differences (Meier-Ruge et al., 1980).

Regionally, it has been reported that capillary density in the aging hippocampus decreases (Iadecola et al., 2009) whereas density of arterioles in this area increases significantly (Bell and Ball, 1981). Density of the capillary network as a whole has been found to increase across the neocortex in general with aging (Meier-Ruge et al., 1980). Both hippocampal and cerebral cortex capillaries are also found to develop thickened and fibrotic basement membranes (Farkas and Luiten, 2001).

Regardless of the mechanisms behind these changes, it is assumed that alterations in vessel size and shape would have the potential to produce substantial hemorheological changes in the microvasculature, decreasing the flow of blood and thereby reducing the delivery of nutrients and oxygen to the brain tissue (Sonntag et al., 2007). Studies have shown that long-term hypoperfusion can predict consequent cognitive decline in both cognitively impaired (Chao et al., 2010) and non-impaired adults (Meyer et al., 2000).
The small arterioles and capillaries of the brain precisely regulate their diameter and tone in order to maintain a constant flow of oxygenated blood to the parenchyma. As these microvessels converge to form capillaries, the thick band of smooth muscle cells that normally surround arteries thins out until there is no more to control vascular tone. The endothelial cells are thus controlled by astrocytic glial end-feet and pericytes, which are in turn altered throughout the aging process (Ojo et al., 2015). As the vascular wall thins out from the pial arteries to the capillaries, pericytes replace the smooth muscle cells to support and elicit control over the vascular diameter and tone (Figure 3). The precise roles of pericytes in the neurovascular unit remain inconclusive, though it has been reported that the loss of pericytes with age can cause vascular damage, leading to reduced capillary function and cerebral blood flow, neurovascular uncoupling, and breakdown of the blood brain barrier (Bell et al., 2010). Pericytes are known to undergo degeneration with aging, potentially augmenting cerebral microcirculation via reduction and dysregulation of CBF in response to brain activation. This disruption of flow and stimulus-driven flow response has the potential to result in chronic perfusion stress and hypoxia (Bell et al., 2010), conditions both associated with subsequent cognitive decline (Liu et al., 2014, Johnson et al., 2005, Ruitenberg et al., 2005).

Perivascular astrocytes surround the outside of the basement membrane with their end-feet to support the blood brain barrier and help regulate capillary diameter. A review on astrocytes and aging reported that while the role of astrocytes in the microvasculature has traditionally been viewed as a passive support cell, these cells potentially influence the function of synaptic transmissions and survival of neurons (Cotrina and Nedergaard, 2002).
### 2.2.3 Summary of structural cerebrovascular changes that occur with age

Over the lifespan, the cerebral vasculature undergoes multiple structural changes from the macroscopic level of the ultrastructure of larger extra-cerebral vessels down to the microvasculature and the components that comprise the neurovascular unit (NVU). These alterations, even in the absence of disease, can impair the capacity of the cerebral circulation to supply blood to regions of the brain parenchyma, potentially leading to hypoperfusion, neural damage and ultimately affecting cognitive performance.

### 2.3 Mechanisms behind cerebral microvascular dysfunction with age

The health of the cerebral microcirculation is fundamental as this is the primary site where exchange of oxygen and nutrients and the removal of carbon dioxide and other waste products occur in the brain. Neural tissue is dependent on sufficient perfusion to operate effectively, thus any alterations in the microvessel system with age will potentially have significant consequences for brain function and ultimately cognition. Several factors can cause microvascular dysfunction across the lifespan, including genetic, environmental and lifestyle, developmental and biological components. The following section focuses on biological causes of cerebral microvascular change with age. Cerebral endothelial dysfunction, changes in biochemical production of the endothelium, arteriosclerosis, and increased blood
pressure in the microvessels are commonly observed in older age, and these alterations impact on the functioning of the microvasculature.

2.3.1 Endothelial dysfunction with age

Endothelial impairment is a main aspect of microvascular change with aging, and is linked to all major cardiovascular diseases (Drexler and Hornig, 1999). The endothelium is a single layer of cells that lines the inside of the blood vessel walls, see Figure 4 above. This serves multiple functions including protecting the wall from damage, maintaining blood circulation and fluidity, and the control and maintenance of vascular tone under the influence of specific vasoactive substances, among numerous other functions (Rajendran et al., 2013). Maintenance of vascular homeostasis is important as changes in the ability of the vasculature, both peripherally and in the brain, to regulate blood flow in response to metabolic demands can result in ischemia and eventually, neurodegeneration and cognitive decline. Endothelial function in the brain can be assessed by tests of cerebrovascular reactivity (CVR), further discussed in Section 2.6 below.

The control of blood flow through larger vessels is dependent on the reciprocal functioning of smooth muscle cells and the endothelial cells. Vasoactive substances are produced by the endothelium resulting in either relaxation or contraction of smooth muscle surrounding the vessel lumen, thus regulating flow. The endothelium can exhibit dysfunction in a variety of ways, and the term ‘endothelial dysfunction’ is broadly defined as a systemic pathological state wherein there is an imbalance between vaso-dilating and vaso-constricting factors produced by the endothelium.
(Deanfield et al., 2007). Reduced production and/or release of nitric oxide (NO) by the endothelium are considered to be the hallmark of dysfunction.

In addition to the reduced capacity for NO production, normal aging is associated with the loss of glucose transporter Na+/K+ ATPase found in endothelial cells, potentially resulting in reductions in the cytoplasm and endoplasmic reticulum, enhanced pinocytosis, dwindling mitochondrial function and changes to tight junctions (Kalaria, 2010). These extensive modifications across the lifespan may precede and contribute to cognitive impairments seen in the elderly. Aging effects on cerebral endothelial function have most often been examined using animal models as few *in vitro* studies assessing isolated human blood vessels have been performed. These, and data collected from limited *in vivo* human studies, are mostly consistent with those obtained from animal research (Farkas and Luiten, 2001).

With age, the functioning of the brain endothelial cells becomes diminished, and can lead to a lowering of the capacity of brain microvessels to respond to changes in blood gas tension and/or metabolic demands. Several mechanisms lead to dysfunctional endothelial cells in the elderly; the most commonly reported change with aging is impaired production and/or release of vasodilatory biochemical products, and the augmentation of vasoconstrictive products (Vanhoutte, 2002). This leads to increased contraction and reduced dilatory capacity, potentially resulting in hypoperfusion, reduced vascular reserve and increased risk of stroke, cardiovascular disease and other ischemic events (Yonas et al., 1993). Cross-sectional studies have indicated that endothelium-dependent dilatation is gradually reduced with age (Sorond et al., 2008, Brandes et al., 2005, Gerhard et al., 1996). This functional
alteration in the tone of the vasculature results in an overall stiffening of the arteries with age.

Older age is the primary risk factor for cardiovascular disease (CVD) (Sutton-Tyrrell et al., 2005). This is, amongst other reasons, due in part to the loss of function of the endothelium. The endothelial cells are responsible for the regulation of blood flow via constriction and dilation of the blood vessels. With increased age the functioning of the endothelium reduces, and its ability to elicit dilation becomes impaired. This dysfunction is a major cause of the development of CVD, which is linked to increased risk of developing dementia (Newman et al., 2005).

Vaso-active chemicals are produced by endothelial cells in response to changes in blood pressure, shear stress and pH level, amongst others. These biochemicals are released to control the diameter of the vessels and capillaries to regulate blood flow. With advancing age, the production of vaso-active substances can become sub-optimal, which will be further discussed below.

2.3.1.1 Age-related alterations in biochemical production in the endothelium

As the brain ages, the capacity for regeneration and angiogenic action is reduced. DNA damage occurs due to alterations in biomolecular expressions of various chemicals. Reviewing the entirety of biochemical alterations in the aging brain is beyond the scope of the current thesis. See Scioli (2014) for an in-depth review of the multitude of biochemical changes in the microvasculature that occur with
advancing age. The current section aims to briefly summarize relevant biochemical changes that occur in the cerebral endothelium with age.

The cells of the endothelium release various chemical products to control smooth muscle tone in the blood vessels. While there are numerous substances involved in the control of endothelial activity, three are most important—nitric oxide (NO), prostacyclin (PGI2) and endothelin-1 (ET-1). NO and PGI2 are vasodilators, whereas ET-1 is a vasoconstrictor and causes the contraction of blood vessels.

NO is a highly reactive molecule naturally produced by the endothelium that causes arterial dilation to regulate blood flow. It is the main ‘endothelium-derived relaxing factor’ (EDRF), and signals to the smooth muscle surrounding the blood vessels to relax, resulting in vasodilation, increased blood flow and lowering of the blood pressure (Kang, 2014). Aging is associated with a deterioration of chemical production by the endothelium, such that there is less bioavailability of NO (Vanhoutte, 2002). This is understood to be resulting from impaired endothelial NO synthase (eNOS) activity and activation of NO.

During the aging process mitochondria produce increasing amounts of reactive oxygen species (ROS), namely superoxide, which reacts with NO and causes it to become inactive. Peroxynitrite is the product of this reaction and is known to decrease the availability of another molecule that is used in the production of NO, thereby further reducing the amount of NO available. Levels of superoxide and other ROS are generally kept in check by the body’s antioxidant enzymes, however with increasing age the production of ROS increases without a compensatory increase in
antioxidant production. This increase in oxidative stress leads to a variety of pathological conditions, including cardiovascular disease.

PGI2, the second EDRF, is a locally active hormone that will relax vascular smooth muscle when released on the abluminal side of the blood vessel, and prevent platelets from clumping together when released into the lumen (Vane et al., 1990). It is formed from the enzyme cyclooxygenase. This pathway is also referred to as the arachidonic pathway because PGI2 is a metabolite of arachidonic acid. The ability of blood vessel tissue to produce prostanoids becomes diminished with age, diabetes mellitus and in atherosclerosis, indicating a direct link between the capacity for biosynthesis of the vasculature and vulnerability to thrombosis and sclerotic plaque build-up with age (Vane et al., 1990).

There are three factors known as the endothelium-derived relaxation factors (EDRFs) - NO, PGI2 and EDHF (endothelium-derived hyperpolarizing factor) - that differentially control vasodilation. The NO and PGI2 pathways are well characterized, but the EDHF pathways is less well understood (Krummen et al., 2005). The PGI2 pathway causes smooth muscle cell relaxation via the actions of prostanoids, including prostacyclin. This pathway acts independently of NO, but it is reasonably limited in its vasodilatory capacity as compared to the others (Deanfield et al., 2007). The NO synthase (NOS) pathway elicits vaso-relaxation via hyperpolarization of the smooth muscle cells surrounding blood vessel walls. While these two pathways explain most endothelium-dependent relaxation, more recent studies identified that a third pathway was also involved. This route involved the hyperpolarization of underlying vascular smooth muscle cells, also independently of NO, and was named endothelium-derived hyperpolarizing factor (EDHF). EDHFs are a group of
substances known to open the K+ channels in endothelial cells to hyperpolarize the smooth muscle cell membrane (Krummen et al., 2005). Endothelin-1 pathways are also implicated in modifying cerebral perfusion with age (Gates et al., 2009). This compound, along with thromboxane A2 and prostaglandin H2 are understood to influence microvascular constriction (Shore, 1996). Augmentation of the production and release of endothelium-dependent vasoconstricting factors (EDCF) is believed to occur with increasing age, leading to hypercontractility of the arteries.

It is suggested that dysfunctions of the vascular endothelium are a mechanism implicated in the initiation and progression of arteriosclerosis, and the progressive build-up of fatty plaques known as atherosclerosis, further discussed below (Gates et al., 2009, Deanfield et al., 2007, Davignon and Ganz, 2004).

### 2.3.2 Arteriosclerosis with age

As in peripheral blood vessels, the vasculature of the brain is also susceptible to age-related damage and endothelial dysfunction, which is a known precursor and determinant of arteriosclerosis. Arteriosclerosis is an umbrella term used to denote any disease in which the artery walls become thicker, harder and lose elasticity. While it can occur naturally with normal aging (Furuta et al., 1991), arteriosclerosis has been implicated as the largely responsible for key indicators of vascular aging, including increased systolic and pulse pressures, increased pulse wave velocity, and lowered diastolic pressure (Barodka et al., 2011). The disorder also often results in decreased vascular responsiveness to changes in arterial gas tension (Ito et al., 2002).
Arteriolosclerosis is a specific type of arteriosclerosis, described as the impairment of dilation of the smaller arterioles in response to changes in flow. It is the result of the stiffening of the arteries, resulting in reduced elasticity of arterioles. It is distinct from atherosclerosis, defined as the stiffening of medium and larger sized arteries caused by atheromatous plaques accumulating in the tunica intima of artery walls (Scioli et al., 2014). Atherosclerosis is common in aging. This build-up of fatty plaques in the interior walls of blood vessels that leads to the hardening and narrowing of the arteries is a common cause of heart attacks, peripheral vascular disease and stroke (Schwartz et al., 1991). Atherosclerosis may result in reduced elasticity of the blood vessels, rendering them less able to respond to changes in blood-gas tension, pressure and demand. Increased stiffness of arteries impairs the capacity for the vessels to react to changes in flow volume and rate in response to changes in metabolism needs can lead to neural damage and in due course will affect cognitive performance (Marshall and Lazar, 2011, Brown and Thore, 2011).

Atherosclerosis is understood to develop, in part, due to the diminished functionality of the cerebral endothelium. As endothelial cells mature and reach the end of their natural life cycle, they will undergo apoptosis and fall away from the blood vessel wall to enter into the bloodstream. This leaves a gap in the vasculature between neighboring endothelial cells, which normally form tight junctions with one another. Remaining endothelial cells regenerate and multiply to close the gap; however, these cells are generally dysfunctional themselves, they exhibit a loss in the capacity to activate nitric oxide synthase (NOS) that normal endothelial cells possess. Areas of the vasculature affected by these regenerated and dysfunctional endothelial cells
suffer from a chronic shortage of NO, and which triggers the atherosclerotic process via various actions including platelet aggregation and adhesion and expression of adhesion molecules (Vanhoutte, 2002, Vanhoutte et al., 2009).

Arteriosclerosis is associated with poorer cognitive performance as evidenced by large-scale epidemiology studies (Bos et al., 2012, Romero et al., 2009) and dementia research (Launer et al., 2000, van Oijen et al., 2007). Clinically silent age-related arterio- and atherosclerosis can lead to alterations in the capacity for the vasculature to regulate blood flow in the brain due to reduced reactivity of the cerebral blood vessels.

2.3.3 Summary of mechanisms underlying microvascular dysfunction with age

Numerous degenerative processes can lead to dysfunctional blood vessels with age. Structural impairments of the cerebral endothelium due to vessel wall stiffening, arteriosclerosis, and reduced production of vasoactive substances can lead to decreased reactivity and compliance in the normal aging process. These alterations can impede the ability for the cerebral circulation to regulate blood flow in the aging brain, further discussed in the following section.

2.4 Changes in cerebral blood flow regulation with age

Cerebral hemodynamics change over the normal aging process (Sorond et al., 2008). An aging cerebrovascular system loses integrity through a variety of
mechanisms. Loss of functionality can lead to disruption in the blood brain barrier, resulting in cognitive and sensorimotor decline. Various age-related alterations in the brain can affect the complex coordination required for flow-metabolism coupling. The vast metabolic demand coupled with relatively low energy storage capacity means that the brain requires a constant stream of blood, as this organ is especially vulnerable to disrupted oxygen and substrate supply (Greene and Lee, 2012).

Normal brain electrical activity is generally lost at flows of 16-18ml/100g/min, and serious ischemic injury and membrane failure will occur when regional cerebral blood flow declines to less than ~10ml/100g/min, as evidenced by experimental animal trials (Markus, 2004, Heiss et al., 1976). Following a lack of blood flow, free radicals begin to accumulate, intracellular enzymes are released, and calcium enters the brain cells. Neurons are for the most part incapable of anaerobic metabolism, so hypoxia for any extended period of time will result in damage and cell death. A drop in cerebral blood flow (CBF) to half of its normal rate would result in unconsciousness in most normal healthy individuals (Clarke and Sokoloff, 1999). Fortunately, a number of reflexes and mechanisms exists that safeguard against extreme changes in arterial blood pressure and CBF.

Constant blood flow in the brain is maintained under a plethora of physiological or pathophysiological conditions. Wide-ranging fluctuations in arterial blood pressure (in the ranges of ~50-160mmHg) (Kalaria, 2010), alterations in venous and intracranial pressure, body temperature, hemorheological (viscosity) properties of the blood, and active vasoconstriction and dilation of the cerebral vessels all have the ability to alter blood flow, yet the ability of the cerebral circulation to autoregulate overcomes these to uphold the constant stream of oxygen and nutrients to the parenchyma.
In the brain, the majority of cerebrovascular resistance is under the control of those outside of the brain parenchyma – the larger cerebral vessels and pial vessels – while the remainder of the resistance is accounted for by the intracerebral arterioles and capillaries (Tarumi and Zhang, 2014). The complex coordination of the extra-cerebral and intra-cerebral vessels maintains perfusion at a relatively steady rate in the face of varying pressures and metabolic demands. Since the brain cannot tolerate any major alterations in its perfusion, several mechanisms regulate the flow of blood into the brain tissue. CBF is sustained by varying directly with cerebral perfusion pressure (CPP), the pressure gradient that drives the blood from the aorta up into the brain. CPP is the difference between the mean arterial pressure (MAP) and the intracranial pressure (ICP), and is normally maintained within the normal limits of 70-100mmHg (Carlson and Nurses, 2008). CPP also varies inversely with the resistance of the cerebral vessels. Therefore, the distribution of the cardiac output in the brain depends upon both the pressure gradients within the brain, and the resistance to blood flow that exists within the small arteries and arterioles. If the CPP drops below the threshold, it will result in ischemia. If it raises too high, hyperemia (excessive blood flow) will result. Cerebrovascular resistance is the consequence of friction between the blood and the vascular walls and exists due to interactions between the viscosity of blood and the radius or diameter and length of the vessels.
Figure 6 Autoregulation maintains constant CBF at mean arterial pressure (MAP) between ~50-160 mmHg, intracranial pressure (ICP) from 0 up to ~15-20 mmHg. Cerebral perfusion pressure (CPP) = MAP – ICP, optimally =~70-100 mmHg.

The diameter of cerebral blood vessels is under the control of four main factors: autoregulation (myogenic, relating to pressure changes), cerebral metabolism (metabolic, relating to changes in metabolic demand), carbon dioxide and oxygen, and neurohumoral factors (referring to changes in neural [autonomic] and humoral – [hormonal] factors, vasopressin and endothelin-1 are some examples). The diameter
of the vessels is of importance as vasodilation also results in increased cerebral blood volume, which leads to increased ICP and reduced CPP.

It is well established that blood pressure tends to increase with advancing age, and this is generally thought to be the result of structural changes to the arteries. Arteriosclerosis occurs during the normal aging process, leading to vessel stiffening (Furuta et al., 1991). Longitudinal data from the Framingham Heart Study demonstrated that systolic pressure tends to increase continuously from about 30 years of age, while diastolic pressure is more variable, increasing up until ~50 years, and then gradually decreasing after approximately the sixth decade (Pinto, 2007). This leads to a steep rise in pulse pressure, which, when coupled with stiffened arteries, may subsequently result in end-organ damage as the cushioning effect of elastic vessels is reduced (Pase et al., 2010). The cerebral circulation by nature has low vascular resistance, and when combined with dramatically increased pulse pressure and reduced elasticity, surges of blood flow can potentially result in injury to microvessels and brain tissue (O'Rourke and Safar, 2005). Thus, there is a two-fold process of cardiovascular assaults at work, increasing the likelihood of neural damage, and subsequently, cognitive decline.

The following section details the age-related changes that may occur in cerebral blood flow regulatory mechanisms. Autoregulation, neurovascular coupling and cerebrovascular reactivity (CVR) are discussed.
2.4.1 Cerebral autoregulation and the effects of aging

Blood pressure increases cause the pre-capillaries in the brain to contract. Myoepithelial cells in the pre-capillaries also contract when they are stretched, thus preventing a large rise in blood flow. Cerebral blood flow remains at a relatively constant rate across a range of arterial blood pressure from 50 to 160 mmHg (Kalaria, 2010, Edwards et al., 2002). This is known as cerebrovascular autoregulation and describes the process that maintains cerebral blood flow close to an optimum level, even though the arterial blood pressure is deviating from what is normal. This pressure-dependent regulation is a mechanoregulatory mechanism, as compared to the chemoregulatory action of neuronal and CO₂-dependent control, discussed further below.

Described as the “intrinsic ability of an organ to maintain a constant blood flow despite changes in perfusion pressure (Klabunde, 2011)”, the autoregulatory process causes CBF to return to the baseline level in response to a change in perfusion pressure due to adjustments to the cerebral resistance. The incitement to autoregulation is the cerebral perfusion pressure (CPP) rather than the mean arterial pressure (MAP). Vascular smooth muscle cells will constrict in response to increased vascular wall tension and relax in response to reduced wall tension (Hill and Gwinnutt, 2007).

Lassen (1959) first used the term ‘cerebral autoregulation’ in a review of over 200 clinical trials that assessed CBF. The review concluded that there was a ‘plateau phase’ which occurred in the CBF response to long-term gradual changes in blood pressure. The ‘plateau’ refers to cerebral autoregulation, whereby the CBF remains
constant over variable blood pressure changes. Age-related alterations in arterial blood pressure coupled with degeneration of upstream vessels may lead to a shift in the autoregulatory upper and lower limits, potentially leading to a multitude of impairments, ranging from damage of the cerebral endothelium, leakage at tight junctions that maintain the BBB or edema (Kalaria, 2010).

![Diagram](image)

*Figure 7* Cerebral autoregulatory process maintains constant cerebral blood flow at perfusion pressures ranging between approximately 50-160 mmHg

When the supply of blood to the brain, or any other organ, is temporarily partially occluded there will be an initial drop in flow, which returns to normal levels once the occlusion is removed. The relationship between cerebral perfusion pressure (blood pressure in the cerebral arteries minus blood pressure in the veins, denoted as $P_{A-P_V}$) flow ($F$) and resistance ($R$) is shown in Equation 1 below.
Reduction of blood flow causes the resistance of the arterial system to drop due to the dilation of arterioles and small intra-parenchymal vessels. The autoregulatory process underpins macroscopic vascular tone changes in response to physiological demand like exercise and in pathological situations such as myocardial infarction (Muoio et al., 2014).

Myogenic vascular regulation refers to the control of blood flow by the vascular smooth muscle surrounding arteries and arterioles. Cerebral vessel walls will relax to dilate the vessel when internal blood pressure drops to maintain vascular tension and contract to constrict the vessel diameter when wall tension increases. Studies have reported no change in autoregulatory functions with normal age in healthy samples without cardiovascular risk factors (Matteis et al., 1998, Kastrup et al., 1998, Carey et al., 2000, Rosengarten et al., 2003, Yam et al., 2005). It is assumed that the vasculature of the brain is able to maintain blood flow at varying pressures in the healthy aging process, though further research is needed to ascertain autoregulatory functions in those over 70 years of age (Carey et al., 2000).

\[ F = \frac{(P_A - P_V)}{R} \]  

2.4.2 Summary of autoregulation in aging

While autoregulatory functions may not change dramatically over the lifespan, blood pressure is known to increase significantly, probably due, in part, to stiffening of the arteries (Pinto, 2007). Increases in systemic blood pressure with age, coupled with
reduced elasticity of blood vessels can result in increased pulsatile pressure (Pase et al., 2010). Low vascular resistance of the brain paired with increased pulsatile pressure and reduced vascular elasticity results in less cushioning of surges of inflowing blood, potentially leading to damage to both microvessels and the brain parenchyma with age (O'Rourke and Safar, 2005). These alterations could have major repercussions for both vascular and cognitive health across the normal aging process. The following section will discuss the effects of aging on neurovascular coupling.

2.5 Neurovascular coupling and the effects of aging

Neurovascular coupling (NVC), or functional hyperemia, refers to the microscopic control of blood flow at the regional level, ensuring the optimum oxygen and nutrient delivery locally in specific brain areas in response to neuronal needs (Filosa, 2010). This process maintains coupling of blood flow to neuronal demands through metabolic and myogenic means. NVC can be assessed by measuring the reactivity of cerebral vessels in response to vasoactive challenges, discussed in further in Section 2.6 below.

The capacity for the cerebral blood vessels to react to changes in neuronal demand is an important regulatory mechanism. Actively metabolizing cells secrete vasodilatory substances which result in dilation of the small arterioles. Vasodilation can also be caused by hypoxia and changes in local tissue metabolite concentration. Changes in the metabolic demands of the brain require dynamic regulation of cerebral blood flow, and this is achieved by control of the degrees of constriction and
dilation of cerebral blood vessels. The tone of the brain vasculature is in turn controlled by local chemical factors, arterial CO$_2$ tension (PaCO$_2$), oxygen tension (PaO$_2$) and pH, numerous vasoactive substances, including ions, various types of neurotransmitters and vasoactive factors that are released in response to the actions of those neurotransmitters, all work in concert to elicit functional hyperemia (Girouard and Iadecola, 2006). High PaCO$_2$, low PaO$_2$ and low pH will dilate the blood vessels and increase CBF, and the opposite will cause blood vessels to constrict and CBF will decrease. These mechanisms regulate the flow of blood to the brain to maintain homeostasis of chemical factors in the surrounding local tissues.

Increased activity of neurons leads to changes in metabolite concentration, lowering of the partial pressure of oxygen, increased partial pressure of CO$_2$ with an associated lowering of pH, increased temperature and an increase in concentration of potassium ions (Girouard and Iadecola, 2006). These alterations in local metabolites cause vasodilation, and vasoconstriction occurs when metabolic activity is slowed and vasoactive materials are washed out. Neurons, astrocytes and pericytes and cerebral capillaries together, form the neurovascular unit where control of the delivery of substrates that support cell function and removal of metabolic wastes takes place. With aging, this unit can become dysfunctional due to a variety of mechanisms, as outlined in Sections 2.2 – 2.3 above. Research suggests that although this unit may undergo significant alterations across the lifespan, in healthy aging not complicated by neurodegenerative disease and/or cardiovascular risk factors, NVC remains relatively stable (Panczel et al., 1999, Rosengarten et al., 2003).
2.5.1 Summary of neurovascular coupling with age

The preservation of neurovascular coupling in the aged brain requires the integrity of the cerebral arterioles to dilate in response to a vasomotor stimulus, a process referred to as cerebrovascular reactivity. These mechanisms all contribute to the decreasing ability of the cerebral microvessels to react to changes in metabolic demand. Maintenance of constant and stable cerebral pulse pressure comes under the responsibility of cerebrovascular reactivity. The following sections provide an in-depth account of the more dynamic aspects of the cerebral circulation, namely cerebrovascular reactivity, blood flow and metabolism of oxygen, and the effect that normal aging has on these parameters. Methodological considerations that need to be considered during assessment are also discussed.

2.6 Cerebrovascular reactivity

Vasoreactivity is the dilatory or constrictive reaction of a blood vessel to a stimulus. This index relates closely to neurovascular coupling (NVC), as impaired vasomotor responsiveness would also impair the capacity for NVC. In the peripheral body, vasoreactivity or vasomotor responsiveness is commonly assessed using flow mediated dilation (FMD) or pulse wave analysis (PWA). While the systemic vasculature undergoes similar changes in reactivity over the lifespan, the main focus of this section is the responsiveness of the cerebral vessels. The reactivity of cerebral blood vessels to changes in arterial tension of carbon dioxide is often studied as this measurement can provide a direct assessment of vascular brain health, providing vital information about the integrity of the vascular system (Lu et al., 2009). Measurement of this index offers data on the dynamic aspects of blood
supply and metabolism that are not able to be obtained via other parameters such as basal perfusion and autoregulation. With regards to the relationship between ageing and cognition, it is known that age-related increases in blood pressure are related to cognitive performance via the effects on neurovascular coupling (Novak and Hajjar, 2010). The capacity for blood vessels to react to changes in arterial blood gases is a crucial function that enables other processes such as autoregulation and perfusion pressure stability to occur optimally. If the vasculature is unable to respond in a timely manner to energetic and metabolic requirements due to increased stiffness and/or impaired CVR, multiple physiological processes will suffer. The reactivity or adjustment of the blood vessels to acute metabolic changes that occur in the body’s tissues is a vital function, and impaired vascular reactivity is associated with greater risk of stroke (Sonntag et al., 2007, Yonas et al., 1993, Webster et al., 1995). Lower blood vessel reactivity to vasodilatory challenges such as hypercapnia (increased CO₂ in the arterial blood) is associated with elevated arterial stiffness via a common mechanism of reduced or dysfunctional distensibility of the vessels (Flück et al., 2014). Vasoreactivity in the brain differs between vascular territories, and is heterogeneous across regions: it has been found to be higher in the parietal and occipital areas when compared to frontal, temporal and insula cortices (Novak, 2012). Regions with a lower vasodilatory capacity could be more affected by reduced perfusion than areas that have greater reactivity (Zhao et al., 2009).

Cerebrovascular reactivity (CVR) reflects the capacity for cerebral blood vessels to dilate and deliver oxygen and nutrients to the brain tissue. Increasing or decreasing the partial pressure of arterial CO₂ (PaCO₂) will increase or decrease CBF respectively, by vasodilation or vasoconstriction in the cerebral vessels,
independently of autoregulation (van Lieshout and Wieling, 2003). This physiological response is implicated in a range of disorders including migraine (Harer and Kummer, 1991), diabetes mellitus (Dandona et al., 1978), coronary artery disease (Webster et al., 1995, Galvin et al., 2010) mild cognitive impairment (Richiardi et al., 2015, Viticchi et al., 2012) and AD (Bar et al., 2007, Cantin et al., 2011, Glodzik et al., 2013, Heun, 1994, Kelleher and Soiza, 2013, Silvestrini et al., 2006, Suri et al., 2015, Yezhuvath et al., 2012). Low CVR indicates poor cerebrovascular integrity and decreased endothelial function. Reduced endothelial function has been linked to poorer cognitive performance (Sinn and Howe, 2008), a concept that will be further explored in the following chapters.

CVR is calculated as the percentage increase in CBF velocity or flow during a vascular challenge divided by the absolute increase in expired CO2 (etCO2) in the same period. Vascular challenges vary, and this can impede the comparability between them. Varying methods can be extrinsic stimulation by vasodilators, such as acetazolamide (Szatmári et al., 2010), hyperventilation (Haussen et al., 2012), or hypercapnic challenge (Sicard and Duong, 2005, Liu et al., 2007, Rostrup et al., 2000). Hypercapnic challenges in CVR assessment can be induced in several ways, including breath-holding, re-breathing exhaled gas, automated computer-controlled targeting of arterial CO2 and CO2 enhanced-gas inhalation (Fierstra et al., 2013, Bhogal et al., 2014, Tancredi and Hoge, 2013, Wise et al., 2007). CO2 inhalation is the most commonly used technique as it enables the researcher to have increased control of the levels of arterial CO2, is independent from subject compliance (in comparison to breath-holding), and is a harmless way to investigate the effects of vascular functioning on the blood flow parameters in the brain (Liu et al., 2012b, van
der Zande et al., 2005, Yezhuvath et al., 2009). Hypercapnia to carbon dioxide results in decreased vascular resistance in both large arteries and arterioles, but because the dilator response is larger in the arterioles than in large arteries, hypercapnia reduces the blood pressure in small vessels.

Widespread factors affecting CVR have been identified. As discussed earlier, evidence suggests that the dynamic components of the vessel walls (elastin and smooth muscle) decrease and the less dynamic components (collagen, basement membrane) tend to increase with age, which results in an overall stiffening of small vessels (Hajdu et al., 1990). This leads to thickening of the endothelial basement membrane which is assumed to contribute the age-related changes in blood vessel reactivity. Endothelial dysfunction, small cerebral vessel disease, inflammation and arteriosclerosis are known to decrease the elasticity and reactivity of the cerebral vasculature (Novak, 2012). These losses in function will potentially result in a lowering of the capacity for the brain to obtain optimum perfusion. Hypoperfusion is associated with decreased cognitive abilities in later life, even in the absence of disease or pathological degeneration (Novak, 2012, Kalaria, 2010).

A clarification of terms may be necessary here. While in the present thesis the acronym CVR is used to symbolize “cerebrovascular reactivity,” other publications have employed the term to stand for “cerebrovascular resistance” (e.g. Ropper (2005), Nation et al. (2013), Dastur (1985)). Cerebrovascular resistance is defined as the ratio of blood pressure to cerebral blood flow (Nation et al., 2013), and given that aging is generally associated with increasing blood pressure and reduced CBF, it would be expected that aging would go hand-in-hand with elevated cerebrovascular
resistance. This is in opposition to what we would expect in terms of cerebrovascular reactivity, which is believed to decrease with advancing age.

Measurement of CVR can be performed by means of several techniques including 15O positron emission tomography (PET), single-photon emission CT (SPECT), the xenon-133 washout technique (Reich and Rusinek, 1989, Davis et al., 1983, Levine et al., 1988) and transcranial Doppler ultrasound (TCD) (Kastrup et al., 1998, Battisti-Charbonney et al., 2011). In more recent years advances in magnetic resonance imaging (MRI) technology have enabled comprehensive structural and functional investigations to be performed to measure CVR on a regional level. These techniques are further discussed in Section 3.2.2.

2.6.1 Methodological considerations for assessing CVR in aging

Reports of CVR changes in normal healthy aging unaffected by vascular or neurodegenerative disease are mixed. Inconsistent evidence has reported that blood vessel reactivity in the brain with age is reduced (Flück et al., 2014, Barnes et al., 2012, Lu et al., 2011), augmented (Zhu et al., 2013), does not change across the lifespan (Davis et al., 1983, Murrell et al., 2013), and is gender-specific (Kastrup et al., 1998, Matteis et al., 1998, Bakker et al., 2004). Discrepancies between findings can be interpreted in the light of a number of factors, such as the age ranges used, varying vasodilatory stimuli and gender differences.
A large epidemiological study of over 1,700 individuals showed that CVR declined progressively at a rate of approximately 0.08%mmHg per year, suggesting that research with older cohorts would be more likely to identify age-related differences than those using a younger aged sample (Bakker et al., 2004). The measurement of CVR is also complicated by use of various vasoactive stimuli. As an example, a gas substance known as carbogen (consisting of 5% CO₂ and 95% O₂) is often used as the vascular challenge (Yonas et al., 1993, Cantin et al., 2011, Gao et al., 2013, Kastrup et al., 1998, Coverdale, 2015). The confounding vasoconstrictive action of hyperoxia would likely dilute the vasodilatory effects of the CO₂. One study compared the inhalation of 5% CO₂ in room air to inhalation of carbogen and determined that the values obtained from differing gas mixtures are not directly comparable (Hare et al., 2013). There are repeatable and standardized techniques available for the measurement of changes in CBF; however, the use of various vasoactive substances for vascular challenge can often hamper the comparisons that can be made between studies. A recent review of the various stimuli currently used to measure cerebrovascular reactivity suggests that CO₂ is the most appropriate vasoactive substance (Fierstra et al., 2013). Various vasoactive challenges are discussed further in Section 3.2.1.

The concentration of CO₂ used also impacts the comparability of results in aging studies. The percentage of inspired-CO₂ concentration ranges from 2% in some experiments to up to 10% in others (Coverdale, 2015, Tominaga et al., 1976). Each mmHg increase in PaCO₂ is expected to increase CBF by approximately 3% (Fierstra et al., 2013), though this relationship is also reported as being sigmoidal in nature, and dependent on the vascular territory studied (Sobczyk et al., 2014).
Additionally, the way in which CVR is reported has implications for the interpretation of aging effects. Some studies report CVR in terms of the absolute change, while others give values in relative terms (Coverdale, 2015), further confusing the generalizability of findings.

Vascular reactivity has been shown to differ between genders (Matteis et al., 1998, Bakker et al., 2004, Kastrup et al., 1997). One study examined changes in cerebral blood flow velocity in the middle cerebral artery (MCA) to 5% CO₂ inhalation (Kastrup et al., 1998). It was found that age did not have an effect on CVR to hypercapnia in men. However, pre-menopausal, but not post-menopausal women had a raised CVR. This finding suggests that estrogens could potentially affect the reactivity of cerebrovascular vessels to CO₂.

2.6.2 Summary of CVR in aging and methodological considerations

CVR reflects the capacity the cerebral microvasculature to respond to changes in arterial gas tension or other metabolic demands. Dysfunctional CVR indicates poor vascular health and integrity and is shown to be impaired in many conditions, including MCI and AD. A variety of methods are used to assess this index, including differing vascular challenges to elicit change in CBF and ways of measuring that change. Recent advances with MRI have enabled measurement of CVR by brain region. The relationship of vascular reactivity to cognition will be discussed in greater detail in Chapters 3 and 4. These chapters will examine the mechanisms behind vasoreactivity and methodological considerations for assessing the effects of aging.
on the CVR response. The influence of advancing age on cerebrovascular reactivity is not completely understood, though it would be expected that CVR would be reduced over the lifespan, given the well-described aging effects on the cerebral vasculature. However, various factors may confound results of aging studies, and hamper the comparability between findings. The following Section 2.7 will discuss cerebral blood flow and oxygen metabolism, and how these parameters may alter over the healthy lifespan.

2.7 Cerebral blood flow and cerebral metabolic rate of oxygen use and the effect of aging

Cerebral blood flow (CBF), or perfusion, is an important indicator of brain health. It refers to the rate of the delivery of oxygen and nutrients to the brain parenchyma, expressed as the amount of blood per 100g of brain tissue per minute. Total blood flow to the brain is approximately 750-1000ml per minute, about 350ml of this enters through each carotid artery and the remaining 100-200ml flows in through the vertebral arteries (Kandel et al., 2000). It has been reported that cerebral blood flow, both globally (Chen et al., 2011) and regionally (Martin et al., 1991) declines with age, though this regional change is heterogeneous across the brain (Parkes et al., 2004). However, some historical studies report that CBF does not change in healthy aging free from cardiovascular or degenerative disease (Dastur et al., 1963).

Cerebral metabolic rate of oxygen use (CMRO2) refers to the rate and amount of oxygen taken up by the brain tissue from arterial blood. The brain uses approximately one fifth of the body’s oxygen supply (Peng et al., 2014). Historically,
CMR\textsubscript{O}2 techniques required invasive dynamic sampling of the arterial blood - as in the Kety-Schmidt (1948b) and 15O PET methods (Aanerud et al., 2012), but more recently the measurement of CMR\textsubscript{O}2 has been performed indirectly. Values of total cerebral blood flow and both arterial and venous blood oxygenation are required to estimate the amount of oxygen perfused into brain tissue. The control and use of oxygen in the brain is of particular importance, as the metabolism of oxygen in the brain is the primary means of energy production in the neural tissue (Magistretti and Pellerin, 1999).

It is most often reported that CMR\textsubscript{O}2 declines over the lifespan, in parallel with CBF decreases (Ropper, 2005). In the aging brain it has been reported that CBF and CMR\textsubscript{O}2 both decrease, although some studies show no change (Aanerud et al., 2012, Dastur et al., 1963). This diminished demand for oxygen by the tissues is not consistently observed in the literature however, with some studies finding that the metabolic rate of oxygen use in the brain increases with advancing age (Peng et al., 2014, Lu et al., 2011). Elucidating the strength and direction of changes in cerebral demand and supply of oxygen is necessary for understanding the mechanisms of age-related decreases in cognition.

Mean cerebral blood flow velocity (CBF\textsubscript{v}), and therefore perfusion, is generally reported as decreasing with healthy aging (Soni et al., 2016, Leoni et al., 2017), although there are some discrepancies depending on the methodology, age range of participants and the regions and tissue types studied. Earlier research using the nitrous oxide technique reported significant reductions in mean CBF in aging individuals (Shaw et al., 1984, Gur et al., 1987, Davis et al., 1983, Melamed et al.,
This technique, described in Kety and Schmidt (1948b), involves inhalation of low concentration of N₂O for the determination of cerebral blood flow, although it has particularly poor spatial resolution compared with more modern imaging methods (Meltzer et al., 2000). Better spatial resolution offered by newer technologies allows for analysis of CBF in different regions of the brain. Regional decreases of both CMRO₂ and CBF have been reported in PET and SPECT studies. Research by Aanerud et al. (2012) reanalyzed previously published results for a PET study with healthy adults aged 21 to 81 years, to determine changes in CMRO₂ and CBF with aging. CMRO₂ and CBF decreased in the majority of the cerebral cortex, although the primary motor and sensory areas were relatively spared.

Martin (1991) used PET to show that regional CBF (rCBF) was significantly reduced in the limbic, or association, cortices with age, and hypothesized that these decreases may comprise the cerebral substrate of age-related cognitive changes. Another PET study found age-related CBF, cerebral blood volume (CBV) and CMRO₂ declined between groups of young (mean age 21 years) and older (mean age 61 years) adults, although there was as significant increase in oxygen extraction fraction (OEF) (Zhu et al., 2011). The increase in OEF with age suggests a greater reduction in CBF than in CMRO₂. The most significant decreases of CBF and CMRO₂ occurred in the frontal and inferior parietal cortices, with minimal change in white matter blood flow. Meltzer (2000) found contradictory evidence suggesting that healthy individuals may not experience age-related CBF decline. PET as a technique for estimating blood flow is somewhat problematic as the majority of studies do not account for individual differences in brain volume. Global brain volume is known to decrease with age (Giorgio et al., 2010) and progressive grey matter atrophy begins
from young adulthood in an approximately linear pattern (Ge et al., 2002). Failing to correct for grey matter volume differences in the aging process may confound the interpretation of previous PET studies that have shown age-related decreases in vascular and metabolic parameters.

Methodological differences between studies have produced some conflicting results. However, in general it has been shown that blood flow in grey matter falls with age while that in white matter, though much lower, is relatively preserved (Pantano et al., 1984). It is important to note that while CBF is widely used as an indirect measure of vascular health, it may not give a good reflection of vessel integrity. This parameter is affected by metabolic factors, and therefore, it may not be the most reliable indicator for assessing vascular function. It has been proposed that other parameters of CVR or CMRO₂ provide a more direct evaluation of the health of brain vascular system (Lu et al., 2009). These dynamic indices show different patterns of changes with age when compared to simple baseline blood flow measures.

2.8.1 Summary of age effects on CBF and CMRO₂

While many reports argue for age-related declines in both CBF and CMRO₂, there are conflicting findings. Differences in methodology, corrections for partial-volume effects and brain region/tissue type studied can ultimately hinder the comparability between studies. CBF is a commonly assessed metric, yet this parameter does not provide a direct measure of vascular integrity. More dynamic indices of CVR and
CMRO$_2$ may present additional information on the functioning of the cerebral vasculature in aging.

2.8 Chapter summary

With advancing age, blood vessels and other components of the microvasculature stiffen and lose their contractibility (Glodzik et al., 2013). At the same time, the pulsatile pressure of blood flow to the brain increases dramatically (Pase et al., 2010). Pulsatile pressure increases, coupled with the stiffening of the arteries and the low vascular resistance in the brain, leads to less cushioning of the pulsing inflow of blood. This may ultimately lead to damage of the microvessels, micro-bleeds into the brain tissue, or injury to neural cells (O’Rourke and Safar, 2005). While autoregulation and neurovascular coupling are generally sustained during normal healthy aging, various structural and functional alterations in the vasculature can affect the integrity of the neurovascular unit, leading to losses in vasomotor reactivity, blood flow regulation and oxygen metabolism. The neurocognitive impacts of these age-related changes in hemodynamics are unclear. Further investigations into the cognitive effects of vascular change in aging will advance the understanding of these associations.

The following chapter will provide more in-depth information on cerebrovascular reactivity (CVR), including how this index is measured using vascular challenges and various assessment tools. It will finish with a discussion of the relationships between CVR and cognition in Alzheimer’s disease and in cognitively healthy adults.
Chapter 3

Cerebrovascular Reactivity and Cognition: Current Concepts, Methods and Background

Advances in medical science are enabling people to live longer, and the population is aging as a result. Increasing life expectancies will continue to burden health services as the prevalence of dementia and age-associated cognitive decline grows. It is becoming more necessary to ascertain the mechanisms leading to neurodegeneration in order to help maintain cognitive functioning with increasing age. Evidence has demonstrated the importance of blood brain barrier integrity and its relationship to cognitive performance in pre-clinical (Montagne et al., 2015) and AD investigations (Takechi et al., 2017; Zlokovic, 2011). This suggests that maintaining cerebral integrity may be the first line of defense against downstream neuropathological processes such as Aβ and tau accumulation in healthy cognitive aging.

The extent to which age-related changes in cerebrovascular function and cognition are linked has not yet been determined. Cerebrovascular reactivity (CVR) is the physiological reaction of the cerebral blood vessels in response to vasoactive stimuli; it reflects the capacity for blood vessels to change their diameter in response to challenges to blood supply. When this capacity is compromised the coupling of blood supply to demand for oxygen is disrupted, leading to hypo- or hyper-perfusion of
regions of the brain, potentially impacting neurological and cognitive functions. This process is referred to as neurovascular uncoupling.

Assessment of CVR depends on robust and reliable methods of inducing repeatable changes in cerebral blood flow (CBF), measuring those changes accurately and performing an appropriate analysis. CVR is widely investigated in a range of instances though vascular challenges, measurement techniques and calculations vary, with some methods more precise than others. Differences in the methodology employed in CVR assessments can hinder the extent to which comparisons between studies can be made. Different types of vascular challenge, assessment tools and calculations are discussed in this review to evaluate the advantages and disadvantages of various methods.

While CVR may be used as a diagnostic tool for cerebrovascular disorders (Ellis et al., 2016), there is strong evidence of its implications in neurodegenerative illnesses and disorders affecting cognition. The contribution of cerebral vasomotor reactivity to cognitive performance in normal aging has yet to be clarified, and characterization of this relationship could greatly advance current understanding of the mechanisms of age-related cognitive decline.

The objectives of this chapter are to 1) provide a brief overview of the mechanisms involved in cerebrovascular reactivity to carbon dioxide 2) evaluate the different vascular challenges and tools used to measure this index, 3) discuss how CVR affects cognition in both healthy aging and cognitive impairment. The following chapter, Chapter 4, will then focus specifically on MRI-based assessments of CVR.
and the relationship with cognition for the purpose of illuminating the potential contributions of vascular integrity to cognitive performance in the form of a systematic review.

### 3.1 Mechanisms of CVR to carbon dioxide

The regulation of cerebral blood flow (CBF) is of paramount importance as neural tissue requires a steady stream of oxygen and nutrients. Regulation of blood flow is generally maintained by alterations in resistance at the level of the arterioles. Two main mechanisms control arteriole diameter and resistance to regulate blood flow. Neurovascular coupling ensures that blood flow increases in areas of the brain that are metabolically active (Magistretti and Pellerin, 1999, Iadecola and Nedergaard, 2007), and autoregulation maintains a constant CBF to changes in arterial perfusion pressure (Lucas et al., 2010). Cerebrovascular reactivity (CVR) reflects the capacity of the small cerebral arterioles to react to changes in arterial gas tension, shear stress and metabolic demand and is a key component in blood flow regulation. Inadequate dilatory or constrictive functioning can increase risk of stroke and other ischemic pathologies (Zhou et al., 2015).

This mechanism is critical as it maintains the constant cerebral blood flow that is necessary for a healthy functioning brain. Assessing the variations in CVR in various brain regions allows investigators to gain knowledge about the ways that the cerebral vasculature controls blood flow, and aids in the detection of cerebral pathophysiology (Schwertfeger et al., 2006). Oxygen (O$_2$) and carbon dioxide (CO$_2$) are particularly
potent vasoactive compounds that adjust the diameter of arteries in the brain to regulate flow of blood in response to changes in metabolic demands (Kety and Schmidt, 1948a). Evaluation of the regulation and distribution of CBF over various brain regions can be undertaken by using vascular challenges with these vasoactive stimuli.

Increases or decreases of CO₂ in the arterial blood will lead to vasodilation or vasoconstriction, and a subsequent increase or decrease in cerebral blood flow respectively, and vice versa for O₂. Increasing concentrations of CO₂ in the blood also results in a rise in arterial blood pressure. It is the combined mechanisms of vasodilation and raised blood pressure that cause the CBF to increase, even after autoregulation is exhausted (Regan et al., 2014). Inhalation of increased concentrations of CO₂ causes hypercapnia, which leads to decreased systemic blood pressure and pH levels. This induces dilation of the small cerebral vessels and capillaries to increase CBF to maintain optimal perfusion pressure (autoregulation).

As outlined in Chapter 2, vascular reactivity is a widely investigated index that reflects the integrity of the cerebral circulatory system. Dysfunctional CVR will impair the blood vessel response to changes in arterial gas tension, leading to reduced capacity for neurovascular coupling and cerebral autoregulation. Failure of the vascular system to maintain the coupling of blood supply with metabolic demand can have momentary or permanent consequences for neurological and cognitive functioning. CVR has been shown to be impaired in multiple pathologies including stroke (Markus and Cullinane, 2001), coronary artery disease (Kuroda et al., 2006), diabetes (Brundel et al., 2012, Dandona et al., 1978) and dementias of both vascular
and Alzheimer’s origin (Glodzik et al., 2013, Keage et al., 2012, Richiardi et al., 2015, Vicenzini et al., 2007). The evaluation of the cerebrovascular reserve allows clinicians to identify groups of individuals who may be at an increased risk of cerebral ischemic events such as stroke (Kleiser and Widder, 1992) and transient ischemic attack (Markus and Cullinane, 2001). Additionally, CVR has been observed as a predictor of the outcome of carotid artery occlusion (Vernieri et al., 1999).

### 3.1.1 The CVR response to increasing levels of CO₂ in the blood (hypercapnia)

The most common vasoactive stimulus used to measure CVR is to induce a state of abnormally heightened carbon dioxide level in the blood, known as hypercapnia. It is generally defined as a blood gas CO₂ tension of over 45mmHg and can lead to a driving of the serum pH down, resulting in respiratory acidosis. Hypercapnia can be induced in several ways, including breath-holding, re-breathing exhaled gas, automated computer-controlled targeting of arterial (PaCO₂) and expired CO₂ (etCO₂) and CO₂ enhanced-gas inhalation (Fierstra et al., 2013, Bhogal et al., 2014, Tancredi and Hoge, 2013, Wise et al., 2007). While it is beyond the scope of the current chapter to give an in-depth examination into the biological processes involved in the vasodilatory actions of CO₂ in the brain, Brian (1998) offers an impressive review of the state of knowledge in this area.

Briefly, when CO₂-enriched gas is inhaled it causes a change in the extracellular pH of the brain to a more acidic concentration. Increased arterial tension of CO₂
(PaCO₂) causes the arteries to dilate, resulting in a lowering of arterial pressure downstream, and increasing the flow of blood to the capillary bed. It was determined by Tominaga and colleagues (1976) that the increase in CBF caused by hypercapnia is in no way dependent on the associated rise in blood pressure. This finding indicates that autoregulation is upheld during periods of hypercapnia and it was suggested that the CBF increase in response to CO₂ inhalation is the result of peri-arteriolar acidosis.

3.1.2 The steal phenomena

The ‘steal’ phenomenon refers to a paradoxical situation in which blood flow decreases in some areas of the brain in response to a vasodilatory stimulus. The cerebral circulation operates such that the capillary beds perfused by the same feeding artery will be filled in parallel. The degree of magnitude of a vasoactive stimulus will affect the degree to which the arterial vessels dilate, as will the flow reserve of the vessels feeding into the vascular beds (Ellis et al., 2016). If the inflow reserve of the feeding vessel and the vasodilatory reserve of the capillary bed are sufficient, a potent vasodilatory stimulus such as CO₂ inhalation will elicit an equal reduction in vascular tone and an increase in CBF in all vascular beds. If the vasodilatory reserve of any given capillary bed is compromised, vasoactive stimuli will cause an imbalanced response. If the demand for oxygen surpasses the inflow reserve, blood flow is redistributed such that the vascular bed with the superior dilatory capacity will receive the majority of flow, a phenomenon known as ‘steal physiology’ i.e. the better vascular bed ‘steals’ the blood supply from a weakened
area. Weakened vasodilatory reserve causes a capillary bed to be solely dependent on the perfusion pressure and changes in vascular resistance of those beds that are filled in parallel that have a sufficient reserve (Fierstra et al., 2010).

It is because of this steal phenomena that even in instances of normal CBF, the brain may still be affected by regional deficits in perfusion. These impairments have been shown at most levels of the cerebral circulation from capillaries to the larger extra-cerebral vessels, as evidenced by neuroimaging investigations. Chronic hypoperfusion may precede or propagate cognitive decline, even in normal aging (De la Torre, 2012).

### 3.1.3 Gender differences in CVR

Studies reporting gender differences in vascular reactivity have been mixed. No gender differences in vascular reactivity were observed in children under 15 years old (Siriussawakul et al., 2011), nor in research with a large sample of adults ranging in age from 20-89 years (Lu et al., 2011). This is in contrast, however, to research with healthy young adults (21-38 years), which reported that males had 22% higher CVR in grey matter and 17% higher CVR in white matter than females (Kassner et al., 2010). However, research on adults aged 21-58 years found that women had a significantly higher vasodilatory response than men (Kastrup et al., 1997).

Investigations of the pattern of CVR changes over the lifespan between genders have provided evidence to suggest that hormones affect vascular responsiveness.
Another study by the same group above used TCD to examine changes in cerebral blood flow velocity in the middle cerebral artery (MCA) to 5% CO₂ (Kastrup et al., 1998). It was found that age did not have an effect on reactivity to hypercapnia in men. Yet premenopausal, but not postmenopausal women had a raised CVR to carbon dioxide. These findings indicate that estrogen may affect the reactivity of cerebrovascular vessels to CO₂. In support of this notion, Matteis et al. (1998) reported that postmenopausal women had significantly reduced CVR compared to premenopausal women of the same age, younger premenopausal women, and young and old men. Due to the substantial reduction of CVR in women post-menopause, which was not equaled in the age-matched male sample, the authors suggest that this effect is not simply due to aging but is most likely due to the effect of hormonal changes. This disparity between genders could help explain the increased prevalence of cerebrovascular disease and stroke observed in postmenopausal women (Bush, 1990).

3.1.4 Regional and tissue differences in CVR

One study has examined the CVR of the white matter, due to potential implications for ischemic events and autoregulation. White matter CVR (WM CVR) was reported as being 0.03 ± 0.002 percentage change in BOLD signal, divided by change in expired CO₂ between the baseline and CO₂ inhalation periods (expressed as %BOLD/mmHg) in fifteen younger and fifteen older healthy adults using BOLD MRI. This figure was significantly lower than that observed in the grey matter (0.22 ± 0.01%BOLD/mmHg). Additionally, the WM CVR had a temporal delay of 19 ± 3
seconds longer than that of grey matter. It was determined from the data that white
matter vascular reactivity increases and becomes faster with increasing age, which
is in opposition to what is observed in the grey matter, where CVR is reduced and
temporally slower (Thomas et al., 2014).

CVR is also heterogeneous across grey matter regions (Lu et al., 2009). Research
indicates that CVR is higher in the cortical lobes compared to the deeper grey matter
structures (Novak, 2012). Perfusion of the brain in response to oxygen and metabolic
demands operates dynamically, wherein different parts of the brain require blood in
differing amounts at various times (Lu et al., 2014). Studies often investigate CVR in
regionally-specific ways, including voxel-wise mapping and region-of-interest
analyses. This enables visualization of the widespread differences in CVR across
brain areas, which can vary widely between individuals. A study involving 150 adults
reported widespread age-related reductions in CVR in the parietal, temporal,
occipital and insula cortices, as well as in the subcortical grey matter (Lu et al.,
2011). It was suggested these aging effects are exponential, with CVR declining
faster than CBF in the same area with increasing age. Regional differences in CVR
have been often been observed in cognitively impaired patients compared to healthy
controls (Cantin et al., 2011, Richiardi et al., 2015, Yezhuvath et al., 2012, Glodzik et
al., 2011, Oishi et al., 1999).

3.2 Measurement of CVR
Numerous techniques have been conceived to measure cerebrovascular reactivity. Essentially, CVR assessment requires valid and measurable vasoactive stimuli that produces consistent effects on the circulation, a reliable method of quantifying changes in blood flow (or a surrogate of blood flow), and standardized method of analyzing the data that is repeatable over time (Ellis et al., 2016). These three parameters vary widely and can impact the comparability of CVR findings. The following section discusses the various vascular challenges used to elicit changes in CBF, tools used to measure the change in CBF or surrogate, and the methods used to quantify CVR from the obtained data.

### 3.2.1 Vascular challenges

There are three key types of vasodilatory challenge: temporary reduction of mean arterial blood pressure; injection of a vasoactive chemical (for example, acetazolamide); and increasing the arterial partial pressure of CO₂ (Fierstra et al., 2013). Repeatable and standardized techniques are available for the measurement of changes in CBF; however, the use of different tools and various vasoactive substances for vascular challenge can often hamper the comparisons that can be made between studies. Additionally, the calculation of CVR can be performed in several ways depending on the methodology used, and slight discrepancies may confuse the interpretation of findings. This section briefly evaluates the different vasoactive challenges to induce hypercapnia and the tools employed to measure CBF used in CVR assessments.
3.2.1.1 Breath Hold Index (BHI)

This technique is a simple test that has the advantage of not requiring any equipment or external sources of stimuli and can thus be performed during any investigation. A disadvantage of this technique is that it requires participant compliance. Due to the nature of the test no measurable inputs of CO₂ concentration can be determined. This is a commonly used test as it is reproducible, non-invasive, and does not require any expensive equipment. Some limitations with the breath-hold Index are that it relies on subject compliance and may not always be executed correctly.

Calculation of the BHI also differs between studies. Zavareo and colleagues (2010) used BHI calculated from the change in blood flow velocity of the middle cerebral artery (MCAv) divided by the length of breath-hold in seconds, whereas Shim et al. (2015) calculated the percentage increase from MCAv at rest to the maximal MCAv during a minimum 30-second breath-hold, divided by the MCAv at rest to give the CVR index. While both methods evaluate CVR as the change in blood flow velocity from baseline to the maximum increase elicited by the breath-hold, inputs to the calculation are somewhat arbitrary as the length of breath-hold time is only one of several factors that can bring about a change in the arterial pressure of CO₂ (Fierstra et al., 2013).

3.2.1.2 Rebreathing
Rebreathing as a stimulus to induce hypercapnia has the clear advantage that no external source of CO₂ is required; the only apparatus needed are a respiratory reservoir with a gas analyzer. One disadvantage is that rebreathing expired air causes the O₂ pressure level to rise. For precise CVR measurements this level should be kept constant (Fierstra et al., 2013). The size of the reservoir will also affect the rate at which arterial pressure of CO₂ (PaCO₂) rises. A rebreathing technique to maintain arterial isoxia has been conceived for use with ultrasound CBF measurements (Duffin, 2011); however, this method is not appropriate for BOLD MRI imaging which is dependent on changes in O₂.

3.2.1.3 Computer-controlled targeting of PaCO₂ and etCO₂

Studies have devised a method of measuring CVR by computer controlling the level of expired CO₂ (Mark et al., 2010) and the arterial partial pressure of CO₂ (PaCO₂). These studies, while allowing a precise control over end tidal (etCO₂) and cerebral hemodynamics, show a remarkable stability and predictability of MR signal variations. To operate correctly, they require complex automated software systems, numerous input gases and specialized analysis algorithms to operate properly. These requirements greatly limit the use of this type of challenge.

3.2.1.4 CO₂ inhalation

A recent review of the various stimuli currently used to measure cerebrovascular reactivity suggests that carbon dioxide is the most appropriate vasoactive substance
(Fierstra et al., 2013). Delivery of carbon dioxide via inhalation of a gas mixture has many advantages as a vasodilatory stimulus. It enables researchers to have a measurable input (expired carbon dioxide, end-tidal CO₂: etCO₂) for calculating CVR, eliminates the influence that participant compliance has on the measurements; and it is non-invasive and easily terminated if necessary. End-tidal CO₂ recording is used for quantifying CVR accurately because it enables both timing and amplitude information for the input function of the vascular system, which is often subject-dependent (Yezhuvath et al., 2009). Using a capnograph attached to breathing apparatus to continuously monitor etCO₂ has been found to give a close approximation of results of direct partial pressure of CO₂ (PaCO₂) measurements taken invasively with arterial puncture (Burki and Albert, 1983). Another study substantiates this claim. It was found that there was a high correlation ($r = 0.91$, $p < .001$) between CVR and changes in CO₂ when calculated from either arterial PaCO₂ or estimated from PetCO₂ values (Young et al., 1991).

### 3.2.1.4.1 Carbogen

A recent study compared the inhalation of 5% CO₂ in room air to inhalation of 5% CO₂ in 95% O₂ (a gas mixture known as carbogen). It was determined that the values obtained from the different gas mixtures are not directly comparable (Hare et al., 2013). The increased oxygen content of the mixture can have a confounding effect of vasoconstriction, which will dampen the dilatory actions of CO₂. It has been suggested that carbogen is a particularly inappropriate stimulus to use in CVR investigations employing blood oxygen level dependent (BOLD) MRI (Hare et al.,
This technique fundamentally operates on the interactions of several factors, including blood flow, volume and metabolism of oxygen, and any increase in PaO$_2$ will have implications for the magnitude of the BOLD signal.

### 3.2.2 Tools for measuring CVR

CVR testing can be performed by means of several techniques including the xenon-133 washout technique (Reich and Rusinek, 1989, Davis et al., 1983, Levine et al., 1988), $^{15}$O positron emission tomography (PET), single-photon emission CT (SPECT) and transcranial Doppler ultrasound (TCD) (Kastrup et al., 1998, Battisti-Charbonney et al., 2011). In more recent years advances in magnetic resonance imaging (MRI) technology have enabled comprehensive structural and functional investigations to be performed. There are numerous MRI techniques that are used to evaluate blood vessel function, CBF and vasoreactivity. While each of the techniques discussed in this section have both advantages and disadvantages (See Urback et al. (2017) for a good summary), developments in MRI have led to its widespread use as the preferred imaging tool for assessment of CVR over and above the other options.

The following section will give an overview of the different technologies used to measure CVR, and discuss the advantages and disadvantages associated with each.
3.2.2.1 CT, PET and SPECT

Xenon computed tomography (CT), positron emission tomography (PET) and single photon emission computed tomography (SPECT) techniques have been utilized to evaluate CVR. The highly accurate xenon CT method utilizes either inhalation or a bolus injection of the radioactive isotope xenon-133 into one of the main cerebral arteries. The arrival and ‘washout’ of the element are monitored over time by an array of high-purity germanium (HPge) detectors that are placed around the head. This technique enables the quantification of the rate of decay of xenon-133 concentration in several brain areas simultaneously, and enables the calculation of CBF to be made in each region separately (Reich et al., 1985), as the amount of the bolus taken up by the brain tissue is equal to the amount in the arterial blood, minus the amount in the venous blood during a given time period (Angevine and Cotman, 1981). This technique was often used prior to the 1990’s, but due to its invasive nature, is less commonly employed in measurements of CVR currently.

The nitrous oxide inhalation (N₂O) technique to determine blood flow was first performed by Kety and Schmidt (1945). This method involves inhalation of low concentration of N₂O for the determination of cerebral blood flow. It has particularly poor spatial resolution compared with more modern imaging methods (Meltzer et al., 2000). Earlier research utilizing this technique has reported significant reductions in mean CBF in aging individuals (Shaw et al., 1984, Gur et al., 1987, Davis et al., 1983, Melamed et al., 1980). This method is invasive, involving the insertion of needles at the femoral artery for estimation of arterial N₂O, and at the superior bulb of the jugular vein for venous N₂O measurement (Dastur, 1985). While the intrusive
nature of these studies does translate into highly precise and accurate measurements, participant comfort is sacrificed. Thus, this type of experimentation is a less practical tool for CVR measurement.

$^{15}$O-labeled water is used in the brain perfusion PET studies. This method enables absolute quantification of CVR when measuring the arterial radioactivity concentration. Xenon-133 and technetium-99m gases are also used in SPECT to measure regional CBF. The use of radioactive substances and inhaled gases limits the extent to which these procedures are employed in experimental investigations. While CVR can be measured accurately and reliably with these techniques, there are several disadvantages, including the necessity of radioisotope use, low spatial/temporal resolution, participant discomfort, limited availability of necessary equipment and the costly nature of examination.

3.2.2.2 Transcranial Doppler ultrasound (TCD)

Transcranial Doppler ultrasound (TCD) is a non-invasive tool has been used for the past two decades to provide inexpensive, reliable and rapid measurement of cerebral blood flow velocity (CBFv) (Willie et al., 2011). It can be used to study intracerebral arteries. In CVR assessments the middle cerebral artery (MCA) is most commonly recorded, though often the left and right common carotids, prior to the bifurcation into internal and external carotids, are investigated. Values for blood flow velocity are given in cm/sec. While this technique is widely used and offers good
temporal resolution, the spatial resolution is low, meaning that impaired reactivity of blood vessels cannot be identified in small regions of the brain (Lythgoe et al., 1999).

3.2.2.3 MRI-based assessments

Magnetic resonance methods improve on this limitation by offering a unique combination of high-resolution anatomic imaging and non-invasive mapping of hemodynamic response. There are two main categories of MRI that measure brain tissue perfusion—those that use an injected contrast agent that changes the magnetic susceptibility of the blood, and thus the MR signal, while it is repeatedly imaged during the bolus passage (dynamic susceptibility contrast (DSC) is an example); and arterial spin labeling (ASL) MRI, where the arterial blood water is tagged magnetically before it enters into the tissue being examined. Blood oxygen level dependent (BOLD) fMRI is also used widely in CVR assessments. In comparison with other techniques used to measure CVR, MRI has the advantages of being highly reproducible, non-invasive, having high temporal and spatial resolution, and is also widely available for clinical purposes.

3.2.2.3.1 Arterial Spin Labeling MRI

Arterial spin labeling (ASL) is a magnetic resonance imaging technique that was conceived over 20 years ago (Williams et al., 1992, Detre et al., 2012, Detre et al., 1998) that is used for measuring tissue perfusion. Perfusion is defined as the amount
of blood delivered to capillary beds in a given tissue per unit time. This technique enables non-invasive imaging and absolute quantification of cerebral blood flow (CBF) in the brain without the use of any exogenous contrast agent.

ASL MRI produces a ‘flow labeled image’ and a ‘control image’ in which the static tissue signals are identical, but the magnetic quality of the inflowing blood is different. In this technique, the water molecules in the arterial blood are magnetically tagged before it enters the tissue of interest. This is performed with a radiofrequency pulse that inverts or saturates the water protons in flowing blood supplying the imaged region. This method uses magnetically-labeled arterial blood water as an endogenous tracer, thereby eliminating the need for an invasive contrast agent to be administered.

In comparison to other perfusion techniques, ASL offers several advantages and is widely available on a standard MRI scanner. It is completely non-invasive and provides a quantitative measurement of tissue perfusion, making ASL ideal for CVR studies. It is often suggested that ASL is the more appropriate MRI-based CVR assessment tool over BOLD as it provides a direct measure of CBF. BOLD MRI has its signal based on a combination of many factors, discussed below. These techniques are both detailed further in Chapter 5.

3.2.2.3.2 BOLD fMRI
Functional MRI using the blood oxygen level dependent (BOLD) technique is also used to examine CVR (e.g. (Kastrup et al., 2001, van der Zande et al., 2005, Vesely et al., 2001). The BOLD signal is an indirect measure of flow which operates on the principles of the magnetic differences between oxygenated and deoxygenated hemoglobin, thus the signal can only be interpreted as a surrogate for blood flow, in much the same way that CBF velocity is used. Use of BOLD MRI to measure CVR assumes that there is no change in oxygen consumption from normocapnia to hypercapnia. The BOLD signal is comprised not only of cerebral blood flow but is also dependent on numerous other factors, including cerebral blood volume (CBV), cerebral metabolic rate of oxygen (CMRO2), oxygen concentration of the arterial blood (Ya) and hematocrit (Mandell et al., 2008).

Calibrated BOLD is a procedure often applied whereby a vascular (e.g. hypercapnia) and a neural (e.g. a motor activation task such as finger-tapping) challenge are performed in the MRI scanner to allow researchers to separate the vascular response from the metabolic response to study the effects of stimuli on the BOLD signal. This thesis will not consider studies that used the calibrated BOLD approach as this is a tool for examining how the BOLD signal is affected by changes in blood flow and neural activity and is not related to the physiological mechanisms of vascular reactivity that we are interested in for the purposes of this research.

While BOLD imaging may involve the use of blood oxygenation as a surrogate for flow per se, this technique has some benefits over that of ASL for the assessment of CVR. BOLD weighted imaging is the more widely used and accessible method for estimation of oxygenation changes, does not require any additional hardware over a
standard MRI scanner and has a slight advantage over ASL in terms of spatial discrimination. It was found that the two methods show significant correlation on CVR when assessed in the same cohort in the same session ($r=.83, p<0.0001$; (Mandell et al., 2008)).

### 3.2.3 CVR calculation

The way that CVR is calculated can also vary. Ultrasound and CT-based research usually assess CVR by dividing the velocity of CBF (CBFv) by expired CO$_2$ (etCO$_2$) in the same time period, in comparison with neuroimaging studies, wherein reactivity is measured based on changes in perfusion (i.e., the amount of blood reaching a given area over a period of time) or change in BOLD signal intensity, which is then divided by the change in etCO$_2$ (if recorded). In works where etCO$_2$ is not recorded, a mathematical estimation of expired CO$_2$ concentration may be used (e.g. Richiardi et al. (2015)). The method of calculation also varies depending on the vasoactive challenge used. As mentioned previously, BHI research may divide the change in CBF (or CBV) by the length of the breath hold, rather than using etCO$_2$ values. One group has suggested that CVR is a heterogeneous response that doesn’t occur uniformly across all brain regions, but relative CVR is a more sensitive biomarker than absolute CVR, as it minimizes inter-subject variations (Yezhuvath et al., 2009).

### 3.2.4 Section summary- Measurement of CVR
While there are a variety of instruments used to evaluate CBF and vasomotor responsiveness, these tools vary in their practicality for CVR measurement. Transcranial Doppler ultrasound (TCD), positron-emission tomography (PET) and single-photon emission CT (SPECT) have also been used with similar stimuli to observe changes in brain perfusion and reactivity, though these techniques lack the high spatial resolution necessary for accurate regional CVR measurements to be made.

MRI with CO₂ inhalation would seem to be the most appropriate technique for assessment of vasomotor reactivity in the brain. In particular, BOLD MRI has high spatial resolution which allows for the assessment of brain activation and perfusion on a regional basis. This method enables localization of function, and the temporal resolution permits imaging of changes in hemodynamic properties that occur in response to vasodilatory stimuli. BOLD fMRI is also widely used to study brain function in relation to cognitive performance.

### 3.3 CVR and cognition

This section will provide an overview of evidence surrounding the connections between CVR and cognition, first in regard to the functioning of the endothelium, and second in regard to the literature on CVR in Alzheimer’s disease and in normal cognitive aging. Chapter 4 will provide a systematic review of all MRI research studies that assessed CVR in relation to cognitive performance. Non-MRI based studies that have assessed the connections between vasomotor reactivity in the brain and cognitive performance have indicated, despite some inconsistencies, that
poorer CVR is generally associated with a poorer cognitive profile (Keage et al., 2012).

Impaired dilatory capacity of the cerebral blood vessels can lead to chronic hypoperfusion of the brain. There is extensive evidence for the case of hypoperfusion preceding cognitive impairments (see De la Torre (2012) for a discussion of possible pathways). Research suggests that long-term cerebral hypoperfusion precedes and potentially promotes the onset of clinical dementia (Poels et al., 2008, Jagust et al., 1997, De la Torre, 2002, De la Torre, 2004, Farkas and Luiten, 2001, Pimentel-Coelho and Rivest, 2012, Ruitenberg et al., 2005). An important review that focused on the reactivity of cerebral blood vessels in response to changes in carbon dioxide in Alzheimer’s disease has suggested that while the results of clinical studies are inconclusive, the measurement of CVR to carbon dioxide is increasingly being seen as useful means for early detection of vascular dysfunction for persons at risk (Glodzik et al., 2013). Poor CVR is suggestive of poor cerebrovascular integrity, as it indicates decreased endothelial function. Reduced endothelial function is linked to poorer cognitive performance (Sinn and Howe, 2008), further discussed below.

3.3.1 CVR, endothelial function and cognition

Endothelial dysfunction impairs the capacity for blood vessels to respond to changes in vasoactive stimuli, i.e., vascular reactivity. These losses to both systemic and cerebral vascular reactivity may impact cognitive performance. Studies have shown
that endothelial dysfunction in the aorta is associated with cognitive decline (Mitchell, 2008). Peripheral endothelial dysfunction is often assessed via the indirect measurement of brachial flow-mediated dilation (FMD). This index has been found to be strongly associated with cognition in elderly individuals with mild cognitive impairment (MCI) (Vendemiale et al., 2013). This relationship was strongest for those showing the most severe impairment; individuals with multiple-domain amnestic MCI had the worst brachial FMD. This finding is supported by extensive research linking both peripheral and cerebral endothelial dysfunction to AD and other types of dementia (Dede et al., 2007, Gorelick et al., 2011, Iadecola et al., 2009, Iadecola, 2010, Iadecola, 2004, Girouard and Iadecola, 2006, Kelleher and Soiza, 2013). The ongoing investigation of endothelial contributions to cognitive performance is required.

Cerebral amyloid angiopathy (CAA) is another age-related disorder of the cerebrovascular endothelium receiving attention for its effects on cognition. Population-based autopsy studies have shown that CAA is an independent predictor of cognitive impairment even when age and AD pathology are accounted for (Greenberg et al., 2004). CAA is associated with attenuation of the CVR response to visual stimuli (Dumas et al., 2012) and hypercapnia (Shin et al., 2007). Evidence links CAA to multiple vascular pathologies, including intracerebral micro-bleeds (Xiong et al., 2016), white matter hyperintensities (WHM) (Viswanathan and Greenberg, 2011), and impaired CBF regulation (Menendez-Gonzalez et al., 2011), all of which are implicated in declining cognition with age.
As outlined in Chapter 2, endothelial dysfunctions commonly occur, even in the normal aging process, impairing the CVR response. The following section will discuss AD and CVR and will then outline the current research as it stands regarding the relationships between normal cognition and CVR.

### 3.3.2 Alzheimer’s disease and CVR

Alterations in vascular function are common in Alzheimer’s disease (AD) (Glodzik et al., 2013, Sabayan et al., 2012, Cantin et al., 2011). While the formation and accumulation of amyloid-β plaques and intra-neuronal neurofibrillary tangles consisting of hyper-phosphorylated tau are central to the pathogenesis of AD (Cees de Groot et al., 2000), this hypothesis does not explain aspects of AD, such as the overlap between neurodegeneration and vascular risk factors (Ogawa et al., 1992, De la Torre, 2004, De la Torre, 2010a). Growing literature provides evidence that vascular disorders (e.g. hypertension, hyperlipidemia, diabetes, vascular disease) contribute to AD pathology (Kivipelto et al., 2001, Silvestrini et al., 2006, De la Torre, 2012).

While CBF is most commonly reported to decrease over the lifespan, AD is associated with more substantial reductions in blood supply, to the point of cerebral hypoperfusion (Kastrup et al., 2001). De la Torre (De la Torre, 2000) conceived the critically attained threshold of hypoperfusion (CATCH) theory to explain the cascade of neurobiological actions that occur when vascular risk factors and advancing age converge, leading to chronic hypoperfusion and a chain of metabolic, cognitive and...
brain tissue impairments which accelerate neurodegeneration and possible AD pathology. The accumulation of beta-amyloid, alterations to vessel constrictors and inflammatory pathways contribute to vascular dysfunction in AD by compromising vessel flow and contractility (Glodzik et al., 2013). This raises the question whether AD and the associated cognitive impairment is at least partially a consequence of vascular dysfunction due to inadequate cerebral blood flow, altered brain metabolism and brain endothelial dysfunction (Chen et al., 2011, De la Torre, 2010a).

There have been increasing reports of impaired cerebrovascular reactivity to breathing carbon dioxide-rich air in AD, and it is suggested that chronic hypercontractility of the cerebral vessels is behind this impairment (Stefani et al., 2009, Glodzik et al., 2013, Bär et al., 2007, Vicenzini et al., 2007, Cantin et al., 2011). Several studies using transcranial Doppler ultrasound (TCD) have found that patients with AD have decreased velocity of blood flow (CBFv) in the middle cerebral artery (Ruitenberg et al., 2005, Franceschi et al., 1995). Reduced CBFv is linked to deficient CVR (Serber et al., 2014). Silvestrini and colleagues (2006) used transcranial Doppler to show that in a group of 53 AD subjects, breath holding index (BHI), a measure of CVR, was the best predictor of change in MMSE and ADAS-Cog scores over a two-year period. This study concluded that there is a concomitant relationship between vascular and degenerative processes in AD progression. Likewise, Ruitenberge et al. (2005) found that patients with cognitive impairment, even mild decline, were more likely to have lower CVR in a large-scale longitudinal study. The authors note that their study possibly failed to find a link between deficits of CVR and clinical AD due to a lack of power. Only 14 participants out of a pool of 1,730 were diagnosed as having dementia.
Further research found that CVR was lower in older individuals with mild cognitive impairment (MCI) versus normal healthy controls (Zavoreo et al., 2010). However, the MCI group had significantly greater cardiovascular risk factors than the controls, including significantly higher levels of C-reactive protein, glucose plasma and cholesterol. Whether these were controlled statistically is not clear. C-reactive protein is strongly associated with increased arterial stiffness even in healthy aging (McEniery et al., 2004), and other risk factors are known to impact on cognitive function (Leritz et al., 2011, Kodl and Seaquist, 2008), potentially confounding these results.

Several MRI-based investigations of CVR showed that cognitive impairment was associated with lower cerebrovascular responsiveness (Richiardi et al., 2015, Metzger et al., 2018, Yezhuvath et al., 2012, Cantin et al., 2011). However, these studies varied widely in sample selection, vascular challenges, neuropsychological assessments and CVR estimation methods, hampering the extent to which they can be compared. The subsequent chapter of the current thesis reviews these works.

While evidence exists for a probable role of CVR in disorders of cognition, continued research is needed. Even less substantive research has been carried out for the effects of CVR in the maintenance of good cognitive function, as in the case of normal cognitive aging.
3.3.3 Normal cognitive aging and CVR

A question that remains unanswered is whether CVR has any direct connection with cognitive decline in normal aging. It would be expected that reduced reactivity of the cerebral blood vessels (i.e. less than optimal functioning of the vascular system in the brain) would be associated with reduced cognitive performance due to either hypoperfusion of the brain tissue, leading to less nutrients and oxygen reaching the tissue, or hyperperfusion, leading to increased metabolic fuel, as hyperoxia can also be damaging to neurons (Diringer, 2008). As people age, large blood vessels, such as the aorta, lose elasticity and become increasingly stiff, leading to greater pulsatile pressure at end organs such as the kidneys and brain (O'Rourke and Safar, 2005, DeCarli, 2012). The brain receives a high-pressure flow, but has low vascular resistance, and increased blood pressure, along with the lack of cushioning due to loss of elasticity, can ultimately lead to damage of the small cerebral arterioles. Damage to arterioles and capillaries in the brain over time could potentially lead to loss of reactivity in the microvasculature, destruction of neurons and eventual cognitive impairments (De la Torre, 2012). Disruption to the regulation of CBF and neurovascular coupling caused by dysfunctional blood vessels may be a fundamental link between cardiovascular risk factors and cognitive decline (Barnes, 2015). Research investigating vascular reactivity in the brains of healthy people is more limited than that of those with cognitive dysfunction. The majority of research investigating CVR and cognition with cognitively-intact adults involves examination of those with some vascular pathology.
One study used the breath holding index (BHI) measured by transcranial Doppler ultrasound (TCD) to investigate cerebral hemodynamic contributions to cognitive performance in patients with unilateral or bilateral carotid stenosis. It was observed that poor verbal fluency was associated with impaired reactivity in the left cerebral hemisphere, and visuospatial and non-verbal abilities were linked with right hemisphere dysfunction (Balucani et al., 2012). These results suggest that in patients with dysfunctional CVR, cognition is affected in a task-specific manner depending on the region of vascular injury. This is not unexpected given that specific neurocognitive functions are known to be localized to certain areas of the brain, though it remains to be clarified whether this same relationship exists in healthy individuals free from vascular conditions. It is likely that the presence of multiple vascular risk factors aggregate to result in more severely dysfunctional CVR, and the cumulative effect is worsening cognitive status, even in a non-clinical setting.

In another study TCD was used to assess CVR in cognitively healthy adults with hypertension. Reduced reactivity in the middle cerebral artery was correlated with poorer executive function (Hajjar et al., 2014). This is corroborated by the findings of a large-scale study with 415 cognitively healthy males (mean age 72 years) with coronary heart disease, in which it was found that dysfunctional CVR was linked to both lower global cognitive function and executive function (Haratz et al., 2015). A report on the large population-based Rotterdam study, which investigated 1,730 adults (aged 55 years +) using TCD and 5% CO2 inhalation to assess CVR, reported that greater reactivity of the middle cerebral artery was associated with less cognitive impairment in the largely non-clinical sample (Ruitenberg et al., 2005). This study
concluded that vascular risk factors, such as cerebral vessel responsiveness, likely contributed to the causes of cognitive decline and dementia.

3.3.4 **Summary of CVR and cognition**

A large body of evidence suggests that CVR is related to performance in cognitive impairment and dementia, and likely plays a role in cognitive processing in normal cognitive aging, although there is a critical lack of research into this relationship in people who are free from both cognitive and vascular dysfunction. Dysfunctional reactivity in the cerebral vessels, stemming from endothelial dysfunction, impairs the delivery of oxygen and nutrients to the brain tissue, disrupting neurovascular coupling and autoregulation, which may result in hypoperfusion, leading to neural deterioration and cognitive decline.

3.4 **Chapter summary**

This chapter gave an overview of the current concepts, mechanisms, background and methods used to measure CVR. Evidence from reviews and meta-analyses suggest that hypercapnia induced via inhalation of CO\textsubscript{2}-enriched gas, measured using MRI is the most robust and easily standardized method to investigate small cerebrovascular territories. Support for the contribution of CVR to cognitive performance in both impaired and healthy aging was discussed. The following chapter will go into greater depth by systematically reviewing all MRI-based studies that assessed CVR and investigated the relationship to cognition.
Chapter 4

Magnetic resonance imaging for assessment of cerebrovascular reactivity and its relationship to cognition: A systematic review

4.1 Objectives

This chapter aims to systematically review all research using MRI to measure cerebrovascular reactivity (CVR) and the relationship to cognition. The main aims of this paper were to 1) Identify and summarize all publications that used MRI to measure CVR, and then investigated the association between cognitive performance and CVR 2) Compare and contrast the specific methods used as a vasoactive challenge, MRI techniques, cognitive assessments and CVR calculation 3) Use this information to make recommendations for future studies aimed at investigating the CVR- cognition relationship.

4.2 Rationale

Both vascular elasticity and cognitive function deteriorate with age. It is not conclusive how significantly cerebral vascular responsiveness contributes to cognitive functioning in either health or pathology. This article provides a review of the currently existing research on the contribution of vascular reactivity to cognitive performance and impairment. The study selection was limited to those utilizing MRI
only for the purposes of gaining greater understanding of whether specific areas of
the brain are reliant on vascular integrity to support cognitive processing.

The study, published in *BMC Neuroscience* (Catchlove et al., 2018c), is reproduced
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4.3 Publication
Magnetic resonance imaging for assessment of cerebrovascular reactivity and its relationship to cognition: a systematic review

Sarah J. Catchlove1*, Andrew Pipingas1, Matthew E. Hughes2,3 and Helen Macpherson4

Abstract

Background: Cerebrovascular reactivity (CVR) refers to the responsiveness of cerebral vasculature to vasoactive stimuli. CVR is an indicator of brain health and can be assessed using vasodilatory techniques and magnetic resonance imaging (MRI). Using such approaches, some researchers have explored the relationship between CVR and cognition; here we systematically review this work.

Results: We extracted information pertaining to: (1) study location and design, participant characteristics, sample sizes, (2) design of vascular challenge, end-tidal CO₂ (etCO₂) concentrations (if applicable), (3) MRI protocol, (4) cognitive assessment, (5) CVR values, and outcomes of statistical analyses with cognitive tests. Five studies assessed participants with cognitive impairment compared to controls, one studied patients with multiple sclerosis with or without cognitive impairment compared to controls, one examined patients with moyamoya disease with or without cognitive impairment, two investigated patients with Type 2 diabetes mellitus (T2DM), and one was a cross-sectional study with younger and older healthy adults. Cognition was typically probed using the MMSE and tests of executive function, while a number of vasodilatory techniques were employed.

Conclusion: CVR was associated with cognition in six of ten studies, but heterogeneity of study samples, designs and vasodilatory methods may have a role in the inconsistent findings. We make recommendations for future research that includes use of a multi-domain cognitive assessment and standardised hypercapnic challenge with MRI.

Keywords: Cognition, Cerebrovascular reactivity, Vasodilation, Magnetic resonance imaging (MRI), Brain

Background

Rising life expectancies, together with declining fertility rates, is leading to rapid global ageing. It is estimated that by the year 2050 the proportion of people aged over 60 years will double from approximately 11 to 22% worldwide [1]. As the population ages, the number of older adults living with impaired cognition and dementia continues to increase. While a variety of mechanisms are thought to contribute to the genesis of cognitive impairment, there is emerging evidence that the signaling between various elements of the neurovascular unit becomes dysfunctional with increasing age, leading to neurovascular uncoupling and dysregulation of cerebral blood flow (CBF) in response to neuronal and metabolic demands [2, 3]. Cerebrovascular reactivity (CVR) refers to the response of cerebral blood vessels to vasoactive stimuli. Dysfunctional CVR impairs blood delivery to brain regions requiring supply, which both precedes and contributes to neuropathology over time. Impaired CVR has been implicated in a wide range of disorders including stroke [4–6], multiple sclerosis [7], hypertension [8], diabetes [9, 10], cardiovascular disease [11] and dementia [12–15]. Further, diminished reactivity has been found to...
contribute to mild cognitive impairment in the non-clinical general population [16].

This potential link between CVR and cognitive impairment is interesting as it suggests that optimal functioning of the cerebral circulatory system is important for maintaining cognitive functions. The relationship between cognitive decline and numerous vascular anomalies, including stiffness of the peripheral arteries and aorta [17, 18], hypoperfusion [19, 20], cerebrovascular disease [21], and pathology of the carotid arteries [22, 23] has been well established in the literature. To date however, the relationship between CVR and cognitive functions has been poorly understood.

CVR is generally measured as a change in some index of blood flow (e.g., blood flow velocity measured with ultrasound or blood oxygen level dependent (BOLD) signal change measured with fMRI) in response to a vasoactive stimulus. Hypercapnia (increased blood carbon dioxide (CO2) concentration) is the most often used stimulus to elicit increased blood flow via vasodilation. Hypercapnia can be induced in several ways including inhalation of CO2-enriched air, breath-holding, and rebreathing. While there are numerous vasoactive challenges that can elicit a change in blood flow required for the assessment of CVR, inhalation of CO2-enriched air is most suitable due to the practicality of its use and the ease with which it can be standardized [24]. Acetazolamide, a carbonic anhydrase inhibitor, has the same capacity to dilate the cerebral microvasculature via increasing carbonic acid in the arterial blood, and is often used to elicit vasodilation in studies of CVR [25, 26].

Likewise, various tools can be employed to measure the change in blood flow. Most frequently used is the transtemporal Doppler ultrasound (TCD). This method is inexpensive, easy to use, non-invasive, is viable for use with large cerebral vessels and has high temporal resolution. However, TCD has low spatial resolution; hence precise regional investigations cannot be performed. Single-photon emission computed tomography (SPECT), positron emission tomography (PET) and other computed tomography (CT)-based technologies also exhibit poor spatial resolution, but are further complicated by the necessity of exposing participants to ionizing radiation. Advances with MRI-based imaging have overcome these limitations whereby CVR assessments can be performed without the use of exogenous contrast agents, and with high spatial resolution so that the responsiveness of blood vessels within discrete brain areas may be studied independently.

Research investigating the relationship between vascular reactivity and cognitive performance has commonly used CT or TCD technology, demonstrating reduced CVR in cognitively impaired patients [12, 14, 27, 28]. Studies using TCD have shown significant relationships between CVR and cognitive status assessed with the mini-mental state examination (MMSE) [28], and with tests of executive function, attention and memory [29]. However, the lack of regional specificity of TCD does not enable an examination of region-specific relationships between CVR and cognitive abilities. To address this apparent gap in the literature, the current work aims to systematically review all research articles investigating the association between cognitive performance and cerebrovascular reactivity to a vasoactive stimulus measured using MRI.

Methods
Search criteria
Searches were conducted using Pubmed and Scopus from earliest record until 15th July 2017. Search terms were entered as follows: Pubmed (cognition OR cognitive OR memory OR attention) AND (“cerebral vascular reactivity” OR “cerebrovascular reactivity” OR “cerebral vasoreactivity” OR cvr OR “cerebral vasomotor reactivity” OR “vasomotor responsiveness” OR “cerebrovascular responsiveness”) AND (Humans [Mesh]); and Scopus (TITLE-ABS-KEY (cognition OR cognitive OR memory OR attention) AND TITLE-ABS-KEY (“cerebral vascular reactivity” OR “cerebrovascular reactivity” OR “cerebral vasoreactivity” OR cvr OR “cerebral vasomotor reactivity” OR “vasomotor responsiveness” OR “cerebrovascular responsiveness”)) AND (LIMIT-TO (DOCTYPE, “ar”)).

Only studies published in English using MRI-based CVR assessments and conducted with adult (> 18 years) humans were included. Exclusion criteria included animal studies, CVR assessed with imaging modalities other than MRI, not performing a cognitive/neuropsychological assessment, or not analysing the associations between CVR and cognition. The reference lists of the included studies were also searched.

Quality assessment and extracted information
Studies deemed eligible were checked for quality using the NIH Quality Assessment Tool for Case/Control Studies and the Tool for Observational Cohort and Cross-Sectional Studies and the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines. Information extracted from the studies related to the country, year of publication, MRI technique and analysis, vasodilatory challenge, CVR values, cognitive/neuropsychological assessment, and participant demographics including age, gender, years of education, cognitive profile and other health status information where available.
Results

10 studies were included in the final review [10, 13, 14, 30–36]. All studies were of a fair to good quality as assessed by two independent researchers (SC and HM; See Fig. 1).

Study demographics and details

Research was conducted in four countries (USA n = 4, Canada n = 2, Switzerland n = 1, France n = 3). Participants in 7 of the studies had an average age of mid-to-late 60’s to early 70’s [10, 13, 30–32, 35, 37], one study investigated adults aged 30–50 years (patients mean age 39 ± 5.91 years, controls mean age 41 ± 6.38 years) [36], one study recruited adults with moyamoya disease aged over 18 years (range 29–73, mean age 40.4 years) [34], while the remaining study involved a cohort of older (mean age 63 ± 5 years) and younger adults (mean age 24 ± 3 years) [33].

Two studies investigated the differences in CVR and cognition between patients with type 2 diabetes mellitus (T2DM) versus healthy controls [10, 32]. Metzger et al. [36] assessed cerebral vasoreactivity and cognitive status in multiple sclerosis patients versus healthy controls. Two studies included patient samples with mild cognitive impairment (MCI) and Alzheimer’s disease (AD) matched with healthy controls [13, 31], while two examined only MCI and healthy controls [35, 37] and one paper examined only AD versus healthy controls [30]. Calviere et al. [34] investigated CVR and cognitive impairment in patients with moyamoya disease. The work by Gauthier et al. [33] was a cross-sectional study assessing differences between groups of healthy younger and older adults.

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**Fig. 1 Flowchart of study selection**
All studies were single-visit examinations, with the exception of Chung et al. [32] which was longitudinal; participants were assessed at baseline and 2-year follow-up. Table 1 displays patient characteristics.

**Vascular challenge paradigm**

Vascular challenges varied between studies. Five elicited hypercapnia via fixed-level CO₂-enriched gas inhalation. Concentrations varied between 5% CO₂ in medical air [30, 35], 7% CO₂ in medical air [31], 7% CO₂ in 93% oxygen (carbogen) [13] and 8% CO₂-enriched gas (BACTAL®) [36]. Two studies used CO₂ rebreathing as the hypercapnic manipulation, though both failed to report the length of the rebreathing period, and the size of the reservoir used [32, 37]. Tchistiakova et al. [10] used the breath-hold technique, wherein participants performed a series of 6 × 15 s breath holds following 3 s of exhalation with 30 s of intermittent regular breathing. Gauthier et al. [33] used a computer-controlled gas delivery system which prospectively targeted the partial pressure of expired CO₂ (etCO₂) to 40 mmHg for normocapnia, and 45 mmHg for the hypercapnia period, whilst maintaining the expired O₂ (etO₂) at 100 mmHg throughout the procedure. The duration of gas delivery also varied across these studies, see Table 2 for details. The remaining study elicited vasodilation via injection of 15 mg/kg acetazolamide [34].

**Cognitive/neuropsychological assessment**

While the majority of the studies reported more than one cognitive assessment, we were primarily interested in the tests that were analysed in connection with CVR. All but three studies [10, 34, 36] reported mini-mental state examination (MMSE) [38] scores. Of the seven studies that reported MMSE, four investigated the relationship between CVR and MMSE score [13, 30, 31, 35, 36]. Results are reviewed in the discussion section.

Assessments of executive function in connection with CVR were included in five studies. Chung et al. [32] composed a composite measure of the average of verbal fluency and Trail Making Tests A & B scores, Gauthier et al. [33] used a modified Stroop task to measure executive function, and Tchistiakova et al. [10] employed the Wisconsin Card Sorting test (WCST). Calviere et al. [34] used a battery of tests examining executive function (letter and category fluency tests, Trail Making Test B, Stroop interference, Brixton test and a modified version of the WSCT that included both number of categories and number of preservations), and attention (Trail Making Test A, and colored dots and words of the Stroop test) to categorize patients as being cognitively impaired or not. Patients scoring below 5th percentile of the normative mean on 3 or more subtests were considered to have dysexecutive cognitive syndrome (DCS), which defined the cognitively impaired sample in this cohort. Metzger et al. [36] used a similar battery, the BCCOG-SEP [39] designed to evaluate cognitive impairment in multiple sclerosis. Tasks included assessments of verbal short-term memory, visual perception, digit spans, working memory, processing speed, go-no-go test and verbal fluency. Cognitive status was defined by this evaluation, patients were classified as cognitively impaired if they scored below the 5th percentile of the normative mean of the BCCOG SEP on at least 4 subtests. Other cognitive tasks that did not overlap between studies are outlined in Table 1.

**MRI data acquisition**

BOLD fMRI was in used in six of the studies [10, 13, 30, 31, 35, 36]. Three papers employed the arterial spin labeling (ASL) MRI technique to measure changes in brain perfusion. Of these, one used pulsed ASL (PASL) [37], one used continuous ASL (CASL) [32] and the final employed pseudo-continuous ASL (pCASL) [33]. However, in the work of Gauthier et al. the images acquired with pCASL sequence were separated into BOLD and CBF time-series data, of which only the BOLD information was used in CVR analysis. Therefore, this work is considered to be a BOLD imaging study. The remaining study used dynamic susceptibility contrast-enhanced (DSC) MRI [34]. All but two studies [13, 34] used an MR scanner with magnetic field strength of 3T. MR protocol information is displayed in Table 2.

**Summary of regional CVR findings**

The studies that employed BOLD imaging assessed CVR in various regions-of-interest (ROIs) with some contrasting findings. Results are shown in Table 2. Cantin et al. [13] observed regional impairment in CVR between healthy controls and patients with cognitive impairment, particularly in posterior brain areas, whereas Yezhuvath et al. [30] reported CVR deficits in more rostral regions in patients with AD compared to healthy controls. Cantin et al. [13] investigated CVR in several regions: frontal, parietal, temporal and occipital lobes, the cingulum, the insula, the striatum and the thalamus. Yezhuvath et al. performed a voxel-wise regression and region-of-interest (ROI) analysis using 6 regions: the occipital lobe, temporal lobe, frontal lobe, parietal lobe, insular cortex and subcortical grey matter. This is in contrast to the work by Thomas et al. [35], who found no differences in reactivity between adults with amnestic mild cognitive impairment compared to controls using a voxel-wise comparison of whole-brain grey matter CVR maps. Metzger et al. [36] calculated CVR in 8 regions of interest (ROIs): occipital, parietal, temporal frontal, insula, cingulum, thalamus.
Table 1  Patient characteristics, cognitive assessment and relationship with CVR

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample (age, mean ± SD)</th>
<th>Cognitive assessment (score ± SD)</th>
<th>CVR in participants</th>
<th>CVR and cognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calviere et al. [34]</td>
<td>10 MMD (404, 8 female), 6 with DCS</td>
<td>Executive function TMT B Letter and category fluency Stroop interference Brixton test WISC-C WISC-P Attention and processing speed TMT A Coloured dots and words Stroop</td>
<td>CVR &lt; DCS than no DCS</td>
<td>Frontal CVR reduced in cognitively impaired patients, temporoparietal CVR was not different between DCS and no DCS</td>
</tr>
<tr>
<td>Cantin et al. [13]</td>
<td>7 MCI (64.1 ± 9.0, 2 female) 9 AD (71.1 ± 6.7, 5 female) 11 HC (65.4 ± 9.3, 6 female)</td>
<td>MMSE MCI 27.4 ± 1.8 AD 21.7 ± 2.2 HC 295 ± 0.5</td>
<td>HC &gt; MCI = AD</td>
<td>CVR correlated with MMSE score in all regions examined</td>
</tr>
<tr>
<td>Chung et al. [32]</td>
<td>35 T2DM (65.1 ± 8.0) 30 HC (67.1 ± 10.4) 33 female whole sample 2-year follow-up: 40ppts, 19 T2DM</td>
<td>MMSE (not analysed with CVR) HVLT-R (verbal learning and memory function) ROCF (visual–spatial ability and visual memory function) TMT A &amp; B (executive function) VF (executive function) IADL scale Composite learning and memory T score (average of HVLT-R and ROCF) Composite executive function (average of VF and TMT) T2DM Baseline: 47.5 ± 8.3 2-year follow up: 44.6 ± 10.5 HC baseline: 52.1 ± 7.6 2-year follow up: 56.5 ± 9.9</td>
<td>No significant CVR differences between T2DM and HC at baseline or 2-year follow up</td>
<td>Controls: no significant association between CVR and executive function Decreased CVR associated with decline in executive function in T2DM Regional CVR associated with executive function in frontal and parietal lobes in T2DM</td>
</tr>
<tr>
<td>Gauthier et al. [33]</td>
<td>31 younger (24 ± 3, 10 female) 54 older (63 ± 5, 37 female)</td>
<td>MMSE (values not reported) Modified Stroop task (executive function)</td>
<td>Frontal CVR lower in older group, but not significant</td>
<td>Frontal BOLD CVR not associated with Stroop performance</td>
</tr>
<tr>
<td>Gloczek et al. [37]</td>
<td>7 MCI (73.4 ± 8.2, 10 female) 17 HC (69.8 ± 6.9, 32 female)</td>
<td>MMSE MCI 27.5 ± 2.4 HC 292 ± 1.0 Brief Cognitive Rating Scale GDS</td>
<td>HC &gt; MCI</td>
<td>CVR not related to MMSE, age, or regional brain volumes, in either the entire sample or in HC and MCI subgroups</td>
</tr>
<tr>
<td>Metzger et al. [36]</td>
<td>33 MS patients, 12 CI (41 ± 6.27, 7 female), 21 CN (39 ± 5.9114 female) 22 HC (41 ± 6.48, 13 female)</td>
<td>BCogSEP (short term memory, visual memory, digit spans, working memory, processing speed, go-no-go test, executive function)</td>
<td>Overall MS = HC CI &lt; CN</td>
<td>CVR lower in cognitively impaired patients in whole brain and all regions examined</td>
</tr>
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</table>


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<thead>
<tr>
<th>Study</th>
<th>Sample (age, mean ± SD)</th>
<th>Cognitive assessment (score ± SD)</th>
<th>CVR in participants</th>
<th>CVR and cognition</th>
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</thead>
</table>
| Richiardi et al. [31] | 15 MCI (71 ± 10, 9 female)  
20 AD (76 ± 7, 10 female)  
28 HC (73 ± 7, 18 female) | MMSE  
MCI 28 ± 2  
AD 25 ± 3  
HC 29 ± 1 | AD and MCI slower CVR velocity | CVR correlated with MMSE in 10 regions of the DMN |
| Tchistiakova et al. [10] | 18 HTN +T2DM (71.8 ± 5.6, 7 female)  
22 HTN only (73.4 ± 6.2, 12 female) | TMT A (processing speed)  
CVLT (memory)  
WCST (executive function) | Compared to HTN, HTN +T2DM had decreased CVR in frontal and parietal areas | No significant associations between CVR and cognitive function |
| Thomas et al. [35]  | 44 MCI (64 ± 6.6, 25 female)  
28 HC (65.6 ± 6.8, 15 female) | MMSE  
MCI 28.9 ± 1.4  
HC 29 ± 1.0  
LM (immediate and delayed recall)  
TMT A & B (executive function)  
CVLT (memory) | MCI = HC | No significant differences in whole brain grey matter CVR between MCI and HC |
| Yezhuvath et al. [30] | 12 AD (68.7 ± 8.4, 10 female)  
13 HC (70.5 ± 8.3, 4 female) | MMSE  
AD 22.8 ± 4.1  
HC 296 ± 0.7  
CERAD battery  
CDR  
BCCogSEP (language ability) | HC > AD  
Compared with controls, AD patients had reduced CVR in rostral brain | CVR in frontal lobe and insula (the primary CVR deficit regions) not related to global cognitive function  
Significant correlation between CVR and Boston Naming Test score in the frontal and insula regions in AD patients |

Mean age and SD in brackets

MMD moyamoya disease, DCS dysexecutive cognitive syndrome, CI cognitive impairment, CN cognitively normal, HC healthy controls, AD Alzheimer’s disease, MCI mild cognitive impairment, HTN hypertension, T2DM type 2 diabetes mellitus, CDR Clinical Dementia Rating, BCCogSEP French language test to evaluate cognitive performance in multiple sclerosis, IADL instrumental activities of daily living, ROCF Rey–Osterrieth Complex Figure, HI T A Hopkins Verbal Learning Test—Revised, GDS Global Deterioration Scale, LM logical memory, TMT A & B Trail Making Test Parts A and B; VF verbal fluency, CVLT California Verbal Learning Test, WCST Wisconsin Card Sorting Test (-C: categories; -P: perseverations), CERAD Consortium to Establish a Registry for Alzheimer’s Disease, BNT Boston Naming Test
<table>
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<tr>
<th>Study</th>
<th>MR method</th>
<th>Vascular challenge</th>
<th>Stimulus effect—CVR values</th>
<th>Stimulus effect—etCO$_2$ values</th>
<th>Brain regions analysed</th>
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<tr>
<td>Calviere et al.</td>
<td>DSC EPI 1.5T TE: 30 ms</td>
<td>15 mg/kg injection of acetazolamide</td>
<td>Frontal CVR DCS $-13.5 \pm 13.2 %$ BOLD/mmHg No DCS Temporoparietal CVR DCS $-7.2 \pm 22.2 %$ BOLD/mmHg No DCS</td>
<td>N/A</td>
<td>ROIs: Frontal, temporoparietal</td>
</tr>
<tr>
<td>Cantin et al.</td>
<td>BOLD EPI 1.5T TR: 3000 ms TE: 45 ms voxel size 4 x 4 x 4 mm$^3$ 32 axial slices</td>
<td>Carbogen (7% CO$_2$ in 93% O$_2$) Air (1 min) — carbogen (2 min) — air (1 min) x 3, Duration 12 min</td>
<td>MCI 0.36 \pm 0.12% BOLD/mmHg AD 0.36 \pm 0.13% BOLD/mmHg HC 0.62 \pm 0.20% BOLD/mmHg</td>
<td>Baseline MCI 39.9 \pm 7.3 AD 34.6 \pm 5.6 HC 42.3 \pm 5.5</td>
<td>ROIs: frontal, parietal, temporal and occipital lobes, cingulum, insula, striatum and thalamus</td>
</tr>
<tr>
<td>Chung et al.</td>
<td>CASL w FAIR 3T Scanning parameters not reported</td>
<td>Vasodilation with CO$_2$ rebreathing, reactivity slope from rebreathing to hyperventilation CVR values reported are from the vasodilation measure</td>
<td>T2DM Baseline 1.1 \pm 0.7 ml/100 g/min 2-year follow up 1.0 \pm 1.0 ml/100 g/min HC Baseline 0.69 \pm 0.43 ml/100 g/min 2-year follow up 0.59 \pm 1.50 ml/100 g/min</td>
<td>Not reported</td>
<td>ROIs: Global, frontal, temporal, parietal, occipital and insula</td>
</tr>
<tr>
<td>Gauthier et al.</td>
<td>pCASL w FAIR 3T simultaneous CBF and BOLD BOLD CVR data reported TR: 3000 ms TE1: 10 ms TE2: 30 ms voxel size 4 x 4 mm$^2$ 11 slices of 7 mm (1 mm slice gap)</td>
<td>Computer controlled gas delivery system 2 x 2 min blocks of hypercapnia with 2 min of air before and after each block.</td>
<td>Older adults 0.25 \pm 0.08% BOLD/mmHg Younger adults 0.25 \pm 0.06% BOLD/mmHg etCO$_2$ targeted to 40 mmHg at baseline 45 mmHg during hypercapnia etO$_2$ targeted to 100 mmHg throughout</td>
<td>etCO$_2$ targeted to 40 mmHg at baseline 45 mmHg during hypercapnia etO$_2$ targeted to 100 mmHg throughout</td>
<td>ROI: frontal lobe</td>
</tr>
<tr>
<td>Study</td>
<td>MR method</td>
<td>Vascular challenge</td>
<td>Stimulus effect—CVR values</td>
<td>Stimulus effect—etCO₂ values (mmHg)</td>
<td>Brain regions analysed</td>
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<tr>
<td>Glodzik et al. [37]</td>
<td>PASL w FAIR 3T TR 3400 ms TE 17 ms Tl 1200 ms voxel size 1.2 x 1.2 x 6 mm³</td>
<td>CO₂ rebreathing</td>
<td>MCI Global cortical 0.31 ± 0.12 ml/100 g/min Averaged hippocampus −0.05 ± 1.7 ml/100 g/min HC Global cortical ±1.5 ml/100 g/min Averaged hippocampus 1.4 ± 2.7 ml/100 g/min</td>
<td>Baseline MCI 38.7 ± 5.9 HC 39.2 ± 3.1 ΔetCO₂ MCI 6.2 ± 1.4 HC 7.1 ± 1.8</td>
<td>ROIs: Global cortical, hippocampus</td>
</tr>
<tr>
<td>Metzger et al. [36]</td>
<td>BOLD EPI 3T TR 3000 ms TE 20 ms voxel size 3 x 3 x 3 mm³ 44 slices</td>
<td>8% CO₂ enriched-gas (BACTAL®) Air (1 min)–BACTAL® (2 min)–air (2 min)–BACTAL® (2 min)–air (2 min)–BACTAL® (2 min)–air (1 min)</td>
<td>Global median CVR Patients CI 0.15 ± 0.06% BOLD/mmHg Patients CN 0.21 ± 0.06% BOLD/mmHg HC 0.207 ± 0.06% BOLD/mmHg</td>
<td>Not monitored, mean etCO₂ obtained from a standard population</td>
<td>Global median grey matter ROIs: frontal, parietal, occipital, temporal, insula, striatum, thalamus, cingulum</td>
</tr>
<tr>
<td>Richiardi et al. [31]</td>
<td>BOLD EPI 3T TR 2970 ms TEs: 12.3, 29.5, 46.8, and 64 ms voxel size 3.44 x 3.44 x 3.5 mm³ 34 axial slices</td>
<td>7% CO₂ in medical air administered via a nasal cannula Air (1 min)–CO₂ (2 min)–air (2 min)–CO₂ (2 min)–air (2 min)–CO₂ (2 min)–air (2 min)–air (1 min) Duration 9 min</td>
<td>Not reported</td>
<td>Not monitored</td>
<td>Lobe level (7 lobes) and regional level analysis (88 regions)</td>
</tr>
<tr>
<td>Tchistiakova et al. [10]</td>
<td>BOLD 3T TR 2000 ms TE 30 ms 32 axial slices</td>
<td>Successive breath holds 6 breath holds lasting 15 s each following 3 s expiration period with intermittent 30 s periods of normal breathing CVR calculated as % change in BOLD signal, not corrected for etCO₂</td>
<td>Not reported</td>
<td>Not monitored</td>
<td>Voxel-wise CVR map Only ROIs that differed between groups specified: left pericalcarine cortex, right inferior parietal, lateral occipital and precuneus, and bilateral cuneus, lingual gyri and superior parietal lobes</td>
</tr>
<tr>
<td>Thomas et al. [35]</td>
<td>BOLD 3T Scanning parameters not reported</td>
<td>5% CO₂ in medical air administered through a mouthpiece, nose-clip fitted CO₂ (1 min)–air (1 min) repeated three times</td>
<td>MCI 0.174 ± 0.04% BOLD/mmHg HC 0.170 ± 0.03% BOLD/mmHg</td>
<td>Not reported</td>
<td>Whole brain grey matter</td>
</tr>
<tr>
<td>Yezhuvath et al. [30]</td>
<td>BOLD EPI 3T TR 3000 ms TE 30 ms voxel size 1.7 x 1.7 mm² 25 axial slices</td>
<td>5% CO₂ in medical air alternating between gas and room air every minute Duration 7 min</td>
<td>Not reported</td>
<td>Baseline AD 33.9 ± 3.5 HC 33.7 ± 5.3 ΔetCO₂ AD 11.9 ± 1.9 HC 12.2 ± 1.4</td>
<td>Voxel-wise map and ROI analysis ROIs: occipital lobe, temporal lobe, frontal lobe, parietal lobe, insular cortex and subcortical grey matter</td>
</tr>
</tbody>
</table>

**Notes:**
- **HC** healthy controls, **AD** Alzheimer’s disease, **MCI** mild cognitive impairment, **MMSE** Mini-Mental State Examination, **BOLD EPI** blood oxygen-level dependent echo planar imaging, **DSC EPI** dynamic susceptibility contrast-enhanced echo planar imaging, **DCS** dysexecutive cognitive syndrome, **PASL** pulsed arterial spin labeling, **pCASL** pseudo-continuous arterial spin labeling, **T2DM** Type 2 diabetes mellitus, **CI** cognitively impaired, **CN** cognitively normal, **etCO₂** end-tidal partial pressure of CO₂, **etO₂** end-tidal partial pressure of O₂.
and striatum, as well as a global median. CVR in all ROIs was significantly reduced in MS patients with cognitive impairment compared to those who were not cognitively impaired.

Another study [31] analysed the BOLD data at the level of overall CVR effect, differences between lobes (7 lobes were delineated as per previous work), brain regions (88 cortical and subcortical regions included), and finally the associations between CVR velocity and the cognitive assessment scores. CVR velocity refers to the temporal dynamics of the CVR response, representing the rate of the vasodilation. It was observed that the largest differences in CVR between AD and healthy controls were seen in the frontal and occipital lobes.

Calviere et al. [34] used 22 ROIs manually drawn on the bilateral frontal and temporoparietal areas of the cerebral cortex, and reference areas in the cerebellum. The mean transit time (MTT) and cerebral blood volume (CBV) values from each area were estimated from perfusion weighted image analysis, and averaged to give one measure from each region. Ratios of CBV in the frontal and temporoparietal areas were calculated relative to the cerebellar CBV, which was used as a control region. The CVR values for each region were estimated from the CBV values relative to the cerebellum. Frontal CVR was lower in cognitively impaired patients with moyamoya disease than those without cognitive impairment.

Gauthier et al. examined CVR using a pseudo-continuous ASL (pcASL) sequence. BOLD data was acquired and intersected with areas of significant signal change in response to the vasoactive stimulus observed with ASL data using cluster analysis to define one frontal ROI [33]. This region was found to be slightly lower in reactivity in older adults compared to younger, yet this difference was not significant, nor was CVR in this region associated with cognitive function.

Chung et al. [32] investigated CVR in the frontal, temporal, parietal, occipital and insula lobes of the brain, as well as calculating a global CVR index. The frontal and parietal lobes were associated with change in executive function in patients with T2DM, but not in healthy controls.

Tchistiakova et al. [10] performed a functional ROI analysis, and reported that there was reduced CVR in several regions in those with both hypertension and T2DM compared to hypertension alone in the left hemisphere (pericalcarine cortex), right hemisphere (inferior parietal, lateral occipital and precuneus) and the cuneus, lingual gyri and superior parietal lobes bilaterally.

CVR and neuroimaging correlates of cognitive dysfunction

Seven studies explicitly mentioned correcting for partial volume effects, grey matter atrophy or white matter hyper-intensities (WMH) in their image analyses [10, 13, 30, 31, 33, 36, 37]. In one remaining paper the authors made mention of normalising the perfusion signal for tissue volume, yet did not give further information on the specifics of this procedure [32].

Several studies examined the relationship of CVR to WMH, with some mixed results. Gauthier et al. [33] showed that age, gender and volume of WMH accounted for a significant amount of variance in frontal CVR. Similarly, Yezhuvath et al. [30] found that lower CVR was associated with greater volume of WMH in their cohort of AD and healthy controls. Yet another study investigating the association of grey matter CVR, cardiovascular risk factors and periventricular WMH found that these parameters were intercorrelated [37]. In contrast, Richiardi et al. [31] reported that there was no significant association between severity of WMH and CVR velocity in their cohort of AD, aMCI and healthy controls. Similarly Metzger et al. [36] found that there was no association between CVR and WMH in MS patients, healthy controls or the cohort as a whole.

Of the reviewed papers, only one investigated hippocampal atrophy in relation to reactivity, and it was found to negatively correlate with CVR in the occipital, parietal, striatum and temporal ROIs [13].

Calculation of CVR

Metzger et al. [36] did not monitor end tidal-CO₂ (etCO₂) throughout their experiments, thus they were unable to use this trace as a regressor in their modelling of CVR. This study used mean etCO₂ obtained from a standard population as a regressor in their general linear model (GLM). Richiardi et al. [31] did not monitor etCO₂ either. In this work two CO₂ regression coefficients were calculated analytically to reflect the CVR amplitude and velocity separately, though the authors only report velocity in this paper. These regression coefficients were calculated from mathematical models of nominal and slow etCO₂ responses to a CO₂ challenge, to represent the expected responses in healthy subjects and those with slower vessel dilation respectively. CVR velocity was defined in this paper as the rate of vasodilation. While the method of CVR estimation here is acceptable, the unavailability of etCO₂ data potentially limits the strength of these findings. Similarly, Tchistiakova et al. [10] did not record etCO₂ throughout the hypercapnic procedure. These researchers calculated CVR as the % change in BOLD signal during 6 × 15 s breath-holds.

Calviere et al. [34] used the regional cerebral blood volume (rCBV) ratio from the regions of interest (ROIs) that was relative to the CBV of the cerebellum (control region). No etCO₂ was recorded in this study as vasodilatation was elicited via injection of acetazolamide, thus the
calculation used in this study was: \( \text{CVR} = \frac{([\text{rCBV ratio before acetazolamide} - \text{rCBV ratio after acetazolamide}] / \text{rCBV ratio before acetazolamide}) \times 100}. \)

Chung [32] used a rebreathing paradigm to assess vasodilation, vasoconstriction and vasoreactivity separately. Vasodilation was measured as the perfusion increase from baseline during \( \text{CO}_2 \) rebreathing normalised to the change in \( \text{etCO}_2 \) between baseline and rebreathing. Vasoreactivity was defined as the best-fitting slope between normal breathing, vasodilation and vasoconstriction. It should be noted that the 'gold standard' for CVR measurement is more likely the whole vasodilatory range of hypocapnia (elicited by hyperventilation) to hypercapnia [40]. However, CVR is most commonly calculated as the difference in CBF (or surrogate) between baseline and during a vascular challenge divided by the change in \( \text{etCO}_2 \) between these conditions, thus the vasodilation measure is taken as CVR, not the vasoreactivity measure in this instance.

The remainder of the studies estimated CVR using the standard calculation:

\[
\text{CVR} = \left( \frac{(\text{MRIparameter}_{\text{dil}} - \text{MRIparameter}_{\text{rest}})}{\text{MRIparameter}_{\text{rest}}} \right) \times 100 \div \Delta \text{etCO}_2
\]

where \( \text{MRIparameter}_{\text{dil}} \) is the CBF or BOLD signal measured during the vasodilated period; \( \text{MRIparameter}_{\text{rest}} \) indicates the CBF or BOLD signal measured at baseline; and \( \Delta \text{etCO}_2 \) is the difference is end-tidal \( \text{CO}_2 \) in mmHg between the two conditions.

**Relationship between CVR and cognition**

Of the four papers that analysed the association of CVR to MMSE score, two reported significant positive correlations [13, 31], and two reported no relationship [30, 37]. Metzger’s work found that CVR was lower in MS patients with cognitive impairment compared to non-impaired patients [36], supporting the findings of Calviere et al. [34], who reported that CVR was significantly reduced in patients with moyamoya disease and dysexecutive cognitive syndrome (DCS) compared to patients without DCS. This is in contrast to the results of Thomas’s study, which concluded that whole-brain grey matter CVR was not significantly different between MCI and healthy control groups [35].

Tchistiakova et al.’s [10] research involved three measures of cognitive function, none of which were found to correlate with CVR. These measures were tests of memory, processing speed and executive function. A second study [33] also reported no significant association between executive function as measured by a Stroop task, yet the work by Chung et al. [32] found that CVR decline was linked to a decrease in executive function in T2DM at 2-year follow-up. These results are further discussed below.

**Discussion**

This paper systematically reviewed research articles that examined the association between cognition and cerebrovascular reactivity (CVR) using MRI. Six out of ten studies described significant relationships between CVR and cognition, including a longitudinal study which reported that lower CVR was predictive of cognitive decline over a 2-year period. The association of CVR to cognition is more established in individuals with cognitive dysfunctions, while this link is less well-known in cognitively normal adults. There was an over-reliance on imprecise measures of cognition, and the vascular challenges used to measure CVR varied widely.

**CVR is reduced in adults with cognitive dysfunction**

CVR was consistently lower in cognitively impaired adults versus healthy controls, or patients without cognitive impairment in the reviewed research (6 of 10 studies). Two studies reported significant correlations between cognition measured by MMSE and CVR in multiple brain regions [13, 31]. These investigations also observed that CVR was significantly reduced in AD and MCI patients, and that AD patients had significantly slower responses to hypercapnia (i.e. CVR velocity was reduced), compared to healthy controls. In contrast, Glodzik et al. and Yezhuvath et al. [30, 37] reported that CVR was not directly related to cognition measured using the MMSE. However, in both of these studies patients with cognitive impairment had lower reactivity than matched healthy controls, seen in the hippocampus in Glodzik et al. [37] and in the prefrontal, anterior cingulate and insular cortices in the study by Yezhuvath et al. [30]. Two other studies reported that CVR was significantly reduced in participants with cognitive impairment compared to those who were cognitively normal [34, 36].

These findings are supported by evidence using other modalities linking dementia severity with cerebrovascular responsiveness [27, 28]. Transcranial Doppler (TCD) ultrasound is often used to measure changes in CBF velocity in investigations of CVR. This method, while temporally precise, lacks spatial resolution, thus its practicality in regional CVR examinations is limited. Nonetheless, research conducted into the relationship between CVR and cognition with TCD has shown interesting results. Silvestrini et al. [28] reported that CVR as measured using the breath-hold index and TCD was the sole predictor of cognitive decline in patients with AD. Moreover, breath-hold index has been found to be associated with early cognitive impairment [41], as well
as an increased risk of conversion from MCI to AD [27]. A systematic review of TCD analyses found that CVR to hypercapnia was a good differentiator of dementia subtypes across multiple studies [42]. Overall, the results of the reviewed studies lend support to the hypothesis that CVR and cognitive functioning are linked, evidenced by findings of reduced reactivity in patients with cognitive impairment compared to cognitively healthy controls.

While the data reviewed is suggestive of reduced vascular reactivity in individuals with cognitive impairment, a definitive relationship between CVR and cognition in cognitively healthy adults was not identified. Only one study focused exclusively on cognitively normal adults without chronic health conditions [33], whilst five studies included healthy controls as compared to patients with cognition impairment, and examined CVR and cognition within these participants [13, 30–32, 35]. Two investigations compared patients with cognitive impairment to those without. Metzger studied MS in relation to healthy controls [36], whilst Thomas et al. [34] investigated only individuals with moyamoya disease (MMD). The remaining study investigated CVR and cognition in cognitively normal individuals with hypertension with or without co-morbid T2DM [10]. Within the reviewed studies, imprecise methods were used for evaluation of cognitive function and CVR. Reliance on the mini-mental state exam (MMSE) as the main assessment of cognition in several studies [13, 30, 31, 37] necessitates some caution, as this measure may not be sufficiently sensitive to variation in cognitive capability, nor does it allow for distinction between different cognitive domains [38]. This is evidenced by the findings of Richiardi et al. [30] in which no evidence of a relationship between CVR and cognitive performance was found using measures of global cognitive function, yet a significant correlation was observed with language ability. The MMSE is specifically designed as a screening tool for distinguishing between individuals with and without gross cognitive impairment [38], and as such its usefulness for precise cognitive assessment is not ideal.

Among research that assessed cognitively healthy cohorts and those assessing the cognitive capabilities of individuals with MS or MMD, tests of executive function were used. However the specific tests used to define this construct varied between the five studies, including tasks of inhibitory control, task-switching, verbal fluency, and processing speed, among others [10, 32–34, 36] (see Table 1 for details). Of the two studies assessing patients [34, 36] batteries of neuropsychological tests examining executive function (amongst others) were used to determine cognitive status. While results of two studies showed that executive function was not directly correlated with CVR in either frontal cortex [33] or averaged across the whole brain [10], the two patient studies both reported that CVR was significantly lower in individuals with cognitive impairment compared to those without. This was observed in the frontal region in MMD [34], and in the whole brain grey matter, as well as in a region-of-interest analysis comprising multiple brain areas in MS [36]. Similarly, Chung et al. [32] observed that global CVR was positively associated with executive function in patients with Type 2 diabetes mellitus (T2DM). In T2DM patients, decreased global, frontal and parietal vasodilation at 2-year follow up was linked to accelerated declines in executive function. The executive function task was composed of separate tasks of verbal fluency and Trail Making Task A, which assesses task-switching and visual attention. The studies that failed to observe any association between CVR and executive function used tasks that assessed interference and flexibility in thinking (Stroop and the Wisconsin Card Sorting Task, respectively), whilst the patient studies that did observe an association defined cognitive status on the basis of multiple executive function tasks. Thus it could be that some aspects of executive function are more related to CVR than others. Notably, it is thought that there are from 3 to as many as 7 distinguishable executive abilities [43], hence a more comprehensive approach to assessment would be necessary to draw definitive conclusions.

**Relationship between CVR and cognition may be mediated by cardiovascular risk factors in cognitively healthy adults**

There is evidence that CVR is related to executive function in populations with cardiovascular risk [10, 32]. One study [10] reported that CVR was significantly lower in those with comorbid hypertension and T2DM versus participants with hypertension alone. Similarly, Chung et al. [32] reported that higher inflammatory markers in T2DM were linked to greater reductions in CVR, which resulted in accelerated cognitive decline over a 2-year period. Gauthier et al. [33] reported an association between cognitive performance and aortic pulse wave velocity (PWV) in their healthy cohort, yet no direct link between CVR and cognition was observed. This finding was interpreted as indicating that declining vascular health, even in primary stages, negatively impacts cognition. Due to the above-average health of the cohort only minor differences in cerebrovascular properties were seen, as compared to larger changes seen in aortic elasticity between younger and older adults. Small blood vessel changes, coupled with the known low signal to noise ratio (SNR) present in BOLD imaging was posited to explain the unexpected lack of relationship observed between CVR and cognition in this study.

The relationship between cardiovascular risk and CVR was more clearly demonstrated by Glodzik et al. [37], who reported moderate negative correlations between
the two in the hippocampus ($r = -0.41$) and cortical grey matter ($r = -0.46$) in both patients and healthy controls. Likewise, there is evidence of a link between reduced CVR and increased vascular risk in previous studies using MRI [44] and TCD [45]. Together, these findings may indicate that decreased reactivity may be the result of poor vascular health in general, and this is the primary factor triggering neurocognitive decline. Extensive evidence indicates that risk factors for cardiovascular disease precede and facilitate cognitive deterioration in aging [46–48].

Cardiovascular factors can result in dysfunctional reactivity in specific brain regions, leading to hypoperfusion which may pertain to cognitive impairment. The discrepancies between these three studies are multifaceted including use of different: executive tasks; methods of inducing hypercapnia; and, different imaging techniques [10, 32, 33]. These discrepancies’ limit the generalisability of the findings to a wider cohort; however, it can be seen that there is a possible association between cardiovascular risk factors and CVR which may mediate the relationship between CVR and executive function in cognitively healthy individuals. Further studies are needed to confirm these associations.

Similarly, there is the possibility that the observed relationships between CVR and cognition could be mediated by the presence of other cerebral pathologies known to disrupt cognition, such as white matter hyper-intensities (WHM) and hippocampal atrophy. It is understood that severity of WMH corresponds to cognitive decline [49, 50], and evidence has shown that normal-appearing white matter that progresses to WMH has lower CVR than areas that do not progress [51]. Within the reviewed articles, the relationship of CVR to WMH was mixed, with three [30, 33, 37] of five studies reported a significant correlation. Interestingly, all three of these papers observed significant relationships between cognition and CVR, thus it is apparent that continued research investigating these associations is necessary.

Methodological considerations
While the results of the reviewed studies are inconsistent, this is likely influenced by heterogeneous samples, imprecise cognitive testing instruments (as outlined above), varying procedures for inducing vasodilation and differences in imaging protocols.

Differences in vascular challenge
All studies induced an increase in cerebral blood flow; however, not all manipulations are equal in their capacity to elicit vasodilation. Whilst the breath-hold method is used widely, is inexpensive and efficient in inducing CBF changes, this technique may produce less reproducible stimuli and/or data due to participant compliance, as well as individual differences in breath hold capacity. Breath-hold and re-breathing procedures during MR imaging also present potential risk of motion artifacts, which may result in undesirable signal differences [52]. It is well established that the strength and duration of the stimulus effects the cerebrovascular response [53]. Inhalation of CO2-enriched gas mixture has been shown to be a more highly reliable means to induce hypercapnia and stimulate the cerebral vasculature [54–56].

Prospective targeting of etCO2 has been deemed the most standardisable stimuli for measuring CVR in a recent review paper [24], yet only one study included in the current work employed this technique [33]. It should be noted however, that the literature is far from a consensus on which vascular challenge is most appropriate for assessment of CVR.

Five studies used the more traditional method of inhalation of fixed-level CO2-enriched gas. Differences may appear somewhat minor—a discrepancy of 2% CO2 concentration between the 7% used by Richiardi et al. [31] and 5% by both Thomas [35] and Yezhuvath et al. [30]; while Richiardi et al. and Cantin et al. [13] used the same concentration of CO2 (7%), the latter study mixed the gas with 93% O2, a substance known as carbogen, rather than medical air, which is balanced with N2. The gas concentration utilised by Metzger [36] was slightly higher again (8% BACTAL®), and it should be noted that the composition of this gas mixture was unreported, and unable to be identified from an internet search.

While these differences in CO2 concentration may seem trivial, the evidence suggests that the relationship between BOLD signal and PaCO2 is non-linear, thus CVR results may be dependent on the CO2 concentration used, as well as baseline PaCO2 [57]. The use of carbogen (and potentially, BACTAL®) as the vasoactive stimulus [13], rather than standard medical air, has implications for the measurement of CVR, particularly when combined with BOLD imaging [54]. The percentage of oxygen present in carbogen is greater than that in the atmosphere, which will result in an increase in arterial partial pressure of O2 (PaO2), and possible vasoconstriction, confounding the vasodilatory response intended for CVR measurement. By nature, BOLD imaging relies on the ratio of oxygenated to deoxygenated hemoglobin in the blood, and any increase in PaO2 in the brain will elicit unwanted changes in the BOLD signal. BOLD is also sensitive to changes in blood flow, volume and oxygen metabolism, whilst ASL measures flow only, and is not affected by changes in blood oxygenation.

Two studies employed CO2 rebreathing [32, 37]. Both of these papers lacked information regarding the volume of the respiration reservoirs used and length of
rebreathing period in the paradigms, hampering comparability. Chung et al. [52] also failed to report the endtidal CO2 values. The speed at which partial pressure of CO2 (PaCO2) rises will be affected by respiration rates and the volume of the rebreathing reservoir, ultimately influencing the measured CVR value. Likewise, breath-holding may produce confounding variables, as the rise in PaCO2 during breath-holding varies between individuals due to differences in lung size and metabolic rate [24]. This method also relies heavily on participant compliance and may be difficult or uncomfortable for some to perform [58].

An acetazolamide challenge was used in one study [34]. Whilst this method is safe, does not rely on subject cooperation and is widely used in clinical settings, administration via injection is invasive, and a standardized dose may not produce the replicable stimulus necessary for CVR estimation due to individual variability [24]. For the purposes of participant comfort, less invasive stimuli would be preferable for measurement of CVR.

There are multiple options for inducing an increase in cerebral blood flow, however future research in this area would benefit from a more standardized and reproducible approach, particularly if the purpose is a simple measure of cerebrovascular response amplitude. Inhalation of CO2-enriched gas is an easily implemented and standardizable method, with fewer contraindications than rebreathing and breath-holding. While computer-controlled etCO2 prospective targeting is the most clinically standardizable technique, it requires expensive equipment which is not readily available in most research facilities. At a minimum, researchers should take care to provide sufficient information regarding vascular challenge techniques so that comparisons may be made between studies.

**Variations in imaging protocol**

ASL, BOLD and DSC imaging methods are all considered valid for measurement of CVR, yet the results from these are not directly comparable. While MR imaging has a clear advantage of spatial specificity over ultrasound and CT-based methods, all three methods present possible drawbacks in regard to measuring CVR. BOLD, the most commonly used method, acquires images via the complex combination of blood volume, flow and oxygenation metabolism in the brain and thus is affected by subtle variations in any of these parameters, despite not directly measuring blood flow per se. BOLD is also known to be more sensitive to the baseline level of vascular tension than perfusion MRI [59]. ASL, while being more physiologically precise, has limited spatial coverage, lower signal to noise ratio, and is generally considered less sensitive for measures of CVR [33]. As BOLD is more commonly used and also more accessible on conventional MRI scanners, it is the currently preferred sequence for CVR measurements, although rapid development of new ASL pulse sequences enabling global brain coverage may render it the favored method in the future. Both ASL and BOLD MRI have been widely used in studies of hemodynamic function and cognitive performance in both healthy [60, 61] and patient samples [62, 63]. DSC is less commonly employed in CVR measurement studies, most likely due to the necessity of an injection of an exogenous contrast agent. Both BOLD and ASL are non-invasive, well tolerated and easily repeatable, thus either of these methods are considered preferable over DSC MRI for measurement of CVR in research studies.

**Conclusion**

The connections observed between hemodynamic dysfunction and cognitive impairment observed in the majority of these studies warrants further investigation. Those affected by cognitive impairment were more likely to exhibit decreased CVR compared to healthy controls, as were individuals with greater cardiovascular risk factors. Previous research using alternative methods have given strong indication of the causal relationship between dysfunctional CVR and cognitive deterioration. Given that vascular risk factors are often modifiable, development of vaso-protective therapies may prevent or slow the progression of cognitive decline.

Due to the fact that there is still so much to investigate regarding which type of vasoactive modulation and imaging protocol provides the richest set of data to assess vascular function, recommendations for measurement of CVR response amplitude include the inhalation of a set concentration of CO2-enriched gas, in combination with either ASL or BOLD MRI, provided that the whole brain is imaged. Future research should also employ more comprehensive neuropsychological examination to further unravel the nature of the association between cerebrovascular reactivity and cognition.

**Authors’ contributions**

SJC came up with the idea, contributed to the design, performed part of the article inclusion process, and drafted the manuscript. HM contributed to the design, performed part of the article inclusion process and revised the manuscript. AP and MEH contributed to the design and revised the manuscript. All authors read and approved the final manuscript.

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4.4 Summary of key findings

Using a systematic review process, it was found that 6 of the 10 reviewed papers showed a significant association between CVR and cognitive function. Heterogeneity between the participant samples, methodologies and measurement tools, and the use of cognitive assessments which lacked sensitivity, limited the extent to which firm conclusions could be made regarding the contributions of regional cerebrovascular reactivity to cognitive performance. Further research which takes these drawbacks into account is recommended.

The review provided the rationale for an empirical study carried out by the authors that forms Chapter 6 of the current thesis- ‘Cerebrovascular reactivity and cognition in healthy aging’. The study was designed to address the limitations of the reviewed works, by employing the use of a comprehensive cognitive testing battery and arterial spin labeling MRI, aiming to improve upon and further the research in this area.

The subsequent chapter of this thesis (Chapter 5) will provide the details of the methodology used in the empirical research.
Chapter 5

Methods

This chapter is divided into two main sections. The first section will outline the cognitive screening and assessment measures and the peripheral cardiovascular measures used in the experiments. The CO₂ inhalation procedure, including safety and participant monitoring information, will follow.

The second section will give a brief history of MRI and the principles of brain imaging, including information on image generation and pulse sequences. The principles of the specific sequences used in the current research will be discussed, and the experimental parameters used will be defined.

Participants were recruited via email, the Centre for Human Psychopharmacology (CHP) research subject database, flyers posted throughout Swinburne University and word of mouth. Participant enrolment occurred between 2014 and 2016 at Swinburne University Hawthorn campus. Participants were aged either 20-45 years old or 55-75 years old. Volunteers’ identities were kept confidential via the use of participant numbers. One experimenter (SC) was responsible for the collection, storage, management and analysis of all participant data.

The rationale for examining differences between older and younger adults was that the researchers were interested in investigating group differences in healthy aging. Previous research has demonstrated that declines in some areas of cognitive
processing can occur as early as the second and third decades of life (Deary et al., 2009; Salthouse, 2009). It is a future aim of the researchers to use the data collected throughout this thesis in larger analysis of impaired ageing, such as in mild cognitive impairment or dementia.

5.1 Cognitive screening

5.1.1 Modified Telephone Interview for Cognitive Status (TICS-m)
The TICS-m is a brief 13-item test of cognitive functioning that is administered over the telephone. The items covered related to questions of orientation, repetition, naming and calculations, with scores ranging from 0 to 50. The modified version of the TICS also includes a 10-item non-semantically related word list that is recalled both immediately and after an approximately five-minute delay filled with distractor questions. The TICS-m has been demonstrated as being as reliable and valid as face-to-face administration, with a sensitivity of 94% and specificity of 100% for distinguishing normal healthy controls from individuals diagnosed with dementia (de Jager et al., 2003). This measure is found to be highly correlated with the more often used measure of global cognition, the mini-mental state exam (MMSE) (de Jager et al., 2003, Folstein et al., 1975).

5.1.2 Memory Assessment Clinics Questionnaire (MAC-Q)
The MAC-Q (Crook et al., 1992) has been designed to quantify subjective memory complaints associated with aging. The MAC-Q consists of six questions which ask the participant to compare their current everyday memory to that of earlier life. Each question is scored on a 5-point Likert scale from “much better now” to “much worse
now." The possible score range is from 7-35. In the current study a cut-off score of >25 is used as evidence of subjective memory impairment. This questionnaire has been used widely in studies of age-associated memory impairment (AAMI) (Koivisto et al., 1995) and healthy aging (Minett et al., 2005).

5.1.3 Wechsler Memory Scale – Revised (WMS-R)

The Verbal Paired Associates Test from the WMS-R assesses verbal immediate explicit memory. In this task three different sets of eight-word pairs are presented to the participant. Each set of words contains four pairs of related and four pairs of unrelated words that are to be memorized. The experimenter then presents eight individual words back to the participant in a different order to which they were presented. For each word presented, the participant is required to reply with the corresponding paired word. Each correct response receives a score of 1, resulting in a total possible score range of 0 to 24 for the three sets of eight word pairs (Wechsler, 1987). This measure is one of the most widely used tests for explicit episodic memory function (Rabin et al., 2005) and has been validated for use with amnestic mild cognitive impairment (Pike et al., 2013) and neuropsychological examinations (Wechsler, 2009).

5.2 Cognitive assessment
5.2.1 Swinburne University Computerized Cognitive Aging Battery (SUCCAB)

The SUCCAB is a battery of screen-based computerized cognitive tasks designed to measure cognitive processes. Participants were given instructions on how to perform each task and complete a practice task prior to performing the main task used for analysis. Tasks were designed to test aspects of spatial and object memory, executive processes, attention and processing speed using tasks similar to those used in previous studies examining the effects of aging on cognition (Pipingas et al., 2010, Scholey et al., 2009). The tasks that were used are described below in the order they will be presented. The SUCCAB took approximately 30 minutes to complete. This measure has been used to investigate cognitive performance in studies of cognitive aging (Simpson et al., 2012), nutraceutical supplementation (Macpherson et al., 2012), and cerebrovascular function (Pase et al., 2014). The SUCCAB displayed a linear change in both accuracy and response time from the age of 20 in a previous validation of the test battery (Pipingas et al., 2010).

*Simple Reaction Time*

This test required participants to respond by pressing a button when a white square appeared on screen with varying inter-stimulus intervals.

*Two-Choice Reaction Time*
Participants responded to either a blue triangle or red square by pressing a corresponding colored button. Stimuli were presented with a varying inter-stimulus interval.

**Stroop Color-Word**

The test consisted of a congruent and an incongruent trial. Stimulus words were randomly presented (RED, BLUE, GREEN, YELLOW) in either congruent or incongruent colors. Participants responded by pressing one of four buttons corresponding to the color of the word, irrespective of what the word reads. This task was used as a measure of executive function and, more specifically inhibition. Participants are required to inhibit the automatic reading response.

**Spatial Working Memory**

Participants were presented with a 4 x 4 white grid on a black background, with six grid positions containing white squares. After three seconds the grid became blank and a series of four white squares are sequentially displayed in various grid positions. Participants responded with a yes/no response to indicate whether each square matched a position that was originally filled. In total, participants completed 14 trials which were separated by a blank screen for a time duration of 2 seconds. Each trial was set such that two out of the four locations in the response series matched the original grid locations, and two did not. The task required participants to hold spatial information in a store that has previously been described as working memory.
Contextual Memory

Participants initially viewed a series of everyday images that were presented at the top/bottom/left/right of the computer screen. On completion of the series, the same images were displayed again in randomized order in the center of the screen. Participants were required to recollect the location where each image was initially presented and thus recall the context of the presented information. Participants responded with a top/bottom/left/right button press to indicate the original location of each image.

Immediate/Delayed Recognition

Participants were asked to study a series of 40 abstract images presented serially in the center of the screen. On completion, another series of images were presented, half of which were from the studied series and half were new (Immediate condition). Participants indicated with a right (yes) or left (no) button press whether or not they recognised the image from the studied series. This task is repeated at the end of the testing session with the remaining 20 images from the studied series and another 20 new images (delayed condition). Because abstract patterns are difficult to verbalize, the task can be described as a measure of non-verbal recognition memory.

5.2.1.1 Method of transforming cognitive tasks into composite measures

Memory and attention composite scores were calculated from the SUCCAB. Response times for immediate and delayed recognition, spatial working and contextual memory tasks were averaged to give the memory composite score.
Simple and choice reaction time, and the two Stroop task response times were averaged to give the attention composite score. The variables are weighted such that greater values indicate slower processing times. The method of composition has been described previously (Macpherson et al., 2012).

5.3 Peripheral cardiovascular measures

5.3.1 Blood pressure

Blood pressure was measured using a self-inflating sphygmomanometer cuff and monitor. Three recordings were taken, and the average blood pressure was calculated. Blood pressure measurements were taken before and after CO₂ gas inhalation familiarization. This was acted as a screening safety measure, if any participants presented with a blood pressure reading of over 140mmHg/ 90mmHg they were considered to be unfit for the trial.

5.4 CO₂ inhalation procedure

A carbon dioxide-enriched gas mixture was used to increase blood flow in the brain. A qualified nurse employed by the Center for Human Psychopharmacology was present in the first instance of each participant inhaling the gas. During this familiarization process, the participant lay supine on a bed for the duration of the gas delivery in order to replicate the conditions in the MRI scanner. The gas, 5% carbon
dioxide (CO₂) mixed with room air (approximately 21% oxygen and 74% nitrogen), was delivered to the participant via a snorkel-like mouthpiece. The participant wore a nose clip to ensure mouth-only breathing, and a pulse oximeter was used for observation of blood oxygen saturation. The procedure utilized an on-off with the following alternation between room air and CO₂-enriched gas: 1 min air/ 2 min CO₂/ 2 min air, and this was performed twice. The study design was reproduced from a similar study completed previously (Chen and Parrish, 2009).

Blood pressure (BP) was taken before and after the gas familiarization. If participant blood pressure was elevated (above 140/90), the study was to be discontinued, although this did not occur with any participants during testing. This cut-off was implemented to primarily exclude those who were hypertensive, as researchers wished to reduce the heterogeneity of the sample. Hypertension may affect cerebrovascular function (Iadecola, Park and Capone, 2009). This cut-off point was also employed as a safety precaution as it is common for blood pressure to rise following CO₂ inhalation (Kety and Schmidt, 1948a). BP was taken supine in all instances as the purpose was to be as similar to the MRI condition as possible, thus considerations for BP postural change were not necessary. The nurse recorded oxygen saturation and heart rate throughout the gas inhalation to ensure that the levels remained steady. If oxygen saturation dropped to below 95%, the participant was instructed to remove the mouth piece and nose clip and breathe deeply to bring the level back up to normal (~98-99%). Participants were informed that if any discomfort was experienced at any time, they could remove the mouth piece and nose clip, and that they were free to discontinue their participation at any time.
In the MRI scanner participants repeated the gas inhalation procedure exactly as in the familiarization. The scanning bed was removed from the scanner briefly to set up the breathing apparatus. Volunteers were informed that if at any time they were experiencing any kind of distress or having trouble breathing they should remove the mouthpiece immediately. The mouthpiece was placed in participants’ mouth and a pulse oximeter attached to the index finger. Once signals were stable the bed was re-entered into the magnet bore and a brief localizer scan was run. A researcher was in the room throughout the hypercapnia scans to change the manual valve on the bag, as instructed by a visual signal from the MR operator, and to monitor the vital signs of the participant. As in the familiarization procedure, the subjects’ vital signs were monitored and recorded, and the study was to be halted if blood oxygen saturation levels dropped to below 95%, although this did not occur with any participants during testing.

The alternation between room air and CO2-enriched gas was achieved through a manual valve located at the top of a non-diffusible bag (See Figure 1 below). End-tidal CO₂ (etCO₂) values were monitored and recorded continuously at a rate of once every 5 seconds during the hypercapnia scans at the end of the snorkel tubing on the exhaust side of a one-way valve.

5.3.1 Safety precautions taken for CO₂ delivery

A 50L Douglas bag containing pure oxygen was on hand during each gas-inhalation procedure in the case of participant distress or reduced arterial oxygen saturation (SpO₂). Constant monitoring of vital signs and pre-test screening for healthy
individuals were additional precautions taken to ensure the safety and comfort of all participants.

External safety measures included hygiene and sanitation of the breathing apparatus, including the use of single-use disposable respiratory filters and nose-clips. The re-usable mouth-piece and tubing was thoroughly sanitized and disinfected using Whitley Medical Viraclean disinfectant following each participant use.

5.3.2 Participant monitoring

Throughout the gas familiarization and MRI hypercapnia scans vital signs of heart rate and oxygen saturation were monitored while the gas mixture was being delivered. A portable infrared finger-clip pulse oximeter was used for the gas familiarization.

MEDRAD Veris Patient Monitor (Bayer HealthCare AG, Leverkusen, Germany) continuously measured heart rate in beats per minute, arterial oxygen saturation (SpO₂) and expired CO₂ (etCO₂) levels, at a rate of once every 5 seconds during the MRI hypercapnia scans. This device was connected wirelessly to a slave monitor in the MRI control room, which was in turn connected to a laptop computer. Software installed on the laptop enabled real-time recording of the vital signs data throughout the experiment. Heart rate and SpO₂ were recorded from a finger-tip pulse oximeter, and etCO₂ was measured from a water line attached to the side valve of the disposable filter.
Figure 1. Breathing apparatus, a) 100L Douglas bag b) 3-way valve c) 2-way non-rebreathing valve d) nose-clip e) filter (gas-sampling tube attaches here, not shown) f) mouth-piece
5.5 MR Imaging

Since its inception in the 1970s, magnetic resonance imaging (MRI) has proven to be a versatile biological imaging technology. MRI uses a combination of a strong static magnetic field, magnetic field gradients, and radio frequency pulses to create detailed images of the human body with excellent tissue contrast. In the neuroimaging context, this allows for the distinction between grey matter, white matter and cerebrospinal fluid. Since no ionizing radiation is involved, there are no known risks to individuals absent from normal MRI contraindications (Dill, 2008).

The development of MRI revolutionized the medical world. Refinement of MRI techniques has enabled its implementation in both medical procedures and research alike. To establish a basis of knowledge for the specific MRI methods used in this thesis the following chapter will outline the history and basic physics of MRI, then go into more detail concerning the MRI sequences employed in the current thesis, namely blood-oxygen-level dependent (BOLD) imaging, arterial spin-labeling (ASL), phase contrast (PC) and T2-relaxation-under-spin-tagging (TRUST).

5.5.1 History of MRI

MRI is built on the principles of nuclear magnetic resonance (NMR). Professor Isidor Rabi from Columbia University first observed NMR phenomena in 1937, when he discovered that atomic nuclei show their presence by absorbing or emitting radio waves when exposed to a sufficiently strong external magnetic field (Rabi, 1937). The term NMR was largely dropped from usage around the late 1970’s, mostly
because of negative connotations regarding the word ‘nuclear’ (Goldberg, 2007). Since then, the use of MRI has thrived, particularly in many biomedical, chemical and engineering applications.

At the time of Rabi’s work, it was well known that atomic nuclei (positively charged due to the presence of protons) behave like gyroscopes, spinning about their axes in random directions, thus generating their own individual magnetic fields. Exposure to an external magnetic field causes the normally random orientations of the spins to all become aligned in distinct orientations. Rabi found that the atomic nuclei absorbed energy when radio waves were applied to nuclei, causing them to flip and change directions, then emit that energy as they returned back to their lower energy equilibrium state. This absorption and re-emission of energy is a phenomenon known as Nuclear Magnetic Resonance. At a given static magnetic field strength, nuclei will absorb energy at a specific radio frequency, permitting identification of different atomic nuclei in nuclear magnetic resonance (NMR) spectroscopy.

In 1946, Felix Bloch (1946) and Edward Purcell (1946) showed that by manipulating and analyzing the movement of the spinning nuclei they could identify the presence of nuclei and thus the structure of molecules in solids and liquids; they received the Nobel Prize in Physics in 1952 for this work. Since then, NMR has become a powerful tool for chemical composition analysis. Another milestone was in 1973 when Paul Lauterbur (1973) and Peter Mansfield (1973) used the principles of NMR to describe a method for manipulating hydrogen nuclei (protons). This technique reduced the time MRI image acquisition from hours to seconds. Following this, the first full-body MRI scanner was built created by Prof. Raymond Damadian in 1977
and took nearly 5 hours to produce the first ever full body scan of a human (Damadian et al., 1976).

5.6 Principles of MRI

5.6.1 Magnetic fields and magnetization

Biological tissues such as the brain’s grey matter and white matter are largely comprised of water molecules, which are comprised of hydrogen and oxygen atoms. Protons at the center of each atom behave like tiny magnets and are thus sensitive to magnetic fields. At rest, hydrogen protons rotate about their own north-south polar axis, but that axis also spins like a top or ‘precesses’ about an external magnetic field in a randomly aligned manner, i.e., out of ‘phase’ (see Figure 2). Upon entering the magnetic field in the MRI scanner (i.e., $B_0$) the protons align with and precess about $B_0$. 
To bring about this process, a tissue of interest is placed inside a strong magnetic field, in the direction of this main stable magnetic field ($B_0$). After a period of time, the magnetic moments of the precessing protons will reach equilibrium in the direction of $B_0$, while a small percentage will align “anti-parallel” to $B_0$, which is a higher energy state (the higher $B_0$ is, the more protons will be aligned in the “parallel” orientation). Since the protons are more likely to be aligned with the lower energy state ($B_0$), the sum of their magnetization fields produces a net magnetization ($M$) that points in that direction (Hanson & Groth, 2009). See Figure 3 below.
Figure 3 (A) Protons at rest have a positive net charge and precess (spin) with angular momentum around an axis. The movement of the charges generates a magnetic field. M represents the magnitude of the magnetic field. (B) At rest, the M's of each proton are oriented in random directions and have no net magnetization. (C) When placed in a stable magnetic field (B₀), protons will become oriented either “parallel” or “antiparallel” to B₀. The “parallel” spin state signifies a lower energy state, and for this reason a greater number of protons will spin parallel to B₀ than those that spin “antiparallel”. The extra protons in the “parallel” spin state creates a net magnetization in the z-direction, which is denoted as the symbol M.
5.6.2 Excitation and the Larmor frequency

The MR signals that are measured to produce MR images have their genesis in the manipulation of $M$, which proceeds via turning on and off of both secondary magnetic fields that vary in each of the x, y and z directions (field gradients), and radio frequency (RF) pulses. RF pulses are switched on causing the protons to ‘flip’ their net magnetization and back to equilibrium (Z direction) when it is switched off again. This process comes about at a precise frequency, known as the Larmor frequency, further explained below.

A useful way to the manipulation of $M$ is to use vectors on a rotating frame of reference with three axes, X, Y and Z. The Z-axis is always the direction of $B_0$, while X and Y axes point away at right angles from Z forming the ‘transverse plane’ (Figure 4).

*Figure 4* Net magnetization in the $B_0$ direction When the net magnetization of the protons is in the same direction as the Z-axis, this is referred to as longitudinal magnetization, or $M_Z$. 
Magnetic resonance is a phenomenon whereby quantum energy (photons) is transferred between atomic nuclei (in the case of MRI, a hydrogen proton) that are precessing (spinning) at the same natural frequency. The nuclei can receive and exchange energy at that precession frequency, termed the resonant or Larmor frequency ($f$, named after Joseph Larmor), which is determined by the gyromagnetic ratio ($\gamma$) of the nucleus (for example, the hydrogen protons will resonate at the specific frequency of $\gamma = 42.58 \text{ MHz/T}$) and the external magnetic field:

$$f = \frac{\gamma}{2\pi} B_0$$

The Larmor frequency is proportional to the main magnetic field strength, i.e., if $B_0$ doubles, the then Larmor frequency doubles. When energy is transmitted into the system at the Larmor frequency, protons spiral away from the direction of $B_0$. If the direction of the radio frequency pulse is perpendicular to $B_0$, the protons - and thus M - will tip out of the z-direction (‘flip’) toward the transverse (X-Y) plane; this is known as ‘excitation’. The angle it moves through (the ‘flip-angle’) depends on the amount of energy transferred which is determined by the length of time the RF pulse is applied. After some time, the net magnetization vector will have rotated through 90 degrees and will lie completely in the transverse plane. When the RF pulse is turned off, the protons will emit energy (‘relax’) and return to the original low energy state (equilibrium) aligned with $B_0$. The energy is emitted at the Larmor frequency, and it is this signal that is measured by a read-out coil. This ‘relaxation’ is a process that is separated into two independent relaxation processes: T1 and T2 relaxation.
Figure 5 Conceptual diagram of T1 relaxation. Net magnetization has been tipped into the transverse plane (X-Y) via the absorption of energy by some of the protons following a RF pulse. Once the excitation pulse has ceased, the protons will release energy (‘relax’), causing the net magnetization to recover along the longitudinal axis, a process which occurs within a few seconds (thin red line). It is assumed that the transverse component has decayed to zero at time zero (left hand side of the diagram).

5.6.3 T1 relaxation

T1-relaxation or recovery is the process whereby the protons that have absorbed energy from the radio frequency pulse emit that energy as they return to equilibrium, in line with the Z-axis ($B_0$). The energy is emitted in the form of a very small amount of heat, and radio frequency waves. It is also known as spin-lattice relaxation, meaning that the energy is emitted back into the lattice (i.e., the surrounding tissue).

T1 relaxation, or T1 recovery as it is also known, is defined as the time taken for the longitudinal relaxation ($M_z$) to recover 63% of the original M, meaning that it is a
measure of how quickly the net magnetization returns to B₀. T₁ times differ between tissue types, as not all molecules are bound in molecules in the same fashion. Protons that are bound very tightly (in fat tissue, the white matter of the brain for example) emit their energy faster than protons bound loosely, such as those in water molecules. This difference in relaxation rates between tissue types is the reason behind the high contrast resolution of MR images. In T₁-weighted images the contrast between light and dark is the measure of the relative difference in the T₁ property of the tissues, wherein fluid such as cerebrospinal fluid (CSF)-filled compartments appear to be black, grey matter shows up as dark grey and white matter is a lighter grey. Table 1 shows the approximate T₁ relaxation values of different tissue types.

5.6.4 T₂ relaxation

T₂-weighted images measure a different property of the tissues called T₂. This contrast is very useful for differentiating tissue from fluids in the brain. With this type of weighting, stationary fluid such as CSF is bright white, blood vessels are black, grey matter is light and white matter appears darker. Table 1 shows the approximate T₁ and T₂ relaxation values of different tissue types.
Table 1. Approximate T1 and T2 relaxation times for differing tissue types. Values from (Stanisz et al., 2005)

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>T1 (ms)</th>
<th>T2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>292</td>
<td>80</td>
</tr>
<tr>
<td>CSF</td>
<td>3120</td>
<td>160</td>
</tr>
<tr>
<td>Gray matter</td>
<td>1470 ± 50</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>White matter</td>
<td>1110 ± 45</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1420 ± 38</td>
<td>32 ±2</td>
</tr>
<tr>
<td>Blood</td>
<td>~1550</td>
<td>275 ± 50</td>
</tr>
</tbody>
</table>

T2 relaxation or decay, also referred to as transverse or spin-spin relaxation, is the process by which the spinning protons progressively 'dephase' following the RF pulse. The two relaxation processes of T1 and T2 simultaneous occur but happen independently of one another. T2 is a much faster process than T1; it is measured in tens of milliseconds, whereas T1 can take seconds to complete.
T2 relaxation is essentially the process of the protons going from a situation whereby they are all in-phase with one another, to being almost completely out-of-phase with one another. When a 90-degree RF pulse excites protons, their orientation will flip from a Z-axis aligned orientation into the X-Y plane, but at the same time the pulse causes the protons to begin rotating exactly in-phase with one another. Almost immediately after the pulse is switched off the spins begin to lose coherence, as some protons will spin somewhat faster than others, this is known as dephasing.
This type of relaxation occurs in the transverse X-Y plane ($M_{XY}$), rather than the Z-plane, as is the case with T1 relaxation. The result of the dephasing effect is that the $M_{XY}$ component of the net magnetization vector decreases exponentially, which is a function of the T2 time constant. Shortly after the RF pulse is switched off the dephasing occurs more slowly, but quickly speeds up until the spins are completely out of phase (Figure 7). The rate of this process is a function of the tissue type, as in T1 relaxation, with fat tissues dephasing much faster than water.

*Figure 7* Conceptual overview of T2 relaxation. Once the net magnetization has been tipped into the transverse plane, it will decay rapidly following the cessation of the RF pulse. This is because of a loss of coherence of the spins, known as dephasing. The decay of the net magnetization back to near zero usually occurs within milliseconds for most types of tissue (thin red line).

As can be seen from Figure 7, immediately following the RF excitation pulse, all the protons are ‘flipped’ into the X-Y plane, so that the net magnetization is now at $M_{XY}$. 
All the spins are in-phase at time=0, but quickly begin to de-phase, i.e., lose coherence with one another. The T2 relaxation time is the time taken for the signal to return to 37% of the original value.

5.6.5 T2*

Following the RF pulse, there is an immediate exponential loss of signal strength in the M_{XY} plane. This depends on both the spin-spin interactions (T2 decay), and the effect of the non-uniformity of the static magnetic fields within each voxel. The construction of MRI machines is imperfect, as are the magnetic susceptibility effects of the object (person) being scanned, and this results in differences in the local magnetic field inside the scanner bore. Spatial inhomogeneities in the magnetic environment cause protons to experience different magnetic field strengths, resulting in them precessing at different rates (Huettel et al., 2004).

Thus, T2 is the time constant of decay in signal in the transverse component of net magnetization resulting from accumulated phase differences from spin-spin interactions, and T2* is the time constant of decay of both T2 and the effect of the local magnetic field inhomogeneities. T2* is always shorter than T2 and is an important component in BOLD fMRI (discussed in Section 5.8.1).
5.4.6 Image Construction

If a loop of wire (a receiver coil) is placed at a right angle to the transverse plane, the protons will induce a voltage in the wire during their precession as they relax. This voltage, which will make up the MR signal, is known as Free Induction Decay (FID). See Figure 8 for a diagram. FID is the MR signal that would be observed in the absence of an external magnetic field, i.e., the response of the net magnetization to an RF pulse over time. It is proportional to the amount of transverse magnetization \( (M_{xy}) \) generated by the pulse. FID is at its maximum when a 90° excitation pulse is used (Brown and Semelka, 2011), and it decays over time as the protons emit the energy that was absorbed via the RF pulse as they lose coherence with each other. The receiver coil then transmits the signal to a computer where it is reconstructed to produce the images.
5.7 Generating MR images- Spatial Encoding

5.7.1 Slice selection

Slice selection occurs by excitation of the spins through that particular plane, or slice, through the object being imaged using an RF pulse along with a gradient known as the slice selection gradient (Gss). Gradients allow for the separation of different
spatial locations by frequency. Three spatial encoding gradient coils are used to create distinct (although weak) magnetic fields inside the scanner, either in the X, Y or Z plane, which are overlaid onto $B_0$. Using the coils in combination with one another can produce a linear gradient in any desired direction in space. The amplitude of the gradient and certain characteristics of the RF pulse determines the thickness and position of the slice (Clare, 1997).

When a gradient coil is switched on, it generates an additional magnetic field in the direction specified by the gradient. Switching on the Z-gradient (denoted as Gz), results in a stronger $B_0$ field at one end of the scanner relative to the center (the z-direction is always in the direction of $B_0$). Since the Larmor frequency is directly proportional to the main magnetic field strength ($B_0$), a stronger $B_0$ results in a higher Larmor frequency. Due to the slope of the gradient field, $B_0$ will have a different strength field at various points, so the protons at one end of the scanner will precess at a slightly different rate than those in different locations. The use of a frequency-selective RF pulse in conjunction with the GSS results in the excitation of only those spins with the exact same Larmor frequency as the pulse. Due to the different rates of proton precession at different spatial locations within the scanner, only protons within a thin slice of the tissue will be excited. This enables the identification of the position of the slice in the Z-direction but does not allow for distinction between protons that are within that slice. Figure 9 shows a diagrammatic explanation of slice selection.
Proton in main magnetic field without RF pulse: all protons precessing with the same frequency, but different phase

Protons in main magnetic field with RF pulse, but without gradient: all protons are affected by RF pulse and precess in resonance with the same frequency and phase

Protons in main magnetic field with the RF pulse and slice selection gradient: protons along the Z axis are subjected to varying magnetic field, so precess with varying frequency. When the RF pulse is applied, only protons in the selected slice that were precessing at the same frequency as the applied RF pulse are affected by the pulse, and these will precess in resonance

Figure 9 Diagrammatic representation of slice selection. Figure adapted from Pinky (2015)
5.7.2 Phase encoding in the y-direction

The next step in image generation is phase encoding, which enables the identification of the row of voxels in the y-direction, within a particular slice. Phase encoding is performed by switching on the y-direction gradient coil (known as Gy) after the selected slice has been excited by the frequency-selective RF pulse. This produces an additional magnetic field in the y-direction (anterior-posterior), causing the protons located more anteriorly to precess slightly faster than those located more posteriorly. When this phase encoding gradient (denoted $G_{PE}$) is turned off there is a phase difference between protons in a single slice, whereby the protons are precessing at the same rate, but each has a different phase, thus creating ‘rows’ of protons, represented in the Figure 10 below.

*Figure 10 Phase encoding and frequency encoding*
5.7.3 Frequency encoding in the x-direction

The final step in spatial encoding is the application of a frequency encoding gradient ($G_{FE}$) when the signal is received by the receiver coil, in the Gx (left-right) direction. This alters the Larmor frequencies in this direction during the time the Gx gradient is on. The protons located on the left side of the slice will precess at a different rate to those on the right, and also adding to the phase shift they had already acquired from application of the $G_{FE}$ previously. It thus creates proton columns, which all have an identical Larmor frequency.

The production of these ‘rows’ and ‘columns’ of protons within the selected slice gives rise to the small cubic volumes known as ‘volume elements’ or ‘voxels’. The number of protons present within each voxel establishes the signal strength picked up by the receiver coil.

*Figure 11 Diagram of voxel rows and columns along the axis in three-dimensional space*
5.5.4 k-space

Very briefly, k-Space refers to a matrix of voxels that contain the raw data. The x- and y- axes (usually- although these are sometimes switched) represent frequency and phase information, respectively. The center of k-space contains data representing the gross form and tissue contrasts, while the outside edges contain information about the spatial resolution (definition of edges and fine structures). The complete sample of k-space is usually necessary to build an image, thus collecting the MR signal is sometimes referred to as ‘filling k-space’. Reconstruction of the raw data from k-space is very computationally complex and requires a Fourier transform, which converts the MR signal into a power spectrum. The fundamentals behind Fourier transforms are beyond the scope of this chapter, but many references on the subject are available (Pinsky, 2002, Bracewell, 1965, Twieg, 1983).

5.7 Pulse sequences

Pulse sequences are programmed sets of changing magnetic gradients and RF pulses that are applied for specific MR image acquisitions. Each pulse sequence will have a number of parameters. Two important parameters that govern the time of the collection of MR images are the repetition time (TR), and the echo time (TE). TR is the time interval (generally expressed in seconds) between the beginning of one 90° RF excitation pulse and the next. TE (usually in milliseconds) is the time interval between the mid-point of an excitation pulse and the mid-point of the data acquisition. This is displayed in Figure 12 below.
Figure 12. Graphical diagram of a pulse sequence. The horizontal axis represents time. The topmost line represents the radio-frequency (RF) pulse, first a 90° pulse is sent by the transmitting coil. The second line represents the slice-selection gradient ($G_{SS}$). The third line indicates the phase selection gradient ($G_{PE}$). Phase selection is performed by switching on the y-direction gradient coil (known as $G_y$) after the selected slice has been excited by the RF pulse. $G_{FE}$ refers the frequency encoding gradient, when the signal is received by the receiver coil, in the $G_x$ (left-right) direction. TR is the repetition time, the time interval between one 90° RF pulse and the next. This bottom line shows the signal emitting from the selected slice, which has a maximum echo signal at a time $TE$ after the initial 90° RF pulse.
Pulse sequences are used to generate images of a wide range of different tissues. MR contrast mechanisms are sensitive to different properties of the objects being imaged. Static contrasts are employed to produce images of brain anatomy and structure, and are particularly sensitive to the type, amount, relaxation and resonance properties of protons. Motion contrasts enable imaging of atomic nuclei as they move through space, and these techniques can be either structural or functional in nature. Motion contrasts are typically sensitive to the dynamic properties of protons, enabling imaging of processes such as blood flow or the diffusion of water (Huettel et al., 2004).

It is important to delineate between derivation of the contrast, which will be either endogenous (the contrast is derived from the properties of the biological tissue), or exogenous (the contrast stems from an external foreign substance that is injected into the body). Improvements in the speed of imaging and the development of exogenous contrast agents initiated the use of MRI for functional brain studies. The first functional MRI (fMRI) experiments were performed in the early 1990’s using a contrast agent, when a visual stimulus was presented to a subject while imaging the visual cortex. Soon after the first experiment using blood as an endogenous contrast agent was performed, rendering it possible to non-invasively image brain function (Clarke and Dewhurst, 1972). The techniques employed in the current research use an endogenous contrast mechanism, which is the magnetic property of the hemoglobin in the blood within the brain. Hemoglobin has different magnetization depending on whether it is oxygenated or not, and these differences alter the MR signal.
The current thesis utilized specific motion-weighted contrasts to investigate vascular function, the details of which are further discussed in the following section. Imaging dynamic indices such as these requires rapid acquisition of data, and specific pulse sequences enable this to occur. Further reading on any of the above information can be found at (Huettel et al., 2004).

5.8 Techniques utilized in the current research

5.8.1 BOLD fMRI

Functional magnetic resonance imaging (fMRI) is a non-invasive neuroimaging technique that is used by neuroscientists to study brain activity. In normal, healthy individuals, changes in neural activity result in alterations in regional cerebral blood flow. It is assumed that neural activity is coupled with alterations in the concentrations of oxygenated hemoglobin in the cerebral arteries. fMRI signal modeling makes use of these variations of the blood paramagnetic properties. As the MRI scanner can detect changes in magnetic resonance, the fMRI signal is considered to be a measure of change in blood oxygenation and thus an indirect measure of neural activity (this is why the fMRI signal is also called blood oxygen level dependent or BOLD response) (Arthurs and Boniface, 2002).

BOLD measures changes in deoxyhemoglobin concentration, which is the result of complex interactions between many physiological parameters, including cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral metabolic rate of oxygen use (CMRO₂) (Ances et al., 2009). Hemoglobin has dual magnetic properties
dependent on whether it is bound to oxygen or not. Oxyhemoglobin is diamagnetic and deoxyhemoglobin is paramagnetic. Increases in CBF that are greater than increases in CMRO$_2$ will lead to a reduction in the deoxyhemoglobin concentration, and subsequently an increase in the brightness on T2* weighted images (Ogawa et al., 1990). BOLD images can be acquired simultaneously with arterial spin labeling (ASL) scans. BOLD images used in the current research were separated from perfusion time-series acquired with ASL, using the surround subtraction and averaging method (Wong et al., 1997) as per Chen and Parrish (2009). This procedure is further discussed in Section 5.8.2.2.

5.8.2 Arterial Spin Labeling MRI

As previously mentioned in Section 3.2.2.3 MRI can be used to characterize hemodynamic changes in the brain. The current research project employed a technique known as arterial spin labeling (ASL). This method is a magnetic resonance imaging pulse sequence that is used for measuring tissue perfusion by flowing blood (Williams et al., 1992, Detre et al., 2012, Alsop and Detre, 1998). Cerebral perfusion is quantified by measuring the amount of blood delivered to the microvascular system and capillary beds in a given tissue per unit of time and is a key indicator of brain health. This technique enables non-invasive imaging and absolute quantification of cerebral blood flow (CBF) in the brain without the use of a contrast agent. CBF is defined as the blood supply to the brain at any given time, and is generally equal to 50ml of blood per 100gm of brain tissue per minute continuously in healthy young adults (Cipolla, 2009).
Compared with other perfusion techniques such as positron emission tomography (PET) (Meltzer et al., 2000, Pantano et al., 1984), single-photon emission computed tomography (SPECT) (Kogure et al., 2000), and computed tomography (CT) (Koenig et al., 1998, Meyer et al., 1994, Mayer et al., 2000), ASL offers several advantages including that it is available for routine clinical practice in many institutions.

MRI often uses contrast agents such as gadolinium to measure CBF. ASL MRI eradicates the need for an external agent, allowing for a completely non-invasive measurement of whole-brain cerebral perfusion which has the ability to quantitatively measure tissue perfusion, qualities that make ASL ideal for research and clinical studies.

The general principle behind ASL is to distinguish the net magnetization of arterial water flowing to the brain from the net magnetization of the background of brain water in the parenchyma (Detre et al., 2012). The goal of ASL MRI perfusion is to produce a ‘flow labeled image’ and a ‘control image’ in which the static tissue signals are equal, but the magnetic quality of the blood flowing through the arteries of interest is different. The water molecules in the arterial blood are magnetically tagged prior to entering the tissue under investigation. A radio frequency pulse magnetically inverts the water protons in flowing blood supplying the imaged region. This method uses the magnetically-labeled arterial blood water as an endogenous tracer, thereby eliminating the need for an invasive contrast agent to be administered.

In comparison with standard blood-oxygen-level-dependent (BOLD) contrast-based MRI, ASL affords a quantitative and highly repeatable measure of cerebral blood
flow (Liu et al., 2007). Also, by employing the use of specific saturation pulses, pulsed ASL sequences can allow for simultaneous acquisition of perfusion and BOLD data that can be easily separated (Wong et al., 1997), as discussed below. The perfusion signal is usually determined by pair-wise comparisons with separate images that are acquired with control labeling (Wang et al., 2008). High spatial and temporal resolutions are some of the benefits of this technique.

Figure 13 Adapted from (Golay et al., 2004). ASL sequences invert the magnetization of arterial water molecules (white circles indicate molecules with inverted magnetization) from upstream in the arterial tree (white arrow shows direction of blood flow). The water molecules diffuse through the blood brain barrier at the capillaries. There, the spins are
exchanged with the tissue magnetization (grey circles), which reduces the signal intensity. The amount of intensity attenuated is a direct measure of perfusion. Remaining inverse magnetized molecules flow out via the venous system (grey arrow).

5.8.2.1 Principles of ASL

In short, ASL uses magnetically labeled arterial water as an endogenous tracer. The basic methodology behind ASL is analogous to that of SPECT and PET scanning. While there are four different types of ASL, -pulsed (PASL), continuous (CASL), pseudo-continuous (PCASL) and velocity-selective (VS-ASL)- these techniques all have the same goal, which is to construct a labeled image and a control image that have identical static tissue signals; following subtraction, the only difference is the signal depicting the blood that entered the image voxel (Buxton et al., 1998).

Cerebral blood flow is measured by calculating the difference between the control signal, wherein the magnetization of the arterial blood is fully relaxed, and the tagged signal, in which the longitudinal magnetization is inverted or saturated (Liu et al., 2007).

Adiabatic inversion pulses are used to invert the magnetization of arterial blood water that supplies the regions of interest. Water molecules in the blood carrying the labeled magnetization flow to the specific brain imaging voxels, where they cross the capillary walls to join the larger pool of water in the brain parenchyma. This process is then repeated without labeling the arterial blood, and the labeled image is then subtracted from the unlabeled (or ‘control’) image. This difference signal is an absolute reflection of the quantitative local perfusion (Buxton et al., 1998).
magnetic tracer that has been applied to the arterial blood water decays with longitudinal relaxation rate $T_1$.

Spatially selective ASL is usually divided into the two most commonly employed methods, PASL and CASL, depending on how the blood water is labeled. PASL has a lower signal to noise ratio than CASL, in addition to higher physiological noise, but is easier to apply. PASL sequences use a short RF pulse to invert all the water magnetization prior to it reaching the region of interest in the brain. This is in contrast to CASL where arterial blood is continuously magnetically inverted in a narrow, well-defined labeling plane (Silva et al., 2006). CASL is the original approach proposed by Williams et al (1992) who labeled blood water using a flow-driven adiabatic inversion. This labeling technique continuously inverts the magnetization of water molecules that have a certain velocity and saturates the stationary tissue. A long (typically greater than 1 second) radio frequency pulse length is used to invert the arterial blood continuously. In contrast, PASL flips the magnetization of the blood water molecules in a large region by applying a short inversion pulse (~10msec). The radio frequency pulse is applied in a way that is spatially selective, i.e., it is slice-specific.

Compared to CASL, PASL generally has higher labeling efficiency. While the labeled blood is flowing in to the region of interest, the magnetization decays with $T_1$, resulting in less signal when longer inflow times are used (Aslan et al., 2010). The inversion delay time (TI) in PASL, the time taken between tagging the blood and the acquisition of the image, allows the tagged blood to flow into the capillaries so that the signal of the cerebral perfusion can be acquired.
There are two main categories of PASL. These are distinguished on the basis of whether the magnetic labeling is applied symmetrically or asymmetrically with respect to the imaging volume. The research detailed in the current thesis utilized an asymmetric technique known as PICORE. PICORE stands for ‘proximal inversion with control for off-resonance effects’. Wong and colleagues (1997) introduced the QUIPSS method to overcome the potential confounding variable arterial transit time,
which is the time that takes for the labeled blood to move from the labeling slab and the measured area. These techniques apply additional saturation pulses to both the control and label imaging planes after the labeling RF pulse. The QUIPSS (quantitative imaging of perfusion using a single subtraction) method features two subtypes, with QUIPSS II being the most commonly employed sub-type. It is mostly associated with transit-delay desensitizing saturation, and used to control the bolus width. Given a specified bolus width, the choice of the appropriate inversion time is what creates the insensitivity to transit delays. A recent improvement of the QUIPSS II scheme is the Q2TIPS, with signifies QUIPSS II with thin-slice TI1 periodic saturation.

The current research used the PICORE/Q2TIPS tagging scheme. This tagging sequence has been used previously to investigate hypercapnia in multiple sclerosis (Ge et al., 2012), diabetes, AD, Parkinson’s disease, WMH and aging (Lu et al., 2011). Figure 14 above shows a diagram of the pulse sequence used in PICORE/Q2TIPS imaging and illustrates the regions of magnetization inversion (tagging) slab, the thin-slice TI1 periodic saturation, in-plane presaturation slab and the imaging slice(s).

5.8.2.2 ASL experiment

The experimental parameters for the ASL sequence are as follows: A sagittal localizer scan was used to identify the A-C P-C commissure. This scan was used to position 14 slices with in-plane resolution of 3.44 x 3.44 x 5mm³, 2.5mm apart. These slices were acquired on a transverse-to-coronal oblique plane in order to
image the visual and motor cortices simultaneously (Figure 15). Hypercapnia scans were acquired using PASL with a PICORE/Q2TIPS tagging sequence, in accordance with similar research on vasoreactivity (Wong et al., 1997, Chen and Parrish, 2009, Luh et al., 1998). Gradient-echo echoplanar (EPI) readout, TI1=700ms, TI2=1500ms, tag-size = 20cm, TR=3000ms, TE= 13ms, flip-angle=90°. The data were separated into CBF and BOLD time-series using the surround subtraction and averaging method (Wong et al., 1997) as per Chen and Parrish (2009). Briefly, this procedure involves separate subtraction methods for CBF and BOLD time series. The CBF data is extracted by subtracting from each image the average of the previous and the next image, while the BOLD signal is separated by adding to each image the average of the preceding and the next image, in the following manner:

Raw: T1, C1, T2, C2, T3, C3…etc.
BOLD: ((T1+T2)/2+C1)/2, ((C1+C2)/2+T2)/2…etc.
CBF: (C1-(T1+T2)/2), (C1+C2)/2-T2…etc.

T1= tagged image #1, C1= control image #1

This method gives two distinct time series, including flow signal data that is insensitive to linear trends in the global signal, and BOLD signal data that is insensitive to flow data trends (Wong et al., 1997), although there may be some residual weighting. The BOLD signal may still be subject to linear data trends (Liu & Wong, 2005). The pre-saturation pulse offers some insensitivity of the BOLD signal to perfusion weighting, however there may still be some residual weighting. The article by Liu and Wong provides more details of these phenomena.
5.8.2.3 CVR estimation and signal shifting

In the research described in Chapter 6, ‘Regional cerebrovascular reactivity and cognitive performance in healthy aging’ hypercapnia was induced in participants in each ASL scan, to assess cerebrovascular reactivity (CVR). End-tidal CO₂ (etCO₂) was sampled continuously from a patient monitor attached to the breathing tube (See Section 5.4 for the CO₂ inhalation procedure and apparatus).

In order to calculate CVR, a measure of change in CBF (or BOLD signal intensity) throughout the hypercapnic period is divided by the change in etCO₂. Due to differences in individual hemodynamic delay, it is necessary to shift the BOLD signal in time against the recorded etCO₂ signal, which accounts for the time that it takes
for the blood to travel from the pulmonary vascular system to the heart and then to the capillaries of the brain (Yezhuvath et al., 2009).

The shifting of etCO$_2$ vs. BOLD signal can be performed in multiple ways. The estimation of CVR on a regional basis was achieved by first averaging the BOLD signal time-courses from the two hypercapnia scans for each region of interest (ROI) to compute the percentage change in BOLD relative to baseline for each individual. In order to consider each individual’s hemodynamic delay, the BOLD signal for each participant’s hypercapnia period was extracted from the point of maximum signal change, averaged across all ROIs, and the 30 seconds preceding. This equated to slightly different sampled time-points for each group (younger hypercapnia period was calculated as 66-96 seconds after switching to the CO$_2$-mixture; older was 72-102 seconds), while the baseline BOLD signal was extracted from the final 30 seconds of the room air inhalation period at the beginning of the scan. This time-shift was applied to each ROI time-course so as to enable sampling of the maximum CVR change for each group across all ROIs.

This averaging and shifting technique is similar to the method used previously in the literature where the maximal cross-correlation coefficient between etCO$_2$ and BOLD signal is calculated to define the time-lag (Yezhuvath et al., 2009); however, it should be noted that other analyses are available and are potentially equally as valid. Future studies could make use of several methods of BOLD CVR calculation and assess the reliability and comparability between different techniques to ascertain the most appropriate.
5.8.2.4 ROI analysis versus whole brain CVR mapping

The present research was aimed toward investigating vascular reactivity differences in specific regions of the brain, and whether CVR in various regions has differential effects on cognitive performance. While the calculation of CVR on a regional basis allows for comparisons between specific brain areas, there are other analyses available that enable voxel-wise mapping, or whole brain analysis of CVR. Studies have shown that cerebral vasodilatory capacity differs from region to region (Lu et al., 2011, Yezhuvath et al., 2009, Lu et al., 2009). The analysis of regional differences in CVR is useful because it allows us to assess the associations of functional areas to the performance of specific tasks, as demonstrated in the findings shown in the following Chapter 6. These findings support the role of regional vascular integrity to certain tasks over others, evidenced by the observations that reactivity in the temporal lobes and hippocampus (areas known for their role in memory function) contributed to performance of memory but not attention tasks.

While performance of a whole-brain CVR mapping analysis may have provided a more easily visualized image of the degree of reactivity in different brain areas, we feel that this method is more suited to clinical examinations. The main objective of the CVR research was to investigate the contribution of region-specific CVR to cognitive function, an aim that would not be possible via mapping of whole-brain CVR.
5.7.2.5 BOLD versus CBF CVR calculation

The study simultaneously acquired BOLD and perfusion data from the arterial spin labeling (ASL) MRI scans. This represents a limitation, as unfortunately, due to insurmountable artefact in the CBF data, these images were unable to be processed, and thus were lost and unable to be analyzed. After surround-subtraction was used to separate the ASL data into distinct time-series representing CBF and BOLD signals, seemingly random and indiscriminate noise impeded the CBF time-series such that no effect of CO₂-enriched gas mixture inhalation could be observed on any given scan. The CO₂ effect was clearly visible on the BOLD data for the majority of subject scans. Several post-acquisition technical approaches were used in an attempt to solve the issue, yet none were successful enough to elicit an outcome that was scientifically reliable enough to allow the data to be used. This issue rendered comparison of BOLD signal-acquired CVR values to CBF signal-acquired CVR values impossible, which was unfortunate, as initially this comparison was a major objective of the research project. To overcome this issue in the future, some recommendations are suggested.

It is apparent that the use of a real-time physiological monitoring system, such as those in-built into Siemen’s MRI scanners, which continuously samples participants’ vital signs, including respiratory effort, blood pressure and cardiac pulsatility, would be of great benefit in filtering the unwanted noise inherent in ASL acquisition. While the present study did employ a patient monitor which recorded expired CO₂ (etCO₂), pulse oximetry, and heart and respiration rates, the device sampled at a rate of every five seconds, which was not sensitive enough to use with retrospective physiological
motion correction software. The addition of chest bands to measure respiratory effort and setting the monitor to sample at a much higher frequency, in the range of tens to hundreds of hertz if possible, would enable motion correction of the fluctuations from respiration and cardiac cycle. It is apparent that the use of the in-built Siemen’s physiological monitoring system would have overcome this, as this would have provided the data necessary to perform the noise correction. This equipment was not used in the current experiments, which was an oversight by the researchers. Future studies in this area should employ a real-time monitor of vital signs to ensure that physiological noise correction can be performed sufficiently. See articles by Restom et al. (2006) and Behzadi et al. (2007) for information on physiological noise correction for arterial spin labeling.

5.8.3 Phase contrast

The following sections will overview the principles behind phase contrast MRI, detail the imaging parameters used in the current research, and describe the method used to calculate grey matter cerebral metabolic rate of oxygen use (gmCMRO$_2$). Phase contrast MRI is an angiographic imaging technique used to image blood flow velocity and vessel anatomy. Phase contrast (PC) can also be used to measure flow in specific arteries, for example the internal carotid and vertebral arteries, which account for most of the cerebral inflow, to use for cross-validation with other MRI blood flow measures (e.g. ASL) as a measure of global CBF (Chen and Pike, 2010). This sequence was used in the current research to measure global CBF, from which the values of grey matter CBF (gmCBF) and gmCMRO$_2$ were calculated. Results are presented in Chapter 7.
5.8.3.1 Phase contrast principles

The PC technique is one of the most widely used angiographic methods in MRI. This type of technique manipulates the phase of net magnetization to produce contrast between flowing blood and static tissue. The phase of the magnetization of spins in the static tissues is zero and the phase of the magnetization from the flowing blood spins in non-zero (Korosec, 1999). Processing the data produces different types of images including phase difference, complex difference and magnitude images (Korosec, 1999). Bipolar gradients are used to modulate the phase of flowing spins and, under the assumption of minimal velocity variation across a voxel and negligible outflow effect, can provide an estimation of flow velocity (Aslan et al., 2010).

5.8.3.2 Phase contrast experiment

In the current study phase contrast MRI was used to provide quantitative whole-brain CBF measurements by quantifying blood flow in each of the 4 feeding arteries of the brain. Separate scans are conducted for each of the arteries (left and right internal carotids, left and right vertebral arteries) feeding into the brain to provide an estimate of the total brain-blood volume.

The parameters for the PC scans were as follows: single slice, peripherally gated, TR = 41.3ms, TE = 4ms, FA = 25°, voxel size 0.5 x 0.5 x 5mm³, FOV 160 x 160 x 5mm³, maximum velocity encoding = 100cm/s. Based on the maximum intensity projection reconstruction of the angiogram, each of the four feeding arteries of the
brain – left internal carotid artery (left ICA), right internal carotid artery (right ICA), left vertebral artery (left VA) and right vertebral artery (right VA) – were scanned separately. Scans were oriented perpendicular to the arteries and positioned so that the center of the FOV was placed at the center of each target vessel.

Additionally, a high-resolution T1-weighted image was acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) image (axial, TR= 2300, TE= 2.52ms, 176 slices, voxel size 1 x 1 x 1mm³, field of view (FOV) 256mm) to provide an estimation of the intracranial volume for calculation of blood flow per unit mass of grey matter tissue, referred to in this thesis as grey matter cerebral blood flow (gmCBF). This accounts for variance in volume and atrophy of grey matter across participants. The calculation of gmCBF is given in more detail in Chapter 7. Briefly, we calculated CBF in the grey and white matter based on the assumption that white matter flow is equal to ~40% that of grey matter based on findings of Leenders et al. (1990). The following equations were used to estimate gmCBF from tCBF values:

\[
t_{CBFv} = \frac{t_{CBF}}{(V_{gm}+V_{wm}) \cdot \rho} \quad [1]
\]

\[
t_{CBFv} = gm_{CBFv} + w_{CBFv} \quad [2]
\]

\[
w_{CBFv} = 0.4 \cdot gm_{CBFv} \quad [3]
\]

\[
t_{CBFv} = gm_{CBFv} + 0.4 \cdot gm_{CBFv} = 1.4 \cdot gm_{CBFv} \quad [4]
\]

\[
gm_{CBFv} = \frac{t_{CBFv}}{1.4} = \frac{t_{CBF}}{(1.4 \cdot (V_{gm}+V_{wm}) \cdot \rho)} \quad [5]
\]

Vgm and Vwm are the volumes of grey and white matter respectively in ml. \( \rho \) is the mass density of tissue used as the scaling factor to convert tissue volumes to mass,
assumed as 1.06g/ml (Herscovitch and Raichle, 1985). tCBFv is the volume-corrected total CBF, normalized to the volumes of grey and white matter, in ml/100g brain tissue/min. gmCBFv and wmCBFv are the volume-corrected blood flows for grey and white matter respectively. A 30-second baseline of arterial oxygen saturation was acquired with a pulse oximeter and averaged for each participant to give the value of \( Y_a \). tCBF was measured using phase-contrast MRI and \( Y_v \) was estimated using T2-relaxation-under-spin-tagging (TRUST) (Lu and Ge, 2008). Total cerebral blood flow (tCBF) is the total amount of blood in ml per minute. The T1-weighted images were also segmented into grey (Vgm) and white (Vwm) matter volumes using an in-house Matlab script (The Mathworks. INC. Natrick, MA).

\[ \text{Internal Carotid Arteries} \]

\[ \text{Vertebral Arteries} \]

*Figure 16* Illustrating the location of phase contrast scans for the left and right ICA and VA.
5.8.3.3 CMRO₂ estimation

Cerebral metabolic rate of oxygen use in the grey matter only (gmCMRO₂) was calculated from measures of cerebral blood flow, venous oxygenation and segmented brain volumes (to incorporate the confounding factor of age-related brain atrophy) then adjusted for age and gender differences in hematocrit concentration, using the method described in Chapter 7. This study is the first to undertake gmCMRO₂ estimation in this specific manner. While it is comparable to that used in previous research (Peng et al., 2014), and we used these authors’ method of calculation as our guide, we expanded upon this by assuming a steady ratio of .4 between grey and white matter blood flow, which we believe leads to a more robust result.

The estimate of the rate of cerebral metabolism of oxygen use (CMRO₂) employed in Chapter 7 was calculated using a modified version of the equation:

\[ \text{CMRO}_2 = \text{CBF} \times (Y_a - Y_v) \times C_a \]  [6]

In this equation, CBF denotes cerebral blood flow in ml/100g grey matter/min, Y_a and Y_v are the percentage of oxygen saturation of the arterial and the venous blood respectively. C_a is the blood’s oxygen carrying capacity, generally established in the literature as 833.7 µmol of O2/100ml of blood for a hematocrit level of 0.44 (Guyton and Hall, 2011). This equation has been validated for the estimation of whole-brain CMRO₂ from measures of CBF, Y_v and brain volume (Liu et al., 2012a).
We believe that the modification of this equation to estimate \( \text{CMRO}_2 \) of the cortical grey matter only is both accurate and appropriate for this study, particularly given that wide-ranging ages of the participants. Grey matter atrophy must be taken into account in studies of brain aging, particularly given that research has shown that oxygen consumption decreases more significantly in the grey matter compared to white across the lifespan (Marchal et al., 1992).

### 5.8.4 T2-Relaxation-Under-Spin-Tagging (TRUST)

The following section will provide the background on the T2-Relaxation-Under-Spin-Tagging (TRUST) technique, and then describe the imaging parameters utilized in the current research.

A recently developed MRI technique termed TRUST was used to estimate the oxygen content of venous blood by quantifying the T2 value. The results can be seen in Chapter 7. Venous oxygenation \( \left( Y_v \right) \) is most often reported as a percentage and denotes the amount of oxygenated hemoglobin in the venous blood. Values typically range between 50-75% in healthy adults (Kety and Schmidt, 1948b). While oxygen content of the venous blood is commonly obtained by direct blood sampling, TRUST allows this estimation to be performed rapidly and non-invasively. TRUST employs the same principles as pulsed arterial spin labeling (PASL) MRI to isolate the blood signal from the surrounding tissues and was developed by Lu and Ge (2008) in order to quantitatively estimate the oxygen saturation by way of the measurement of pure blood T2 in the superior sagittal sinus (SSS).
The superior sagittal sinus is the largest dural venous sinus and receives blood from
the cortical veins over the cerebral hemispheres. It extends sagittally from the
anterior portion of the falx cerebri to the torcular herophili at the occipital
protuberance, where it connects to the straight and occipital sinuses. From this
confluence of sinuses, the blood then drains into the left and right transverse
sinuses. Generally, the SSS will drain into one transverse sinus, most often the right,
with the straight sinus draining into the other.

Intravascular T2-based measurements of venous blood oxygenation such as TRUST
are performed with a calibration plot and offer a straightforward method of
quantification of $Y_v$. One challenge that researchers face when measuring the
oxygenation of blood is isolating the pure blood signal from that of surrounding
tissues. The development of the TRUST technique addressed this challenge by
applying a spin-tagging principle similar to that of ASL MRI on the venous side of the
circulation (i.e., the labeling slab is above the imaging slice) so that the venous blood
inside the labeling slab flows down to enter the slice that is imaged (See Figure 17).
The spin-labeling principle separates flowing blood signals from static tissue to
obtain a pure blood signal by subtraction of control and label images. Venous
oxygenation is estimated by comparing the experimentally determined venous blood
T2 to a calibration plot specifying the relationship between T2 and $Y_v$ (Lu and Ge,
2008, Xu et al., 2009, Devor et al.).

The TRUST method is increasingly being used in studies that require whole-brain
measurement of $Y_v$ due to its simplicity and ability to minimise partial volume effects
(Krishnamurthy et al., 2014, Xu et al., 2011, Xu et al., 2015, Yablonskiy et al., 2013).
Figure 17 Diagram of TRUST labeling and acquisition. The location of the labeling slab region (orange block) of the TRUST scans upstream from the imaged slice. Red line indicates the plane of acquisition (imaging slice), localized to the A-C P-C commissure, T2 blood signal was acquired from the superior sagittal sinus (SSS)

5.8.4.1 TRUST principles

The TRUST technique uses a pulse sequence consisting of interspersed acquisitions of tag and control scans. Each image type is acquired with four different effective echo times (eTEs) which range from 1-160ms. In each scan, a pre-saturation radio frequency (RF) pulse is used to suppress the static tissue signal. This is followed by a labeling or control RF pulse which magnetically labels the incoming blood. After a short waiting period (1.2s) the blood will have flowed into the imaging slice. A non-
selective T2-preparation pulse train is applied prior to data acquisition to achieve T2-weighting. The duration of these is denoted eTE. T2-preparation rather than the conventional spin-echo sequence is used to minimize the blood outflow effect in T2-measurement. T2-preparation pulses accentuate the T2 contrast and exploit the difference between arterial and venous blood T2 times (Lu and Ge, 2008). Arterial blood has a T2 time of 250ms, compared to venous blood which has a T2 of only 35ms.

In this technique, a 90° pulse is first applied, which tips all protons into the transverse plane. If nothing else was done here, the differences in the decay would reflect T2* relaxation. Instead, T2 preparation uses a series of 180° refocusing pulses, so the decay is based on T2 relaxation. A -90° pulse tips the magnetization vectors back to the longitudinal plane, concluding the preparation. The outcome of this preparation is that the arterial blood protons have significantly higher magnetization than venous protons (Lu and Ge, 2008). The complete dataset for TRUST MRI includes labeled and control images that are acquired at different T2 weightings (Lu and Ge, 2008). Pair-wise subtractions, performed in the same way as in ASL data processing, allows for the subtracted image to contain only the venous blood signal (Lu and Ge, 2008).

5.8.4.2 TRUST experiment

TRUST was performed with the following parameters: single-shot echo-planar imaging (EPI), axial plane, repetition time (TR) = 4000ms, echo time (TE) = 18ms, inversion time (TI) = 1400ms, tagging slab thickness= 50mm, gap between imaging
slab and tagging slab = 25mm, FOV 180 x 180 x 5mm$^3$, voxel size 1.4 x 1.4 x 5mm$^3$.

Four different T2 weightings with effective echo times (eTE) of 0ms, 40ms, 80ms and 160ms, corresponding to 0, 4, 8, and 16 refocusing pulses in the T2 preparation (Carr-Purcell-Meiboom-Gill (CPMG) = 10ms), 1 average, scan duration = 2.20 min:s.

To improve the signal to noise ratio (SNR), four pairs of tag and control images were acquired each eTE. The slice was positioned to be parallel to the anterior-commissure posterior-commissure (AC-PC) line with a distance of approximately 1cm above the confluence of the sinuses, however in some cases this was tilted slightly to section the SSS at an angle closer to 90° in order to acquire more distinct flow images. Slice positioning was based on a middle sagittal survey image (Figure 18). The tagging slab was selected based on areas chosen in previous literature (Xu et al., 2009), so that all blood upstream of the imaging location was tagged. It was found that partial volume effects were reduced with higher in-plane resolution than that employed previously by Xu et al (2009).
Figure 18 Quantification of the venous oxygenation using T2- relaxation-under- spin- tagging (TRUST). Raw axial images of the control and labeled scans, SSS region is shown in the orange box. The label inverts the venous blood signal so it appears darker in the image.

5.9 Chapter summary

This chapter provided information on the methods used in the current research. The descriptions and utilization of several cognitive screening and assessment measures (TICS-m, WMS-r, MAC-Q and SUCCAB), and cardiovascular testing measures (blood pressure sphygmomanometer) were reported. The following section gave an overview of the history of magnetic resonance imaging (MRI), including the basic principles and applications, physics including the processes of excitation and relaxation, and image construction. Generation of MR images was explained, including slice selection, phase encoding, frequency encoding and k-space. This was followed by an overview of pulse sequences, which led to the description of the MRI sequences used in the current research. These were BOLD imaging, ASL with
Q2TIPS tagging scheme, phase contrast and TRUST. The principles and the experimental parameters of each sequence were outlined.

The following chapters present the experiments conducted as part of this thesis. Chapter 6 is an empirical study using PASL to investigate cerebrovascular reactivity (CVR). Subsequently, Chapter 7 presents a publication using phase contrast and TRUST sequences to measure CMRO$_2$, CBF and $Y_v$. These research articles aimed to examine age-related differences in CVR, CMRO$_2$, CBF and cognitive performance in healthy adults, and to investigate the associations between regional CVR, CMRO$_2$, CBF and cognitive performance across the lifespan.
Chapter 6

Regional cerebrovascular reactivity and cognitive performance in healthy aging

6.1 Objectives

This paper is a cross-sectional study aimed at investigating the differences in regional cerebrovascular reactivity (CVR) in healthy adults aged between 21-44, and 55-75 years of age. Further, the associations between CVR in specific regions of the brain and performance on tasks of attention and memory are considered.

6.2 Rationale

As outlined in the systematic review presented in Chapter 4 of this thesis, there is a lack of conclusive evidence regarding the association between MRI-measured cerebrovascular reactivity and impairments of cognitive function. This is even more apparent for adults who are cognitively intact. While non-MRI methods of measuring global CVR (e.g. transcranial Doppler ultrasound, CT and SPECT scans) have indicated a probable link with Alzheimer’s disease (AD) (Silvestrini et al., 2006, Lee et al., 2007) and vascular dementia (Vicenzini et al., 2007), as well as in mild cognitive impairment (MCI) (Viticchi et al., 2012), these tools are relatively spatially insensitive, and cannot easily distinguish regional reactivity variation.

Thus, it is of interest to examine the regional and age-related differences in vascular reactivity and uncover whether there are links between vascular responsiveness and
cognition in a healthy aging population. Identification of a connection between CVR and cognition could present an opportunity for early detection of those at risk for vascular or cognitive impairments. It was hypothesized that CVR would be reduced in the older group, and that this decline would vary between brain regions. A positive association between CVR and cognition was also expected, such that greater reactivity would correlate with better performance. This paper was accepted for publication in the Journal for Experimental Neuroscience on the 3rd June 2018.

6.3 Publication
**Introduction**

Maintaining good cognitive health is of critical importance in a time where there are increasing numbers of older individuals. Aging is associated with alterations in the structure and function of the brain and the vascular system that supports it. There is growing evidence that age-related declines in cardiovascular and neurocognitive function are intrinsically linked.\(^1\)\(^-\)\(^4\) Arterial health has emerged as a significant predictor of cognitive performance, and both arterial health and cognitive performance have been shown to decline with increasing age, an indication that there is an age-dependent association between the two.\(^5\) Cerebral blood flow (CBF) decreases with advancing age,\(^6\) and this reduction is associated with cognitive decline.\(^7\) Peripheral arterial stiffness has been shown to predict cognitive performance in a group of healthy middle-aged adults.\(^8\) The connection between brain and vascular health is undoubtedly significant; however, the intrinsic nature of this connection is unclear.

Blood-oxygen-level dependent contrast imaging (BOLD) functional magnetic resonance imaging (fMRI) studies of cognitive aging have provided an abundance of information about the brain. Normal physiological aging in the absence of pathology is linked with various alterations in the vasculature of the brain, both functionally and structurally.\(^9\) Endothelium-dependent cerebrovascular reactivity (CVR) is a main regulatory mechanism that controls CBR.\(^10\)\(^-\)\(^11\) CVR refers to a vasodilatory or vasoconstrictor response of the blood vessels to a stimulus, and this measure provides a direct assessment of vascular brain health.\(^12\) Impaired CVR indicates microvascular hemodynamic dysfunction, which is implicated in many brain disorders, including stroke\(^13\) and Alzheimer’s disease (AD),\(^14\)\(^-\)\(^16\) as well as mild cognitive impairment.\(^17\)

The CVR index reflects the response of cerebral blood vessels to extrinsic stimulation by vasodilators such as acetazolamide\(^18\),\(^19\) or hypercapnic challenge.\(^20\),\(^21\) Hypercapnia can be induced through inhalation of CO\(_2\)-enriched gas, which is a harmless way to investigate the effects of vascular functioning on the blood flow parameters in the brain.\(^22\),\(^23\) Vasodilation in response to CO\(_2\) enhances CBR through diameter enlargement in the small arterioles, making it possible to evaluate the function of small intra-cerebral vasomotor vessels.\(^28\)

**Age differences in CVR**

Increasing evidence shows that the cerebral vessel contractility and dilation becomes diminished in later life, consequently impacting neurovascular coupling.\(^29\) Multiple biochemical elements have been suggested as responsible for this loss of function, including...
Cognition and CVR

Given the predictive relationship of vascular health and cognitive performance, CVR has been examined for its relationship to cognition. Changes to the integrity of the cerebrovascular system can bring about cognitive change due to dysfunctional neurovascular coupling. Vascular disorders have been shown to contribute to neurodegenerative and cognitive illnesses. Associations between impaired CVR and severity of dementia have been established. CVR assessed by the breath-holding index (BHI) was found to be the best predictor of cognitive performance in AD over and above gender, age, education, and vascular risk factors, and pathological BHI is also predictive of conversion from MCI to AD. One study in cognitively healthy patients with peripheral artery disease reported that CVR was linked to executive function, memory, global cognition, and attention scores. While the vessel reactivity–cognition link has often been investigated in cardiovascular and neurodegenerative disorders, particularly AD, the association between cerebrovascular function and cognition in healthy aging is yet to be clarified. If impaired vascular reactivity is found to be implicated in cognitive performance, vaso-protective therapies may be a target for prevention of age-related cognitive decline.

The goal of the present study was to further this research by using MRI to measure the BOLD signal during CO₂ inhalation in healthy, normally aging adults to assess differences in CVR across the lifespan. A cognitive assessment was performed to ascertain the relationships between cognition and cerebrovascular function in healthy aging. Second, it was of interest to investigate whether reactivity was the same across different brain regions, that is, was the age-related change in CVR heterogeneous throughout the brain, or are some regions more affected than others. Additionally, the relationships of regional CVR to cognitive performance on measures of memory and attention were examined to explore whether these cognitive domains are differentially affected by CVR in distinct brain regions.

Based on previous research, it was expected that CVR values would vary across regions and CVR would decline with age. Also expected was a positive association between CVR and cognition, such that greater reactivity would be correlated with better performance. 

Results

Characteristics of sample are shown in Table 1 below. The younger group was significantly more educated, with lower body mass index (BMI) and larger grey matter volumes than the older group. Reaction times on the memory and attention tasks were significantly faster in the younger group.

CVR is reduced in some regions with age

When using age as a continuous variable, CVR in the temporal lobe was found to significantly decrease with age ($r = -0.43$, $P = .002$). An independent t-test revealed that CVR in the
temporal lobes was higher in the younger compared to the older group, $F(1, 50) = 2.58, \ P = .006$. No other regions-of-interest (ROIs) showed significant change with age in the sample as a whole.

After separating the cohort into age groups, it was observed that CVR was significantly reduced in the cingulum ($r = -0.54, \ P = .008$), grey matter ($r = -0.54, \ P = .006$) and temporal ($r = -0.52, \ P = .006$) areas in the older group. The grey matter and temporal areas still showed a marginally significant reduction after correction for multiple comparisons. A graphical representation of the percentage change in BOLD signal in the temporal lobes plotted with the change in etCO$_2$ is shown in Figure 1, separated by age group. CVR did not change significantly with age in any of the ROI in the younger group. Figure 2 shows the mean regional CVR values for each group. There were no significant differences between genders in CVR in any region studied across the lifespan, nor when separated into age groups.

**Temporal lobe CVR predicts cognition across the lifespan**

In the sample as a whole, correlations between cognitive variables and CVR were observed. CVR in the temporal lobes was associated with both memory ($r = -0.46, \ P = .001$) and attention performance ($r = -0.41, \ P = .003$). Table 2 displays results of separate unadjusted linear regressions for temporal lobe CVR and cognitive score. Greater CVR was associated with faster reaction times. Linear regression analyses showed that CVR in the temporal lobes significantly predicted memory score, and this effect was independent of age, gender and years of education. Figure 3 shows the relationship between temporal lobe CVR and memory task reaction times. A second linear regression showed that attention was not significantly predicted by temporal lobe CVR when adjusted for age, gender and education, though the model itself was significant. Table 3 shows the results of separate adjusted linear regression analyses.

**Hippocampal CVR predicts memory score in older adults**

When separated by age group, separate linear regressions showed that in the younger sample there were no significant associations between CVR in any brain region and either cognitive measure. In the older group however, CVR in the hippocampus significantly predicted memory score, $F(1, 20) = 5.83, \ P = .026$, with an $R^2$ of .24. CVR in the hippocampus was negatively associated with memory score such that worse CVR reflected longer

### Table 1. Means, standard deviations for characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>WHOLE SAMPLE</th>
<th></th>
<th>YOUNGER AGE RANGE 21-44</th>
<th></th>
<th>OLDER AGE RANGE 55-75</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AGE RANGE 21-75</td>
<td></td>
<td>(M: 47.22, SD: 18.59)</td>
<td></td>
<td>(M: 65, SD: 5.57)</td>
</tr>
<tr>
<td></td>
<td>N=59</td>
<td></td>
<td></td>
<td>N=30</td>
<td>N=29</td>
</tr>
<tr>
<td>Years of education</td>
<td>17.18 (3.69)*</td>
<td>18.37 (3.22)</td>
<td>15.81 (3.77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TICS-m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29.36 (2.40)</td>
</tr>
<tr>
<td>MAC-Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.83 (3.57)</td>
</tr>
<tr>
<td>BMI</td>
<td>23.98 (4.03)*</td>
<td>22.61 (3.21)</td>
<td>25.45 (4.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>66.98 (9.87)</td>
<td>67.46 (9.63)</td>
<td>66.50 (10.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>127.11 (16.31)</td>
<td>124.11 (16.32)</td>
<td>129.90 (16.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>75.82 (10.76)</td>
<td>73.56 (10.69)</td>
<td>77.93 (10.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gmCBF, mL/100g/min</td>
<td>45.04 (9.05)</td>
<td>45.54 (9.01)</td>
<td>44.52 (9.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMV, cm$^3$</td>
<td>703.99 (85.42)**</td>
<td>753.44 (73.77)</td>
<td>652.83 (64.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMV, cm$^3$</td>
<td>481.68 (58.46)</td>
<td>487.34 (60.66)</td>
<td>475.83 (52.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline etCO$_2$, mmHg</td>
<td>38.27 (4.55)</td>
<td>39.06 (4.18)</td>
<td>37.42 (4.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercapnia etCO$_2$, mmHg</td>
<td>46.09 (4.33)</td>
<td>46.88 (4.03)</td>
<td>45.24 (4.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$etCO$_2$, mmHg</td>
<td>7.82 (2.26)</td>
<td>7.81 (2.32)</td>
<td>7.82 (2.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory mean RT, ms</td>
<td>908.19 (113.16)**</td>
<td>839.05 (87.08)</td>
<td>982.10 (88.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention mean RT, ms</td>
<td>529.65 (84.30)**</td>
<td>472.78 (62.32)</td>
<td>586.51 (62.31)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TICS-m: modified Telephone Interview for Cognitive Status; MAC-Q: Memory Complaint Questionnaire; BMI: body mass index; gmCBF: grey matter cerebral blood flow; GMV: grey matter volume; WMV: white matter volume; etCO$_2$: end-tidal CO$_2$.

Values displayed are M (SD).

* $P < .01$, ** $P < .001$, significant differences between younger and older groups.
response times (i.e., slower processing). Attention was not associated with CVR in any brain region in either age group.

Scores on the MAC-Q were significantly correlated with hippocampal CVR ($r = -0.439, P = .046$), lower CVR was associated with greater subjective memory complaints in the older group. Additionally, there was a marginally significant correlation between hippocampal CVR and TICS-m score in the older group ($r = 0.46, P = .056$), suggesting a trend toward a relationship between global cognitive impairment and regional reactivity in elderly individuals.

**Summary of findings**

As a whole, (1) CVR declined with age in the temporal lobes across the lifespan, (2) CVR in the temporal lobes was associated with memory and attention scores, and (3) when adjusted for age, gender, and education, temporal lobe CVR predicted memory score across the entire sample.

When the sample was split into age groups, (1) there were significant declines in CVR in several regions with age in the older group, but these declines were not seen in the younger group; (2) there was a significant association between CVR in the hippocampus and memory score in the older sample, but memory was not related to CVR in any region in the younger group; (3) CVR was not associated with attention score in either group; and (4) lower CVR was associated with greater subjective memory concerns and global cognitive impairment in the older group.

**Discussion**

The present study investigated age-related differences in regional CVR to carbon dioxide in healthy younger and older adults, and the relationships between regional CVR and cognitive performance. It was expected that an age-related CVR decline would be observed between the younger and older groups, and that this decline would vary between brain regions. Also expected was a positive association between CVR and cognition, such that greater reactivity would be correlated with better performance.

Temporal lobe CVR predicted memory score independent of age, gender, and education across the entire sample. Hippocampal CVR significantly predicted memory score in the older group and was negatively associated with subjective memory complaints. Consistent with literature, both measures of cognition declined with increasing age. This is the first study to examine the differences in regional CVR between younger and older cognitively healthy individuals in relation to performance on memory and attention tasks. Findings demonstrate that reactivity is linked to cognition in both regionally and task-specific ways in older healthy adults.

**Age and regional differences in CVR**

While CVR was lower in the older group in several regions studied, these declines were not significant in all areas. The temporal lobes showed significant reductions in reactivity between the younger and older groups. Reactivity declined significantly in the cortical grey matter, the cingulum and the temporal lobes in older adults with age, but no age effects were seen in the younger adults. These findings are in line with...
previous research that reported heterogeneous declines from younger to older age.12

Mechanisms underlying the decreasing reactivity of cerebral vessels with age can be explained by well-described age-associated vascular stiffening.29 The more distensible, elastic properties of blood vessel walls are known to deteriorate, and regenerate more slowly in aged individuals, while the more rigid wall components, such as collagen and the basement membrane, may be augmented.43 This results in the vessel wall becoming stiffer with time, even in the absence of pathology. Arteriosclerosis can also be prevalent to some degree even in healthy aging, adding to the rigidity of vessels.9 A third insult that occurs with aging is the over-production or release of vasoconstrictive substances such as endothelin-1, and the diminished release of vasodilatory chemicals such as NO.30 Underproduction of vasodilators by the endothelium will result in reduced capacity for dilation or possibly even hyper-constriction, observed as impaired responsiveness. Most MRI-based reports of regional CVR decline suggest that diminishing vasomotor responses are widespread and generally will encompass most areas of the brain, and that the decline in CVR is more abundant and pervasive both spatially and in percentage than the decline in CBF.32

CVR was heterogeneous across brain regions. Reactivity was higher in the parietal, temporal, and frontal areas, and lowest in the hippocampus and cingulate. This is corroborated by previous research indicating that CVR is greater in the cortical grey matter when compared to the deeper grey matter structures.43 Studies investigating the spatial distribution of small vessel reactivity have observed widely varying values between areas, variation even between vascular territories supplied by the same major arteries has been observed.35 Variability between regions is likely due to differences in vascular structure and density, and occurs due to the spatial inhomogeneity and temporally dynamic nature of blood flow, in addition to differing neural and metabolic demands in different parts of the brain.44 ROIs assessed in the current work were intersected with grey matter masks, which we expected would reduce any atrophy-related variability between younger and older adults, thus providing a relative measurement of reactivity not confounded by age-related volume differences.

CVR in the temporal lobes was associated with both memory and attention

To our knowledge, this is the first study to assess the contribution of CVR in specific brain regions to attention and memory in cognitively healthy adults with ages ranging across the adult lifespan. It was observed that better vascular reactivity in the temporal lobes was associated with faster reaction times on both memory and attention tasks. These associations were region-, task- and age-dependent. When the effects of age, gender and education were controlled for, the relationship between temporal CVR and memory remained significant. These findings are corroborated by research aligning vascular reactivity with cognitive scores in patient groups of AD,15,51 vascular dementia,56 and mild cognitive impairment.53 Elucidating the patterns of change in reactivity that occur with cognitive change is important; one study observed that there was a 33% chance of conversion from MCI to AD within a year for those MCI patients who had pathologically low CVR.53

CVR in the hippocampus significantly predicted memory score in older adults

Additionally, we aimed to assess differences between younger and older individuals, and found that when comparing the groups, there were significantly different relationships in
region CVR effects on cognition. Vascular reactivity in the bilateral hippocampus was linked to memory performance in the older group, yet this relationship was not observed in younger adults. Previous research found that vasoreactivity in the hippocampus is reduced in cognitively impaired individuals compared to healthy controls; however, this is the first study to show a direct correlation between memory function and hippocampal CVR in cognitively healthy individuals. Much evidence has shown the association of hippocampal volume and perfusion to various memory functions, though the impact of integrity of the small vessels supplying this structure are less well understood. This novel finding highlights the contribution of vascular integrity to a distinct cognitive domain both regionally and functionally. This result is not surprising, given the evidence showing that impaired vessel function can lead to changes in perfusion, possibly culminating in damage to brain regions that are strategic for memory functions. The hippocampus is particularly vulnerable to damage from ischemia and is notably damaged by AD pathology. Dysfunctional reactivity reduces perfusion, potentially resulting in greater cognitive losses in those at risk.

Another notable finding is the significant relationship between subjective memory complaints and hippocampal CVR. Greater reactivity was associated with less memory concerns. Previous research has linked smaller hippocampal volume to various memory functions, though the impact of integrity of the small vessels supplying this structure are less well understood. This novel finding highlights the contribution of vascular integrity to a distinct cognitive domain both regionally and functionally. This result is not surprising, given the evidence showing that impaired vessel function can lead to changes in perfusion, possibly culminating in damage to brain regions that are strategic for memory functions. The hippocampus is particularly vulnerable to damage from ischemia and is notably damaged by AD pathology. Dysfunctional reactivity reduces perfusion, potentially resulting in greater cognitive losses in those at risk.

Highly responsive blood vessels are essential for optimum flow-metabolism coupling. Impaired CVR is an indication of underlying microvascular dysfunction wherein the metabolically active regions of the brain will not be supplied adequately to meet demands. This hypoperfusion can lead to chronic ischemia over time, potentially preceding neurological and cognitive impairments. Indeed, research has shown strong evidence of vascular origins in the development and progression of AD.

**Limitations**

The results of this study should be viewed in light of some limitations. Common to all neuroscientific aging research, brain atrophy can present some issues with comparability between age groups. In the present study, CVR values were calculated such that they are absolute measures; however, care was taken to ensure that all ROIs from which the data were extracted were masked with the grey matter probability map to account for individual differences in partial volume and tissue atrophy. This validated procedure has been utilized previously. Additionally, it should be noted that inhalation of CO₂-enriched gas does not provide a precise standard arterial partial pressure of CO₂ (PaCO₂) stimulus, due to variability in individual respiratory responses. While more exact methods of increasing PaCO₂ exist (eg, computer-controlled targeting of end-tidal CO₂ partial pressures), these are expensive and require specific equipment and are thus not as accessible. A recent review of MRI-based CVR studies by the authors found that inhalation of a fixed concentration of CO₂-enriched gas was the most commonly used vasoactive stimuli.

The regional CVR values were calculated from the difference in etCO₂ signal from the first 30 seconds of room air breathing to the last 30 seconds of CO₂ inhalation, while the BOLD values were calculated on the basis of age group. BOLD signal traces were shifted backward to account for the hemodynamic lag, in order to align the expired gas signal with the increase in BOLD signal. It was found that the younger group had slightly shorter hemodynamic lag than the older group (9 seconds versus 18 seconds), thus the data points sampled for the CVR calculation were different based on age group. While this shifting is valid and has been used in previous research, various other sampling methods are available. Future studies may benefit from analyzing CVR using other sampled

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**Table 3.** Showing results of adjusted linear regression for temporal lobe CVR contribution to memory and attention score for the entire sample.

<table>
<thead>
<tr>
<th></th>
<th>MEMORY</th>
<th></th>
<th>ATTENTION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>B</td>
<td>SE B</td>
</tr>
<tr>
<td>Age</td>
<td>3.14</td>
<td>0.76</td>
<td>-0.51***</td>
<td>3.02</td>
</tr>
<tr>
<td>Gender</td>
<td>-46.07</td>
<td>25.12</td>
<td>-0.20</td>
<td>-6.31</td>
</tr>
<tr>
<td>Education (years)</td>
<td>1.17</td>
<td>3.50</td>
<td>0.04</td>
<td>5.61</td>
</tr>
<tr>
<td>Temporal lobe CVR</td>
<td>-626.24</td>
<td>265.20</td>
<td>-0.29**</td>
<td>-331.54</td>
</tr>
<tr>
<td>R²</td>
<td>0.52</td>
<td></td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>11.75***</td>
<td></td>
<td>12.16***</td>
<td></td>
</tr>
</tbody>
</table>

*P < .05, **P < .01, ***P < .001.
Conclusions
In this study, we examined the differences in regional CVR between older and younger adults and investigated the associations of regional CVR to cognitive performance. The current data highlight the association between brain vascular reactivity and cognitive function; better performance was associated with greater blood vessel responsiveness, particularly in regions directly associated with memory function. The identification of cerebrovascular impairment in aging could help to distinguish those who would benefit from vascular-specific therapeutic approaches, and could potentially lead to detection of early dysfunction of the vascular system in people at risk. These findings could inform other areas of research for implications of vascular stiffening in the brain, and provide impetus for vascular-specific therapeutic targets to help maintain cognitive functions over the lifespan.

Experimental Procedure
Participants
In all, 30 younger (aged 21–44, mean age 30 years, SD: 6.22 years, 12 females) and 29 older (aged 55–75, mean age 65 years, SD: 5.57 years, 18 females) healthy (i.e., active, independently-living, asymptomatic) adults were recruited from the community. Participants underwent telephone screening and had no contraindications for MRI scanning (no pacemaker, metallic implants, or claustrophobia). Participants were of generally good health, with no self-reported history of neurological, psychiatric, cardiovascular or inflammatory diseases, and not suffering from asthma or other respiratory problems. The Modified Telephone Interview for Cognitive Status—(TICS-m) was used to screen for mild cognitive impairment and dementia for participants aged over 45 years. Participants scoring 23 or below were omitted from the final analysis (n = 3). The Memory Complaint Questionnaire (MAC-Q) was used to quantify subjective memory complaints in the older participants. After receiving an explanation of the study procedure, participants provided written informed consent. The research protocol was approved by both the Swinburne University Human Research Ethics Committee and the Alfred Hospital Human Research Ethics Committee, and was conducted in accordance with the Australian Code for the Responsible Conduct of Research. Regional CVR was measured in a total of 54 participants; some data were lost due to motion in the scanner. Table 1 lists the demographic information for the participants.

Experimental design and procedure
Data were acquired in one testing session of approximately 3 hours’ duration. Participant’s blood pressure was taken before and after an initial 5-minute gas-breathing procedure conducted outside the scanner. A registered nurse was present for this familiarization in which each subject’s SpO2 was constantly monitored. Participants then underwent magnetic resonance imaging (MRI) scanning during which they inhaled CO2 twice over a period of 10 minutes (see Figure 4). After MRI scanning, participants underwent a 30-minute computerized cognitive assessment.

MRI data acquisition
Data were collected using a 3T Siemens Tim Trio MRI scanner (Siemens, Erlangen, Germany) fitted with a 32 channel head coil at Swinburne University of Technology (Hawthorn, Australia). Participants were requested to minimize head movements, and foam padding was inserted around the head to aid this.

Data from two hypercapnia scans were sampled and averaged for analysis. Hypercapnia was induced by inhalation of gas mixture containing 5% CO2, 21% O2, and 74% N2 that was delivered from a 100 L non-diffusing Douglas bag through a mouth-piece with nose-clip to ensure mouth-only breathing. A two-way valve allowed the researcher to manually switch between CO2-enriched gas and room air for the 2 x 5-minute scans, as instructed by a visual signal from the MR operator. Gas delivery was alternated: 1 min room air, 2 min CO2, and 2 min room air. The breathing apparatus is shown in Figure 5. An MRI-safe patient monitor (Medrad Veris system, Bayer HealthCare AG, Leverkusen, Germany) continuously recorded a digital output of participants’ vital signs (heart rate, arterial oxygen saturation, respiratory rate), as well as end-tidal CO2 (etCO2).

In all, 14 axial slices with in-plane resolution of 3.44 x 3.44 mm were acquired oblique to the commissural plane (shown in Figure 6A and B). Hypercapnia scans were acquired using PASL with a PICORE/Q2TIPS...
tagging sequence, in accordance with similar research on vasoreactivity.\textsuperscript{70–72} Gradient-echo echoplanar (EPI) readout, $T1 = 700$ ms, $T2 = 1500$ ms, tag-size = 20 cm, $TR = 3000$ ms, $TE = 13$ ms, flip-angle = 90°. T1-weighted structural images were obtained using a high resolution MPRAGE 3D anatomical scan (axial, $TR = 2300$ ms, $TE = 2.52$ ms, voxel-size = 1 x 1 x 1 mm$^3$, FOV = 256 mm, 176 slices).

**Data analysis**

CVR data were processed using SPM12 (SPM12, University College of London, UK) and associated toolboxes, in addition to in-house tools, all run on MATLAB 2014b (The MathWorks). All data were motion-corrected, co-registered to individual T1-weighted images, normalized to the stereotactic space defined by the Montreal Neurologic Institute (MNI space), and then spatially smoothed with 8 mm FWHM Gaussian filter. The data were separated into CBF and BOLD time-series using the surround subtraction and averaging method\textsuperscript{70} as per Chen and Parrish.\textsuperscript{71} CVR based on BOLD were calculated. CBF-based CVR values are not reported in this paper.

Age-related changes in CVR were explored using ROI analysis. Seven ROIs were chosen based on previous reports in the literature.\textsuperscript{7,21} These regions were the bilateral superior parietal, mid-occipital, frontal, mid-temporal, insula, hippocampus and the cingulum (ROIs are shown in Figure 6C) and were applied to the data to extract BOLD time-courses. Mean whole brain grey matter was also used as an ROI. These regions were defined from the AAL package in WFU PickAtlas toolbox\textsuperscript{73} for SPM12.

Grey and white matter volumes were obtained using standard segmentation in VBM8 software from SPM12. Individual grey matter masks from the VBM analysis were smoothed to the resolution of the BOLD time-series. To account for partial volume and atrophy effects, the grey matter masks were set with a threshold of .5 (>50% probability to be grey matter) and the intersection with each ROI was used for final CVR analysis. CVR in the whole grey matter was also estimated using the thresholded grey matter mask as the ROI for each individual. This adjustment corrects for brain tissue volume differences. CBF in the grey matter at baseline was calculated from the ASL scans, values shown in Table 1.

**CVR calculation**

The two hypercapnia blocks (Figure 1) were averaged for each subject prior to computing the percentage change in BOLD relative to baseline for each ROI. Baseline etCO$_2$ data were calculated from the final 30 seconds of the room air period at the beginning of each scan, and etCO$_2$ for the hypercapnia period was calculated from the final 30 seconds of each CO$_2$ inhalation block. Due to variable hemodynamic delays, each subject’s BOLD signal for the hypercapnia period were extracted from the point of average maximum signal change across all ROIs and the 30 seconds preceding, which equated to time-points 66–96 seconds for younger and 72–102 seconds for the older group, following the beginning of the CO$_2$ inhalation period. Baseline BOLD data were calculated from the final 30 seconds of the room air period at the beginning of each scan. Data were sampled from different points in order to
obtain the maximum CVR change for each group across all ROIs. Both BOLD and etCO2 data were temporally smoothed by a 5-point moving average filter using the "smooth" function in MATLAB. CVR was calculated as the %change in BOLD/mmHg change in etCO2.

To assess differences in hemodynamic delay between the age groups, the time to 50% of the maximum value from beginning of the CO2 mixture inhalation was computed for each group and each ROI separately.

Cognitive assessment

Screening tool- Modified Telephone Interview for Cognitive Status (TICS-m). The TICS-m is a reliable and validated brief 13-item test of cognitive functioning that is administered over the telephone. The items covered related to questions of orientation, repetition, naming, calculations and immediate and delayed recall with scores ranging from 0 to 35. Participants who scored 23 or below were omitted from the final analysis (n = 3).

Memory Complaint Questionnaire (MAC-Q). The MAC-Q is designed to quantify subjective memory complaints associated with aging. It consists of six questions which ask the participant to compare their current everyday memory to that of earlier life. Possible score range is from 7 to 35. Greater scores indicate greater subjective memory concerns.

Swinburne University Computerized Cognitive Aging Battery (SUCCAB). The SUCCAB is described previously in Pipingas et al. Participants were given instructions on how to perform each task and completed a practice task prior to performing the main task used for analysis. Tasks were designed to test aspects of spatial and object memory, executive processes, attention and processing speed using tasks similar to those used in previous studies examining the effects of aging on cognition. Tasks were presented in the order of simple reaction time, choice reaction time, immediate recognition, congruent Stroop, incongruent Stroop, spatial working memory, contextual working memory, delayed recognition. The SUCCAB took approximately 30 minutes to complete. The method of composition has been described previously. Response times for the immediate and delayed recognition, spatial working memory and contextual memory tasks were averaged to give the memory composite score. Simple and choice reaction time, and the two Stroop task response times were averaged to give the attention composite score. Higher scores reflected slower response time.

Statistical analyses

IBM SPSS statistics v.23 (Chicago, IL, USA) was used to conduct analysis. Pearson’s correlations determined the association between age, CVR in various brain regions and composite cognitive scores. Linear regressions were performed to assess the contribution of CVR in different brain regions to the cognition measures separately. Cognitive score was entered as the dependent variable, age, gender, and years of education were entered as the independent variables in model one, and CVR region entered in model 2. P values of .05 or less were considered statistically significant in all analyses; however, a correction for multiple comparisons (Bonferroni’s correction) was applied to investigate CVR differences between the age groups for the ROI analysis. The level of significance was adjusted on eight ROIs (P ≤ .00625).

Acknowledgements

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Author Contributions

SJC, AP and TBP conceived and designed the experiments; SJC performed the experiments; SJC, YC, TBP, and MEH analyzed the data; AP, MEH, HM, and SJC worked on funding acquisition; AP, TBP, YC, and MEH contributed materials/analysis tools; and SJC, TBP, YC, MEH, HM, and AP wrote the paper.

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De la Torre JC. Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. *Cardiovascular Psychiatry Neurol.* 2012;2012:36716.


6.4 Summary of key findings

In the preceding publication it was demonstrated that in CVR in the temporal lobes is associated with attention and memory performance across the healthy lifespan. Assessment of the two age groups separately revealed that CVR was not related to either memory or attention in younger adults as a whole. However, in the older group, even when correcting for age, gender and education level, greater CVR in the temporal lobes was associated with faster responses on memory function tests. We also demonstrated that in cognitively healthy older adults, greater CVR in the hippocampus was related to faster response on memory tasks. Greater incidence of subjective memory concerns was also correlated with vascular reactivity in the hippocampus, and scores on a global measure of cognition was marginally significantly related to CVR in this region. This study supports research indicating that blood vessel function in specific regions of the cerebral cortex may contribute to specific cognitive changes in older adults.
Chapter 7

An investigation of cerebral oxygen utilization, blood flow and cognition in healthy aging

7.1 Objectives

The preceding chapter showed that regional cerebrovascular reactivity (CVR) contributes to cognitive function in healthy adults in an age- and task- specific manner. In this chapter this notion is expanded on by examining the role that cerebral blood flow and metabolism play in maintaining cognition in healthy older and younger adults.

This paper is a cross-sectional study in which various MRI-based techniques were used to examine the cerebrovascular physiology of cognitively healthy participants. Specifically, the aim of this paper was to investigate the differences in cerebral blood flow and metabolic parameters between younger (<50 years of age) and older (>50 year) adults in relation to cognitive performance. It was published in PLOS One. The paper has been reproduced with permission from PLOS One below.

7.2 Rationale

The energy homeostasis of the brain is maintained via multiple processes. Blood flow, metabolism and oxygenation are three measurable indices used to provide information regarding the delicate balance between supply, demand and
consumption of oxygen in the cerebral tissues. While cerebral blood flow (CBF) is commonly shown to decline over the lifespan, reports of age-related change in metabolic rate of oxygen use (CMRO$_2$) and venous oxygenation ($Y_v$) are less conclusive.

Likewise, the impact of physiological parameters on cognitive functioning are not completely understood, particularly in normal healthy aging (Ruitenberg et al., 2005, Steffener et al., 2013). Based on previous research it was expected that age-related reductions in CMRO$_2$, CBF, and performance on attention and memory tasks would be observed. The associations between CMRO$_2$, $Y_v$ and CBF with cognitive function were also investigated.

7.3 Publication
An investigation of cerebral oxygen utilization, blood flow and cognition in healthy aging

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Abstract

Background
Understanding how vascular and metabolic factors impact on cognitive function is essential to develop efficient therapies to prevent and treat cognitive losses in older age. Cerebral metabolic rate of oxygen (CMRO₂), cerebral blood flow (CBF) and venous oxygenation (Yv) comprise key physiologic processes that maintain optimum functioning of neural activity. Changes to these parameters across the lifespan may precede neurodegeneration and contribute to age-related cognitive decline. This study examined differences in blood flow and metabolism between 31 healthy younger (<50 years) and 29 healthy older (>50 years) adults; and investigated whether these parameters contribute to cognitive performance.

Method
Participants underwent a cognitive assessment and MRI scan. Grey matter CMRO₂ was calculated from measures of CBF (phase contrast MRI), arterial and venous oxygenation (TRUST MRI) to assess group differences in physiological function and the contribution of these parameters to cognition.

Results
Performance on memory (p<0.001) and attention tasks (p<0.001) and total CBF were reduced (p<0.05), and Yv trended toward a decrease (p = .06) in the older group, while grey matter CBF and CMRO₂ did not differ between the age groups. Attention was negatively associated with CBF when adjusted (p<0.05) in the older adults, but not in the younger group. There was no such relationship with memory. Neither cognitive measure was associated with oxygen metabolism or venous oxygenation in either age group.

Conclusion
Findings indicated an age-related imbalance between oxygen delivery, consumption and demand, evidenced by a decreased supply of oxygen with unchanged metabolism resulting
in increased oxygen extraction. CBF predicted attention when the age-effect was controlled, suggesting a task-specific CBF-cognition relationship.

**Introduction**

Older age is accompanied by deteriorating cognitive abilities that occurs even in the absence of neurological or neurodegenerative disease [1]. Neuropsychological studies have reported a significant decline in the speed of information processing [2], attention [3, 4], working memory and executive functions [5, 6]. Vascular dysfunction and cardiovascular disease (CVD) also become increasingly prevalent and severe with age, and have been linked to both dementia and cognitive impairment [7–10]. Current evidence suggests that CVD and CVD risk factors such as diabetes, smoking and hypertension can accelerate cognitive decline by causing hypoxia and cerebral hypoperfusion, amongst other pathophysiological processes [11–15]. Likewise, age-related cognitive decline has been attributed to compromised oxygen and nutrient delivery from the cerebral circulation [16, 17], which is highlighted by the observation that inspiration of oxygen-rich gas can significantly improve cognitive performance [18, 19].

In humans, the brain consumes roughly one-fifth of oxygen intake, while only making up about 2% of body weight. Cerebral metabolic rate of oxygen (CMRO\textsubscript{2}), cerebral blood flow (CBF) and venous oxygenation (Y\textsubscript{v}) are measurable indices that reflect brain energy homeostasis. These parameters represent oxygen demand, supply and the portion of oxygen that remains after demand has been met, respectively. Studies of age-related changes in supply and metabolism of oxygen have produced inconsistent results. CMRO\textsubscript{2} reflects the amount of oxygen used by the brain tissue over time. A number of studies have reported that CMRO\textsubscript{2} is diminished in older adults [16, 20–28], while other work suggests there is little or no change over the lifespan [29–31]. More recent studies examining aging-related changes in CMRO\textsubscript{2} reported that the rate actually increased, perhaps due to decreased tissue volume of the elderly brain [32], indeed, remaining neurons would need to expend more energy to maintain the same level of function due to a greater workload [33].

Other research suggests that CMRO\textsubscript{2} alterations across the lifespan may differ between genders, with males showing an age-related increase while females showed no significant change [32]. Similarly, there are conflicting reports of age-associated changes to the supply of blood to the brain. CBF has been found to decrease with age [24, 25, 34–39], yet others report no differences between younger and older individuals [23, 30, 40–42]. Gender differences [43], brain region [21], and tissue atrophy with age [44], can confound findings of aging effects on CBF. One study reported that cerebral venous oxygenation declined by 1.4% per decade from young adulthood [33], indicating that the oxygen supply/demand equilibrium undergoes a gradual change across the lifespan. It has been demonstrated that age-related changes in CBF and oxygen metabolism are related to performance in healthy adults in calibrated fMRI studies [28, 45]. The present work aims to build upon limited healthy aging studies by examining separate cognitive abilities of memory and attention to investigate whether CBF and CMRO\textsubscript{2} contribute to cognitive performance in a task-specific manner.

Research indicates that decreased perfusion of the brain may precede and contribute to the onset of clinical dementia [46]. The direct impact of changes in oxygen delivery, consumption and metabolism on performance of memory and attention tasks in cognitively healthy individuals remains to be clarified [47, 48]. Therefore, it is important to establish the magnitude and direction of alterations in vascular and metabolic factors that occur over the healthy lifespan,
and the contribution of these parameters to cognition. The present study aimed to investigate the relationships between CBF, CMRO₂ and cognitive performance in a cognitively intact population. To this end, we used a combination of magnetic resonance imaging and tests of neuropsychological functions to examine differences in CMRO₂, CBF and cognitive performance between younger (<50 years) and older (>50 years) healthy adults. Based on previous studies [24, 33, 48, 49], it was expected that age-related reductions in CMRO₂, CBF, as well as cognitive function would be evident. Moreover, we investigated whether CMRO₂ and CBF were related to cognitive performance on tests of memory and attention.

Results

Table 1 shows the characteristics of the sample. The two age groups did not differ significantly on measures of blood pressure, arterial oxygen saturation or heart rate. One-way ANOVAs revealed that the groups differed on BMI, tCBF, intracranial and grey matter volumes, and response times on both of the cognitive composite measures, while the differences in venous oxygenation approached significance ($p = 0.06$). Older adults had higher average BMI and response times on the cognitive tasks (i.e. slower processing), yet lower average tCBF, intracranial and grey matter volumes than younger adults.

Table 1. Means, standard deviations for characteristics of the entire sample, and each age group separately.

<table>
<thead>
<tr>
<th></th>
<th>Whole sample (n = 60)</th>
<th>Younger (n = 31) Ages 21–44 years (M29.97 SD6.17)</th>
<th>Older (n = 29) Ages 55–75 years(M65.00 SD5.56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td>21–75 years (M 46.9 SD 17.5)</td>
<td>21–44 years (M29.97 SD6.17)</td>
<td>55–75 years (M65.00 SD5.56)</td>
</tr>
<tr>
<td>Years of education</td>
<td>17.2 (3.7)</td>
<td>18.37 (3.22)</td>
<td>15.81 (3.77)</td>
</tr>
<tr>
<td>TICS-m</td>
<td></td>
<td>22.61 (3.21)</td>
<td>25.45 (4.34)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.0 (4.0)**</td>
<td>22.61 (3.21)</td>
<td>25.45 (4.34)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>67.0 (9.9)</td>
<td>67.46 (9.63)</td>
<td>66.50 (10.26)</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>127.1 (16.3)</td>
<td>124.11 (16.32)</td>
<td>129.90 (16.09)</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>75.8 (10.8)</td>
<td>73.56 (10.69)</td>
<td>77.93 (10.57)</td>
</tr>
<tr>
<td>SpO₂, %</td>
<td>98.3 (0.8)</td>
<td>98.45 (0.64)</td>
<td>98.21 (.92)</td>
</tr>
<tr>
<td>tCBF, ml/min</td>
<td>786.7 (157.1)*</td>
<td>827.33 (147.33)</td>
<td>743.18 (157.99)</td>
</tr>
<tr>
<td>gmCBF, ml/100g/min</td>
<td>50.6 (10.2)</td>
<td>51.17 (10.12)</td>
<td>50.02 (10.36)</td>
</tr>
<tr>
<td>wmCBF, ml/100g/min</td>
<td>20.25 (4.07)</td>
<td>20.47 (4.05)</td>
<td>20.01 (4.14)</td>
</tr>
<tr>
<td>gmCMRO₂ μmol/100g/min</td>
<td>148.3 (28.2)</td>
<td>145.98 (30.94)</td>
<td>151.29 (25.18)</td>
</tr>
<tr>
<td>Yv, %</td>
<td>62.7 (5.4)†</td>
<td>63.94 (5.72)</td>
<td>61.38 (4.77)</td>
</tr>
<tr>
<td>ICV, cm³</td>
<td>1184.4 (126.1)***</td>
<td>1237.04 (127.34)</td>
<td>1128.09 (98.96)</td>
</tr>
<tr>
<td>Vgm, cm³</td>
<td>703.8 (84.7)***</td>
<td>751.46 (73.36)</td>
<td>652.83 (64.40)</td>
</tr>
<tr>
<td>Vwm, cm³</td>
<td>480.9 (56.3)</td>
<td>485.58 (60.44)</td>
<td>475.83 (52.18)</td>
</tr>
<tr>
<td>Memory mean RT, msec</td>
<td>908.2 (113.2)***</td>
<td>839.05 (87.08)</td>
<td>982.10 (88.92)</td>
</tr>
<tr>
<td>Attention mean RT, msec</td>
<td>529.7 (84.3)***</td>
<td>472.78 (62.32)</td>
<td>586.51 (62.31)</td>
</tr>
</tbody>
</table>

Note: Values displayed are M (SD). BMI, body mass index; tCBF, total cerebral blood flow; gmCBF, grey matter cerebral blood flow; CMRO₂, grey matter cerebral metabolic rate of oxygen; Yv, venous oxygenation; ICV, intracranial volume; Vgm, volume of grey matter; Vwm, volume of white matter; TICS-m, modified Telephone Interview for Cognitive Status. Asterisks indicate statistically significant differences between the groups as revealed by one-way ANOVAs.

†$p = 0.06$

*p<0.05

**p<0.01

***p<0.001

https://doi.org/10.1371/journal.pone.0197055.t001
Age and gender effects on physiological measures

Univariate General Linear Models (GLMs) were performed on the data to assess the influences of age and gender on physiological parameters for each age group separately.

In the younger group the main effect of gender approached significance, while age had no association with tCBF. In older adults, gender was again approaching significance, whilst the main effect of age was marginally significant (p = .056). Parameter estimates for the older age group revealed that for each additional year of life, tCBF decreased by approximately 9.70ml/min. The model for the younger group had an $R^2$ of .12, while the model for the older group had an $R^2$ of .29. Results are shown in Table 2.

The same analysis was performed for the grey matter corrected blood flow measure (gmCBF). As revealed in Table 3, in both groups there was a significant main effect of gender, whilst age did not contribute significantly. On average, females had higher average gmCBF than males in both groups (younger females 12.4ml/100g/min higher; older females 11.1 ml/100g/min higher) when the effect of age was controlled for. The younger group model had an $R^2$ of .39 and the older group model had an $R^2$ of .42.

Univariate GLM showed that there was a significant main effect of gender on gmCMRO2 in both age groups, but age did not have a significant effect. On average females had higher gmCMRO2 than males in the same age group when the age effect was controlled (younger = 38.7μmol/100g/min higher; older = 24.1μmol/100g/min). $R^2$ for the younger model was .41, and the older model $R^2$ was .22. Table 4 shows the results.

Univariate GLMS performed for venous oxygenation showed that in the younger adult group there were no significant main effects of gender (p = .61) or age (p = .83) on $Y_v$ (Model $R^2 = .01$). The older group showed a significant main effect of age (F (1, 26) = 10.09, p = .004), but not gender (p = .68) on $Y_v$, $R^2 = .31$. In older adults, parameter estimates showed that for each additional year of life $Y_v$ decreased by .46% when gender effects were controlled.

Relationships between physiological measures and cognitive performance

Several univariate GLMs were performed to assess the contribution of physiological processes to memory and attention in each age group separately. Each model assessed the main effects of age, gender and years of education, and the physiological parameter in question, on the cognitive composite score for each age group. Ratios of tCBF: $Y_v$ and gmCBF: $Y_v$ were calculated to assess the rate of change in these parameters relative to one another. The use of this measure

Table 2. Univariate GLM for each age group, showing the age and gender main effects on tCBF.

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
<td>Age</td>
<td>.07</td>
<td>.796</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>3.74</td>
<td>.063</td>
</tr>
<tr>
<td>Older</td>
<td>Age</td>
<td>3.99</td>
<td>.056</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>3.76</td>
<td>.063</td>
</tr>
</tbody>
</table>

Table 3. Univariate GLM for each age-group, showing age and gender main effects on gmCBF.

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
<td>Age</td>
<td>.09</td>
<td>.769</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>16.76</td>
<td>.000</td>
</tr>
<tr>
<td>Older</td>
<td>Age</td>
<td>2.59</td>
<td>.120</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>12.28</td>
<td>.002</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t002

https://doi.org/10.1371/journal.pone.0197055.t003
enabled investigation of how the balance of CBF and $Y_v$ changes with age. One-way ANOVA indicated that there were no significant differences in the ratio of tCBF: $Y_v$ between groups (F $(1, 59) = 11.52, p = .097$) or in the ratio of gmCBF: $Y_v$ between groups (F $(1, 59) = .075, p = .79$) indicating that the two parameters decrease at roughly the same rate in each age group, i.e. as tCBF/ gmCBF decreases, there is a concomitant decrease in $Y_v$.

Memory, blood flow and ratios of blood flow to venous oxygenation. There were no significant effects of tCBF, age group, gender or years of education on memory score for either younger or older adults. S1 Table in the Supplementary Material shows the F, p and partial eta squared values for separate univariate GLMs for tCBF. Likewise, there were no significant effects of gmCBF, age, gender or years of education on memory score in either younger or older adults. S2 Table in Supplementary Material shows the values of statistical tests for gmCBF and memory. The ratios of tCBF and gmCBF to $Y_v$ were found to have no significant impact on memory scores for either age group.

Attention and tCBF. There were no significant effects of tCBF, age, gender or education on attention score in the younger group. In the older group however, tCBF had a highly significant main effect on attention, as did education. Parameter estimates for the older group revealed that for each additional 10ml/min of blood flow, reaction time on the attention tasks increased by approximately 2.2msecs on average when the demographic characteristics were controlled. R² for the younger group was .08, whilst the older group R² was .46. Table 5 below shows the results.

Attention and gmCBF. There were significant main effects of gmCBF and years of education on attention score in the older group, yet none of the variables contributed to attention score significantly in the younger group. Parameter estimates for the older group revealed that for each additional 10ml/100g grey matter/min of blood flow, reaction time on the attention tasks increased by approximately 34.8msecs on average when the demographic characteristics were controlled. R² values were .07 for the younger group, and .43 for the older group. Table 6 displays the results.

Attention and tCBF: $Y_v$ ratio. Univariate GLM revealed that in the older group the ratio of tCBF: $Y_v$ significantly contributed to attention score, as did years of education, see Table 6

### Table 4. Univariate GLM for age-groups separately, showing age and gender main effects on gmCMRO₂.

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age</td>
<td>.26</td>
<td>.618</td>
<td>.009</td>
</tr>
<tr>
<td>Gender</td>
<td>18.27</td>
<td>.000</td>
<td>.395</td>
</tr>
<tr>
<td>Older</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>.75</td>
<td>.393</td>
<td>.028</td>
</tr>
<tr>
<td>Gender</td>
<td>7.33</td>
<td>.012</td>
<td>.220</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t004

### Table 5. Univariate GLM showing age, gender, education and tCBF main effects on attention for younger and older adults.

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
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<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.35</td>
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<td>.055</td>
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<td>Gender</td>
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<tr>
<td>Education (years)</td>
<td>.05</td>
<td>.833</td>
<td>.002</td>
</tr>
<tr>
<td>tCBF</td>
<td>.85</td>
<td>.365</td>
<td>.036</td>
</tr>
<tr>
<td>Older</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>3.37</td>
<td>.081</td>
<td>.138</td>
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<tr>
<td>Gender</td>
<td>1.22</td>
<td>.282</td>
<td>.055</td>
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<tr>
<td>Education (years)</td>
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<td>.273</td>
</tr>
<tr>
<td>tCBF</td>
<td>8.56</td>
<td>.008</td>
<td>.289</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t005
for statistics. Parameter estimates for the older group revealed that for each additional unit of tCBF: Yv, attention score increased by approximately 15msecs on average when age, gender and education were controlled. Age and gender did not predict any significant amount of variance. None of the variables contributed to attention score significantly in the younger group. R² values were .06 for the younger group, and .45 for the older group. Table 7 displays the results.

**Attention and gmCBF: Yv ratio.** Univariate GLM revealed that in the older group the ratio of gmCBF: Yv significantly contributed to attention score, as did years of education. Age and gender did not predict any significant amount of variance. Parameter estimates for the older group revealed that for each additional unit of gmCBF: Yv, attention score increased by approximately 217msecs on average when age, gender and education were controlled. None of the variables contributed to attention score significantly in the younger group. R² values were .05 for the younger group, and .40 for the older group. Table 8 displays the results.

**Cognition, gmCMRO2 and Yv.** Neither memory nor attention scores were significantly related to gmCMRO2, age, gender or education in either the younger or the older group. It was also found that there were no significant effects of Yv, age, gender or years of education on either memory or attention score.

### Summary

This study revealed the main findings that cognitive performance was significantly reduced in older compared to younger adults; Older adults had significantly lower tCBF and grey matter volume compared to younger adults, yet there were no differences between the groups on measures of gmCBF and gmCMRO2, whilst the difference in Yv was approaching significance; In

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**Table 6. Univariate GLM showing age, gender, education and gmCBF main effects on attention for younger and older adults.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.20</td>
<td>.285</td>
<td>.049</td>
</tr>
<tr>
<td>Gender</td>
<td>.36</td>
<td>.554</td>
<td>.015</td>
</tr>
<tr>
<td>Education (years)</td>
<td>.04</td>
<td>.852</td>
<td>.002</td>
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<tr>
<td>gmCBF</td>
<td>.51</td>
<td>.482</td>
<td>.022</td>
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<tr>
<td>Older</td>
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<tr>
<td>Age</td>
<td>2.36</td>
<td>.139</td>
<td>.101</td>
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<tr>
<td>Gender</td>
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<td>.163</td>
<td>.090</td>
</tr>
<tr>
<td>Education (years)</td>
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<td>.284</td>
</tr>
<tr>
<td>gmCBF</td>
<td>6.97</td>
<td>.015</td>
<td>.249</td>
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</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t006

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**Table 7. Univariate GLM showing age, gender, education and tCBF: Yv main effects on attention for younger and older adults.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.14</td>
<td>.298</td>
<td>.047</td>
</tr>
<tr>
<td>Gender</td>
<td>.21</td>
<td>.653</td>
<td>.009</td>
</tr>
<tr>
<td>Education (years)</td>
<td>.01</td>
<td>.911</td>
<td>.001</td>
</tr>
<tr>
<td>tCBF: Yv</td>
<td>.37</td>
<td>.551</td>
<td>.016</td>
</tr>
<tr>
<td>Older</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.48</td>
<td>.237</td>
<td>.066</td>
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<tr>
<td>Gender</td>
<td>1.04</td>
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<td>Education (years)</td>
<td>8.10</td>
<td>.010</td>
<td>.278</td>
</tr>
<tr>
<td>tCBF: Yv</td>
<td>7.76</td>
<td>.011</td>
<td>.270</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t007
the older group, tCBF was marginally associated with age, and this association was attenuated when corrected for grey matter volume; In the older group tCBF, gmCBF, tCBF: Yv ratio and gmCBF: Yv ratio were associated with reaction times on attention tasks when age, gender and education effects were controlled, yet no such associations were observed in the younger group; In the older group, tCBF and the ratio of tCBF: Yv contributed greater amounts of variance to attention score than gmCBF in adjusted models; There were no significant contributions to memory tasks in either group, with any parameter.

**Discussion**

The present study investigated the relationships between brain oxygen metabolism, blood supply and cognitive function between healthy younger and older adults. It was hypothesized that age-related declines in CBF, CMRO₂ and cognition would be evident. Total blood flow in the brain decreased with age; however when this was corrected for grey matter volume, grey matter blood flow did not differ between young and older adults. The rate of cerebral oxygen utilization did not differ between groups, and the amount of oxygen extracted from the blood showed a trend toward being increased in the older group compared to younger. Taken together, these results suggest that there is some imbalance between the supply, consumption and demand for oxygen in the brain with older age, a pattern demonstrated in previous research [50]. A further aim of this study was to investigate the association of both CBF and grey matter oxygen utilization with performance on specific cognitive domains of attention and memory in healthy younger and older adults.

Advanced age in this sample was characterized by poorer performance on both the attention and memory measures of cognition and a reduced supply of blood to the neural tissue, yet when corrected for grey matter volume this reduction in CBF was no longer apparent. This was coupled with a trend toward increased oxygen extraction in the older group. In the older group total CBF, grey matter CBF and the ratios of both total and grey matter CBF to venous oxygenation contributed to attention score. Higher blood flow and ratios were associated with slower reaction times. Memory function was not associated with any physiological variable in the either group, and no relationships between physiological measures and cognitive performance were observed in the younger group.

**Age differences**

*Whole brain CBF was reduced in the older group, yet grey matter CBF was comparable between groups.* As hypothesized, total CBF was reduced in older adults compared to younger. These findings are supported by numerous reports of MRI-based [33–35, 44, 49, 51, 52].

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**Table 8. Univariate GLM showing age, gender, education and gmCBF: Yv main effects on attention for younger and older adults.**

<table>
<thead>
<tr>
<th>Age group</th>
<th>F</th>
<th>p</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
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<td>.329</td>
<td>.003</td>
</tr>
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<td>.795</td>
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<tr>
<td></td>
<td>.05</td>
<td>.834</td>
<td>.002</td>
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<tr>
<td>Older</td>
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<tr>
<td></td>
<td>5.72</td>
<td>.026</td>
<td>.214</td>
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</tbody>
</table>

https://doi.org/10.1371/journal.pone.0197055.t008
and specifically phase contrast MRI research [48, 53]. However, when total flow was normalized to the volume of grey matter, blood flow did not differ between groups. It is understood that cerebral grey matter undergoes significant atrophy with age [54], a finding corroborated in the present data. To account for this atrophy, we corrected for tissue volume in our estimation of gmCBF. Once this volume reduction was accounted for, blood flow in the remaining grey matter was found to be unchanged over the lifespan. This result, despite being in line with some previous findings (e.g.[21, 50]), was unexpected, as grey matter CBF is generally understood to decline with age, though CBF reduction across the brain is heterogeneous across brain regions [35]. Thus, the current data may have failed to observe this decline by assessing gmCBF as a derivative of grey matter volume, rather than regionally. According to the data presented here, we can infer that while the total flow entering the brain may decrease with age, blood supply to grey matter specifically does not change per se. This finding, coupled with age-related decline in grey matter volume, suggests that the observed decrease in total flow may be due to an overall reduction in volume with aging.

It was observed that there were differing patterns of age-related changes between the groups. In the younger adults, there were no observed alterations of any vascular measure with age, yet the older group showed significant age-related decreases in both blood flow and venous oxygenation, whilst gmCMRO$_2$ remained the same. This pattern of hemodynamic changes suggests that in older age, neural tissue extracts greater amounts of oxygen from the blood when the supply is reduced, in order to maintain the same level of oxygen consumption.

**CMRO$_2$ did not differ between age groups.** Cerebral metabolic rate of oxygen use did not differ between age groups. Previous PET studies of brain aging and CMRO$_2$ have been inconsistent. Most often oxygen utilization is observed to decrease with age [16, 20–28], yet some studies have found an increase in oxygen demand with age [32, 33], and, in-keeping with the current findings, other researchers have reported no change in CMRO$_2$ over the lifespan [21, 29, 30, 40, 55, 56].

The majority of this previous research utilized PET technology, making correction for brain parenchyma volume reduction difficult and possibly resulting in underestimation of CMRO$_2$. Developments in MRI capabilities allow for greater sensitivity in detecting partial volumes and accounting for brain atrophy, thus providing more robust estimation of oxygen use [57]. The method employed in this study calculated the rate of oxygen use relative to the size of the grey matter on an individual basis to overcome this potential limitation.

Lu et al. [33] reported that CMRO$_2$ increased across the lifespan; however potential age-related changes in hemoglobin concentration were not accounted for. The ability for blood to carry oxygen, and thus the hemoglobin content, can decrease by a rate of 0.0079mmol/L per year [21]. Researchers [32] re-analysed the data from Lu et al. [33] to include the potential age-effect on hematocrit to demonstrate that the CMRO$_2$ dependence on age was no longer significant. Therefore, the present study adjusted for the effect of age on hemoglobin content, replicating this result, which suggests that some previous findings may have overestimated the change in CMRO$_2$ that is truly attributable to aging rather than that which is dependent on age-associated hemoglobin decrease.

**Hemodynamic differences between groups.** Consistent with others [33, 50, 58], oxygenation of the venous blood declined with age in older adults (i.e. an increase in the oxygen extraction fraction (OEF)), and this was coupled with a decrease in cerebral blood flow, while CMRO$_2$ remained constant. Overall decrease in blood supply without a proportionate fall in metabolism would likely have this result [50]. The ratio of tCBF: Yv was trending towards a decline in the older group as compared to the younger adults, suggesting that the equilibrium between oxygen demand and whole brain blood supply is gradually altered across the lifespan. This is possibly due to diminished efficiency of the neural tissue with aging; the brain tissue...
requires more oxygen from the blood supply to maintain the availability of oxygen in the brain parenchyma, and thus, energy homeostasis. Previous research by Dastur et al. [50] using the nitrous oxide method [59] showed a similar pattern of results wherein CBF and CMRO₂ remained the same with age, matched with a trend toward decreased venous oxygenation. The authors concluded that oxygen extraction from the arterial blood is increased over the lifespan in order to maintain optimum CMRO₂ levels due to relative circulatory insufficiency. Note however, that no correction for brain volume was performed in this previous work, limiting the comparability of results with the present findings, in which total CBF was reduced in the older group compared to the younger, but CBF specific to grey matter tissue did not differ between groups. Moreover, the ratio of grey matter blood flow to venous oxygenation was not statistically different between the two age groups; these two parameters thus vary at the same rate with age. This highly non-significant result would indicate that the loss of gmCBF is in line with oxygen extraction in both younger and older adulthood. This suggests that in normal, healthy aging the cerebrovasculature is functioning beyond what is required to compensate for decreases in flow and oxygen extraction, particularly when adjusted for the grey matter atrophy.

Regardless, if the ability of the circulatory system to adjust to local changes in supply and requirement for oxygen is compromised by age-related pathologies such as hardening of vessels or accumulation of arteriosclerotic plaques, then increased extraction of oxygen from the circulating blood is the last remaining mechanism that can accommodate further demand, even when blood flow is maintained.

Summary of age group differences. The current data showed a decrease in supply of blood across the whole brain, yet flow and oxygen metabolism in the grey matter alone were maintained. This was coupled with a trend toward an increase in oxygen extraction between the age groups. This gradual shift in maintenance of energy homeostasis was accompanied by declining grey matter volume. The measured decrease in total CBF appears to be primarily due to atrophy of cortical tissue, rather than a true decline in flow. Overall, the data shows that aged brains have less volume, yet receive the same amount of blood flow per 100g of tissue as younger. This, matched with the observed trend towards an increase in oxygen extraction from the blood, suggests that the aged brain requires greater amount of oxygen per unit of tissue in order to maintain optimum metabolism.

Association between vascular factors and cognitive performance

The present study expanded on previous research by assessing the contributions of vascular and metabolic factors to attention and memory in physically and cognitively healthy adults. Consistent with previous research, attention and memory performance were lower in older adults compared to younger [3, 5]. Statistical analyses showed differences in the contributions of vascular factors to attention performance between the younger and older groups. While in the older group there were significant associations of CBF and the ratio of CBF to venous oxygenation (a measure of the coupling of flow and consumption) to the attention composite measure, these relationships were not observed in the younger adults. Memory was not associated with any physiological parameter in either group. This is in support of findings using phase contrast MRI to show a task-specific CBF- cognition relationship, wherein CBF predicted attention and information processing speed [48, 52], but not memory measures [60].

In the older group, when adjusted for participant characteristics, attention task performance was inversely related to blood flow, such that faster responses were associated with lower blood flow. While these findings are consistent with previous research in healthy participants [e.g. 48, 52], this is opposite to the expected outcome, given that both CBF and cognitive performance decrease with age.
This finding is in line with those of previous healthy aging studies. For example, it was found that blood flow in the carotid and basilar arteries in healthy older adults was negatively correlated to cognitive processing speed [48]. Bertsch et al. [52] reported a similar inverse relationship between resting grey matter blood flow and selective attention and tonic alertness in healthy individuals. The authors related their findings to the hypothesis of neural efficiency [61]. This theory posits that general intelligence is not a reflection of how hard a brain works, but due more to proficiency of neural circuits, as less ‘fuel’ i.e. oxygen and nutrients from the circulation, is required when the brain is working more efficiently. Faster reaction time on the attention tasks is assumed to represent more efficient processing. Attention and intelligence measures have been shown to be highly associated [61,62], lending support to the notion that better performance on attention tasks could be linked to lower global blood flow.

Another study reporting significant correlations between cognition and resting CBF found that once brain size was taken into account these associations disappeared [63]. In the present study, global CBF predicted attention score, yet this association was attenuated when volume of grey matter was accounted for, suggesting that some of blood flow effects are partially due to brain atrophy [47]. Likewise, in the present research the ratio of total CBF: venous oxygenation was found to predict attention score (a higher ratio was associated with slower responses on attention tasks) when age, gender and education were controlled, however this relationship was statistically weaker when corrected for grey matter volume. While this is difficult to interpret, a possible explanation is that oxygen extraction may be driving this association; a lower venous oxygen percentage (i.e. higher oxygen extraction) for the amount of blood flow could signify that the brain tissue is more at risk, and this would present as a smaller ratio. The attenuation of the relationship between CBF: Yv ratio and attention score once brain tissue volume is accounted for suggests that at least some portion of this flow/consumption coupling impact is due to grey matter volume differences.

**CMRO₂ and Yv did not predict cognition.** Contrary to expectations, grey matter CMRO₂ did not contribute to either memory or attention score, which could be the result of the compensation between CBF and Yv. These findings have been corroborated in previous literature in studies of healthy aging [40,63–65]. One study [64] found no association between cerebral metabolism of glucose and visual memory performance in healthy adults. Researchers concluded that resting brain metabolism is not linked with mental ability in the absence of disease. This notion is corroborated by Dastur et al. [50], who found a connection between mental abilities and CMRO₂ in normal older men with asymptomatic, sub-clinical vascular disease, but not in men who were optimally healthy. The present study employed a rigorous health screening questionnaire to exclude those presently or previously suffering disease or poor health, lending support to these hypotheses. Similarly, oxygenation of the venous blood was not associated with either cognitive measure. Previous research has linked lower oxygen content in the jugular vein to post-operative cognitive dysfunction in patients [66], however this is the first study to examine the association of venous oxygenation and cognitive performance in a healthy population of younger and older adults.

**Summary of vascular and metabolic contributions to cognitive performance.** Taken together, these results suggest that declining blood supply and grey matter volume results in increased extraction of oxygen from the blood in order to maintain normal oxygen consumption, a process that likely protects against neural impairment. Cognitive performance slows even in healthy aging, and there are differences in the contribution of vascular factors to cognition from younger to older adulthood. Physiological factors were not found to contribute to cognitive performance in the younger group. In older adults however, oxygen supply has an inverse effect on attention when demographic characteristics are accounted for, such that lower flow is associated with faster cognitive speed, perhaps indicating better neural efficiency.
Furthermore, the ratio of blood flow to oxygen extraction also contributes to attention score. Interestingly, these relationships are attenuated when grey matter atrophy is corrected for, indicating that for these older, cognitively intact individuals, brain volume has some impact on cognitive performance.

Limitations
The findings of the current study should be interpreted in light of some limitations. Participants were both healthier and more educated than what may be expected in the general population of adults, and were selected for the purposes of reducing any unwanted confounding variables in terms of biological aging. Care was taken to exclude individuals with cardiovascular risk factors that may confuse findings resulting from normal healthy aging.

Much of the previous research on CBF with aging has employed analysis based on brain region, due to the acknowledged heterogeneity of blood flow across different brain areas [21]. Previous studies indicate that there is a greater decrease in CMRO₂ in the grey compared to white matter across the lifespan [67], and others [68] reported that CBF and CMRO₂ reductions occur primarily in the parietal and temporal cortices, with other areas remaining unaffected. The method employed in the present study is considered to be valid and reliable for whole-brain estimation of CBF [63], and CMRO₂ [32], yet does not allow for regional analysis to be performed. Measurement of grey matter CMRO₂ without analyzing by specific region could underestimate the alteration in grey matter oxygen use whilst overestimating the oxygen use in the white matter, thus cancelling out any age effect in some regions versus others. The current study corrected for this by using the VBM toolbox in SPM12 (SPM12, University College of London, UK) which uses an iterative method to optimally segment different tissue types of the brain to calculate CMRO₂ in the grey matter only. However, this approach doesn’t allow for the distinction between grey and white matter contributions to the aging effect in vascular and metabolic function. If there is a differential rate of change in tissue volume with age, and this leads to changes in the ratio of grey to white matter across the lifespan, then CMRO₂ could appear to be modified without any true change in either grey or white matter rate of oxygen use, yet even after accounting for this gmCMRO₂ was not found to change across the lifespan, a finding corroborated in the literature [24]. The data showed that both grey and white matter was reduced in the older group compared to the younger adults, yet only the grey matter difference was significant. It has been suggested that alterations of tissue microstructure are the more commonly detected age-related change in white matter over volume reduction, and these structural changes are not easily observed using conventional T1-weighted imaging [69].

Additionally, the CMRO₂ values obtained in this study show some discrepancy when compared to literature values (e.g. 146 μmolO₂/100g/min on average in the younger group in the current work, versus 171μmolO₂/100g/min for an average 20 year old male reported in Aanerud et al. [21], versus ~135μmolO₂/100g/min for healthy controls reported in Thomas et al [70]). Design differences between these studies (e.g. samples, measurement tools, variation in hematocrit values) would limit the extent to which the specific values of CMRO₂ can be compared.

CBF change over the lifespan is heterogeneous across different brain regions, and distinct areas of the brain may be more responsible for cognitive performance than others [60,63]. Cerebral hypoperfusion can result in neural deterioration, and subsequently impaired cognition; however it is also possible that brain atrophy causes hypoperfusion due to decreased demand for oxygen and nutrients. The cross-sectional design of the study limits the extent to which causality can be deduced, further study is required to address whether blood flow changes are a cause or a consequence of brain shrinkage with age.
Conclusion

In summary, the current research showed that healthy aging is linked to inferior memory and attention, reduced total cerebral blood flow and a trend towards increased oxygen extraction, whilst blood flow and oxygen metabolism in the grey matter is maintained. These results indicate that there is some imbalance between the demand and the supply for oxygen and nutrients in the brain, even in successful aging. CBF was shown to contribute to attention task performance, though this relationship was in an unexpected direction with higher flow associated with poorer performance. The results of this study suggest that the association between CBF and cognition is age-dependent and task-specific. Overall deterioration in the aging brain results in losses of synaptic and vascular density and viability, and these diminishing functions may be responsible for the progressive slowing of cognitive speed, particularly in the realm of attention. Understanding how vascular and metabolic factors impact on cognition in normal, healthy adults is essential to develop efficient therapies to prevent and treat cognitive losses in older age. Future research employing regional brain area analyses and incorporating larger sample sizes is necessary to continue to investigate these relationships, in addition to the inclusion of cognitively impaired elderly to assess whether there are differential associations between CMRO$_2$, $Y_v$, CBF and cognitive performance between impaired and healthy aging.

Materials and methods

Participant characteristics

Vascular, metabolic and cognitive function was assessed in 60 healthy (active, asymptomatic, independently-living) adults. Two separate age groups were formed, the younger group <50 years ($n = 31$, ages 21–44, M 29.97 years, SD 6.17, 13 females) and the older aged >50 ($n = 29$, ages 55–75, M 65.00 years, SD 5.56, 16 females). Exclusion criteria included contraindications for MRI, left handedness, existing or previous cardiovascular or psychiatric disorders, blood pressure above 140/90mmHg, diabetes, pregnancy, use of medication for cardiovascular, neurologic or psychiatric disorders, and any other chronic illness. A telephone-based assessment (the Modified Telephone Interview for Cognitive Status- TICS-m [71]) was used to screen for mild cognitive impairment and dementia for participants aged over 45 years. Participants scoring 23 or below were omitted from the final analysis ($n = 3$). After receiving an explanation of the study procedure, participants provided written informed consent. This study was granted ethical approval by: Alfred Hospital Ethics Committee (338/13) and Swinburne University Human Research Ethics Committee (2013/316). Participant characteristic information is presented in Table 1.

Experimental design and procedure

Data was acquired in one testing session, approximately 3 hours length. Each participant had their blood pressure taken by a registered nurse, and then underwent a magnetic resonance imaging (MRI) scan. A 30-minute computerized cognitive assessment followed.

   MRI data acquisition. Data was collected using a 3T Siemens Tim Trio MRI system (Siemens, Erlangen, Germany) fitted with a 32-channel head coil at Swinburne University, Hawthorn, Australia. Participants were requested to minimize head movements, and foam padding was inserted around the head to aid this.

   Total and grey matter cerebral blood flow. Phase contrast MRI uses the phase of acquired images to determine the velocity of moving spins to obtain quantitative whole-brain blood flow measurements. Based on the maximum intensity projection reconstruction of the TOF angiogram, each of the four feeding arteries of the brain (left and right internal carotid
arteries and left and right vertebral arteries) were scanned separately [72]. Scans were oriented perpendicular to the arteries, and positioned with the center of the FOV placed at the center of each target vessel. Imaging parameters were: single slice, peripherally gated, TR 41.3ms, TE 4ms, FA 25°, FOV 160 x 160 x 5mm³, voxel size 0.5 x 0.5 x 5mm³, maximum velocity encoding 100cm/s. The flow (velocity x area) through the four arteries was summed together and multiplied by 60s/min to give the total CBF (tCBF) in ml/min. Mean grey matter CBF (gmCBF) was calculated by dividing the total CBF by grey matter volume relative to white matter volume. White matter flow was assumed to be 40% of grey matter flow [25]. This ratio and the grey and white matter volumes obtained from the segmentation procedure were used to calculate tissue-specific flow and metabolism rates. Equations for gmCBF are supplied in Supplementary Material.

**Measurement of gmCMRO₂.** Grey matter CMRO₂ was estimated due to differing rates of volume loss and oxygen metabolism between tissue types with age. This study employed a technique developed by Xu et al. [73], improved upon by Liu et al. [72], using independent MRI estimates of venous blood oxygenation (Yₐ) and cerebral blood flow. A 30-second baseline of arterial oxygen saturation was acquired with a pulse oximeter and averaged for each participant to give the value of Yₐ. Total CBF (tCBF) was measured using phase-contrast MRI and Yₐ was estimated using T2- relaxation-under-spin-tagging (TRUST) [74]. Grey matter CBF (gmCBF) was calculated from tCBF values as shown in Supplementary Material. Fick’s principle of arteriovenous difference [75] was used to quantify the rate of cerebral oxygen metabolism as per previous research [33, 76–79]. The equation was adapted for grey matter:

\[
gmCMRO₂ = gmCBF \cdot (Ya - Yv) \cdot Ca
\]  

Where gmCBF is the cerebral blood flow in ml/100g grey matter/min, Yₐ and Yₐ are the percentage of oxygen saturation of the arterial and the venous blood respectively, and Cₐ is the blood’s oxygen carrying capacity, generally established in the literature as 833.7 μmol of O₂/100ml of blood for a hematocrit level of 0.44 [80]. Previous research [21] reports age- and gender-dependence in hemoglobin concentration. To overcome this confound, Cₐ was set at 856 μmol of O₂/100ml blood for young males and 815 μmol of O₂/100ml of blood for young females, for hematocrit levels of 0.42 and 0.40 respectively, as per previous research [21], and was adjusted for the decline rate of 0.0079 μmol of O₂/ml/year [21]. This value had little effect on the calculation as a whole; experimental substitution with a single average value of 833.7 μmol of O₂/100ml of blood for all participants did not affect the outcome of statistical analyses.

All data processing was performed off-line with MATLAB 2014b (The MathWorks Inc., Natick, MA, 2014) using SPM12 (SPM12, University College of London, UK) or software developed in house. MATLAB scripts were used to segment the T1-weighted images of the brain into grey and white matter volumes using SPM12.

A high-resolution T1-weighted image was acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) image (axial, TR 2300ms, TE 2.52ms, voxel size 1 x 1 x 1mm³, FOV 256mm, 176 slices) to provide an estimation of the intracranial, grey, and white volumes for calculation of blood flow per unit mass of grey matter tissue. This accounts for variance in brain size and atrophy across participants.

A whole-brain 3D time-of-flight (TOF) angiogram enabled visualization of the four feeding arteries of the brain for phase-contrast MRI slice positioning (Fig 1). Imaging parameters were TR 20ms, TE 3.59ms, flip angle 18°, FOV 200 x 180 x 1mm³, voxel size 0.5 x 0.5 x 1mm³, 149 slices.
Global venous oxygenation. TRUST quantitatively estimates the oxygenation of the venous blood by measurement of pure blood T2 in the superior sagittal sinus (SSS). Imaging parameters: single-shot echo-planar imaging (EPI), axial plane, TR 4000ms, TI 1400ms, four effective TEs 0, 40, 80, 160ms; tagging thickness 50mm, gap 25mm, voxel size 1.4 x1.4 x 5mm³, 1 average, scan duration 2.20 min. The slice was parallel to the anterior-commissure-posterior-commissure (AC-PC) line with a distance of approximately 1cm above the confluence of sinuses. Slice positioning was based on a middle sagittal survey image (Fig 2A). The control and tagged images were subtracted pairwise to yield the venous blood signal, which was fitted a mono-exponential function to obtain the \( T2 \). The \( T2 \) was then in turn converted to \( Y_v \) via a calibration plot as described previously [74, 81, 82].

Computerized cognitive assessment. Cognitive function was measured using the Swinburne University Computerized Cognitive Assessment Battery (SUCCAB) [83]. This validated computerized test battery of 8 tasks is designed to capture the range of cognitive functions that decline with age. Tests were administered in the following order: simple reaction time, two-choice reaction time, immediate recognition memory (testing immediate memory of abstract images), congruent and incongruent color-word Stroop tasks, spatial working memory (testing working memory of spatial locations), contextual memory (testing recall memory of objects in spatial locations), and delayed recognition memory (testing the delayed recall of images from the immediate recognition memory component). Details of the tasks have been described previously [83]. Participants completed the SUCCAB once with duration of approximately 30 minutes. Participants responded to each task using a button box [83]. The researcher read the instructions aloud to ensure consistent explanation of all tasks. Each task was preceded by a brief practice trial. Memory and attention composite scores were calculated from the SUCCAB. Response times for immediate and delayed recognition, spatial working and contextual memory tasks were averaged to give the memory composite score. Simple and choice reaction time, and the two Stroop task response times were averaged to give the attention composite score. The variables are weighted such that greater values indicate slower processing times. The method of composition has been described previously [84].

Modified Telephone Interview for Cognitive Status (TICS-m). The TICS-m is a reliable and validated brief 13-item test of cognitive functioning that is administered over the telephone [71]. The items covered related to questions of orientation, repetition, naming and calculations, with scores ranging from 0 to 35. The modified version of the TICS also includes
immediate and delayed recall. Participants who scored 23 or below were omitted from the final analysis (n = 3).

**MRI data analysis.** An in-house Matlab script was used for quantification of CBF, which involved a region-of-interest (ROI) mask being manually drawn onto each target artery based on the magnitude image of each PC MRI scan. The phase signals (i.e. velocity values) within the masks were summed and combined with the cross-sectional area of each artery to calculate blood flow in ml/min for each artery. Blood flow values of each artery along with the diameter to convert to blood flow (ml/min). Velocities of the four arteries were added together to give the total CBF. Brain volume differences were accounted for by normalizing total CBF in ml/min to total grey matter (Vgm) and white matter (Vwm) in grams to give estimates of CBF in the grey and white matter separately. Grey and white matter volumes were estimated from the T1 structural MPRAGE scan using voxel-based morphometry (VBM8) segmentation with SPM12 software (SPM12, University College of London, UK). CMRO₂ per unit mass of grey matter was calculated according to Eq 1 above. Eq 1 was used to obtain unit-mass grey matter CMRO₂ μmol of O₂ per 100g of grey matter per minute as per previous literature [32].
Statistical analyses

IBM SPSS statistics v.23 (Chicago, IL, USA) was used to conduct analysis. Means and standard deviations for patient characteristics, baseline vascular and metabolic (tCBF, gmCBF, Yv and gmCMRO2) structural markers (ICV, Vgm and Vwm), and cognitive composite scores are shown in Table 1 for the whole sample and separately by age group. Differences between age groups were assessed using one-way independent samples analyses of variances (ANOVAs). Univariate general linear models (GLMs) were used to assess the main effects of age and gender on physiologic variables for each age group separately.

Separate univariate GLMs were performed for each age group to assess the main effects of vascular and metabolic factors to the cognition measures separately. Age, years of education and the vascular, ratio or metabolic factors were entered as fixed factors, and age was entered as a covariate. P values of 0.05 or less were considered statistically significant in all analyses; however a correction for multiple comparisons (Bonferroni’s correction) was applied to adjust for the inclusion of two physiological measures in the hypotheses. Significance level was adjusted for two comparisons in the univariate linear regression analyses (p ≤ 0.025). P values between 0.06 and 0.05 were considered to be marginally significant prior to correction, and reported as such.

Calculation of grey matter cerebral blood flow (gmCBF). Calculation of CBF in the grey and white matter on the assumption that white matter flow is approximately 40% that of grey matter as per previous literature [25]. The following equations were used to estimate gmCBF from tCBF values:

\[ \text{tCBFv} = \frac{\text{tCBF}}{\text{Vgm} + \text{Vwm}} \]
\[ \text{tCBFv} = \text{gmCBFv} + \text{wmCBFv} \]
\[ \text{wmCBFv} = 0.4 \cdot \text{gmCBFv} \]
\[ \text{tCBFv} = \text{gmCBFv} + 0.4 \cdot \text{gmCBFv} = 1.4 \cdot \text{gmCBFv} \]
\[ \text{gmCBFv} = \frac{\text{tCBFv}}{1.4} = \frac{\text{tCBFv}}{(1.4 \cdot (\text{Vgm} + \text{Vwm}))} \]

Where Vgm and Vwm are the volumes of grey and white matter respectively in ml. ρ is the mass density of tissue used as the scaling factor to convert tissue volumes to mass, assumed as 1.06g/ml [85]. tCBFv is the volume-corrected total CBF, normalized to the volumes of grey and white matter, in ml/100g brain tissue/ min. gmCBFv and wmCBFv are the volume corrected blood flows for grey and white matter respectively. A 30-second baseline of arterial oxy-gen saturation was acquired with a pulse oximeter and averaged for each participant to give the value of Yv, tCBF was measured using phase-contrast MRI and Yv was estimated using T2-relaxation-under-spin-tagging (TRUST) [74].

Supporting information

S1 Table. Table showing the results of univariate GLM on age, gender, education and tCBF contributions to memory score for younger and older adults. R² values for younger adults was .17, and older adults was .10.

(S1 Table. Table showing the results of univariate GLM on age, gender, education and gmCBF contributions to memory score for younger and older adults. R² values for younger adults was .17, and older adults was .10.

Supporting information

S1 Table. Table showing the results of univariate GLM on age, gender, education and tCBF contributions to memory score for younger and older adults. R² values for younger adults was .17, and older adults was .10.

(DOCX)

S2 Table. Table showing the results of univariate GLM on age, gender, education and gmCBF contributions to memory score for younger and older adults. R² values for younger adults was .17, and older adults was .10.

(DOCX)
adults was .17, and older adults was .06.

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References


7.4 Summary of key findings

Results of the current study indicated that there was some imbalance between physiological parameters in the older adults compared to the younger group. It is suggested that this imbalance in the delicate interrelationship between blood supply, demand and consumption of oxygen is involved in the deterioration of cognitive function in some way.

Whole brain CBF was predictive of attention performance in the older group; however, the amount of variance predicted was attenuated when corrected for individual grey matter volume, suggestive of some impact of brain atrophy in this effect. Oxygen metabolism in the grey matter of the brain was not related to cognitive performance in either younger or older adults, which was unexpected.

The subsequent chapter will discuss the overall findings of this thesis in greater detail in relation to the literature, and what is known regarding the effects of vascular aging on cognitive function. Clinical implications and limitations of the current research will be examined, followed by recommendations and directions for future research. This Chapter will finish with a concluding section, drawing the discussion points together.
Aging is coupled with declines in both cognitive and vascular functioning. How strongly these processes are related remains unclear, despite wide-ranging research in the area. The brain requires a constant supply of oxygen and glucose to maintain proper functioning, consequently, deterioration of the cerebral vascular system supporting the brain can impact neuronal and eventually cognitive functioning. Impairment of the cerebral microvasculature in the normal aging process can result from multiple structural and functional changes, as outlined in Chapter 2. While the physiological mechanisms underlying cognitive impairment in dementia and other neurodegenerative disorders have been examined extensively (See Kalaria (1996), Farkas & Luiten (2001), de la Torre (2004) or Iadecola (2004) for excellent reviews on the subject), cerebrovascular mechanisms that support healthy cognitive aging have not been explored to the same extent. The current research offers a unique insight into the contributions of vascular health toward maintaining good cognitive performance with age. This thesis investigated several questions relating to cerebrovascular function, oxygen utilization and blood flow regulation, and its effects on cognition in normal, healthy aging.

This chapter discusses the major findings of the research and highlights the contributions of these findings to the wider literature. Strengths and limitations of the research presented in this thesis including discussion of the novelty of methods, measures and analyses used are discussed in relation to recommendations for
future studies. Subsequently the clinical implications of this work are presented, followed by conclusions.

8.1 Key findings

Chapter 4 of this thesis presents a review published in BMC Neuroscience (Catchlove et al., 2018c). This paper systematically reviewed all published work that used MRI to assess cerebrovascular reactivity (CVR), and examined the relationship between this parameter and cognition. This chapter was aimed at gaining an understanding of the region-specific relationships between CVR and cognitive abilities. The findings from this review highlighted the lack of research investigating the impact of CVR on specific cognitive functions in healthy aging, which informed the design of the investigation presented in Chapter 6.

Ten research articles were included in the review. Quantitative synthesis of this evidence was not possible due to the heterogeneous nature of the articles; however, it was determined that cerebrovascular reactivity was lower in individuals experiencing some cognitive dysfunction, including MCI and AD, when compared to healthy controls, and in patients with cognitive impairment compared to those with normal cognition. CVR was also found to be lower in those with greater vascular risk factors (Tchistiakova et al., 2014, Glodzik et al., 2011). Cognitive performance was found to be directly associated with CVR in six (Yezhuvath et al., 2012, Chung et al., 2015, Richiardi et al., 2015, Calviere et al., 2010, Metzger et al., 2018, Cantin et al., 2011) out of the ten reviewed papers. While the data reviewed was suggestive of reduced vascular reactivity in individuals with cognitive impairment, a definitive
relationship between CVR and cognition in cognitively healthy adults was not identified. To address this gap in the literature, this thesis explored the extent to which CVR contributes to memory and attention in normal cognitive aging.

The review also emphasized that differences between the studies limited the comparability and reliability of the findings, including the use of non-standardized methods of hypercapnic challenge such as rebreathing, and imprecise cognitive assessment tools such as the mini-mental state examination (MMSE) (Folstein et al., 1975). This evidence guided the design of the empirical study ‘Regional cerebrovascular reactivity and cognitive performance in healthy aging’, in which a group of healthy adults with a wide range of ages completed a standardized hypercapnic challenge using CO₂-enriched gas, and underwent a detailed cognitive battery constructed to measure specific cognitive domains.

Chapter 6 presented this study, which explored the contributions of vascular reactivity to cognition in normal aging. The novel approach of this research was that to date no investigations have focused on CVR in specific regions of the brain in relation to memory and attention in cognitively healthy people with ages ranging the adult lifespan. Principal findings were that regionally, better CVR in the hippocampus was significantly related to greater performance on memory tasks in people aged over 50. CVR in the hippocampus was also inversely related to subjective memory concerns in the older group, such that greater CVR was associated with fewer memory complaints. Reactivity in the temporal lobes was shown to be predictive of memory and attention across the whole sample of individuals aged 21-75. Findings from this investigation add to the findings of the review, which suggested that CVR is
related to cognition in those with cognitive impairment, by demonstrating a relationship between CVR and cognition in healthy adults.

The second major investigation of the current thesis (Chapter 7; Catchlove et al., 2018a) also investigated mechanisms underlying healthy cognitive aging. Age-related differences in cerebral metabolic rate of oxygen use (CMRO₂), cerebral blood flow (CBF) and memory and attention performance in healthy adults were examined to investigate the associations between these parameters across the lifespan. Novel findings included the contribution of both whole brain and grey matter-specific blood flow to attention in older cognitively healthy adults. There was no relationship between any physiological parameter and memory. These data demonstrate that when age, gender and education are considered, lower grey matter blood flow is associated with faster responses on cognitive function tests in older, but not younger adults. Neither CMRO₂ in the cerebral grey matter (gmCMRO₂), nor venous oxygenation was found to contribute to memory or attention task performance. gmCMRO₂ did not differ between the younger and older adults, while the venous oxygen content was reduced on average in the older group, this was only approaching significance ($p=0.06$).

### 8.1.1 Importance of findings

The findings of the systematic review highlight the need for further investigation into the associations of CVR to cognition, particularly in the field of healthy cognitive aging. The purpose of reviewing MRI-based CVR studies was to gain a deeper understanding of whether there are precise areas of the brain that are reliant on
vascular integrity to support effective mental processing. Non-compliance and dysfunctional vasodilation of small cerebral vessels impairs the delivery of blood to the areas of the brain that require it. Region-specific relationships between CVR and cognition were inconclusive from the reviewed publications; however, research using non-MRI based estimates of CVR have shown promising results regarding larger vascular territories (Keage et al., 2012), as have the findings of our own study presented in Chapter 6.

The observation that the reactivity of blood vessels in the hippocampi and temporal lobes contribute to memory function in older adults are somewhat to be expected; these important findings reiterate the well-known localization of memory-related function in these areas (Petersen et al., 2000, Squire et al., 2004, Schacter and Wagner, 1999). Importantly, it was observed that lower CVR in the hippocampus was also associated with greater subjective memory complaints. This offers an insight into the notion of a biological basis for pre-clinical memory problems. This is a valuable finding, given that often subjective memory complaints may be the initial hallmark of impending cognitive impairment (Schmand et al., 1996, Jonker et al., 2000). This concept is discussed further in Section 8.4.

Previous research has shown that a number of brain regions are more vulnerable to age- and disease-related damage than others. The hippocampus and medial temporal lobes are areas known to be affected early in dementia (Kaye et al., 1997, Visser et al., 1999). The degeneration of these areas by accumulation of amyloid-β and hyperphosphorylated tau leads to accelerated atrophy and neuronal death in AD (Huang and Jiang, 2009). Studies have indicated the interrelationship between
vasodilatory impairment and cerebral amyloid angiopathy (CAA) in aging. CAA causes accumulation of amyloid-β in blood vessels, attenuating the CVR response (Shin et al., 2007, Han et al., 2015).

Dysfunctional CVR is also known to correlate with white matter lesions (Marstrand et al., 2002, Conklin et al., 2010, Sam et al., 2016). Population studies have found a strong connection between white matter hyperintensities (WMH) and cognitive decline (Kearney-Schwartz et al., 2009, Au et al., 2006). While there is currently no treatment available for either CAA or WMH, vascular function is to some extent modifiable through interventions with vasoactive nutrients, exercise, or medications.

Various alternative vascular and physiological indices have been used to explore the impact of cerebrovascular health on cognitive performance, such as CBF velocity (Vingerhoets and Stroobant, 1999), global and regional CBF (Dai et al., 2009, Alsop et al., 2000), and the hemodynamic changes resulting from oxidative stress and amyloid-β build-up (e.g. blood viscosity) (Butterfield et al., 2007, Ajmani et al., 2000, Liu and Zhang, 2012), amongst others. The assessment of these parameters has been crucial in gaining understanding into the processes underlying vascular cognitive impairments, and it is clear that further investigation is required.

8.2 Differential effects in younger and older adults

The current research showed that there were significant differences in reaction times on cognitive tasks between younger and older adults, and these were in part associated with blood delivery or regulatory mechanisms. CBF (blood supply) was
inversely related to attention processes but not memory, and CVR (blood flow regulation and neurovascular coupling) was positively associated with memory but not attention. These patterns were exhibited in the older group only, while the younger group showed no associations of physiological function with either cognitive domain.

Impairment of the small cerebral blood vessels with increasing age is common, even in healthy aging uncomplicated by illness or disease. Damage occurs to blood vessel walls over time, elastic components break down and regenerate more slowly, while the more rigid constituents like collagen and calcium increase in concentration, and atherosclerotic plaques build up, leading to significant increases in stiffness (Farkas and Luiten, 2001, Kalaria, 1996). This stiffening can cause the vessels to become less compliant to changes in blood-gas tension, metabolic demands and shear stress (O’Rourke and Safar, 2005). When small vessels are less reactive to energy and mechanical demands, hypoperfusion, microbleeds and damage to neural tissue may result.

This is evidenced by the observations of the current research that regional CVR and CBF were found to contribute to cognitive abilities in older people, but these relationships were not observed in the younger adults. These differences across the lifespan suggest that older-aged adults are more affected cognitively by age-related changes in vascular function than their younger counterparts. Increasingly, research is moving toward the examination of the role of chronic hypoperfusion and vascular non-compliance in progressive age-related cognitive decline. Cross-sectional and longitudinal studies provide considerable evidence for the contribution of arterial
stiffness to cerebral hypoperfusion and ultimately cognitive decline (De la Torre, 2012, Mitchell et al., 2011).

Growing literature suggests that age-related vascular disorders (e.g., hypertension, hypercholesterolemia, arteriosclerosis) and hypoperfusion contribute to cognitive impairments (Gorelick et al., 2011). Research has shown there is a significant role of peripheral vascular dysfunction in cognitive decline (Pase et al., 2012, Pase et al., 2014). Stiffening of the systemic blood vessels – the aorta, femoral and brachial arteries in particular – leads to a two-fold process whereby the mean arterial pressure (MAP) increases, due to less cushioning effects of the more rigid arteries. This creates greater pulsatile pressure within the vasculature, which then may damage the delicate neural tissue (Steppan et al., 2011). Central artery stiffness (carotid-femoral and carotid-heart; measured by pulse wave analysis) was linked to cognitive deficits in a large population-based study (Hughes et al., 2018).

These findings reiterate the common observation that some domains of fluid intelligence (memory and attention) decline over the normal aging process, even in the absence of objective cognitive impairment (Glisky, 2007). Evidence from functional neuroimaging investigations suggest that brain regions activated during memory and attention tasks differ between healthy older adults and younger people (Cabeza, 2001, Rypma and D'Esposito, 2000) as older brains reorganize to accommodate for anatomical and physiological alterations over the lifespan (Grady et al., 1999). The cognitive domains assessed by the current research were measured by combining individual tasks, so that different aspects of memory (contextual and spatial working memory, immediate and delayed recall) and attention
(congruent and incongruent Stroop) were tested. Composite scores were reflective of the reaction time taken to respond to these tasks, which provides an additional aspect of processing speed which is also known to decline with age (Glisky, 2007, Salthouse, 1996).

The findings of the systematic review also raise the possibility of using vascular reactivity as a marker of mental processing. CVR represents a more dynamic measure of vascular functioning than CBF; CVR is a physiological response which not only regulates the flow of blood but also maintains homeostasis of central pH, affecting the chemoreceptor ventilatory response (Ainslie and Duffin, 2009). The process reflects the functioning of the vessels, but also the coordinated actions of neurons, astrocytes, smooth muscle and endothelial cells, and biochemical messengers underlying the neurovascular unit. One systematic review of 34 TCD studies concluded that CVR was a better predictor of AD severity than other TCD indices such as resting cerebral blood flow velocity (Keage et al., 2012). The current findings indicate that CVR is related to memory loss pre-clinically. Reactivity was shown to correlate with subjective concerns about memory in a sample that was objectively unimpaired.

Elucidating the patterns of change in reactivity that occur with age-related cognitive change is important; it has been reported that pathologically low CVR was linked to a 33% greater risk of conversion from MCI to AD within a year than those with normal CVR (Viticchi et al., 2012). Our findings suggest that regional vascular integrity may be a part of the early pathological process and serve as a first step in disentangling the relationship between memory deficits and vascular change in aging.
The findings presented in this thesis show that the responsiveness of the microvasculature affects memory performance in a region-specific manner, both subjectively and objectively, in older, but not younger adults. An unexpected observation was that in older people greater CBF was linked to reduced attention performance. Global CBF decreased with age, yet when corrected for grey matter volume blood flow was comparable between younger and older adults. This highlights the importance of adjusting for brain size when interpreting blood flow indices, particularly in investigations of aging effects. Moreover, the older group showed an altered pattern of vascular function to the younger adults. Cerebral oxygen extraction (measured via venous oxygen content) was reduced with age in the older group, coupled with decreased blood supply and maintained oxygen metabolism (CMRO$_2$). Overall decline in CBF without a proportionate drop in CMRO$_2$ requires the extraction of greater amounts of oxygen from the blood in order to keep CMRO$_2$ constant. We suggest that it is likely this gradual shift in hemodynamics which results in progressive slowing of cognitive speed, particularly in the domain of attention.

8.2.1 Differential effects of physiological measures on separate domains of memory and attention in older adults

The observation that the effects of CBF on attention were attenuated when flow was adjusted for grey matter volume is interesting as it suggests that cognitive performance is also in some way impacted by brain volume. These findings are consistent with those of Poels et al. (2008), who used phase contrast MRI to
measure total CBF in a large study of 862 older participants (55 years+). Whole brain CBF (in ml/min) correlated with tests of global cognition, executive function and processing speed; however, when the authors adjusted CBF for brain volume, these relationships were no longer significant. These results indicate some imbalance between the demand and the supply for oxygen and nutrients in the brain, even in non-pathological aging free from cognitive dysfunction. Previous research investigating CBF and attentional abilities has revealed an inverse relationship between the two (Rabbitt et al., 2006, Bertsch et al., 2009), consistent with the findings of the current study.

In the present research, CMRO₂ corrected for grey matter was found to have no impact on cognitive performance in either older or younger adults. This was unexpected, given that calibrated fMRI studies have shown that CBF and CMRO₂ are related to performance (Hutchison et al., 2013, Mohtasib et al., 2012). These previous studies both focused more on the change in CMRO₂ in relation to the change in CBF to reflect the supply/demand relationship of neurovascular coupling. CMRO₂ is assumed to reflect neural activity, as increased neural activity creates a metabolic demand, which is met by increases in glucose utilization (Hutchison et al., 2013, Raichle and Mintun, 2006). Oxygen metabolism is usually tightly coupled to the cerebral metabolism of glucose. Reduced regional glucose use (CMRglu), measured with PET technology, predicts future conversion from cognitive impairment to AD (Anchisi et al., 2005, Arnáiz et al., 2001, Silverman et al., 2001) and has been shown to progressively decline for years prior to onset of clinical AD symptoms (Mosconi et al., 2009, Minoshima et al., 1997). In a study using the nitrous oxide method Dastur et al. (1963) reported that CMRO₂ and cognitive abilities were
associated in cognitively normal older males with asymptomatic, subclinical vascular disease, but not in those who were unaffected by vascular conditions. The exclusion criteria in the present study ensured that all participants were unaffected by current or past cardiovascular disease or poor health, which may provide a possible explanation as to why we did not observe a relationship between CMRO$_2$ and cognition.

By examining the effects of specific vascular functions on memory and attention performances separately we were able to uncover differential relationships evident in these domains. Improving vascular reactivity may provide a vital clue toward reducing or delaying decline in memory abilities. Vasoactive compounds that improve the functioning of any of the multitude of components that act in concert to dilate the cerebral vessels could help ameliorate dysfunctional CVR, possibly slowing memory loss over the aging process.

8.3 Regional specificity of CVR predicted memory performance in healthy older adults

It was observed that in the entire cohort of adults aged 21-75, temporal lobe CVR and memory were significantly associated, in which greater vascular response was linked to faster reaction times. The medial temporal lobes are known to have distinct functions relating to memory formation and storage (Squire et al., 2004). Analyzing the age groups separately also revealed a noteworthy relationship between the blood vessel reactivity in the hippocampus and memory in the older group, yet this
relationship was not observed with younger adults. Better reactivity was associated with greater performance on the memory tasks.

As discussed in Chapters 2 and 3, the aging vasculature of the brain undergoes notable changes in its structure and functioning. These changes can lead to stiffening of the vessels, resulting in a reduced capacity to contract and dilate in response to changes in metabolic demand or neural activity, hindering, or even possibly augmenting the delivery of vital nutrients and oxygen to the brain tissue. Not surprisingly, this situation can result in functional losses to affected brain areas. The present research showed that there were significant differences in brain blood vessel integrity between younger and older healthy adults. Older adults also showed significant reductions in vascular reactivity with age in various regions, and these changes were not seen in the younger cohort.

Positron emission tomography (PET), single-photon emission CT (SPECT) and transcranial Doppler ultrasound (TCD) investigations have indicated that vascular responsiveness does indeed impact cognitive performance in region-specific ways (Silvestrini et al., 2006, Viticchi et al., 2012, Glodzik et al., 2013). Yet due to the limited spatial resolution of these techniques the regions identified are not clear-cut and much ambiguity remains. Detection of specific lobes or cerebral structures that are more affected by decrements in reactivity would enable better-tailored approaches for treatment. Likewise, if certain aspects of cognition are found to be explicitly affected by functional losses in certain regions of the brain, this could drive personalized therapeutic programs aimed at reducing impairments on a case-by-case basis.
While there are numerous research papers dedicated to the contribution of vascular reactivity to global cognitive function in individuals with impaired cognition, it is apparent that this area of research in normal cognition is limited. Of the studies reviewed in Chapter 4, only one utilized a sample of people who had unimpaired cognitive and cardiovascular functioning (Gauthier et al., 2015). This study investigated the differences between younger and older healthy adults using a single test of executive function, measured using a modified Stroop task. While the results suggested that performance of this particular executive function task was not related to CVR, the current study, presented in Chapter 6, employed a more comprehensive neuropsychological assessment to further investigate whether CVR was related to other aspects of cognition that are known to decline with age, namely memory and attention (Cabeza et al., 2004).

Across most studies reviewed in Chapter 4 healthy cognitive aging exhibited greater vascular reactivity than in those with cognitive impairment. This is supported by broader research of CVR and cognition using non-MRI investigations. One large scale population-based study using TCD reported CVR was a good discriminator of future decline in cognitively healthy adults (Ruitenberg et al., 2005), while a smaller study of 406 MCI patients found CVR a strong predictor of conversion to AD within a year (Buratti et al., 2015). Our own research revealed that CVR was correlated not only with better memory task performance, but also with concerns of memory in healthy older adults.
Hippocampal and temporal lobe CVR were predictive of memory task performance in healthy older adults. The present research used a novel approach to assess vascular reactivity in localized regions of interest, in relation to specific cognitive processes. The hippocampus is particularly vulnerable to both structural and functional decline with age (Morrison and Hof, 2002). Of particular interest is the selective and widespread damage to this structure in neurodegenerative diseases, with the classic example of AD, in which specific areas of the medial temporal lobe gradually deteriorate early in the disease. The hippocampus is one of the first cerebral structures to show damage in AD, and is also affected in diabetes, hypertension, sleep apnoea and hypoxic brain injury (Fotuhi et al., 2009).

To the authors' knowledge, this is the first study to show that responsiveness of small vessels in the hippocampus and temporal lobes, well-established for functions in memory-formation and processing (Davachi et al., 2003, Schacter and Wagner, 1999), impact scores on memory tests in cognitively-intact older individuals. These observations are noteworthy; CVR is a dynamic measure of vascular health which may help to explain additional variability in cognitive change when considered with changes in structural volume losses. Inadequate dilatory capacity of cerebral vessels increases stroke risk, amyloid-β accumulation in the brain (senile plaques) and microvasculature (cerebral amyloid angiopathy; CAA), oxidative stress and inflammation, white matter hyperintensities and multiple other ischemic pathologies, and has been linked to numerous vascular disorders including hypertension, diabetes and cardiovascular diseases (De la Torre, 1997, De la Torre, 2012). Interestingly, these pathologies are all known to be linked to some extent in cognitive impairment when considered in isolation.
A goal of the current research was to uncover the reasons why less reactive cerebral vessels may impact cognition. Small vessel damage may result from stiffened microvasculature, which limits the capacity for vascular responsiveness. It was observed that reactivity of the microvasculature was not heterogeneous across all areas, though CVR did correlate well across regions, i.e., if CVR was higher in one area it tended to be higher in other areas also. These heterogeneous findings are supported by previous research in which age-related changes in CVR were found to be region-specific (Lu et al., 2011, De Vis et al., 2015, Liu et al., 2013). In the current study, reactivity was higher in the parietal, temporal and frontal areas and lowest in the hippocampus and cingulate across the entire cohort, in keeping with research reporting that CVR is greater in the cortical grey matter when compared to the deeper grey matter structures (Novak, 2012, Last et al., 2007).

8.4 Reduced CVR in the hippocampus was associated with subjective memory concerns in healthy older adults

Research presented in Chapter 6 is the first study to show that vascular reactivity to CO₂ in the hippocampus is related to subjective memory concerns (SMC) in older adults with normal cognitive function. Subjective concerns about memory are common in older adults, and are associated with increased risk for developing dementia (Clarnette et al., 2001). Population-based studies have reported that peripheral vascular stiffness is reduced in both vascular- and Alzheimer’s dementia (VaD and AD, respectively), and in subclinical mild cognitive impairment (MCI)
(Hanon et al., 2005, Keamey-Schwartz et al., 2009). Similarly, vascular stiffness, measured as reduced CVR, has been associated with poorer cognitive functions in studies of clinical cognitive impairment and AD (Silvestrini et al., 2006, Viticchi et al., 2012). It appears that subtle vascular structural changes that occur in normal aging effect memory performance, not only objectively, but also contributing to subjective concerns.

Multiple structural and functional indices of the hippocampus have been associated with cognitive performance (Petersen et al., 2000). In healthy older adults (mean age ~71 years), reduced resting hippocampal CBF has been shown to significantly correlate with spatial memory; greater flow was linked to faster reaction times (Heo et al., 2010). In a similar vein, while large hippocampal volume is associated with better memory performance and cognition (Schuff et al., 1999), atrophy of this structure is linked to the onset of cognitive impairment and dementia (Driscoll et al., 2009, Mueller et al., 2010).

The hippocampus is a heterogeneous structure comprised of several smaller ‘subfields’ that are histologically distinct. Measuring the volume of the whole hippocampus provides information on the overall integrity in aging or disease states; however, it is reported that investigation of the smaller subfields is superior to predict normal from impaired cognitive profiles (Mueller et al., 2010). In a sample of 138 adults aged 64-86 years, decreased hippocampal volume over a 10-year period differentiated MCI from normal aging (Driscoll et al., 2009), although only 18 participants were given a diagnosis of MCI over this time. Importantly, it was found that specific regions of tissue within the hippocampus may deteriorate at different
rates, in concert with functional changes in cognitive abilities (Mueller et al., 2010). This research showed that while there are distinct patterns of cell and volume losses that occur in the global hippocampus in healthy aging, early stages of cognitive impairment and AD, measurement of atrophy in smaller subfields of hippocampal regions may be better to distinguish normal aging from MCI and pre-clinical AD. A review and meta-analysis of the literature has shown that, in fact, the predictive utility of hippocampal volume to cognitive functions changes considerably across the lifespan and is particularly variable in older adults (Van Petten, 2004).

The results presented in this thesis contribute to the existing knowledge surrounding the established relationships between dysfunctional CVR and cognitive impairment in patient populations (Richiardi et al., 2015, Silvestrini et al., 2006, Cantin et al., 2011). While these studies have elucidated that there is a relationship between CVR and cognition in patients with advanced memory decline, ours is the first study to show a significant correlation between CVR and memory worries prior to any instance of objective dysfunction. This research highlights the potential for development of specific vascular-targeted therapies, improving the possibility of ameliorating age-associated cognitive decline and potentially the conversion to mild cognitive impairment and Alzheimer’s disease.

Patterns of regional neurodegeneration observed in cases of Alzheimer’s disease impact specific functions, and episodic memory is often the first sign of pathology (Ewers et al., 2011). Hippocampal atrophy has been shown to correspond with worsening memory abilities in longitudinal (Petersen et al., 2000, Gorbach et al., 2017, den Heijer et al., 2006) and cross-sectional studies (Ward et al., 2015).
Studies have demonstrated that blood flow in the hippocampus is likely to decline in normal aging, yet in AD has been reported to become hyperperfused (Alsop et al., 2008, Zlatar et al., 2014). This is in contrast with the findings of a longitudinal study which revealed that reduced glucose metabolism in the hippocampus was predictive of the decline to AD compared to normal cognitive aging with over 80% accuracy (Mosconi et al., 2009). While these findings are not straightforward, they do support the notion that localized alterations to vascular function in the hippocampus can affect cognitive functions. Changes in reactivity of the smaller cerebral vessels can impact brain function in various ways. Sections 2.6 and 3.1 provide more detail.

Vascular risk factors are associated with increased risk for dementia in large-scale population studies (Luchsinger et al., 2005). Studies have shown that the less-compliant nature of the aged and stiffened vasculature can cause damage to end-organs that receive steady high flowing surges of blood (O'Rourke and Safar, 2005, DeCarli, 2012). The brain is particularly at risk, as the blood flow required for constant neural activity is up to 20% of the cardiac output of the entire body (Owen and Sunram-Lea, 2011). Continuous surges of high pressure blood flow have the potential to damage small cerebral vessels, cause micro-bleeds or infarctions in the brain, likely resulting in implications for cognition. Watershed regions of the brain are at greater risk for damage from stroke and hypoperfusion due to their distal location from the major cerebral arteries. The findings of the current research show that the functioning of small cerebral vessels impacts on cognitive performance, even in healthy older people.

8.5 Strengths, limitations and future directions
A gap in the CVR-cognition research identified by the systematic review was that most studies in this area have relied on either dementia screening tools, namely the mini-mental state exam (MMSE), or tests of executive function, to assess cognitive performance. These approaches lack sensitivity for assessing mild neuropsychological changes, as subtle decrements in cognitive abilities could be potentially overlooked. Changes in cognitive ability in the normal aging process is individualistic, it depends on many factors (including education and social support) and there is often much variation from person to person (Glisky, 2007). As we continue to delve into the physiological mechanisms underlying cognitive health, rather than gross impairment, it is evident that more discerning assessment tools that are precise enough to observe slight alterations are necessary. It is for this reason that we chose to use a computerized cognitive testing battery with highly sensitive tasks focused on age-associated cognitive decline.

We addressed limitations of previous research by employing the Swinburne University Computerized Cognitive Aging Battery (SUCCAB; Pipingas et al. (2010)) outlined in Section 5.2.1. This battery consists of 8 tasks that assess specific neuropsychological functions of processing speed and differing aspects of memory and attention. This assessment tool has millisecond accuracy and has been validated by previous research in aged individuals (Pipingas et al., 2010) and numerous research studies investigating the effects of various dietary interventions on cognition, including multivitamins (Macpherson et al., 2012), green tea extract (Scholey et al., 2012), fish oil supplements (Pase et al., 2011) and cocoa flavanols (Massee et al., 2015), amongst others, as well as interventions with cognitive training (Simpson et al., 2012). A further investigation used the SUCCAB to assess the
effects of multivitamin supplementation on cognitive function (Pipingas et al., 2014). This study demonstrated the sensitivity of the battery, particularly the Stroop task (an assessment of speeded attention) to changing B vitamin levels in healthy adults aged 20-50 years. This assessment tool is highly sensitive to the subtle effects of normal cognitive aging, particularly in comparison to the mini-mental state exam (MMSE) (Folstein et al., 1975) which has been often used to assess the CVR-cognition relationship. We consider the use of this cognitive battery a strong point of the current research.

A number of strengths of this research are relevant to the MRI techniques utilized. For example, using MRI instead of the more commonly employed transcranial Doppler ultrasound (TCD) has the added benefits of regional examination and enables acquisition of perfusion data from the small cerebral arterioles and capillaries. While TCD is less expensive, the lack of spatial resolution was deemed to be too limiting for the purposes of this investigation. The particular MRI sequences employed in the current research are a valid and reliable means to assess the various physiological indices presented here, including regional perfusion, whole brain blood flow, venous oxygen content and structural brain volumes. These techniques have been used previously in numerous investigations of brain vascular health and functioning in aging (Buijs et al., 1998, Jain et al., 2011, Liu et al., 2012b, Vernooij et al., 2008, Xu et al., 2009). ASL and phase contrast MRI techniques are often used in clinical examinations of vascular alterations in small vessel disease, stenosis and occlusion (Stalder et al., 2008, Johnson et al., 2005, Alsop et al., 2015). It should be noted, that the results produced by varying methods of cerebrovascular assessment are not necessarily interchangeable; different assessments may reflect
distinct processes or functions. For example, assessments of cerebral blood flow (CBF) are not comparable to measurements of CVR, as these reflect separate physiological processes.

The present research had several limitations which were addressed in the experimental and methods chapters (Chapters 5, 6 and 7). Some further methodological considerations should be taken into account. A potential limiting factor is the size of the cohort. While the size of the samples used in this research is sufficient in terms of effect sizes, a greater number of participants are generally desirable in any study on cognitive aging (Salthouse, 2010). Future research on CVR, CMRO$_2$, CBF and the contributions of these parameters to cognitive performance over the healthy lifespan would benefit greatly by larger numbers of participants, particularly those ranging between different cultures, education and fitness levels. Additionally, greater amounts of subjects would allow better control of these other contributing factors.

Difficulties in recruitment are common in aging research particularly when the sample are required to have good cognitive and cardiovascular health in older age, however very few issues were present in the current investigations. This was aided by the use of the Centre for Human Psychopharmacology (CHP) participant database. Those in this database had previously participated in studies and were willing to be involved in future research. The participants were self-rated ‘cognitively healthy,’ yet some in the older group reported subjective memory concerns, which indicates some heterogeneity of the sample. The combination of subjective concerns about memory and older age classifies these individuals as being at risk of cognitive
decline. We attempted to recruit a healthy sample of older adults who were free from objective cognitive impairment by using screening tools – the verbal pairs component of the Weschler Memory Scale-revised (WMS-R; (2009)) and the Telephone Interview for Cognitive Status-modified (TICS-m; (de Jager et al., 2003)) – to ensure that the older sample were not cognitively impaired. Three older volunteers scored 24 or below on the TICS-m and were excluded from participating in the study. It is not possible to determine whether some of the older individuals may have premorbid AD. Further research that targets recruitment of aged individuals with mild objective memory impairments may be the next step in uncovering the mechanisms behind early detection of vascular correlates of cognitive change.

As discussed in Chapter 6, it is noteworthy that inhalation of CO$_2$-enriched gas does not provide a precise standard arterial partial pressure of CO$_2$ (PaCO$_2$) stimulus. This is due to differences in individual respiratory responses, lung size and breathing rate (Fisher, 2016). Although there are more exact methods of increasing PaCO$_2$ available (computer-controlled targeting of end-tidal CO$_2$ partial pressures, for example), these are expensive, necessitate the purchase of specific equipment, require training for operation and are thus not as accessible as CO$_2$-gas delivery. The review on MRI-based CVR studies by the authors (Catchlove et al., 2018c), presented in Chapter 4, found that inhalation of a fixed concentration of CO$_2$-enriched gas was the most commonly-used vasoactive stimuli for assessing CVR in MRI investigations, hence our selection of this method was deemed appropriate.

Another possible limitation is that our MRI analysis methods used a number of assumptions to calculate CVR, CBF, grey matter CBF (gmCBF), venous oxygen
content and CMRO$_2$. For instance, the technique used to estimate gmCBF from separate measures of grey and white matter volume and total cerebral blood flow (tCBF) has not previously been reported in any published work that the author is aware of. This technique was the innovation of co-authors (YC & TBP) who have firm standing in the fields of brain imaging and vascular function. Mean grey matter CBF (gmCBF) was calculated by dividing the total CBF by grey matter volume relative to white matter volume. White matter flow was assumed to be 40% of grey matter flow as per previous research (Leenders et al., 1990). This ratio and the grey and white matter volumes obtained from the segmentation procedure were used to calculate tissue-specific flow and metabolism rates, which we believe to be an improvement over less sensitive global measures. These assumptions are not age-dependent, and thus, may reduce the power of the data, yet should not result in false positive findings.

The method of grey matter cerebral metabolic rate of oxygen use (gmCMRO$_2$) estimation outlined in Section 5.7.3.3, used indices of gmCBF, venous oxygenation and segmented white and grey tissue volumes to account for individual differences in brain size, which we adjusted for age and gender differences in hematocrit concentration. The method is described in Chapter 7. This study is the first that we know of to measure gmCMRO$_2$ in this manner. While the equation we used is comparable to previous research (Peng et al., 2014), and we used these authors’ method of calculation as our guide, we expanded upon this method by assuming a steady ratio of .4 between grey and white matter blood flow, which we believe leads to a more robust result. However, there is the possibility that this ratio varies with age, potentially confounding the results. Future research may make use of separate
Another factor to be considered is that T1-based segmentation algorithm is known to have greater trouble differentiating between grey and white matter in older adults (i.e. the effects of grey matter atrophy). Analysis of the impact of grey matter atrophy and gm/wm CBF ratio differences on cognitive performance in the older adults was not possible given the data acquired did not allow for exact measurement of these parameters. Forthcoming research should ensure that more sensitive measurement of volumes of grey and white matter are made. A whole-brain axial dual-echo fast spin-echo (FSE) MRI sequence can be used to acquire both proton-density and T2-weighted images to overcome this issue (Ge et al., 2002), however this was not used in the current investigation. Estimation of the oxygen consumption rate of the cortical grey matter only is both accurate and appropriate for this study, particularly given the wide-ranging ages of the participants. Grey matter volume differences must be taken into account in studies of brain aging, as research shows that oxygen consumption decreases more significantly in the grey matter compared to white across the lifespan (Marchal et al., 1992).

The CVR estimation and signal shifting procedures (discussed in Section 5.8.2.3), while not completely novel, were not the most common methods used in research. The method we employed to align the end-tidal CO₂ (etCO₂) and BOLD signals to account for hemodynamic delay was similar to that used previously (Yezhuvath et al., 2009); however, there are other possible techniques to do this. Prospective studies could benefit from time-locking the etCO₂ recording to the MRI scan to avoid
the pitfalls of manually shifting the time-series. It was unfortunate that we were unable to analyze the CBF data obtained from the ASL scan (discussed in Section 5.8.2.5). This drawback could have been avoided by real-time continuous sampling of participants’ physiological signals (i.e., respiratory effort, blood pressure and cardiac pulsatility), to allow filtering of unwanted biological noise. Moment-to-moment sampling of vital signs can be used as an input into post-processing software.

Another methodological consideration relates to the sampled data points for the CVR measurement. The CVR estimation technique that was employed sampled 30 seconds of the data in each hypercapnia scan, which accommodated for the rise and fall times of the responses. In retrospect, it would have been useful to have considered longer plateau periods. Given the inherent low SNR of ASL methods, it is advantageous to use longer plateau periods (e.g. 4 minutes) in order to have more data to average over. Future reproducibility studies should consider increasing the sampled periods to increase sensitivity of the measurement.

8.6 Implications for future research

The research presented in this thesis aims to address gaps in the current literature surrounding the extent to which certain vascular and metabolic processes support healthy cognitive aging. Task- and region-specific analyses enabled a thorough and comprehensive assessment of the differential contributions of oxygen metabolism, arterial and venous oxygen concentration, whole brain and cortical blood flow, and
regional vascular integrity to two distinct cognitive domains in healthy younger and older individuals.

These findings have implications for the wider clinical and pre-clinical population of people experiencing cerebrovascular damage, memory problems or cognitive decline. Barnes and Yaffe (2011) reported that up to 18% of dementia risk factors are related to vascular health. This statistic is alarming, given that many dysfunctions of the vasculature are either preventable or modifiable (de la Torre, 2010b). This information may help inform the development of targeted therapies to maintain vascular health, potentially delaying the onset of cognitive impairments. It is estimated that delaying the onset of symptoms of Alzheimer’s dementia for up to one year could potentially reduce worldwide prevalence by over nine million cases by the year 2050 (Brookmeyer et al., 2007). Persons presenting with dysfunctional CVR or hypoperfusion relating to deficits in vascular function could be at risk for cognitive decline in the future; thus, the early detection of these pathological age changes could assist in the prevention of further impairments. The potential to use these parameters as preclinical indicators of brain dysfunction in older age is also a possibility.

Therapies targeted at improving the responsiveness of small cerebral vessels by enhancing their ability to dilate and constrict when faced with changes in metabolic demand would improve perfusion by increasing regulation of cerebral blood flow. Vascular dysregulation leading to neurovascular uncoupling is an important aspect of neural dysfunction in disease states (Girouard and Iadecola, 2006). Research is being conducted to identify compounds that may improve vascular function
pharmacologically. Studies with AD models using aged transgenic mice with overexpression of the amyloid precursor protein (APP) have shown that antioxidant and other compounds can completely reverse cerebrovascular dysfunctions (Nicolakakis et al., 2008, Park et al., 2007, Tarantini et al., 2015). Nicokolakis et al. (2008) used antioxidants and pioglitazone as a late-stage intervention to treat aged APP mice with severe cerebrovascular and memory deficits with remarkable results—CBF response to sensory stimulation was completely normalized to that of normal wild-type mice. This improvement in NVC did not translate to improved cognitive performance, although antioxidant interventions have been shown to improve or even reverse cognitive deficits in other investigations (Liu et al., 2003, Yin et al., 2013). Likewise, inhibition of NADPH oxidase (Park et al., 2007) and production of reactive oxygen species (ROS) have been shown to successfully restore endothelial function in the microvasculature and improve overall functioning of CVR.

Studies investigating human cognitive function have revealed promising results with numerous nutrients that act on the vascular system. Certain phytochemical-rich foods, such as grapes, tea, cocoa and blueberries, contain high amounts of flavonoids, which are powerful antioxidants. These compounds have been demonstrated to elicit specific beneficial actions in the brain to protect neurons from damage, enhancing the functioning of vascular and neural structures that commonly deteriorate in the aging process, thereby improving cerebral blood flow and stimulating neurogenesis (Spencer, 2010). Flavonoids are understood to improve vascular function via numerous mechanisms of action including augmenting endothelial function and increasing peripheral blood flow (Schroeter et al., 2006). The effects of acute and long-term supplementation with flavonoid-rich foods on
cognition and neurodegeneration have been widely investigated and reviewed (see Lamport et al., 2012; and Macready et al., 2009), however further research is necessary to resolve questions such as the optimum duration of intervention, dosages, and when in the lifespan is the ideal time-point to consume vasoactive nutrients to receive the most benefits (Spencer, 2010).

Resveratrol is one polyphenolic compound gaining weight for its impressive vasoprotective effects, in both animal models and humans (Tomé-Carneiro et al., 2013, Wong et al., 2016b). A meta-analysis of randomized controlled trials involving 247 participants reported that a high dose (>150mg/day) of resveratrol could significantly reduce systolic blood pressure (Liu et al., 2015). An exciting large-scale study (the Systolic Blood Pressure Intervention Trial; SPRINT- MIND; Ambrosius et al., 2014) showed promising results relating to intensive blood pressure lowering and cognitive functioning. Significant improvements in mild cognitive impairment (MCI) risk and combined risk of MCI/dementia outcomes were observed following treatment that lowered systolic blood pressure to a target of less than 120mm Hg as compared to the clinical standard of 140mm Hg. This finding further corroborates the cardiovascular-cognition connection, and highlights the potential for use of vasoactive compounds in maintaining cognitive health in the aging process.

This thesis has identified a link between cognitive abilities and cerebrovascular integrity. Modification of the functioning of the microvasculature in the brain could potentially improve cognitive functions, delaying or even preventing neurodegeneration and ultimately dementia. Delaying the onset of dementia symptoms for even 12 months is estimated to reduce global prevalence by almost 10
million cases over the next 30 years (Brookmeyer et al., 2007). This raises the question, if vascular function can be improved via lifestyle, diet or antioxidant interventions, at what point will the introduction of these interventions be most successful?

The results of the current empirical studies suggest that there is a connection between vascular function and cognition in older people without cognitive impairment. Numerous investigations have been focused toward identifying whether hemodynamic processes are modifiable, and to what extent. Momentum is gaining for lifestyle interventions which may help improve vascular function. Diet, cardiovascular exercise, social support and education are key factors associated with mental decline and dementia prevalence. Vasoactive nutritional interventions can aid in the prevention and treatment of cognitive slowing. Resveratrol (Wong et al., 2016a), cocoa flavanols (Massee et al., 2015), green tea extract (Scholey et al., 2012), fish oil supplements (Pase et al., 2015) and curcumin (Akazawa et al., 2012) amongst others may enhance vascular function or CBF to elicit improvements in memory, processing speed and/or mood. A study by Guiney et al. (Guiney et al., 2015) reported that CBF regulation predicts better cognitive inhibitory control in young healthy adults. Better CBF regulation was linked with more frequent physical activity and better aerobic fitness. This suggests that regular physical exercise benefiting the cerebrovascular system improves the capacity for cerebrovascular vessels to respond to changes in stimuli, thus driving the physical activity-related cognitive improvements (Guiney et al., 2015).

8.7 Conclusion
The current thesis sought to investigate the extent to which normal age-associated alterations in vascular integrity and cerebral blood flow affect neurocognitive function. Findings suggest that cerebrovascular health is an important contributor to cognitive performance in healthy aging. Significant novel results expand previous findings that CVR and CBF are related to cognitive performance, with the additional observations that reactivity of the small cerebral blood vessels in memory-specific brain regions impacts memory functioning, and subjective memory concerns in cognitively intact, healthy, community dwelling adults. These findings have significant implications for the wider community, who are an increasingly aging demographic.

The research presented in this thesis provides empirical evidence that the reactivity of the vasculature in specific regions of the brain is related to memory performance, emphasizing the potential for development of interventions that will target blood vessel function. Not only was the responsiveness of hippocampal blood vessels related to memory performance, but also to subjective memory concerns. The identification of these relationships is advantageous as it paves the way for future research into whether reversal of cerebrovascular dysfunction via therapeutic means would improve memory functions, both subjectively and objectively.

It is clear that the pursuit for improved treatments will require greater understanding of the mechanisms that underlie the aging process. Identifying those who are at risk for vascular or hemodynamic impairments may enable health professionals to better target vascular-based therapies to aid in prevention or even treatment for cognitive decline.
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Appendices

Appendix A. Ethics clearance and final report acknowledgment from the Alfred Human Research Ethics Committee

![Ethics Committee Certificate of Approval]

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Please quote project number and time in all correspondence.
The Alfred
Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: 23813 Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in Alzheimer’s disease

Principal Researcher: Dr Andrew Pipingas

Amendment: Revised protocol and PICF

- Change in title from ‘Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in Alzheimer’s disease’ to ‘Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in memory impairment’
- Request for approval of part 2
- Addition of Telephone Interview for Cognitive Status Modified (TICS-m), Memory Complaint Questionnaire (MAC-Q), Verbal Paired Associates Test from the Weschler Memory Scale-revised (WMS-r); all tests are to be conducted over the phone for participants who already had their MRI scan
- Extension of study until end of June 2015

Protocol, Version 3, June 2015;
Participant Information and Consent Form — healthy volunteers, Version 5;
Participant Information and Consent Form — AD, Version 1

have been approved in accordance with your amendment application dated 22/6/2015 on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil
Chair, Ethics Committee

Date: 22/7/2015

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007). The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).
Alfred Health
Alfred Hospital Ethics Committee

PROGRESS or FINAL REPORT AKNOWLEDGEMENT

I hereby acknowledge receipt of the report relating to Project 338/13

This acknowledgement is applicable to:

☐ Progress report – site form:
☐ Progress report – project form covering sites:
☒ Project final report
☐ Site closure report:

Signature: __________________________ Date: 13/06/2018

Angela Herjak (Manager, Ethics & Research Governance)

NOTE: For clinical trials involving an investigational drug or device an Annual Safety Report is required. The sponsor is responsible for completing the annual safety report and submitting this to the reviewing HREC. For investigator initiated studies, the report should be completed by the Principal Investigator from the institution responsible for the trial.
Appendix B. Ethics clearance and final report acknowledgment from the
Swinburne University Human Research Ethics Committee

Dear Andrew and Sarah,

SUHREC Project 2013/316 Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in Alzheimer's disease (Alfred HREC 338/13)

Dr A Pipingas, Ms S Catchlove (student) et al

Approved duration to 30/06/2014

I refer to your application for Swinburne ethics clearance for a supervised Swinburne student project given ethics clearance by The Alfred Human Research Ethics Committee (Alfred HREC 338/13).

Relevant documentation pertaining to your application was emailed on 28 and 29 October and 27 November 2013 with attachments. The documentation was given expedited ethical review on behalf of Swinburne's Human Research Ethics Committee (SUHREC) by a SUHREC delegate, significantly on the basis of the ethical review conducted by Alfred HREC.

I am pleased to advise that, as submitted to date, Swinburne ethics clearance has been given for the project to proceed in line with standard on-going ethics clearance conditions (as applicable) and special conditions here outlined and on the understanding that appropriate insurance arrangements are in place to cover the Swinburne-sanctioned research activity. (Nb Alfred HREC may need to be apprised of the Swinburne ethics clearance.)

Standard conditions:

- All human research activity undertaken under Swinburne auspices must conform to Swinburne and external regulatory standards, including the current National Statement on Ethical Conduct in Human Research and with respect to secure data use, retention and disposal.

- The named Swinburne Chief Investigator/Supervisor remains responsible for any personnel appointed to or associated with the project being made aware of ethics clearance conditions, including research and consent procedures or instruments approved. Any change in chief investigator/supervisor requires timely notification and SUHREC endorsement.

- The above project has been approved as submitted for ethical review by or on behalf of SUHREC. Amendments to approved procedures or instruments ordinarily require prior ethical appraisal/clearance. SUHREC must be notified immediately or as soon as possible thereafter of (a) any serious or unexpected adverse effects on participants and any redress measures; (b) proposed changes in protocols; and (c) unforeseen events which might affect continued ethical acceptability of the project.

- At a minimum, an annual report on the progress of the project is required as well as at the conclusion (or abandonment) of the project. (A copy of any progress, annual or final report submitted to Alfred HREC also being submitted to the Research Ethics office should meet this requirement; similarly with any request to modify the approved protocol.)

- A duly authorised external or internal audit of the project may be undertaken at any time.

Special Conditions:
- Ethics clearance is limited to the younger cohort (20-45 yrs. old). Request for ethics clearance for the older cohort (55-80yrs old) will only be considered after submission to the Research Ethics Office, the approvals from the Neuroimaging Committee and Biosafety Committee of standard operating procedures (SOPs) for the older cohort (55-80 yrs. old) in the MRI.

- Ethics clearance is limited to the first part of the project. Request for ethics clearance for the second part (involving participants with AD) will be considered after funding has been obtained.

**The approved documentation includes:**

- Study Protocol Version 2, October 2013
- PICF Healthy participants Version 4
- PICF AD participants Version 4

Please contact me if you have any queries about Swinburne on-going ethics clearance and if you need a signed Swinburne ethics clearance certificate, citing the SUHREC project number. Copies of clearance emails should be retained as part of project record-keeping.

Best wishes for the project.

Yours sincerely

Ann

____________________________________
Dr Ann Gaeth
Administration Officer (Research Ethics)
Swinburne Research (H68)
Swinburne University of Technology
P O Box 218
HAZTHORN VIC 3122
Ph +61 3 9214 8356
To: Dr A Pipingas, Ms S Catchlove FHAD

Dear Andrew and Sarah,

**SUHREC Project 2013/316 Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in memory impairment (Alfred HREC 338/13)**

Dr A Pipingas, Ms S Catchlove (student) et al

Approved duration to 30/06/2014; extended to 30/6/2015; [August 2014]; extended to 30/06/2016 [July 2015]


I refer to your e-mail of 30 July 2015 in which you requested a modification to the project by extending the study to 30/06/2016, changing the project title, addition of telephone interviews/questionnaires, changes to protocol (updated to version 3, Jun 2015) and updated consent instruments (version 1 (AD) and version 5 (healthy volunteers)). The documentation (including further information provided on 31 July 2015) has been reviewed.

I am pleased to advise that, as modified to date, the project/protocol may continue in line with standard ethics clearance conditions previously communicated and reprinted below.

Please contact me if you have any queries about on-going ethics clearance, citing the SUHREC project number. Copies of clearance emails should be retained as part of project record-keeping.

As before, best wishes for the project.

Kind regards,

Astrid Nordmann

---------------------------------------------

Dr Astrid Nordmann

**Research Ethics Executive Officer**

Swinburne Research (H68)

Swinburne University of Technology

PO Box 218, Hawthorn, VIC 3122

Tel: +613 9214 3845

Fax: +613 9214 5267

Email: anordmann@swin.edu.au
Dear Andrew Pipingas,

Re: Final Report for the project (Report Date: 08-08-2016)

2013/316 'Alterations in cerebral blood flow, perfusion and vascular function across the adult lifespan and in memory impairment (Alfred HREC 338/13)'

The Final report for the above project (Report Date: 08-08-2016) has been processed and satisfies the reporting requirements set under the terms of ethics clearance.

Research Ethics Team

Swinburne Research (H68)
Swinburne University of Technology
PO Box 218
HAWTHORN VIC 3122
Tel: 03 9214 3845
Fax: 03 9214 5267
Email: resethics@swin.edu.au
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With kind regards,

---

Joel Lagmay
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Global Open Research Support

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Tessa Gregory
Journal Media Manager, PLOS
1160 Battery Street, Suite 225, San Francisco, CA 94111

Case Number: 05795290
Appendix E. Acceptance email from Journal of Experimental Neuroscience

From: Journal of Experimental Neuroscience  
Sent: Monday, 4 June 2018 12:02:09 PM (UTC+09:30) Adelaide  
To: Sarah Catchlove  
Subject: Journal of Experimental Neuroscience - Decision on Manuscript ID EXN-18-0025.R1  

03-Jun-2018  

Dear Miss Catchlove,  

It is a pleasure to accept your manuscript entitled "Regional cerebrovascular reactivity and cognitive performance in healthy aging" in its current form for publication in Journal of Experimental Neuroscience. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.  

Thank you for your fine contribution. On behalf of the Editors of Journal of Experimental Neuroscience, we look forward to your continued contributions to the Journal.  

Sincerely,  
Dr. Elaine Ellerton  
Editor in Chief, Journal of Experimental Neuroscience  
elaine.ellerton@sagepub.com
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- Robin Wright rwright@swin.edu.au
  Manager, Licensing, Acquisitions and Copyright

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Best wishes,
Joe Cho
Morgan & Claypool Publishers
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Subject: Re: Permission to re-use diagram from your webpage

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Thanks for letting us know.

Regards
Alex
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Title: QUIPSS II with thin-slice TI1 periodic saturation: A method for improving accuracy of quantitative perfusion imaging using pulsed arterial spin labeling

Author: Wen-Ming Luh, Eric C. Wong, Peter A. Bandettini, et al

Publication: Magnetic Resonance in Medicine

Publisher: John Wiley and Sons

Date: Jun 9, 1999

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Licensed Content Date Jun 9, 1999
Licensed Content Volume 41
Licensed Content Issue 6
Licensed Content Pages 9
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<td>Title of your thesis / dissertation</td>
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<td>Expected completion date</td>
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<td>Expected size (number of pages)</td>
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Ms. Sarah Catchlove  
Swinburne University  

Requestor Location  
Hawthorn, VIC 3122  
Australia  
Attn: Ms. Sarah Catchlove  

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DECLARATION
We hereby declare our contribution to the publication of the 'paper' entitled:

Magnetic Resonance Imaging for assessment of CUE and relationship to cognition

First Author
Name: Sarah Catchlove  Signature:  
Percentage of contribution: 40%  Date: 15/06/2018
Brief description of contribution to the 'paper' and your central responsibilities/role on project:

Second Author
Name: Andrew Pippingas  Signature:  
Percentage of contribution: ___%  Date: 15/06/2018
Brief description of your contribution to the 'paper':

Third Author
Name: Matthew Hughes  Signature:  
Percentage of contribution: ___%  Date: 15/06/2018
Brief description of your contribution to the 'paper':

Fourth Author
Name: Helen Macpherson  Signature:  
Percentage of contribution: ___%  Date: 15/06/2018
Brief description of your contribution to the 'paper':

Principal Coordinating Supervisor: Name: Andrew Pippingas  Signature:  
Date: 15/06/2018

In the case of more than four authors please attach another sheet with the names, signatures and contribution of the authors.
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We hereby declare our contribution to the publication of the ‘paper’ entitled:

Cerebrovascular reactivity and cognitive decline in healthy aging

First Author
Name: Sarah Catelini Signature:
Percentage of contribution: 60% Date: 15/06/2013
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Second Author
Name: Todd Parrish Signature:
Percentage of contribution: __% Date: __/__/2013
Brief description of your contribution to the ‘paper’:

Third Author
Name: Yufan Chen Signature:
Percentage of contribution: __% Date: 15/06/2013
Brief description of your contribution to the ‘paper’:

Fourth Author
Name: Matthew Hughes Signature:
Percentage of contribution: __% Date: 15/06/2013
Brief description of your contribution to the ‘paper’:

Principal Coordinating Supervisor: Name: Andrew Pizzagalli Signature:
Date: 15/06/2013

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First Author
Name: Helen Macpherson
Signature: [Signature]
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Brief description of contribution to the ‘paper’ and your central responsibilities/role on project:

Second Author
Name: Andrew Pippingas
Signature: [Signature]
Percentage of contribution: ___% Date: 15/06/2018
Brief description of your contribution to the ‘paper’:

Third Author
Name: [Name]
Signature: [Signature]
Percentage of contribution: ___% Date: [Date]
Brief description of your contribution to the ‘paper’:

Fourth Author
Name: [Name]
Signature: [Signature]
Percentage of contribution: ___% Date: [Date]
Brief description of your contribution to the ‘paper’:

Principal Coordinating Supervisor: Name: Andrew Pippingas
Signature: [Signature]
Date: 15/06/2018

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DECLARATION
We hereby declare our contribution to the publication of the 'paper' entitled:
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First Author
Name: Sarah Catchlove Signature: [Signature]
Percentage of contribution: 80.0 % Date: 15 Oct 2018
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Second Author
Name: Helen Macpherson Signature: [Signature]
Percentage of contribution: ___ % Date: 15 Oct 2018
Brief description of your contribution to the 'paper':

Third Author
Name: Matthew Hughes Signature: [Signature]
Percentage of contribution: ___ % Date: 15 Oct 2018
Brief description of your contribution to the 'paper':

Fourth Author
Name: Vicente Chen Signature: [Signature]
Percentage of contribution: ___ % Date: 15 Oct 2018
Brief description of your contribution to the 'paper':

Principal Coordinating Supervisor: Name: Andrew Papagrigoriou Signature: [Signature]
Date: 15 Oct 2018

In the case of more than four authors please attach another sheet with the names, signatures and contribution of the authors.
We hereby declare our contribution to the publication of the ‘paper’ entitled:

An investigation of cerebral oxygen utilisation, cerebral cognition in healthy young adults.

Fifth Author
Name: Todd Parrish
Percentage of contribution: ___% 
Date: 13/06/2018
Signature: 

Brief description of contribution to the ‘paper’ and your central responsibilities/role on project:

Sixth Author
Name: Andrew Pipangas
Percentage of contribution: ___% 
Date: 13/06/2018
Signature: 

Brief description of your contribution to the ‘paper’:

Third Author
Name: 
Percentage of contribution: ___% 
Date: _/__/____
Signature: 

Brief description of your contribution to the ‘paper’:

Fourth Author
Name: 
Percentage of contribution: ___% 
Date: _/__/____
Signature: 

Brief description of your contribution to the ‘paper’:

Principal Coordinating Supervisor: Name: Andrew Pipangas
Signature: 
Date: 13/06/2018

In the case of more than four authors please attach another sheet with the names, signatures and contribution of the authors.
Appendix K. Supplementary Material for PLoS One publication- Tables S1 and S2

**Table S1** Table showing the results of univariate GLM on age, gender, education and tCBF contributions to memory score for younger and older adults. $R^2$ values for younger adults was .17, and older adults was .10

<table>
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<td>tCBF</td>
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<td>Education (years)</td>
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<td>tCBF</td>
<td>2.11</td>
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**Table S2** Table showing the results of univariate GLM on age, gender, education and gmCBF contributions to memory score for younger and older adults. $R^2$ values for younger adults was .17, and older adults was .06

<table>
<thead>
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<td>Education (years)</td>
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Calculation of grey matter cerebral blood flow (gmCBF)

Calculation of CBF in the grey and white matter on the assumption that white matter flow is approximately 40% that of grey matter as per previous literature (25). The following equations were used to estimate gmCBF from tCBF values:

$$tCBFv = \frac{tCBF}{(V_{gm} + V_{wm}) \cdot \rho} \quad [1]$$

$$tCBFv = gmCBFv + wmCBF \quad [2]$$

$$wmCBFv = 0.4 \cdot gmCBF \quad [3]$$

$$tCBFv = gmCBFv + 0.4 \cdot gmCBFv = 1.4 \cdot gmCBFv \quad [4]$$

$$gmCBFv = \frac{tCBFv}{1.4} = \frac{tCBF}{(1.4 \cdot (V_{gm} + V_{wm}) \cdot \rho)} \quad [5]$$

Where $V_{gm}$ and $V_{wm}$ are the volumes of grey and white matter respectively in ml. $\rho$ is the mass density of tissue used as the scaling factor to convert tissue volumes to mass, assumed as 1.06g/ml (85). $tCBFv$ is the volume-corrected total CBF, normalized to the volumes of grey and white matter, in ml/100g brain tissue/ min. $gmCBFv$ and $wmCBFv$ are the volume corrected blood flows for grey and white matter respectively. A 30-second baseline of arterial oxygen saturation was acquired with a pulse oximeter and averaged for each participant to give the value of $Y_a$. $tCBF$
was measured using phase-contrast MRI and $Y_v$ was estimated using T2- relaxation-under-spin-tagging (TRUST) (74).