

**Influence of age, exercise and atrial fibrillation on the  
cerebrovascular function**

by

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## **ABSTRACT**

Ageing is associated with morphologic and functional changes in the brain, and is a major risk factor for cerebral vascular disease and dementia. Exercise is well known to promote cardiovascular health and reduce the age-related cognitive decline, but the mechanisms underlying this protective effect are not fully understood. Cardiovascular diseases, such as atrial fibrillation (AF), can exacerbate the risk of brain disease. This thesis aimed to assess the influence of ageing, exercise and AF on the cerebral blood flow and its regulation. It was observed that a difference of  $\approx 55\%$  in daily physical activity levels in a cohort of healthy old individuals did not influence internal carotid and vertebral artery blood flow. However, a high cardiorespiratory fitness was associated with increased bilateral internal carotid artery blood flow in young and old individuals when compared to their sedentary counterparts, but the influence on cerebral vasodilatory reserve is still unclear. AF patients had lower cerebral vasodilatory reserve, but preserved dynamic cerebral autoregulation, when compared to healthy controls. Future research is needed to elucidate whether cerebral haemodynamics is modified by exercise training in AF patients.

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# TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION .....	1
CHAPTER 2: LITERATURE REVIEW .....	4
2.1. Overview of the cerebral anatomy and circulation.....	6
2.2. Methods for the assessment of cerebral blood flow .....	10
2.3. Cerebral metabolism .....	13
2.4. Cerebral blood flow regulation – from phenomena to mechanisms.....	15
2.4.1. Neuro-glia-vascular coupling and cerebral metabolism .....	15
2.4.2. Arterial blood pressure.....	17
2.4.3. Arterial blood gases .....	20
2.4.4. Autonomic nerves and intrinsic pathways in the brain .....	25
2.4.5. Cardiac output .....	27
2.5. The ageing brain: structure and function .....	30
2.6. Cerebral blood flow and exercise .....	32
2.6.1. Acute effects of exercise on the cerebral blood flow.....	32
2.6.2. Cerebral metabolism during exercise .....	34
2.6.3. Chronic effects of exercise on cerebral blood flow.....	35
2.7. Atrial fibrillation – from the heart to the brain.....	37
2.7.1. Definition, epidemiology and pathophysiology .....	37
2.7.2. Diagnosis, treatment and potential complications.....	39
2.7.3. Cerebral blood flow in atrial fibrillation .....	41
2.8. Aims and hypotheses of this thesis .....	43
CHAPTER 3: GENERAL METHODS .....	44
3.1. Ethical approval and consent .....	45
3.2. Variables measured .....	46
3.2.1. Haemodynamic and respiratory .....	46
3.2.2. Cerebral blood flow .....	47
3.2.3. Physical activity .....	50
3.2.4. Cognition .....	51
3.3. Interventions.....	52
3.3.1. Hypercapnic and hypocapnic tests .....	52
3.3.2. Assessment of cerebral autoregulation.....	55
3.4. Data acquisition and analysis.....	60
3.5. Reliability of the methods .....	61
3.5.1. Doppler ultrasound of the extracranial vessels .....	62
3.5.2. Conclusions .....	63
CHAPTER 4: INFLUENCE OF DAILY PHYSICAL ACTIVITY ON CEREBRAL BLOOD FLOW IN HEALTHY OLD INDIVIDUALS.....	64
4.1. Introduction .....	65
4.2. Methods .....	68
4.2.1. Cohort characteristics.....	68

4.2.2. Measured variables .....	69
4.2.3. Data analysis .....	69
4.2.4. Statistical analysis .....	70
4.3. Results .....	71
4.4. Discussion.....	77
CHAPTER 5: IMPACT OF AEROBIC FITNESS ON CEREBRAL BLOOD FLOW AND CEREBRAL VASCULAR RESPONSIVENESS TO CO <sub>2</sub> IN YOUNG AND OLDER MEN .....	83
5.1. Abstract.....	84
5.2. Introduction .....	85
5.3. Methods .....	88
5.3.1. Measurements .....	88
5.3.2. Protocol .....	89
5.3.3. Data analysis .....	90
5.3.4. Statistical analysis .....	91
5.4. Results .....	93
5.4.1. Participant characteristics.....	93
5.4.2. Resting cerebral blood flow .....	95
5.4.3. Cerebral vascular reactivity .....	97
5.5. Discussion.....	101
CHAPTER 6: CEREBRAL BLOOD FLOW REGULATION IN ATRIAL FIBRILLATION .....	107
6.1. Introduction .....	108
6.2. Methods .....	111
6.2.1. Ethics.....	111
6.2.2. Participants.....	111
6.2.3. Measurements.....	112
6.2.4. Protocol .....	112
6.2.5. Data analysis .....	114
6.2.6. Statistical analysis .....	114
6.3. Results .....	116
6.4. Discussion.....	130
CHAPTER 7: GENERAL DISCUSSION AND FUTURE DIRECTIONS .....	139
LIST OF REFERENCES .....	147

## LIST OF ILLUSTRATIONS

<b>Figure 2.1.</b> Principal fissures and lobes of the cerebrum viewed laterally .....	6
<b>Figure 2.2.</b> Arteries of the brain, seen caudally .....	8
<b>Figure 2.3.</b> Limits of the cerebral autoregulation: historical view and current understanding .....	18
<b>Figure 2.4.</b> Putative mechanisms of cerebral vessel dilatation during hypercapnia .....	22
<b>Figure 2.5.</b> Electrocardiogram of atrial fibrillation (AF, top) and normal sinus rhythm (bottom) .....	38
<b>Figure 3.1.</b> Ultrasound images of the internal carotid and vertebral arteries ..	50
<b>Figure 3.2.</b> An original record of MCA V, blood pressure and $P_{ET}CO_2$ responses to the hypercapnic and hypocapnic tests in a representative individual .....	54
<b>Figure 3.3.</b> An original record of MCA V, blood pressure and $P_{ET}CO_2$ responses to the single sit-to-stand test in a representative individual .....	56
<b>Figure 3.4.</b> An original record of MCA V, blood pressure and $P_{ET}CO_2$ responses to the repeated squat-to-stand test in a representative individual .....	59
<b>Figure 3.5.</b> Bland-Altman plots of the between days reproducibility of blood flow measurements .....	62
<b>Figure 4.1.</b> Global and regional cerebral blood flow (CBF) in low and high physical activity groups .....	75
<b>Figure 5.1.</b> Bilateral internal carotid artery (ICA) blood flow (A), vertebral artery (VA) blood flow (B) and mean middle cerebral artery blood flow velocity (MCA $V_{mean}$ , C) in young and old, trained and untrained participants .....	96
<b>Figure 5.2.</b> Cerebral vascular reactivity to carbon dioxide ( $CVR_{CO_2}$ ) at the internal carotid artery (ICA, A and C) and middle cerebral artery (MCA, B and D) in young and old, trained and untrained participants .....	98



**Figure 6.1.** Resting cerebral vascular and respiratory characteristics in healthy controls, patients with atrial fibrillation (AF) and hypertension (HT) ..... 118

**Figure 6.2.** Graphical representation of the cerebral and systemic haemodynamic and ventilatory responses to the hypocapnic and hypercapnic challenges ..... 120

**Figure 6.3.** Hypercapnic and hypocapnic cerebral vascular reactivity to CO<sub>2</sub> (CVR<sub>CO2</sub>) slopes in healthy controls, patients with atrial fibrillation (AF) and hypertension (HT) ..... 121

**Figure 6.4.** Middle cerebral artery blood velocity responses to a single sit-to-stand manoeuvre in healthy controls, patients with atrial fibrillation and hypertension ..... 123

## LIST OF TABLES

<b>Table 4.1.</b> Participant characteristics .....	72
<b>Table 4.2.</b> Physical activity as measured by 7-day accelerometry .....	73
<b>Table 4.3.</b> Arterial diameter and blood velocity measured in the internal carotid and vertebral arteries .....	76
<b>Table 5.1.</b> Participant characteristics .....	94
<b>Table 5.2.</b> Changes in end tidal CO <sub>2</sub> , blood pressure, arterial diameter and blood flow velocity at the right ICA in response to hypercapnia .....	99
<b>Table 6.1.</b> Participant characteristics and baseline data .....	117
<b>Table 6.2.</b> Changes in haemodynamic and respiratory parameters during the hypercapnic and hypocapnic challenges .....	122
<b>Table 6.3.</b> Changes in haemodynamic, respiratory and cerebral vascular parameters during the single sit-to-stand manoeuvre .....	125
<b>Table 6.4.</b> Dynamic cerebral autoregulation quantified by transfer function analysis of MAP and MCA $V_{\text{mean}}$ signals with participants resting supine .....	126
<b>Table 6.5.</b> Dynamic cerebral autoregulation quantified by transfer function analysis of MAP and MCA $V_{\text{mean}}$ signals during a repeated squat-to-stand manoeuvre .....	128

## **LIST OF ABBREVIATIONS**

ANOVA – Analysis of variance

AF – Atrial fibrillation

BMI – Body mass index

CMRO<sub>2</sub> – Cerebral metabolic rate of oxygen

CV – Coefficient of variation

CVCi – Cerebral vascular conductance index

CVR<sub>CO<sub>2</sub></sub> – Cerebral vascular reactivity to carbon dioxide

ECG – Electrocardiogram

ICA – Internal carotid artery

MAP – Mean arterial pressure

MCA – Middle cerebral artery

MoCA – Montréal cognitive assessment

NO – Nitric oxide

OCI – O<sub>2</sub>-carbohydrate index

OGI – O<sub>2</sub>-glucose index

PaCO<sub>2</sub> – Partial pressure of arterial carbon dioxide

PaO<sub>2</sub> – Partial pressure of arterial oxygen

SD – Standard deviation

VA – Vertebral artery

V<sub>mean</sub> – Mean blood flow velocity

VO<sub>2</sub>max – Maximal oxygen consumption

## **CHAPTER 1: INTRODUCTION**

The maintenance of a steady cerebral blood flow is of paramount importance for optimal function of the brain. Even a few seconds to minutes of ischaemia can cause significant damage to the brain, either directly or via a secondary mechanism (such as falls and trauma), due to the necessity of maintaining homeostasis and very limited energy reserves (Madsen *et al.*, 1998). To prevent hypo- or hyperperfusion, the cerebral circulation responds to changes in brain metabolism, arterial blood pressure, partial pressures of arterial oxygen (PaO<sub>2</sub>) and carbon dioxide (PaCO<sub>2</sub>) and a complex network of nerve fibres that control the inflow of blood to the encephalic structures (Lassen, 1959; Fox & Raichle, 1986).

Ageing is associated with a reduction in cerebral blood flow, as well as aspects of cognition such as signal processing speed, even in healthy individuals (Leenders *et al.*, 1990; Burgmans *et al.*, 2011). Furthermore, old age is one of the main risk factors for heart and neurodegenerative diseases, both of which can affect the brain (Sacco *et al.*, 1997; Lindsay *et al.*, 2002). Exercise has been proposed as a means of enhancing quality of life and reducing morbidity and mortality in a myriad of diseases (e.g., hypertension, dementia, diabetes), and a high cardiorespiratory fitness in old individuals is reported by some to improve cerebral vascular function and cognition (Bailey *et al.*, 2013b; Barnes *et al.*, 2013). However, little is known about the effects of physical activity and exercise on the cerebral blood flow and the mechanisms of its regulation in health and disease in ageing individuals.

Atrial fibrillation (AF) is a heart disease strongly associated with age that substantially increases the risk of cerebrovascular disease and dementia (Wolf

*et al.*, 1991). Even with proper optimal anticoagulant therapy, people with AF have an increased risk of stroke and the mechanisms of this increased risk are not fully understood.

This thesis aims to assess the influence of ageing, habitual physical activity, cardiorespiratory fitness and AF on the cerebral blood flow and its regulation. A literature review (Chapter 2) will lay the foundation and identify the gaps in knowledge that will be addressed along the thesis, while Chapter 3 will present the methodology used and assess its reliability. The experimental chapters aim to assess the influence of healthy ageing, physical activity, cardiorespiratory fitness and disease on cerebral blood flow, its regulation and cognition. Chapter 4 will determine the influence of daily physical activity on the cerebral blood flow and cognition in a cohort of healthy elderly individuals. Chapter 5 will extend this work by comparing not only cerebral blood flow but also cerebral vascular reactivity to CO<sub>2</sub> in young aerobically trained and untrained and old aerobically trained and untrained individuals. Chapter 6 will investigate the impact of AF on the cerebral blood flow and its regulation, as well as cognition. The findings of this thesis will be summarised and integrated in Chapter 7, along with the provision of a perspective on the broader implications of these findings from this thesis and possible directions for future studies.

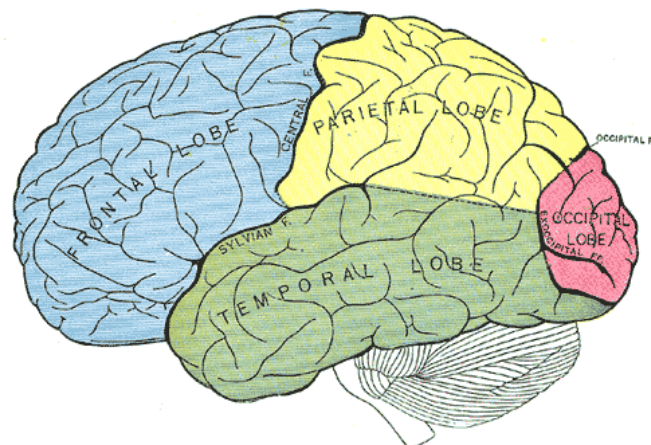
## **CHAPTER 2: LITERATURE REVIEW**

This literature review will provide basic information about brain structure, present the methods commonly used for the assessment of cerebral blood flow, and outline the current understanding of the key mechanisms implicated in the regulation of cerebral blood flow. In addition, the effects of age, acute exercise, cardiorespiratory fitness and heart disease on the regulation of cerebral blood flow will be evaluated, with an emphasis on the limits of present understanding highlighted with reference to the experimental chapters contained within this thesis. Parts of this work have been published in *The Journal of Physiology* (Braz & Fisher, 2015).



## 2.1. Overview of the cerebral anatomy and circulation

The brain is the central signal-processing unit in humans, encased by the cranium and considered to be the most complex organ in the body. It is composed by a myriad of cell types, including but not limited to neurons, astrocytes, oligodendrocytes, microglia, vascular and precursor cells (Darmanis *et al.*, 2015). The cerebral cortex, formed mainly by the cell bodies of neurons and glial cells can be divided in lobes according to its topography as shown in Figure 2.1. These regions may be divided in sensory, motor and association areas which occupy specific gyri and sulci, and each area have zones related to a specific task.

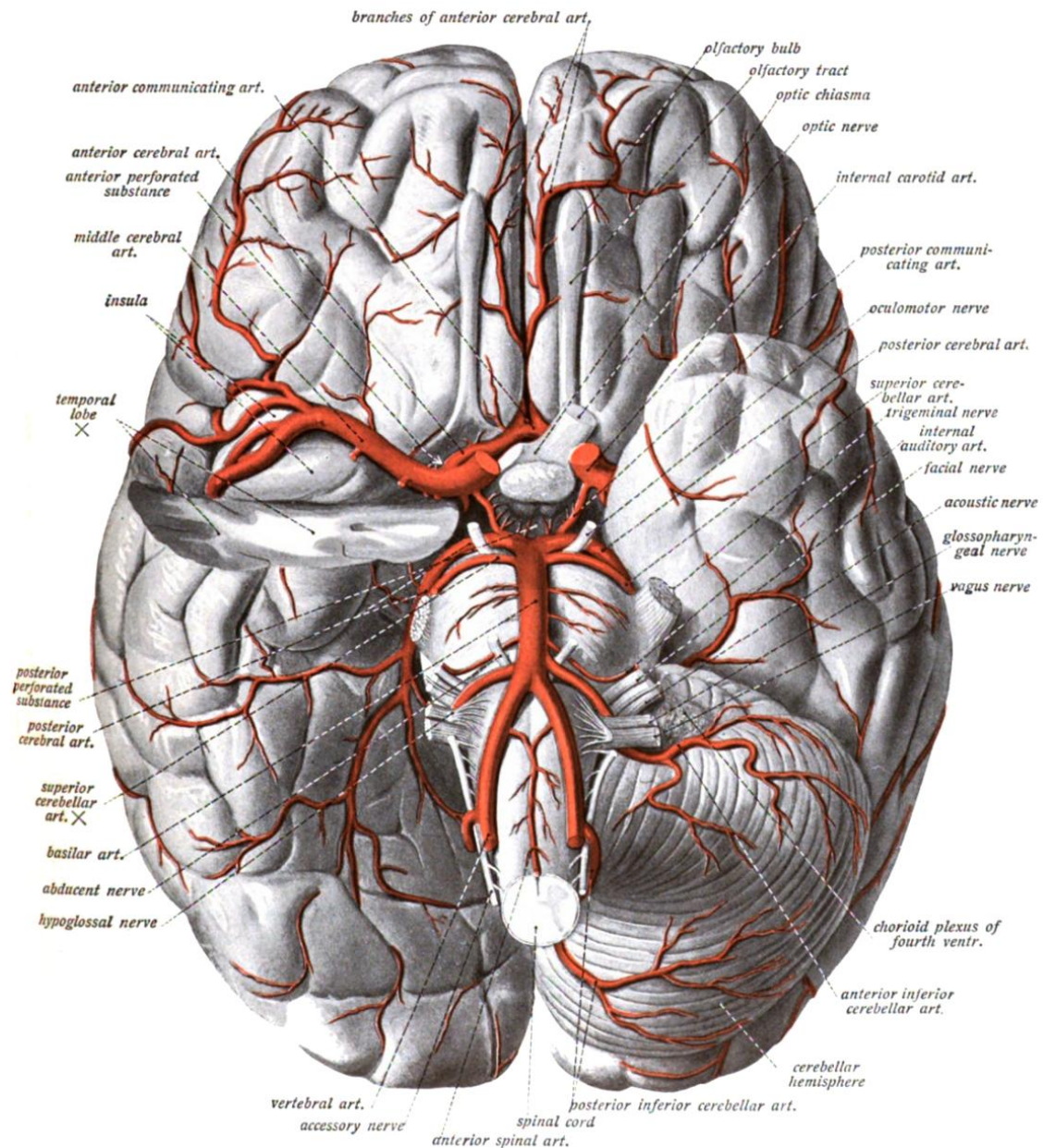


**Figure 2.1.** Principal fissures and lobes of the cerebrum viewed laterally  
From: Henry Gray (1918) *Anatomy of the Human Body*

The main inflow of blood to the brain comes from two pairs of extracranial arteries: the internal carotid arteries (ICA) anteriorly accounting for ~72% of the global cerebral blood flow and the vertebral arteries (VA) posteriorly accounting for ~28% of the cerebral blood flow (Zarrinkoob *et al.*, 2015). The ICA arises

bilaterally from the common carotid artery as it bifurcates to form the ICA and the external carotid artery approximately at the level of the fourth cervical vertebra. It then enters the skull through the carotid canal located in the temporal bone and, after providing several small branches, penetrates the *Dura mater* when exiting the cavernous sinus. In the subarachnoid space, the ICA provides branches to the orbit (ophthalmic artery), pituitary gland (meningohypophyseal and superior hypophyseal arteries) and posterior cerebral circulation (posterior communicating artery), before dividing into its terminal branches: the middle cerebral artery (MCA) and the anterior cerebral artery.

The VA arises bilaterally from the subclavian arteries, proceeding cephalically through the transverse foramen of each cervical vertebra from C6 to C1, when they circle C1 and enter the cranium through the *foramen magnum*. Both VAs combine at the brainstem level to form the basilar artery, which provides several branches to provide blood to the brainstem via the complex vertebrobasilar system. The basilar artery provides branches to the cerebellum and proceeds to divide into the terminal branches of the posterior cerebral arteries. The posterior cerebral arteries receive branches from the ICA and the series of arterial anastomoses at the base of the brain connecting the anterior and posterior circulations called the circle of Willis (Figure 2.2).



**Figure 2.2.** Arteries of the brain, seen caudally  
 From: "Sobo 1909 3 548" by Dr. Johannes Sobotta - Atlas and Text-book of Human Anatomy Volume III Vascular System, Lymphatic system, Nervous system and Sense Organs. Licensed under Public Domain via Wikimedia Commons

The anterior cerebral arteries supply blood for the medial portion of the frontal and parietal lobes including the leg motor and sensory cortex, the anterior portions of most midline structures such as *corpus callosum*, basal ganglia and internal capsule. The MCAs are responsible for the irrigation of most of the

brain, including the frontal, temporal and parietal lobes (except from areas supplied by the anterior and posterior cerebral arteries), insula and deep structures. The posterior cerebral arteries deliver the blood to the occipital lobe, inferior areas of the temporal lobe and some deep structures such as the thalamus and cerebral peduncle.

The cerebral arteries (i.e. MCA) provide smaller branches penetrating the cerebral tissue until themselves end up penetrating the brain (Cipolla, 2009). At the surface of the brain, the pial vessels are surrounded by the leptomeninges and cerebrospinal fluid until they penetrate into the brain, where they continue into the Virchow-Robin space. With increasing depth, the arteriolar pia-mater sheath gives way to the formation of the parenchymal arterioles, capillaries and the neurovascular units, which are comprised of the interface between the parenchymal arterioles and capillaries with neurons and glial cells such as the astrocyte end-feet (Rennels & Nelson, 1975). The neurovascular unit is where the glia and neurons receive nutrients and dispose of products of cerebral metabolism.

## **2.2. Methods for the assessment of cerebral blood flow**

The ability to measure cerebral blood flow and understand how it is regulated has long attracted scientists. Records from the eighteenth century show the use of invasive techniques to understand the cerebral circulation. Albrecht von Haller observed exposed pia mater to assess the cerebral volume variation and cerebral movement inside the skull (Haller, 1757). In the nineteenth century new methods were proposed including the measurement of cerebral venous outflow, the measurement of pressures within the systemic and cerebral circulations, and plethysmography of the brain in animals and people with cranial defects, as will be described below (Mosso, 1881; Roy & Sherrington, 1890). Other methods developed in the early twentieth century used local changes in tissue temperature, arterial-venous differences for oxygen and metabolites, and bubble flowmetry, which was the first technique used to quantitatively measure blood flow to the brain in monkeys (Dumke & Schmidt, 1943). Although the latter was technically challenging and needed extensive surgery and monitoring, meaning that it was unsuitable to be performed in humans.

The first direct measures of cerebral blood flow in non-anesthetized humans were made by Kety and Schmidt (1945; 1948b) using the nitrous oxide inhalation technique. This technique is non-invasive, using inhalation of an inert gas (nitrous oxide in this case) and it is based on the fact that the rate of the uptake of the inert gas depends on the blood flow to that organ. Although it was responsible for a great contribution on the generation of knowledge on cerebral blood flow and its regulation, some limitations of the technique stimulated its improvement and the development of more sophisticated means of assessing

the brain circulation. In order to address the limitation of only having the global cerebral blood flow with the nitrous oxide inhalation technique, several modifications were described over the 1950s and 1960s using inert radioactive gases or venous and arterial blood tracers, ranging from [<sup>131</sup>I] trifluoriodomethane in association with autoradiographic recordings to [<sup>85</sup>Kr] or [<sup>133</sup>Xe] with external scintillation detectors, to provide the detection of regional cerebral blood flow changes (Landau *et al.*, 1955; Lassen & Ingvar, 1961; Hoedt-Rasmussen, 1965). Over the next decade, new techniques allowed for the assessment of regional cerebral blood flow as well as cerebral metabolism using tracers such as [<sup>18</sup>F] fluorodeoxyglucose or [<sup>15</sup>O] water associated with single-photon emission computed tomography or positron emission tomography (Phelps *et al.*, 1979; Reivich *et al.*, 1979; Raichle, 1981).

The transcranial Doppler technique was introduced in the early 1980s (Aaslid *et al.*, 1982), and it was non-invasive with a high temporal resolution in measuring beat-by-beat blood velocity at the cerebral arteries. This technique rapidly became an important tool to assess cerebral perfusion in situations where bulky radiation detectors were a problem, such as in many exercise settings (Jorgensen *et al.*, 1992) as well as enabling the continuous monitoring of cerebral blood flow during surgical procedures (Spencer *et al.*, 1990). Although the transcranial Doppler showed clear advantages over the known techniques in several situations, the main limitation of this technique is that it cannot measure the diameter of the insonated artery. In order to extrapolate that the changes in velocity are proportional to changes in blood flow, it is assumed that the arterial diameter is unchanged, which may not be true in some situations

(Wilson *et al.*, 2011). This led to the establishment of duplex Doppler ultrasonography of the extracranial vessels as an equally powerful alternative of non-invasive measurement of cerebral blood flow for bedside or even exercise settings (Hellstrom *et al.*, 1996). In contrast, new techniques were developed in order to achieve great spatial resolution, culminating with the development of blood oxygenation level-dependent contrast and arterial spin labelling used with magnetic resonance imaging (Ogawa *et al.*, 1990; Williams *et al.*, 1992), as well as using exogenous contrast agents to facilitate the measurement of cerebral blood flow (Villringer *et al.*, 1988). The combination of these techniques, each with its own strengths and limitations, provided the tools to greatly improve the understanding of the cerebral circulation and metabolism, and how the cerebral microenvironment and systemic factors can influence cerebral blood flow. These are discussed in more detail below.

### 2.3. Cerebral metabolism

The mass of the brain only accounts for  $\approx 2\%$  of total body mass, but remarkably the brain receives  $\approx 15\%$  of cardiac output during rest and the cerebral metabolic rate for oxygen (CMRO<sub>2</sub>) is  $\approx 25\%$  ( $\approx 60$  mL/min) of whole-body resting oxygen consumption (Kety & Schmidt, 1946; Lassen, 1959). Although there is a small contribution from anaerobic glycolysis ( $\approx 10\%$ ), the oxidation of glucose is the principal mechanism by which the energy demand of the brain is met during resting wakefulness. As such, the molar ratio between the cerebral consumption of oxygen and glucose (O<sub>2</sub>-glucose index: OGI) during rest is slightly lower than 6:1 ( $\approx 5.7$ ) (Siesjö, 1978). However, lactate supplementation during euglycemia promotes a reduction in glucose utilization and may be a source of energy to cerebral tissue while sparing glucose, although the impact of this energy source during rest is still unclear (Smith *et al.*, 2003).

A decrease in cerebral oxygen content is associated with a reduced cerebral metabolism and presyncopal symptoms that may lead to syncope if not reverted promptly (Madsen *et al.*, 1998). Hypoxia decreases neuronal excitatory capacity at first by causing neuron membrane hyperpolarisation followed by depolarisation that becomes permanent after a few minutes, as well as increasing synthesis and release of inhibitor neurotransmitters and neuromodulators such as  $\gamma$ -aminobutyric acid, adenosine, lactate and endogenous opioids (Neubauer *et al.*, 1990). As the period of oxygen deprivation becomes longer, there is a higher proportion of neuro-glial damage, cell death and possibly long-term functional impairment. Not only lower oxygen supply can cause neuronal damage, but glucose itself also plays an important



role. Neurons lack storage of energy substrates and rely on the constant supply of glucose from the blood. Although the amount of glycogen stored primarily in astrocytes might be higher than of free glucose, under normal circumstances the glycogen utilization is slow (Oz *et al.*, 2007). Stepped acute reductions in blood glucose with insulin infusion also lead to impaired brain metabolism with reduced glucose uptake and cognitive function (Boyle *et al.*, 1994), and extremely low levels of blood glucose (e.g. lower than 1.36 mM) cause coma, an isoelectric electroencephalogram and, after a few minutes, neuronal death (Auer, 2004). On the other hand, cerebral hypermetabolism leads to lactate accumulation and adenosine triphosphate depletion, causing neuronal necrosis (Wasterlain *et al.*, 1993) and may be associated with glutamate excitotoxicity, seizures and cerebral damage (Alkonyi *et al.*, 2011). In order to maintain a steady blood flow supply and keep the delivery of oxygen, glucose and other nutrients relatively constant, and to allow adequate washout of substances such as CO<sub>2</sub> and metabolic products, the brain has several mechanisms to control its vascular resistance and blood flow. These are described in detail below.

## **2.4. Cerebral blood flow regulation – from phenomena to mechanisms**

Acute reductions in cerebral perfusion, such as those that can occur during head-up tilt and cause a decrease in cerebral activity within a few seconds, characterized by light-headedness, blurred vision or a complete syncope, parallel to the change in perfusion (Njemanze, 1992). In contrast, cerebral hyperperfusion syndrome is associated with headache, vomiting, confusion, orbital alterations, seizures and even intracranial haemorrhage, which may occur in situations such as hyperperfusion after carotid endarterectomy (van Mook *et al.*, 2005). The main mechanisms of cerebral blood flow regulation are activated by changes in cerebral metabolism, arterial blood pressure, PaCO<sub>2</sub> and PaO<sub>2</sub>, neural mechanisms, metabolites and cardiac output. All these mechanisms are tightly connected to keep the cerebral blood flow in a relatively constant level, which ranges around 50-60 ml·min<sup>-1</sup>·100g<sup>-1</sup> (Leenders *et al.*, 1990).

### **2.4.1. Neuro-glial-vascular coupling and cerebral metabolism**

More than a century ago, it was shown that cerebral blood flow would increase in response to peripheral nerve stimulation in dogs as measured with changes in cerebral volume (Roy & Sherrington, 1890) and even in humans as assessed using brain plethysmography in people with cranial defects (Mosso, 1881). In fact, after visual stimulation, an increase in blood flow at the occipital lobe was detectable by a localized bruit in a patient with *angioma arteriale racemosum* of the left occipital visual cortex (Fulton, 1928). This regional increase in blood flow was later observed with modern methods of cerebral perfusion assessment

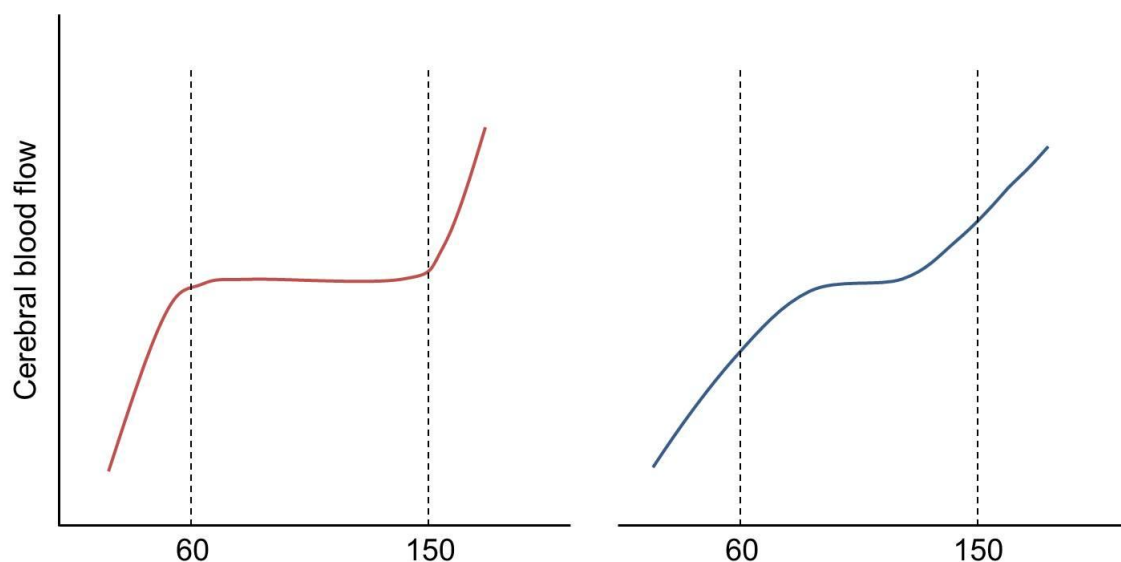
such as positron emission tomography using [ $^{15}\text{O}$ ]  $\text{H}_2\text{O}$  as a tracer (Fox & Raichle, 1984). It was proposed that the increase in regional cerebral blood flow was caused by the increase in cerebral activity. The phenomenon whereby an increase in neuronal energy expenditure is associated with an increase in regional blood flow is commonly known as neurovascular coupling (Attwell *et al.*, 2010). Although it was originally described that the neuronal consumption of oxygen and glucose, and increase in metabolic by-products such as  $\text{CO}_2$ , would eventually lead to the increase in perfusion in a negative feedback manner (Attwell & Laughlin, 2001), current evidence suggests that there is a feedforward mechanism in which the neurons and astrocytes regulate the local blood flow and that may even be actively modulated by pericytes and microglia (Filosa *et al.*, 2015). In fact, synaptic glutamatergic stimulation can promote nitric oxide (NO) synthesis in the neurons which is released and diffuses to the vascular smooth muscle cells causing dilatation (Busija *et al.*, 2007). In addition to NO, energy utilization is characterized by the metabolism of adenosine triphosphate into adenosine, which can bind to its  $A_{2A}$  receptors and also cause cerebral vessel dilatation (Ko *et al.*, 1990). Along with a direct effect on the blood vessels, neuronal activation also causes an increase in  $\text{Ca}^{2+}$  in neighbouring astrocytes, producing arachidonic acid and vasoactive metabolites such as prostaglandins, especially prostaglandin  $E_2$  (in neurons as well as astrocytes), and epoxyeicosatrienoic acids, which cause dilatation of the cerebral arterioles (Zonta *et al.*, 2003). Synaptic glutamatergic stimulation also increases astrocytic intracellular  $\text{Ca}^{2+}$  in a similar rate that of neurons and through the arachidonic acid cascade and possibly through  $\text{K}^+$  release and

glutamate uptake mechanisms (Attwell *et al.*, 2010). Ideally located with end-feet around blood vessels, astrocytes are proposed to be the main drivers of cerebral vessel dilatation, being modulated primarily by neurons and also possibly by microglia and vascular cells (Filosa *et al.*, 2015). Oxygen also influences the functional hyperaemia as a decrease in oxygenation will alter the production of vasoactive messengers such as NO and interfere with cerebral metabolism, causing an increase in adenosine due to adenosine triphosphate substrate utilization and lactate which will, in turn, reduce the clearance of prostaglandins resulting in potentiation of dilatation (Ido *et al.*, 2001; Gordon *et al.*, 2008).

#### **2.4.2. Arterial blood pressure**

The brain possesses the capacity to regulate its own supply of blood flow by changing the luminal diameter of the cerebral arteriolar beds. This phenomenon was first observed in cats with open cranial windows by Mogens Fog while studying the relationship between the arterial blood pressure and pial vascular tone that was independent of neurogenic stimulus (Fog, 1937, 1939). Whenever there is a rise in blood pressure, the smooth muscle in the arterial wall increases its tone, and the opposite happens with a fall in blood pressure, in order to keep cerebral blood flow in an optimal range for the nutrition and protection of the brain over a wide range of physiological arterial pressures. The term coined to describe this process was cerebral autoregulation (Lassen, 1959). As shown in Figure 2.3, a plateau in cerebral blood flow was originally described between a blood pressure range of  $\approx 60$  mmHg to  $\approx 150$  mmHg. However, more recently this view has been contested in favour of a slope or a

plateau with a very limited range over the full autoregulatory range, (Heistad, 1983; Rosenblum, 1995; Tan, 2012). Within these limits, there is a myogenic response from the cerebral arteries that counteract the increased pressure as demonstrated in isolated bovine cerebral arteries over a wide range of pressures with and without functioning smooth muscle (Vinall & Simeone, 1981). When the smooth muscle was eliminated, the arteries behaved as passive conduit arteries increasing the diameter with increases in intraluminal pressure, while the diameter remained constant in the intact isolated arteries until pressures above 150 mmHg caused the arteries dilatation, often with discontinuous regions of smooth muscle contraction (“bead-string” or “sausage” patterns). While it may not account directly for cerebral blood flow regulation, this myogenic mechanism may protect the brain from the deleterious effects of increased blood pressure on the delicate vascular bed (Smeda, 1992).



**Figure 2.3.** Limits of the cerebral autoregulation: historical view and current understanding. Schematic representation of cerebral blood flow over a range of blood pressure levels. The red curve shows the historical view of cerebral autoregulation, with a plateau of the cerebral blood flow ranging from 60 mmHg to 150 mmHg arterial blood pressure, while the blue curve shows a modern view of a varying but relatively constant cerebral blood flow in the autoregulatory range, with a much more limited range plateau observed.

Several cellular mechanisms may contribute to the phenomenon of cerebral autoregulation. The vascular smooth muscle cells have in their membranes stretch-activated cation channels that increase the membrane permeability to  $\text{Ca}^{2+}$  ions with an increase in luminal pressure, promoting vessel constriction (Davis *et al.*, 1992). Several other ion channels may be directly implicated in the mechanism of vascular smooth muscle control of arteriolar tone (Jackson, 2000), but their influence on the human cerebral vasculature remains to be proven. Other mechanisms that may influence cerebral autoregulation are autonomic innervation (Hamner *et al.*, 2010; Hamner *et al.*, 2012) and NO (White *et al.*, 2000; Zhang *et al.*, 2004), although these are subject of debate and their impact on cerebral autoregulation remains unresolved.

An impairment in cerebral autoregulation is, by itself, a risk factor for the development of brain disease and poor outcome in several clinical conditions and surgical procedures such as cardiopulmonary bypass (Ono *et al.*, 2014; Otite *et al.*, 2014). Impaired cerebral autoregulation may be present, contribute to and potentiate the mechanism of diseases that affect the brain such as Alzheimer's (Di Marco *et al.*, 2015), ischaemic stroke (Aries *et al.*, 2010), type 2 diabetes mellitus (Vianna *et al.*, 2015) and hypertension (Zhang *et al.*, 2007). In fact, pharmacological treatment of hypertension or diabetes ketoacidosis significantly improves cerebral autoregulation in these patient groups, showing the importance of maintaining a good control of the underlying diseases that may prevent brain damage (Zhang *et al.*, 2007; Ma *et al.*, 2014).

### **2.4.3. Arterial blood gases**

The PaCO<sub>2</sub> and PaO<sub>2</sub> are strong mediators of blood flow responses in the cerebral circulation. In an evolutionary context these phenomena were hypothesised to be responsible for “washing out” excessive CO<sub>2</sub> and hydrogen ions as well as increasing oxygen delivery when cerebral perfusion is insufficient, although this remains to be proven. The PaCO<sub>2</sub> has been implicated in cerebral blood flow regulation in humans for over 80 years, even before the quantitative cerebral blood flow assessment techniques emerged (Gibbs *et al.*, 1935). Over a decade later, there was the first direct evidence that increases in PaCO<sub>2</sub> promoted a profound increase in cerebral perfusion that was associated with a reduction in cerebral vascular resistance (Kety & Schmidt, 1948a), whereas hyperventilation, and consequent hypocapnia, increased the cerebral vascular resistance and reduced cerebral perfusion (Wasserman & Patterson, 1961). This phenomenon was hypothesised to take place only at the smaller cerebral arteries, such as the vessels of the pia (Wolff *et al.*, 1930) and cerebral surface, with dilatation of the distal middle and anterior cerebral arteries of nearly 29% in response to hypercapnia in contrast to the 4% increase in diameter of the internal carotid and vertebral arteries (Giller *et al.*, 1993). However, recent evidence shows that even the large conduit arteries in the neck can also respond to CO<sub>2</sub> with changes in diameter of ≈25% over stepped changes in PaCO<sub>2</sub> from ≈15 mmHg to ≈65 mmHg (Willie *et al.*, 2012). Furthermore, a lower blood flow reactivity to inhalation of 3% and 6% CO<sub>2</sub> was observed in the posterior circulation of the brain, with the vertebral artery responding ≈30% less than the internal carotid artery and it was

proposed that this lower reactivity to CO<sub>2</sub> would be advantageous to the protection of the brain regions controlling vital functions (Sato *et al.*, 2012b), although this has not been a universal finding (Willie *et al.*, 2012).

Although the exact mechanisms by which CO<sub>2</sub> modifies cerebral vascular smooth muscle tonus are still unclear, it is currently accepted that CO<sub>2</sub> itself lacks direct vascular action and exerts its vascular effects through changes in tissue pH and vasoactive substances (Figure 2.4). It has been shown that, with direct measurement of cerebral arterial diameters in cats through a cranial window, synthetic cerebral spinal fluid with high or low CO<sub>2</sub> levels did not modulate arterial diameter when pH was maintained constant, while cerebral spinal fluid alkalosis or acidosis would cause arterial constriction and dilatation respectively regardless of maintained or altered CO<sub>2</sub> levels (Kontos *et al.*, 1977). The cerebral tissue changes in pH that are followed by an increase or decrease in CO<sub>2</sub> levels may cause potassium channels in the endothelium and vascular smooth muscle to be activated or closed (causing hyperpolarization or reducing the depolarization threshold, respectively), therefore modulating vascular tonus and resistance (Faraci & Sobey, 1996; Kinoshita & Katusic, 1997). Increased PaCO<sub>2</sub> may also contribute to cerebral vasodilatation via a NO-mediated mechanism, and NO molecular pathways are present in neurons, endothelium, and glial cells (Faraci & Brian, 1994). Pharmacological blockade of the NO pathways impairs cerebral vascular reactivity to CO<sub>2</sub> and also interferes with the pH-mediated vascular responses (Wang *et al.*, 1992). However, even after blockade of NO pathways (such as blocking NO synthase enzyme), severe hypercapnia (PaCO<sub>2</sub> >100 mmHg) still promoted an increase in cerebral blood





*al.*, 1997; Gao *et al.*, 2013; Len *et al.*, 2013), and it may be an independent risk factor for cerebral vascular disease (Molina *et al.*, 1999; Reinhard *et al.*, 2014). Furthermore, manipulation of CO<sub>2</sub> has long been used to control cerebral perfusion in a myriad of clinical situations such as traumatic brain injury, cerebral ischaemia and during surgical procedures (Brian, 1998). Although therapeutic hypocapnia has been used clinically for treating patients with acute traumatic brain injury, it has been shown that hyperventilation can cause marked cerebral vasoconstriction and increase the incidence of cerebral ischaemia (Muizelaar *et al.*, 1991).

Arterial oxygen can also influence cerebral perfusion, both directly and indirectly. Cerebral blood flow tends to remain constant with mild to moderate reductions in PaO<sub>2</sub>, reflecting a balance of the hypoxic-mediated cerebral vessels dilatation and hypocapnic-mediated vessel constriction, the latter of which is a result of a hyperpnoeic ventilatory response driven by the carotid body chemoreceptors (Teppema & Dahan, 2010). However, when PaO<sub>2</sub> is reduced to lower than ≈50 mmHg, there is a progressive increase in cerebral perfusion associated with a reduction in cerebral vascular resistance in dogs (Kogure *et al.*, 1970). In humans, cerebral perfusion remains constant until a decrease in peripheral oxygen saturation to 90%, beyond this a progressive increase in perfusion occurs (Gupta *et al.*, 1997). With the normal haemoglobin/oxygen dissociation curve, the threshold would be at ≈ 58 mmHg PaO<sub>2</sub>. In contrast, even moderate hyperoxia leads to cerebral vasoconstriction and consequent reduction in cerebral perfusion, although this effect is less pronounced than the hypoxic response (Bulte *et al.*, 2007). Several

mechanisms have been implicated in the cerebral vascular responses to changes in arterial oxygen content. Adenosine is released in response to hypoxia and is reportedly one of the main stimulators of hypoxic-mediated cerebral vessel dilatation (Phillis, 1989). The blockade of adenosine receptors by theophylline blunts the cerebral vascular dilatation to hypoxia (Bari *et al.*, 1998), although the magnitude of the contribution of this mechanism is still controversial (Haller & Kuschinsky, 1987; Morii *et al.*, 1987; O'Regan, 2005). Prostacyclin, a prostaglandin with dilatory properties, has also been implicated in the cerebral vascular response to hypoxia (McCalden *et al.*, 1984). However, there is evidence that the relative cerebral vascular response to hypoxia is maintained after cyclooxygenase inhibition, and effect of prostaglandins on the cerebral vascular response to hypoxia requires clarification (Hoiland *et al.*, 2015). Hypoxia may also have a direct effect on the smooth muscles surrounding the cerebral arteries. Impaired oxidation of energetic molecules and consequent adenosine triphosphate depletion will result in a reduced contractile capacity by inhibition of cytosolic calcium influx and increased potassium efflux (Tomiyama *et al.*, 1999; Phillis, 2004). Prolonged hypoxia will cause extracellular fluid acidification and potassium release, which will promote cerebral vascular dilatation in the same way as with the hypercapnic response.

In contrast to hypoxia, isocapnic hyperoxia at a target partial pressure of end-tidal oxygen of 500 mmHg, mean blood flow at the carotid arteries and cerebral blood volume measured by magnetic resonance imaging remained constant (Croal *et al.*, 2015). Similarly, at 320 mmHg to 430 mmHg PaO<sub>2</sub>, the ICA and VA blood flow remained unchanged from baseline values as measured by

Doppler ultrasound (Willie *et al.*, 2012). However, other studies have found that the cerebral perfusion is significantly reduced with isocapnic hyperoxia by increasing the inspired fraction of oxygen to 100% (Kolbitsch *et al.*, 2002) or achieving PaO<sub>2</sub> of ≈482 mmHg (Tajima *et al.*, 2014). One possible explanation for this contradictory evidence is that after an initial hypoventilatory response, hyperoxia may cause a mild progressive hyperventilation, which can reduce the PaCO<sub>2</sub> leading to cerebral vasoconstriction (Marczak & Pokorski, 2004).

#### **2.4.4. Autonomic nerves and intrinsic pathways in the brain**

The blood vessels at the surface of the brain are innervated with fibres from the superior cervical, sphenopalatine, otic and trigeminal ganglia, and neurotransmitters such as acetylcholine, nitric oxide, vasoactive intestinal peptide, norepinephrine, neuropeptide Y and others have been implicated in this signalling (Hamel, 2006). Although many animal models are used to assess the influence of the autonomic nervous system on the cerebral blood flow regulation, the invasiveness of most studies prevent the translation of these methods for human use, and this literature review will focus on the data in humans.

The sympathetic nervous system's contribution to the control of the cerebral circulation is still highly debated (Strandgaard & Sigurdsson, 2008; van Lieshout & Secher, 2008b). Routine upper thoracic sympathectomy has been shown to increase arterial diameter and blood flow at the ICA in a cohort of 68 patients with palmar hyperhidrosis (Jeng *et al.*, 1999) whereas bilateral stelletomy promoted a reduction in cerebral vascular resistance with a non-significant increase in cerebral blood flow (Shenkin *et al.*, 1951). However, studies using

pharmacological blockade show conflicting results. Stellate ganglion blockade has been shown to increase cerebral perfusion both at rest measured by scintigraphy (Umeyama *et al.*, 1995) and during exercise with transcranial Doppler (Ide *et al.*, 2000a). In contrast to these findings, other studies showed the cerebral blood flow may be unaffected or even reduced after stellate blockade when measured by the [<sup>133</sup>Xe] technique or magnetic resonance imaging respectively (Ohta *et al.*, 1990; Kang *et al.*, 2010). Thus, if the sympathetic nervous system has any effect on the cerebral circulation, the driving factor that commands the responses may be linked to changes in cardiac output, as described in the next section (van Lieshout & Secher, 2008a). The sympathetic nervous system has also been implicated as a modulator of cerebral autoregulation as  $\alpha$ -adrenergic blockade with phentolamine was associated with an increased transfer function coherence between arterial blood pressure and cerebral perfusion (Hamner *et al.*, 2010). This suggests that the buffering provided by the myogenic mechanism to changes in blood pressure is lost without the influence of the sympathetic nervous system.

Parasympathetic fibres arising from the sphenopalatine, internal carotid and otic ganglions, and the nucleus basalis of Meynert innervate cerebral blood vessels (Biesold *et al.*, 1989; Suzuki *et al.*, 1990) and, although there is evidence that the parasympathetic nervous system plays an important role in modulating cerebral blood flow in non-human animal species (Biesold *et al.*, 1989; Toda & Okamura, 1990), current evidence in humans is contradictory. Administration of glycopyrrolate, a cholinergic blocker that does not cross the blood-brain barrier,

impaired cerebral autoregulation at higher frequencies of oscillatory lower body negative pressure (Hamner *et al.*, 2012) and abolished the exercise-induced increase in MCA  $V_{\text{mean}}$  using transcranial Doppler (Seifert *et al.*, 2010). However, it was shown that an increase in regional cerebral blood flow of the motor cortex evoked by a visual or motor task was not influenced by cholinergic blockade with glycopyrrolate in one recent study using the arterial spin labelling and blood oxygen level dependent techniques (Rokamp *et al.*, 2014). Additional studies are required to further investigate the influence of intrinsic and extrinsic autonomic fibres on the regulation of cerebral blood flow.

#### **2.4.5. Cardiac output**

There is mounting evidence that cardiac output regulates cerebral perfusion independently from changes in arterial blood pressure and  $\text{PaCO}_2$  and  $\text{PaO}_2$ . In healthy young individuals, the application of graded lower body negative pressure to reduce central blood volume and cardiac output, also reduced cerebral perfusion even before any decrease in arterial blood pressure (Levine *et al.*, 1994). During head-up tilt, a manoeuvre also used to reduce central blood volume, cerebral perfusion was decreased despite an increase in mean arterial pressure and a maintained  $\text{PaCO}_2$  (Jorgensen *et al.*, 1993). Conversely, increasing central blood volume with venous infusion of a 25% albumin solution caused an increase in cardiac output that was coupled with an increase in cerebral perfusion (Ogoh *et al.*, 2005). A similar result was observed when 15 and 30 mL/kg of normal saline solution were infused in healthy young individuals, which increased cardiac output by  $\approx 0.57$  L/min (15 mL/kg) and

≈0.92 L/min (30 mL/kg) leading to an increase in MCA  $V_{\text{mean}}$  of 5 cm/s (15 mL/min) and 6 cm/s (30 mL/min), respectively (Ogawa *et al.*, 2007).

The mechanisms responsible for the association between cardiac output and cerebral perfusion remain hypothetical. It has been proposed that small vessel vasoconstriction may be the mechanism underpinning a reduced cerebral perfusion caused by the reduced cardiac output (Levine *et al.*, 1994). This would be explained by the increased pulsatility index detected, as this index is related to intracranial pressure and may be indicative of cerebral vascular resistance, although this last assumption is not always true (de Riva *et al.*, 2012). Most of the above studies related the change in cerebral perfusion with indices of cerebral vascular resistance, implying that the changes in cardiac output are coupled with resistance vessels tone (Levine *et al.*, 1994; Ogoh *et al.*, 2005). In fact, one study hypothesised that the cerebral responses to a change in cardiac output is mediated by the sympathetic innervation of the brain vessels (Ogoh *et al.*, 2005). However, direct evidence for the autonomic regulation of the cerebral circulation in response to changes in cardiac output is lacking.

Cardiac output is reduced in diseases such as heart failure and AF and this reduction may explain several of the brain-related symptoms often perceived by the patients (Petersen *et al.*, 1989b; Fraser *et al.*, 2015). Cerebral perfusion was improved in patients with heart failure after treatment with captopril (inhibitor of the angiotensin converting enzyme), cardiac transplantation or electrical therapies such as cardiac resynchronization therapy, all of which improve ventricular function (Meng *et al.*, 2015). However, the contribution of changes in

blood pressure and direct effects of the medication on the cerebral circulation cannot be excluded.



## 2.5. The ageing brain: structure and function

Ageing is a natural biological process characterized by a series of cumulative alterations that can be observed from the molecular to the organism level. There are several reasons why these alterations are thought to occur, ranging from DNA replication flaws, errors on protein synthesis, damage caused by increased oxidative stress and free radicals, disruptive binding of macromolecules and deleterious immune responses (Harman, 1981). After achieving the peak function, which can happen at any time from the early adolescence to adulthood, the changes that occur during the ageing process becomes progressively clearer and the functional limitations also become more apparent (Bafitis & Sargent, 1977). Public health interventions along with health care and education improvements over several decades, have led to an increase in life expectancy and global population ageing (Salomon *et al.*, 2012). Age is one of the most important risk factors for brain diseases such as dementia and stroke (Sacco *et al.*, 1997; Lindsay *et al.*, 2002), but even in 'healthy ageing' brain structure and function are altered (Scahill *et al.*, 2003; Burgmans *et al.*, 2011). Grey and white matter blood flow decrease by ~0.5% per year from early adulthood, and despite a small increase in oxygen extraction, the CMRO<sub>2</sub> may also be decreased (Leenders *et al.*, 1990). In fact, resting CMRO<sub>2</sub> is reported to be reduced with ageing in some (Kety, 1956; Pantano *et al.*, 1984), but not all studies (Burns & Tyrrell, 1992). Ageing also causes a reduction in cerebral metabolic rate for glucose (Nugent *et al.*, 2014), which is estimated to decline by ~6% per decade globally with most cerebral regions affected, except for the occipital cortex and cerebellum (Petit-Taboue *et*

*al.*, 1998). However, this age-related impairment in cerebral perfusion and metabolism may be confounded by the incidence of subclinical disease such as arteriosclerosis, cognitive impairment, hypertension and further changes in cerebral metabolism due to neuronal loss that can also be implicated in a lower cerebral blood flow in ageing. Advancing age is also associated with cerebral atrophy (particularly in frontal and temporal regions), altered neural signalling, and impairments in aspects of cognition (e.g., working memory and processing speed) (Martin *et al.*, 1991; Jagust, 2013).

Despite the scientific progress in understanding the cerebral circulation and its regulation over the last few decades, the mechanisms of structural and functional cerebral vascular changes seen in ageing and disease are still unclear.

## **2.6. Cerebral blood flow and exercise**

Physical activity comprises any bodily movement made by skeletal muscles that result in energy expenditure. Exercise is defined as a planned, structured physical activity which has a goal of maintaining or improving physical function such as cardiorespiratory fitness as measured by oxygen consumption (Caspersen *et al.*, 1985). Physical activity and exercise have long been proposed as a means of improving cerebral health (Dustman *et al.*, 1984; Clarkson-Smith & Hartley, 1989; Paillard *et al.*, 2015) and exercise has also been used to assess cerebral vascular responses to a physiological stressor (Davenport *et al.*, 2012). The prevalence of physical activity and exercise has been shown to decrease  $\approx 33\%$  over a 10-year period in men aged 65 or older (Bijnen *et al.*, 1998), and promoting at least moderate levels of physical activity may protect this segment of the population against chronic diseases (DiPietro, 2001). However, the effects of physical activity and exercise on the cerebral blood flow and its regulation in old individuals are not completely understood and will be presented in section 2.6.3. The following section pertaining to cerebral blood flow (2.6.1) and metabolism (2.6.2) during exercise, have been published as part of a recent *Journal of Physiology* review article (Braz & Fisher, 2015).

### **2.6.1. Acute effects of exercise on the cerebral blood flow**

Studies using the Kety and Schmidt technique reported that global cerebral blood flow was unchanged during exercise (Scheinberg *et al.*, 1954; Kleinerman & Sancetta, 1955; Lambertsen *et al.*, 1959). However, a change in subject position from rest (supine) to exercise (upright), an associated change in the

anatomy of cerebral drainage, confounding alterations in PaCO<sub>2</sub>, and a reduced activation in some brain regions during exercise, may in part explain these observations (Secher *et al.*, 2008).

The administration of dissolved inert radioactive gases such as [<sup>133</sup>Xe] and [<sup>85</sup>Kr] via the common carotid artery and measurement of the emitted radiation by extracranial scintillation detectors permitted the earliest determination of regional cerebral blood flow responses to exercise (Hoedt-Rasmussen, 1965). Accordingly, Olesen (1971) observed a regional increase in perfusion of the cortical sensorimotor area corresponding to the hand during contractions with the contralateral hand. A ~10-30% increase cortical blood flow is also elicited by leg cycling, as determined with the [<sup>133</sup>Xe] clearance initial slope index (Jorgensen *et al.*, 1992). This is paralleled by a comparable increase in MCA  $V_{\text{mean}}$  (Jorgensen *et al.*, 1992) measured using the transcranial Doppler technique introduced by Aaslid and colleagues (Aaslid *et al.*, 1982). In fact, leg cycling bilaterally increases  $V_{\text{mean}}$  in the MCA and anterior cerebral artery, whereas rhythmic handgrip exercise performed with the right hand principally increases the left MCA  $V_{\text{mean}}$ , and calf exercise performed with the right leg predominantly increases  $V_{\text{mean}}$  in the left anterior cerebral artery (Linkis *et al.*, 1995). Positron emission tomography and single-photon emission computed tomography have confirmed the exercise-induced regional increase in cerebral perfusion and activation in the sensorimotor and premotor regions, as well as the supplementary motor area, cerebellum and insular cortex (Williamson *et al.*, 1999; Hiura *et al.*, 2014), and highlights the coupling between regional cerebral activation and perfusion during exercise. Along with an increase in MCA  $V_{\text{mean}}$ ,

blood flow in the internal carotid and vertebral arteries increases by ~17% during moderate intensity leg cycling (Hellstrom *et al.*, 1996; Sato *et al.*, 2011). However, at exercise intensities above ~60% maximal oxygen uptake ( $\text{VO}_2\text{max}$ ) MCA  $V_{\text{mean}}$  and internal carotid artery flow plateau and then return toward resting levels as exercise intensity increases, whereas in contrast vertebral artery flow continues to increase up to 80%  $\text{VO}_2\text{max}$  (Sato *et al.*, 2011).

### **2.6.2. Cerebral metabolism during exercise**

Early reports that global cerebral blood flow was unchanged during exercise, also suggested that cerebral metabolism was unaltered (Madsen *et al.*, 1993), and in fact it was even concluded that "*during vigorous physical exercise the brain behaved as a steady-state organ with little or no change in cerebral circulation or metabolism*" (Zobl *et al.*, 1965). However, cerebral activation with tactile stimulation increases regional cerebral blood flow and  $\text{CMRO}_2$  (Fox & Raichle, 1986), and the same appears to be true for  $\text{CMRO}_2$  during exercise (Seifert *et al.*, 2009; Smith *et al.*, 2014) although this has not been universally observed (Trangmar *et al.*, 2014). The cerebral metabolic rate for glucose and the OGI tend to be similar at rest and during exercise (Ide *et al.*, 2000b), although OGI can be reduced by very strenuous exercise, such as prolonged maximal exercise in the heat (Nybo *et al.*, 2003). Along with glucose, lactate also plays an important role as a substrate during exercise (Smith *et al.*, 2003), particularly when arterial lactate concentration is elevated such as during high intensity exercise. The combined uptake of glucose and lactate relative to oxygen remains stable at low-to-moderate exercise intensities, but when exercise becomes more strenuous glucose and lactate uptake increase in

excess of oxygen, in an intensity dependent manner. This means that the 'oxygen-to-carbohydrate consumption index' (OCI;  $O_2/[\text{glucose} + \frac{1}{2} \text{lactate}]$ ) is reduced, and during all-out rowing the OCI can decrease to <35% of the baseline value (Volianitis *et al.*, 2008). As the increase in cerebral uptake of lactate does not result in an accumulation of this substance in brain structures or in the cerebral spinal fluid, it is seemingly metabolized by the brain during exercise (Dalsgaard *et al.*, 2004).

### **2.6.3. Chronic effects of exercise on cerebral blood flow**

It is well known that regular exercise reduces cardiovascular risk (Mora *et al.*, 2007) and improves systemic arterial function via a series of mechanisms including NO (DeSouza *et al.*, 2000) and arterial compliance (Ashor *et al.*, 2014). However, the chronic effects of regular daily physical activity or exercise training on the cerebral circulation are still unclear. It has been previously shown that a higher  $VO_2\text{max}$  was associated with a higher resting MCA  $V_{\text{mean}}$  in young individuals and throughout the lifespan (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b), although this is not a universal finding (Zhu *et al.*, 2013; Brugniaux *et al.*, 2014). When assessed by magnetic resonance imaging, whole brain averaged blood flow in sedentary elderly individuals was not different when compared to masters athletes, although there was a significantly increased cerebral blood flow to brain regions such as the posterior cingulate cortex (Thomas *et al.*, 2013). Furthermore, the effects of cardiorespiratory fitness on the cerebral vascular reactivity to  $CO_2$  ( $CVR_{CO_2}$ ) are also controversial, with evidence showing that  $CVR_{CO_2}$  was improved (Bailey *et al.*, 2013b; Barnes *et al.*, 2013), unchanged (Zhu *et al.*, 2013) or even reduced (Thomas *et al.*, 2013)

in trained individuals when compared to sedentary age-matched controls. Potential mechanisms proposed to mediate improved cerebral haemodynamics resulting from chronic exercise may include, brain-derived neurotrophic factor, insulin-like growth factor 1, or prostaglandin activation (Bailey *et al.*, 2013b; Barnes *et al.*, 2013). Neurotrophic factors such as the nerve growth factor and brain-derived growth factor have been shown to reduce infarct area in rats that exercised in a treadmill for 12 weeks (Ang *et al.*, 2003). Furthermore, insulin-like growth factor 1 also plays a role by promoting angiogenesis by the stimulation of vascular endothelial growth factor, as well as stimulating the production of brain derived neurotrophic factor (Carro *et al.*, 2000; Lopez-Lopez *et al.*, 2004). Finally, inhibition of the cyclooxygenase enzyme with indomethacine caused fit old adult humans to have a reduced response to inhaled CO<sub>2</sub> in contrast to a control condition, suggesting that prostaglandins may be partially responsible for the cerebral vascular adaptations to exercise (Barnes *et al.*, 2013). The effects of daily physical activity and cardiorespiratory fitness on the cerebral blood flow and CVR<sub>CO2</sub> will be discussed in detail in Chapter 4 and 5.

## **2.7. Atrial fibrillation – from the heart to the brain**

AF is the most common sustained heart rhythm abnormality and has a lifetime incidence risk of 26% in men and women aged over 40 years old (Lloyd-Jones *et al.*, 2004). The incidence and prevalence of AF is strongly correlated with advanced age. There are several potential mechanisms through which AF may influence cerebral blood flow and its regulation. These will be discussed below and will be further detailed in chapter 6.

### ***2.7.1. Definition, epidemiology and pathophysiology***

The defining characteristics of AF are the presence of an absolutely irregular heart rhythm and absent P waves in the electrocardiogram (ECG, Figure 2.5). This disease can present itself in different ways according to the duration of the bouts of fibrillation: paroxysmal, when the episode resolves spontaneously within 48h; persistent, when the abnormal heart rhythm persists for longer than 7 days, needs cardioversion or rhythm controlling medications; and permanent, when the patient persists in long-standing AF even after cardioversion is attempted or is considered inappropriate and rhythm control is not pursued (Camm *et al.*, 2010). The prevalence of AF in the general population is  $\approx$ 2% in Europe and is steadily increasing (Zoni-Berisso *et al.*, 2014). While the younger segments of the population experience very low rates of AF prevalence (0.2 to 0.6% in people up to 49 years old), older segments of the population face much higher prevalence of AF (over 10% in individuals  $\geq$ 80 years old). It is more common in men, with a ratio 1.2:1 when compared to women (Camm *et al.*, 2010; Zoni-Berisso *et al.*, 2014).



**Figure 2.5.** Electrocardiogram of atrial fibrillation (AF, top) and normal sinus rhythm (bottom).



The P wave is observed in the sinus rhythm (lower trace, arrow) while the AF shows no identifiable P waves (upper trace, arrow).

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Structural changes in the atria increase the arrhythmogenic potential and may precede the onset of AF. These changes include alterations in the extracellular matrix, myocytes, with endocardial and microvascular remodelling (Camm *et al.*, 2010). Furthermore, an increased vagal tone which prolongs the PR interval, added to the increased cardiac stress during vigorous physical activity have been proposed as mechanisms of a higher incidence of AF in endurance athletes, although this remains controversial (Wernhart & Halle, 2015). After its onset, AF causes inflammatory changes that may fulfil criteria for the diagnosis of myocarditis, noninflammatory cardiomyopathy and fibrosis even in patients with lone AF (Frustaci *et al.*, 1997). The pulmonary veins are the source of the arrhythmia in the majority of patients, possibly due to shorter refractory periods and a different myocyte arrangement (Haissaguerre *et al.*, 1998). The high ventricular rates achieved during episodes of AF may cause a ventricular cardiomyopathy, which in turn reduces cardiac output and is associated with poorer long-term outcomes (Lin *et al.*, 2010). Even within the normal heart rate

range, the chaotic atrial electrical activity causes a reduction in cardiac output of  $\approx$ 5-15%, with some patients presenting overt heart failure (Camm *et al.*, 2010). Furthermore, the disrupted atrial systole and atrial enlargement in AF causes stasis, combined with increase of pro-inflammatory cytokines and a general shift of the coagulation cascade to a prothrombotic state results in intravascular activation of coagulation, which is one of the most damaging complications of AF (Watson *et al.*, 2009). In addition, it has been shown that AF patients have systemic endothelial dysfunction as characterized by impaired flow-mediated dilatation, increased plasma von Willebrand factor and E-selectin (Freestone *et al.*, 2008). NO is one of the most important vasoactive substances produced by the endothelium and it is thought to be the major endothelial factor regulating cerebral vascular tone (Faraci & Heistad, 1998). In fact, administration of a NO donor (glyceryl trinitrate) to AF patients increased flow-mediated dilatation responses to levels similar to that observed in a healthy control group, indicating a major role of the NO mechanism to arterial dilatation in AF (Freestone *et al.*, 2008). The endothelial dysfunction/damage as characterized by increased plasma von Willebrand factor has also been shown to be a predictive factor for cerebrovascular events (Conway *et al.*, 2003). However, little is known about how these mechanisms interact at the cerebral circulation and this knowledge may assist in the development of strategies to prevent complications of the disease.

### **2.7.2. Diagnosis, treatment and potential complications**

This section is based on the NICE (2014) and European (Camm *et al.*, 2010) clinical guidelines.

AF is usually suspected on the basis of a patient presenting with an irregular pulse that may or may not be accompanied by symptoms. Common symptoms are palpitations, dizziness, fatigue, dyspnoea, chest pain or even heart failure. In some cases, patients will have as first symptoms cerebral vascular manifestations such as a transient ischaemic attack or stroke. The diagnosis is made with the electrocardiographic identification of AF whether symptoms are present or not. In patients in whom AF is strongly suspected but is not confirmed by routine ECG (i.e. in patients with paroxysmal AF in sinus rhythm during the consultation), a longer recording period such as a 7-day Holter ECG may be warranted.

The treatment options for AF include cardioversion (electric or pharmacological), rhythm control medication, rate control medication and anticoagulation, and should be individually tailored in order to reduce the symptoms (if any) and prevent stroke, one of the main long-term complications of the disease. Of note, the relative risk of stroke ranges from 2.6 to 4.5 in patients with AF when compared to healthy controls (Wolf *et al.*, 1991). Strategies targeting stroke prevention, haemodynamic improvement and reduction of symptoms are the cornerstones of AF therapy. Prevention of stroke is achieved with the use of oral anticoagulants and haemodynamic and symptomatic improvement is attempted with rhythm or rate control medication. Treatment with anticoagulants reduces the risk of stroke in AF to a level near that observed in the general population (Petersen *et al.*, 1989a).

AF is associated with cognitive impairment and dementia (e.g. Alzheimer's disease) even in patients without evidence of stroke (Ott *et al.*, 1997). One

proposed mechanism for this increase in incidence of dementia is the occurrence of silent cerebral infarcts, as a result of small vessel damage causing subcortical lesions (Vermeer *et al.*, 2003). A relative cerebral hypoperfusion due to the lower cardiac output or other unknown mechanism in AF patients has also been proposed as another mechanism that may contribute to the cognitive decline in this group (Alosco *et al.*, 2015). Although resting steady-state cerebral perfusion assessments provide valuable information to understand the pathophysiology of the complications of AF, dynamic studies on the cerebral vascular function in humans are warranted to further knowledge on the status of the cerebral vasculature in response to different stressors.

### **2.7.3. Cerebral blood flow in atrial fibrillation**

Cerebral blood flow is reported to be lower in chronic AF patients when compared to controls especially in younger age groups (Lavy *et al.*, 1980). During an episode of paroxysmal AF, MCA  $V_{\text{mean}}$  is acutely lowered and increases once the arrhythmia was resolved (Totaro *et al.*, 1993). Similarly, cerebral blood flow is improved after cardioversion in patients with AF when measured by [ $^{133}\text{Xe}$ ] (after correction for changes in partial pressure of end-tidal  $\text{CO}_2$  [ $P_{\text{ETCO}_2}$ ]), becoming similar to values obtained in healthy individuals (Petersen *et al.*, 1989b). In contrast to these findings, one study did not find a significant difference in MCA  $V_{\text{mean}}$  between paroxysmal AF patients and controls (Porebska *et al.*, 2007). In the same study, cardioversion was associated with a significantly increased MCA  $V_{\text{mean}}$  when compared to values before the procedure. In AF the ability to increase cerebral perfusion during exercise is diminished, along with the ability to increase cardiac output.

Whereas control participants had a 23% increase in MCA  $V_{\text{mean}}$  during intense cycling exercise, in AF patients it was only 9% (Ide *et al.*, 1999). The reduction in cerebral perfusion in AF, secondary to a reduction in cardiac output, can further be complicated by other diseases such as heart failure. Patients with AF and heart failure (NYHA  $\geq 2$ ) had a lower MCA  $V_{\text{mean}}$ , and poorer memory and global cognition, when compared to patients with heart failure without AF (Alosco *et al.*, 2015). Furthermore, although endothelial function and NO are known to be impaired in AF and are important modulators of cerebral haemodynamics, the impact of the endothelial dysfunction on the cerebral vasculature in this patient group remains to be elucidated. Therefore, studying the cerebral blood flow and its regulation in patients with AF is imperative in order to identify mechanisms of cerebral vascular damage and devise strategies for prevention and treatment of the much feared complications of this disease.

## **2.8. Aims and hypotheses of this thesis**

Understanding of how healthy ageing and disease affect the regulation of cerebral blood flow, and how this may be modified by physical activity, is incomplete. The specific questions that are to be addressed in this thesis are: 1) How does normal daily physical activity influence global cerebral blood flow in healthy older individuals? 2) Does a higher cardiorespiratory fitness elevate cerebral blood flow and improve  $CVR_{CO_2}$  in healthy young and elderly individuals? 3) Is cerebral vascular function impaired in patients with AF?

These questions will be addressed in the experimental chapters of this thesis, with the following specific aims and hypotheses:

Chapter 4: To investigate if normal daily physical activity influences resting cerebral blood flow in a cohort of elderly individuals. It was hypothesised that people who had higher levels of daily physical activity would have higher cerebral blood flow when compared to people with the lowest levels of physical activity from the cohort.

Chapter 5: To investigate if age and cardiorespiratory fitness influence resting cerebral blood flow and  $CVR_{CO_2}$ . It was hypothesised that bilateral ICA and VA blood flow and  $CVR_{CO_2}$  would be greater in aerobically trained young and old individuals compared to their untrained counterparts.

Chapter 6: To investigate whether AF influences resting cerebral perfusion,  $CVR_{CO_2}$  and cerebral autoregulation. It was hypothesised that people with AF would have lower cerebral perfusion,  $CVR_{CO_2}$  and impaired cerebral autoregulation when compared to healthy age-matched controls.

## **CHAPTER 3: GENERAL METHODS**

### **3.1. Ethical approval and consent**

All procedures were approved by local ethical committees and conformed to the Declaration of Helsinki. The studies presented on chapters 4 and 6 were approved by the Health Research Authority (formerly National Research Ethics Service) of the United Kingdom, while chapter 5 had approval from the Swiss Federal Institute of Technology Zurich. Potential participants received an information sheet containing details of the research and, after having all the procedures explained and being given the opportunity to ask questions, written informed consent was obtained prior to the participation in each study.



## 3.2. Variables measured

### 3.2.1. Haemodynamic and respiratory

Resting blood pressure was assessed on the brachial artery using the oscillometric principle (Dinamap ProCare 200, GE Medical Systems, Milwaukee, WI, USA – Chapters 4 and 5; M2, Omron, Kyoto, Japan – Chapter 6) or an automated auscultatory R-wave gating algorithm (Tango+, SunTech Medical, Eynsham, UK – Chapter 3). Furthermore, beat-by-beat changes in blood pressure were continuously recorded non-invasively using finger photoplethysmography (Portapres, Chapter 5; Finometer MIDI, Chapter 6; all made by Finapres Medical Systems, Amsterdam, the Netherlands). This methodology has been shown to agree with intra-arterial measurement of blood pressure, allowing the real-time beat-by-beat monitoring of blood pressure changes during dynamic manoeuvres such as tilt test (Petersen *et al.*, 1995). Systolic and diastolic blood pressures were recorded, and mean arterial pressure (MAP) was calculated according to the formula:  $MAP = 1/3 SBP + 2/3 DBP$ , where SBP denotes systolic blood pressure and DBP denotes diastolic blood pressure. Heart rate was calculated using the R wave peaks from a continuously recorded lead II ECG (BioAmp, ADInstruments, Dunedin, New Zealand). The choice of using lead II ECG was made because the placement of the electrodes on the base of the right upper limb (negative) and left lower limb (positive) provide a superior-inferior plane of reference of the heart, allowing a best possible visualization of the upward P waves and a well-defined R wave peak in people with normal cardiac axis (Meek & Morris, 2002). Respiratory variables were recorded continuously through a face mask and two-

way valve (Hans Rudolph, Kansas City, KS, USA – Chapters 5 and 6) or, when only  $P_{ET}CO_2$  was feasible, through a nasal cannula (PROBreathe nasal cannula, PROACT Medical, Corby, UK – Chapter 4). Respiratory flow was measured using a heated pneumotach (Hans Rudolph, Kansas City, KS, USA – Chapter 6; Cosmed Quark CPET, Rome, Italy – Chapter 5), which was calibrated with a 3 L syringe before each testing session. Respiratory rate, tidal volume and minute ventilation were calculated online by the Spirometry module from Labchart (ADInstruments, Dunedin, New Zealand). For the measurement of the partial pressure of  $CO_2$ , the nasal cannula or mask was connected to a capnograph which continuously sampled the gas (Moxus modular, AEI Technologies, Pittsburgh, PA, USA – Chapter 4; Cosmed Quark CPET, Rome, Italy – Chapter 5, RespSense, Nonin medical, Plymouth, MN, USA – Chapter 6). In Chapter 4, the  $P_{ET}CO_2$  was derived from the relative percentage of expired  $CO_2$  as the maximum value at the end of the expiration, calculated as:  $P_{ET}CO_2 = PCO_2 \times (P_0 - 47)$ , where  $PCO_2$  is the recorded relative percentage of  $CO_2$  and  $P_0$  is the atmospheric pressure measured on the day of the experiment. The devices in Chapters 5 and 6 already provided corrected partial pressures of  $CO_2$  and no calculation was required.

### **3.2.2. Cerebral blood flow**

Transcranial Doppler ultrasound (Multidop X or Doppler BoxX, DWL, Sippligen, Germany) acquired MCA  $V_{mean}$  was used as a surrogate index for the assessment of cerebral blood flow (Chapters 5 and 6) and volumetric cerebral blood flow was assessed at the ICAs and VAs with duplex Doppler

ultrasound (Sonos 7500 – Chapter 3 and iE33 – Chapter 4, Philips healthcare, Best, The Netherlands; Mindray M7, Shenzhen, GNG, China – chapter 5).

The transcranial Doppler technique was performed using a 2 MHz pulsed wave transducer placed over the temporal window, a region superior to the zygomatic arch and anterior to the tragus where the temporal bone is less dense allowing the insonation of the MCA. The probe was fixed in place using an adjustable headgear and the interface between the probe and skin was made with ultrasound gel in order to transmit the waves directly from the probe to the skin reducing static. An optimal signal would then be acquired searching for a bright M-mode signal towards the probe, with a depth range from 40 to 65 mm. Gain and power would then be adjusted to allow for a high quality signal with minimal artifacts. The systolic, diastolic and mean blood flow velocity parameters were expressed in  $\text{cm}\cdot\text{s}^{-1}$  and of the cerebral vascular conductance index (CVCi) was calculated as  $CVCi = MCA V_{mean}/MAP$ .

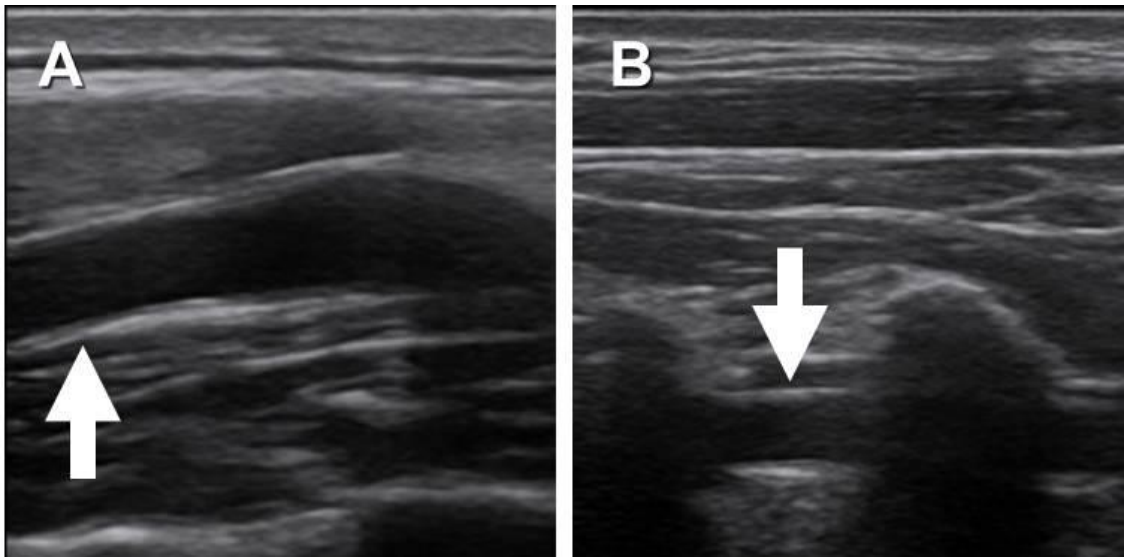
Cerebral blood flow was measured with duplex Doppler technique using a 10 MHz (7.5 MHz – Chapter 5) linear array transducer that was placed over the anterolateral surface of the sternocleidomastoid muscle with ultrasound gel as the interface between the probe and the skin. The arterial diameter was determined using bidimensional B-mode and was composed of the average over five to ten cardiac cycles (Figure 3.1). Images were stored for offline analysis and diameter was measured using the NIH ImageJ 1.49v software (Chapter 5), which has been widely used for a diverse range of applications including ultrasound (Schneider *et al.*, 2012). The arterial diameter over each cardiac cycle was defined as the average of systolic and diastolic phases, with

three measurements being acquired during systole and three during diastole. ICA measurements were performed  $\approx 1.5$  cm distal from the bifurcation of the common carotid artery and VA measurements were performed between the transverse processes of C3 to C6.

The blood velocity was acquired using the PW-mode and defined as the time averaged mean velocity over the cardiac cycle as calculated by the machine. To ensure the best possible velocity spectral waveform was captured, care was taken to maintain the Doppler angle between 30-60° from the direction of the flow, the sample volume width was adjusted to cover the width of the luminal diameter and appropriate setup of Doppler gain, angle correction and scales. Blood flow in each artery was calculated as:

$$Flow = \pi \times \left(\frac{diameter}{2}\right)^2 \times velocity \times 60$$

Total cerebral blood flow was calculated as the sum of the blood flow in both ICAs and both VAs. All ultrasound images, measurements and analyses were performed by the candidate (IDB).



**Figure 3.1.** Ultrasound images of the internal carotid and vertebral arteries. Internal carotid artery (Panel A, arrow) and vertebral artery (Panel B, arrow) as visualized by ultrasonography. Images acquired from the study presented in Chapter 5.

### **3.2.3. Physical activity**

Physical activity was measured using an accelerometer (GT3X, Actigraph, Pensacola, FL, USA – Chapter 4). Participants were instructed about the placement of the device, and asked to wear them for 7 days. The accelerometer was able to detect movement in 3 orthogonal planes and also measured the time spent in sedentary, mild, moderate, vigorous and very vigorous activity throughout the day. Counts of movement in each orthogonal plane were used to calculate tri-axial vector magnitude, total daily counts and steps. Physical activity levels were calculated by the Actigraph accelerometer software and defined as light ( $\leq 2.99$  metabolic equivalents), moderate (3.0-5.99 metabolic equivalents), hard (6.0-8.99 metabolic equivalents), and very hard ( $\geq 9.0$  metabolic equivalents) as typically used in the literature (Freedson *et al.*, 1998). The phenomenon of reactivity is when study participants change their normal behaviour when they are being monitored and may result in an overestimation

of  $\approx 15\%$  on the variable measured (McCarthy & Grey, 2015). However, the occurrence of this phenomenon is controversial (Behrens & Dinger, 2007) and, if present in the participants from Chapter 4, it is likely that both groups were affected equally.

#### **3.2.4. Cognition**

In Chapters 4 and 6, participants underwent cognitive screening using the Montréal Cognitive Assessment (MoCA). The MoCA aims to assess global cognitive function and is used to assess the major cognitive domains, being composed of visuospatial/executive, naming, memory, attention, language, abstraction and orientation tests (<http://www.mocatest.org>). It has a high sensitivity (0.89, 95% confidence interval of 0.84 to 0.92) and specificity (0.75, 95% confidence interval of 0.62 to 0.85) to detect mild cognitive impairment (Tsoi *et al.*, 2015). The MoCA is considered to have comparable performance against the most widely used global cognition assessment test, the mini-mental state exam (Tsoi *et al.*, 2015).

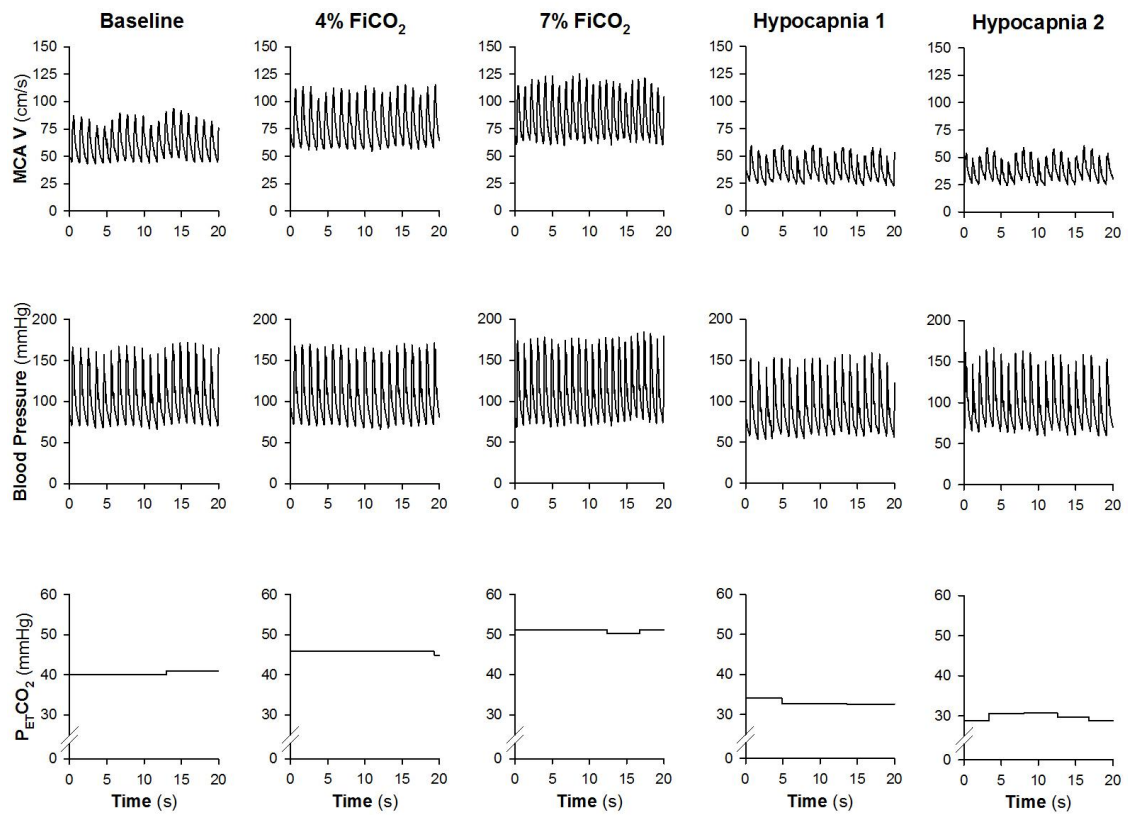
### 3.3. Interventions

#### 3.3.1. Hypercapnic and hypocapnic tests

The atmospheric relative content of CO<sub>2</sub> is currently ≈400 parts per million (0.04%), and changes in arterial CO<sub>2</sub> were required to allow the determination of hypercapnic or hypocapnic CVR<sub>CO<sub>2</sub></sub> in Chapters 5 and 6. In order to provoke hypercapnia, supplemental CO<sub>2</sub> was added to the inspired normoxic air in 4% and 7% concentrations (Chapter 6, Figure 3.2) or dynamically adjusting the fraction of inspired CO<sub>2</sub> to achieve a specific P<sub>ET</sub>CO<sub>2</sub> target (Chapter 5). In Chapter 5, this dynamic control of P<sub>ET</sub>CO<sub>2</sub> was performed manually as elsewhere in the literature (Verbree *et al.*, 2014). Participants would be given supplemental CO<sub>2</sub> to achieve an increase of +1.5 and +5.5 mmHg for 3 minutes in each step after a stable plateau was reached. This small increase in baseline (+1.5 mmHg) was performed as it has been shown to reduce the resting cerebral perfusion variability (Harris *et al.*, 2006). In Chapter 6, Douglas bags were used to deliver increased inspired CO<sub>2</sub> at concentrations of 4 % CO<sub>2</sub> (≈21 % O<sub>2</sub>) and 7 % CO<sub>2</sub> (≈21 % O<sub>2</sub>) for 4 minutes in each step. These concentrations of gas have been previously used in the literature to assess cerebrovascular reserve (Ringelstein *et al.*, 1988; Vernieri *et al.*, 2004). This increase in the inspired fraction of CO<sub>2</sub> caused hypercapnia without the potentially confounding presence of altered arterial O<sub>2</sub>, which happens when hypercapnia is achieved with breath-hold and rebreathing without oxygen supplementation. It is also an advantage over protocols that give gas mixtures with hyperoxia, as seen in studies using carbogen (usually 5% CO<sub>2</sub> in 95% O<sub>2</sub>). Although widely used, caution is needed to interpret CVR<sub>CO<sub>2</sub></sub> tests when

monitoring cerebral perfusion with transcranial Doppler, as it has been shown that a  $\approx 6.8\%$  increase in MCA diameter was observed while the  $P_{ET}CO_2$  was set  $\approx 15$  mmHg above the baseline (Verbree *et al.*, 2014). If unaccounted for, this dilatation may underestimate in  $\approx 14\%$  the actual  $CVR_{CO_2}$  in that artery. Although hypercapnic dilatation of the cerebral arteries insonated in  $CO_2$  reactivity tests using transcranial Doppler may result in an underestimation of the actual cerebral blood flow, this was not an issue in Chapter 5 as the main outcome variable was the blood flow through the ICA. In Chapter 6, it is expected that this underestimation affected all groups with the same magnitude, and the statistical approach with multiple pairwise comparisons can reduce the clinical and physiological impact of this aspect of the test. Hypocapnic  $CVR_{CO_2}$  (Chapter 6) was achieved by asking the participant to hyperventilate in a controlled fashion, in order to maintain  $P_{ET}CO_2$  within a predetermined range that was defined as the same magnitude of change achieved by the hypercapnic steps, but in the hypocapnic range. Participants were continuously instructed to adjust their respiration by increasing or decreasing rate or depth in a similar way to the hypercapnic changes.

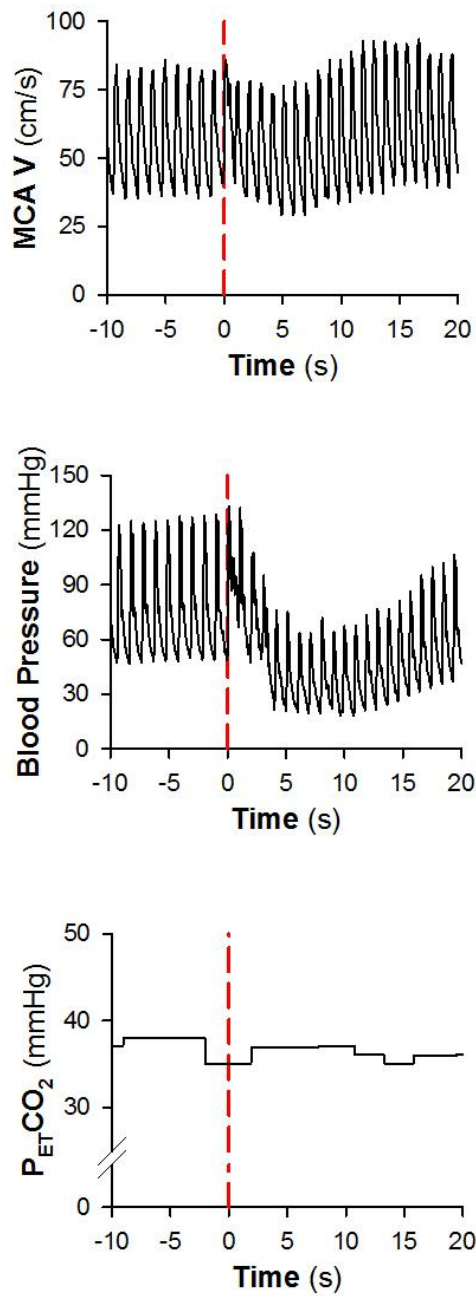




**Figure 3.2.** An original record of MCA V, blood pressure and P<sub>ET</sub>CO<sub>2</sub> responses to the hypercapnic and hypocapnic tests in one representative individual. MCA V, middle cerebral artery blood velocity; FiCO<sub>2</sub>, fraction of inspired CO<sub>2</sub>; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of end-tidal CO<sub>2</sub>.

### **3.3.2. Assessment of cerebral autoregulation**

A single sit-to-stand was performed to assess dynamic cerebral autoregulation (Chapter 6), in which the participant was instrumented, asked to rest comfortably seated and, after 5 minutes, the participant was asked to stand up in a single movement (Figure 3.3). This procedure evoked a drop in blood pressure which was coupled with a drop in cerebral blood velocity, and the ability of the cerebral circulation in maintaining the perfusion despite this drop in blood pressure is one aspect of the cerebral autoregulation. The MCA  $V_{\text{mean}}$ , MAP and  $P_{\text{ET}}\text{CO}_2$  were calculated as the average of the 20 s before the participant stood up for the baseline, while the response was the average value of the 5 cardiac cycles surrounding the nadir of the blood pressure.

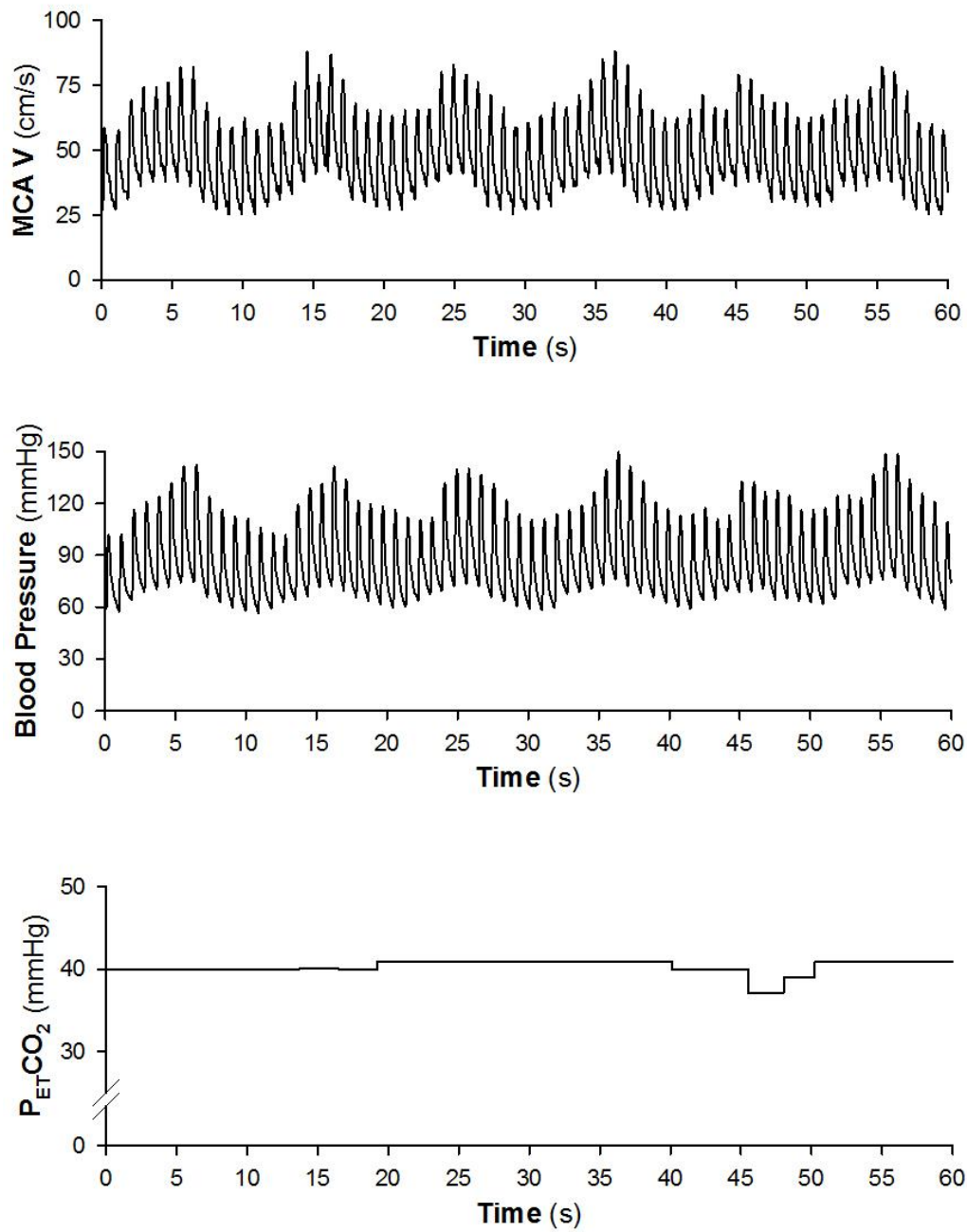


**Figure 3.3.** An original record of MCA V, blood pressure and P<sub>ET</sub>CO<sub>2</sub> responses to the single sit-to-stand test in one representative individual. MCA V, middle cerebral artery blood velocity; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of end-tidal CO<sub>2</sub>. The red dashed line denotes the moment of standing.

Cerebral autoregulation was also assessed in a frequency-dependent manner, as slow oscillations in blood pressure ( $<0.2$  Hz) are dampened by the cerebral vessels (Aaslid *et al.*, 1989; Zhang *et al.*, 1998). Higher frequency oscillations ( $>0.2$  Hz) are transferred to the cerebral vasculature unimpeded, characterizing the cerebral autoregulation as a high-pass filter (Smirl *et al.*, 2016). Transfer function analysis (TFA) is the most widely used method for this assessment and recent guidelines were published in an attempt to standardize this approach (Claassen *et al.*, 2016). TFA is usually computed using a fast Fourier transform to quantify the relationship between input (MAP) and output (MCA  $V_{\text{mean}}$ ) signals with respects to amplitude (gain), timing (phase) and their linear association (coherence). A lower gain is usually indicative of better buffering of changes in MAP by the cerebral vasculature, but it can also suggest a greater cerebral vascular resistance (Claassen *et al.*, 2009). Phase (also called phase shift or phase lead) is representative of the delay between input and output, and a phase shift close to zero in the lower frequency range may indicate an impaired autoregulation (van Beek *et al.*, 2008). Coherence is a measure of the linearity of the relationship between input and output signals, and low coherence values (typically  $<0.5$ ) may indicate: 1) nonlinear relationship between input and output; 2) poor signal-to-noise ratio; and 3) presence of other factors influencing the output parameter (Panerai *et al.*, 2006; van Beek *et al.*, 2008). As TFA requires equidistant data points, beat-by-beat data undergo cubic polynomial interpolation and resampling at 10 Hz. To improve the spectral estimations, the Welch method is used to smooth the data after it is broken down using Hanning tapered windows to prevent spectral leakage. Segments of

data are superimposed by 50% in order to maximise smoothing. Then, auto- and cross-spectral analysis between MAP and MCA  $V_{\text{mean}}$  are used to derive gain, phase and coherence between the signals. MCA  $V_{\text{mean}}$  and MAP spectral power, gain, phase and coherence were averaged over the very low frequency (0.02–0.07 Hz), low frequency (0.07–0.2 Hz) and high frequency (0.2–0.5 Hz) ranges as recommended by current guidelines (Claassen *et al.*, 2016).

A low coherence may compromise the assessment of gain and phase, therefore manoeuvres such as repeated squats can be employed to increase coherence, and arguably allow a better interpretation of TFA (Claassen *et al.*, 2009). In this thesis, participants were requested to perform a repeated squat-to-stand after a baseline period of 2 minutes standing. The squat-to-stand protocol consisted of 5 s squatting with the knees bent at  $\approx 90^\circ$ , followed by 5 s standing straight (i.e. at a frequency of 0.1 Hz), a process which was repeated for 5 minutes. During the squat phase, contraction of the leg muscles causes an increase in blood pressure due to an increased venous return by the muscle pump and increased resistance caused by compression of the surrounding vessels. When standing, the reduction in venous pressure at the legs causes blood to pool in the lower extremities, which generates a reduction in blood pressure. These cyclic changes in blood pressure lead to an increased coherence which optimizes the TFA analysis at that frequency range. Typical recordings are presented in Figure 3.4.



**Figure 3.4.** An original record of MCA V, blood pressure and P<sub>ET</sub>-CO<sub>2</sub> responses to the repeated squat-to-stand test in a representative individual. MCA V, middle cerebral artery blood velocity; P<sub>ET</sub>-CO<sub>2</sub>, partial pressure of end-tidal CO<sub>2</sub>.

### **3.4. Data acquisition and analysis**

In all studies, signals from haemodynamic, respiratory variables and transcranial Doppler were sampled by a high-performance data acquisition hardware (Powerlab, ADInstruments, Dunedin, New Zealand) at 1 kHz and recorded using a multi-channel data acquisition software (Labchart 7 and 8, ADInstruments, Dunedin, New Zealand). The acquired data were then transferred to a digital spreadsheet (Excel 2010 and 2013, Microsoft, Redmond, WA, USA), where the beat-by-beat data was converted into averages for each time point. Statistical analysis was performed using scientific data analysis and graphing software (SPSS 22, IBM, Armonk, NY, USA – Chapters 3 and 4 or Sigmaplot 12.5, Systat software, London, UK – Chapters 4, 5 and 6). Normality was assessed with the Shapiro-Wilk test and non-normally distributed data underwent  $\log_{10}$  transformation. If after the  $\log_{10}$  transformation the data was still non-normally distributed, a non-parametric test was used. The statistical approach used for each variable is described in the methods section of each chapter. Statistical significance was set at  $p < 0.05$  to all tests performed.

### **3.5. Reliability of the methods**

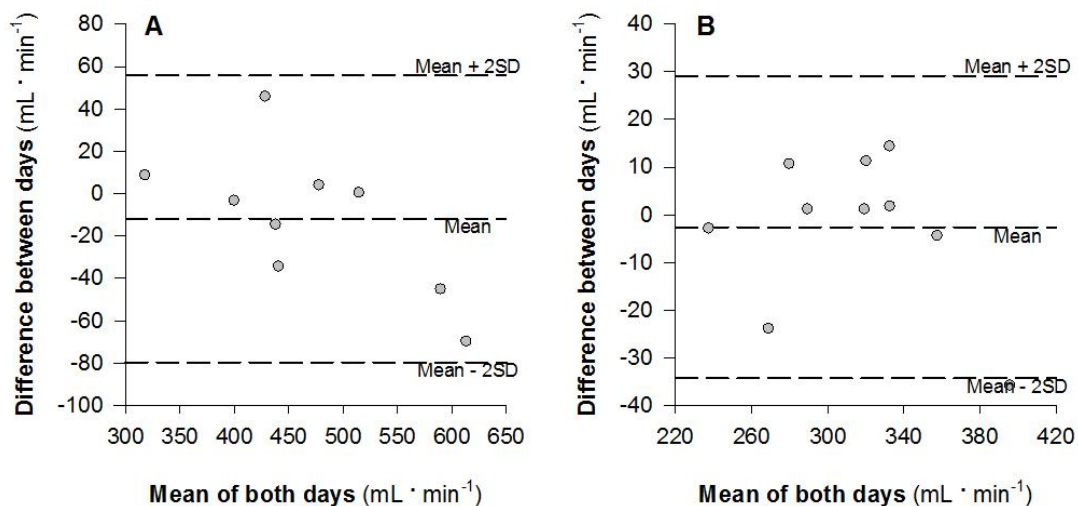
In order to assess if the methodology described above can be used in future studies aiming interventions to improve the described parameters (i.e. repeated measures), it is essential that the results obtained are reproducible and reliable in the laboratory where the assessment is taking place. Although the reproducibility of the duplex Doppler techniques were reported to be good at rest (Schoning & Scheel, 1996), training and appropriate assessment of the reliability of the examiner prior to the use of a technique in research is warranted. This section aims to assess the between-day reliability of the cerebral blood flow measurement used in the studies described in Chapters 4 and 5.

Measurements were performed in healthy young individuals (age range from 21 to 32 years) in two separate occasions at the same time of the day, at least 24 hours apart. Participants were asked to refrain from caffeinated beverages, alcohol or high intensity physical activity for 12h prior to the test. On the study day, the participant was positioned supine, resting comfortably in a bed with external noise kept to a minimum. Agreement between the two measurements was assessed using intraclass correlation coefficient with a one-way analysis of variance (ANOVA) random model (Shrout & Fleiss, 1979), coefficient of variation (CV) and Bland-Altman plots (Bland & Altman, 1986). The CV was calculated in Excel, as:  $CV = SD/\bar{x}$ , where SD is the standard deviation of the sample and  $\bar{x}$  is the sample mean.



### 3.5.1. Doppler ultrasound of the extracranial vessels

Doppler ultrasound assessment of 19 arteries (9 ICAs and 10 VAs) was performed in two occasions as described above and stored for offline analysis. Arterial diameter was measured using the device calliper function and the time averaged mean blood velocity was calculated by the machine. Blood flow in each artery was calculated as described previously. The overall correlation between days was very high, with an intraclass correlation coefficient of 0.934 (95% CI 0.756 to 0.985) for the ICAs and 0.947 for the VAs (95% CI 0.812 to 0.986), and the CV showed acceptable results at 19.5% at the ICAs and 14.5% at the VAs. The Bland-Altman plots (Figure 3.5) also show a good agreement, with a mean difference of  $-11.87 \text{ mL} \cdot \text{min}^{-1}$  and limits of agreement ranging from  $-79.65$  to  $55.92 \text{ mL} \cdot \text{min}^{-1}$  at the ICAs and a mean difference of  $-2.58 \text{ mL} \cdot \text{min}^{-1}$  and limits of agreement ranging from  $-34.27$  to  $29.11 \text{ mL} \cdot \text{min}^{-1}$  at the VAs.



**Figure 3.5.** Bland-Altman plots of the between days reproducibility of flow measurements. The between days reproducibility in blood flow is shown at the ICA (panel A) and at the VA (panel B). Dashed lines indicate the mean of the differences and limits of agreement.

### **3.5.2. Conclusions**

In this thesis, the assessment of cerebral blood flow via ultrasonography of the extracranial vessels ICA and VA was shown to be reliable, with substantial agreement between days, and have variability parameters within expected limits in all arteries measured (Quan & Shih, 1996).  $P_{ET}CO_2$  was not measured during this assessment and may account for some of the measurement variability.

**CHAPTER 4: INFLUENCE OF DAILY PHYSICAL ACTIVITY ON  
CEREBRAL BLOOD FLOW IN HEALTHY OLD INDIVIDUALS**

#### 4.1. Introduction

The global population has recently experienced an unprecedented increase in life expectancy, and the segment of the population aged more than 60 years is expected to increase the most, from 871 million people in 2013 to more than 2 billion by 2050 (United Nations, 2013). Even in the absence of disease, ageing is associated with detrimental changes in brain structure and function (Scahill *et al.*, 2003; Burgmans *et al.*, 2011), and it is one of the most important non-modifiable risk factors for cerebral vascular disease, cognitive decline and dementia (Lindsay *et al.*, 2002; Bos *et al.*, 2012). The ageing process is coupled with a reduction in cerebral blood flow in grey and white matters that can be as much as 0.5 % per year during adult life (Leenders *et al.*, 1990). This reduction has been proposed to be linked to the age-related decrease in cerebral mass or neuronal density (Kety, 1956; Leenders *et al.*, 1990), although the exact mechanisms for the cerebral blood flow decline due to the healthy ageing process remain unknown.

Physical inactivity increases the burden of disease, contributing to  $\approx 9\%$  of the overall premature mortality and to the loss of life expectancy with conditions such as coronary heart disease, diabetes and cancer (Lee *et al.*, 2012). Regular physical activity is a well-known strategy to reduce the age-related decline in physical (Chodzko-Zajko *et al.*, 2009) and mental (Paillard, 2015) function, although the mechanisms of this protection are not fully understood. Animal studies show that an increase in the number of new hippocampal cells (van Praag *et al.*, 1999) and enhanced cerebellar vascularization (Black *et al.*, 1990) were associated with physical activity in rats. Furthermore, exercise, which is a

planned and structured form of physical activity, has also been associated with increased synaptogenesis, as exercising rats had increased dendritic complexity and number of dendritic spines when compared to control animals (Eadie *et al.*, 2005). However, some cerebral adaptations to chronic exercise training, such as the functional connectivity in cerebral association networks, are independent of physical activity levels (Voss *et al.*, 2016). This may implicate that different mechanisms can be stimulated by high levels of physical activity and cardiorespiratory fitness. In general terms, molecular messengers proposed to mediate these changes include brain-derived neurotrophic factor, insulin-like growth factor 1, uncoupling protein 2, and neurotransmitters such as serotonin, noradrenalin and acetylcholine (Lista & Sorrentino, 2010).

Human studies show that higher physical activity is associated with an improvement in several cognitive areas, such as working memory, attention and task-switching (Kramer *et al.*, 1999; Weuve *et al.*, 2004), although the mechanisms are still unclear. In one study with 18766 women, participants in the highest quintile of physical activity ( $> 26$  metabolic equivalent  $\cdot$  h  $\cdot$  wk<sup>-1</sup>), had a 20 % lower risk of cognitive impairment when compared to the ones in the lowest physical activity quintile ( $< 5.2$  metabolic equivalent  $\cdot$  h  $\cdot$  wk<sup>-1</sup>) (Weuve *et al.*, 2004). It has been shown that a one year walking intervention increased the neuronal connectivity between cognitive-related areas such as the fronto-temporal cortices in humans (Voss *et al.*, 2010). It has been previously demonstrated that old individuals that keep working or remain physically active after reaching retirement age have higher cerebral blood flow and superior cognition when compared to sedentary retired individuals (Rogers *et al.*, 1990).

Furthermore, old individuals who are highly aerobically fit are reported to have higher cerebral perfusion than their untrained counterparts (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b), although it has also been shown that a high cardiorespiratory fitness was associated with a reduced cerebral vasodilatory reserve (Thomas *et al.*, 2013). Despite being methodologically sound, the choice of recruiting highly fit individuals (Ainslie *et al*  $VO_{2max}$ : 52.4  $ml \cdot kg^{-1} \cdot min^{-1}$ , Bailey *et al*  $VO_{2max}$ : 39  $ml \cdot kg^{-1} \cdot min^{-1}$ , Thomas *et al*  $VO_{2max}$ : 40.6  $ml \cdot kg^{-1} \cdot min^{-1}$ ) may not be representative of the general population, limiting the findings to this specific group. On the other hand, the studies with less fit but still active individuals assessed physical activity using questionnaires (Rogers *et al.*, 1990; Weuve *et al.*, 2004), and as these self-assessment tools were not objectively validated, discrepancies between the report and the actual physical activity levels may limit the interpretation of these findings (Shephard, 2003).

Understanding how daily physical activity influences cerebral blood flow is important to identify the mechanisms by which it confers protection against cognitive decline and dementia. This Chapter aimed to determine the influence of objectively measured daily physical activity on the cerebral blood flow using duplex Doppler ultrasound and cognition in a cohort of community dwelling healthy old individuals. This study tested the hypothesis that physically active individuals would have higher cerebral blood flow and cognition than their inactive counterparts.

## **4.2. Methods**

All the procedures performed in this study were approved by the National Research Ethics Service Committee West Midlands - Black Country (10/H1202/77) and conformed to the declaration of Helsinki. Potential participants were given an information sheet and a detailed verbal explanation of the tests. If satisfied with the explanation and given the opportunity to ask questions about the procedures, volunteers would provide written informed consent to participate in the study. This study is a part of a larger study that assessed cardiovascular and immune system function in a cohort of healthy elderly individuals (Bartlett *et al.*, 2016).

### **4.2.1. Cohort characteristics**

Participants were recruited from a cohort of 217 healthy individuals aged 60-80 years. After written informed consent was obtained, participants were screened at the Wellcome Trust Clinical Research Facility, Birmingham, UK for their blood glucose (Accu-Chek Performa, F. Hoffmann-La Roche AG, Basel, Switzerland) which excluded 6 participants due to possible undiagnosed dysglycemic pathology. Participants were free from diabetes, neurologic, cerebral vascular, heart, liver, inflammatory disease, or cancer. Physical activity was assessed by 7-day accelerometry (GT3X, Actigraph, Pensacola, FL, USA). Participants wore the accelerometer around the waist above the left leg, except for water based activities such as showering and swimming, 24 h/day for 7 consecutive days and the numbers of daily steps, counts, vectors and time in each intensity of physical activity were calculated. The participants were then grouped in physical activity quintiles according to the accelerometry data, and participants from the

least (n = 16, low physical activity group) and most (n = 17, high physical activity group) physically active quintiles were recalled for further assessment, as detailed below.

#### **4.2.2. Measured variables**

On a separate visit to the laboratory, blood pressure and heart rate (Dinamap ProCare 200, GE Medical Systems, Milwaukee, WI, USA) were measured after at least 5 minutes of resting in a supine position. Cognition was determined with the MoCA. A nasal cannula was connected to a capnograph (Moxus modular, AEI Technologies, Pittsburgh, PA, USA) to measure  $P_{ET}CO_2$ . Arterial diameter and time averaged mean blood velocity at the ICAs and VAs were assessed bilaterally using duplex Doppler ultrasound (iE33, Philips Healthcare, Best, the Netherlands). The blood flow in each artery was calculated as described in section 3.2.2.

Global cerebral blood flow was the result of the sum of the bilateral ICAs and bilateral VAs blood flow. Due to insufficient image quality, 3 participants were excluded from the final analysis, resulting in 14 participants on the low physical activity group and 16 participants on the high physical activity group.

#### **4.2.3. Data analysis**

Capnograph data was recorded digitally and stored for offline analysis (Powerlab and LabChart Pro, ADInstruments, Dunedin, New Zealand) and ultrasound data was stored and analysed offline (Xcelera, Philips healthcare, Best, The Netherlands).



#### **4.2.4. Statistical analysis**

Normality was assessed with the Shapiro-Wilk test, and non-normal data was  $\log_{10}$  transformed to achieve normal distribution. Independent samples *t*-tests were used for between groups comparisons and significance was set on  $\alpha = 0.05$ . Mann-Whitney U test was used to analyse the cognition and non-normally distributed data. Statistical analysis was performed using the SPSS software (version 22, IBM, Armonk, NY, USA) and results are presented as mean (95% confidence interval).

### 4.3. Results

Participant characteristics are presented in Table 4.1. There were no significant differences in age, blood pressure, heart rate,  $P_{ET}CO_2$  or MoCA score between the low and high physical activity groups. Accelerometry data is presented in Table 4.2. The low physical activity group was on average  $\approx 55\%$  less active than the high physical activity group when assessed by the number of daily steps. The time over the 7-day period spent in moderate activity (from 3 to 5.99 metabolic equivalents) in the low physical activity groups was 107 (69-145) min while the high physical activity group spent 382 (258-505) min ( $p < 0.001$ ) over the 7 days. Four participants (2 low and 2 high physical activity group) did not complete the 7-day period (range: 4 to 6 days) and were excluded from this analysis.

**Table 4.1.** Participants characteristics

	<b>Low activity</b>	<b>High activity</b>	<b><i>t</i> and <i>p</i>-value</b>
<b>Age</b> (years)	68 (65-71)	66 (65-68)	$t(28) = 0.85$ $p = 0.400$
<b>Sex</b> (males/females)	7 / 7	7 / 9	-
<b>Height</b> (cm)	169 (163-174)	169 (163-174)	$t(28) = 0.01$ $p = 0.995$
<b>Weight</b> (kg)	75 (65-85)	68 (62-73)	$t(28) = 0.30$ $p = 0.766$
<b>BMI</b> (kg·m <sup>-2</sup> )	26 (24-29)	24 (22-26)	$t(28) = 1.80$ $p = 0.084$
<b>SBP</b> (mmHg)	133 (122-144)	126 (119-133)	$t(28) = 1.12$ $p = 0.272$
<b>DBP</b> (mmHg)	77 (71-82)	77 (73-81)	$t(28) = 0.01$ $p = 0.989$
<b>MAP</b> (mmHg)	96 (89-102)	93 (88-98)	$t(28) = 0.58$ $p = 0.568$
<b>HR</b> (bpm)	65 (59-72)	68 (63-73)	$t(28) = -0.64$ $p = 0.525$
<b>P<sub>ET</sub>CO<sub>2</sub></b> (mmHg)	35 (33-36)	35 (33-36)	$t(28) = 0.22$ $p = 0.827$
<b>MoCA</b> (score)*	27 (25.5-29)	26.5 (25-28)	$U = 95.0$ $p = 0.498$

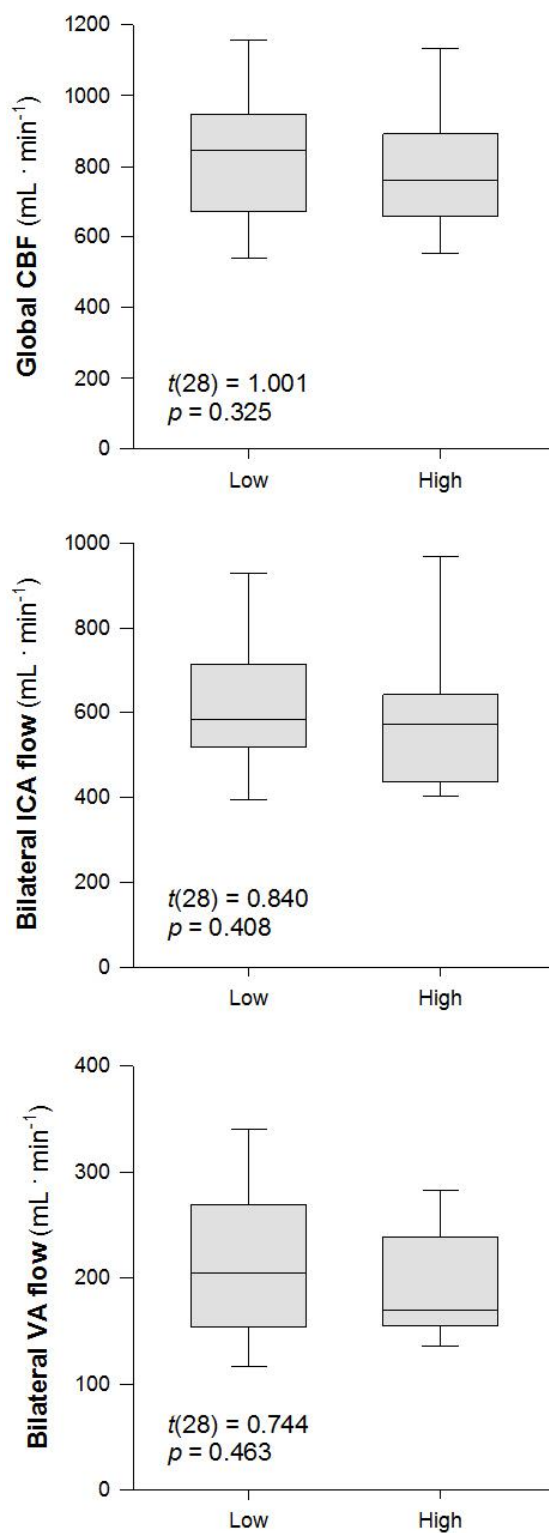
SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of arterial carbon dioxide; MoCA, Montréal Cognitive Assessment. Values are reported as mean (95% confidence interval). \*Reported as median (interquartile range).

**Table 4.2.** Physical activity as measured by 7-day accelerometry

	<b>Low activity</b>	<b>High activity</b>	<b><i>t and p-value</i></b>
<b>Daily steps (n)</b>	4923 (4168-5677)	11063 (9445-12681)	$t(28) = -7.90, p < 0.001$
<b>Daily counts (x1000)</b>	614 (558-670)	1400 (1287-1513)	$t(28) = -14.49, p < 0.001$
<b>Daily vectors (x1000)</b>	361 (328-394)	820 (755-885)	$t(28) = -12.89, p < 0.001$
<b>Validated time in light PA (min)</b>	285 (217-352)	719 (627-811)	$t(28) = -7.97, p < 0.001$
<b>Validated time in moderate PA (min)</b>	107 (69-145)	382 (258-505)	$t(28) = -4.29, p < 0.001$
<b>Validated time in vigorous PA (min)*</b>	0 (0-0.5)	2.5 (0-33.5)	$U = 57.50, p = 0.013$

PA, physical activity. Values are reported as mean (95% confidence interval). \*Reported as median (interquartile range).

As presented in Figure 4.1, global cerebral blood flow was not significantly different between low and high physical activity groups. Low physical activity group had a bilateral ICA flow of 617 (515-719) mL · min<sup>-1</sup> while in the high physical activity group, bilateral ICA flow was 595 (500-690) mL · min<sup>-1</sup> with no significant difference between groups ( $t(28) = 0.84$ ,  $p = 0.408$ ). Similarly, the bilateral VA flow was 211 (168-253) mL · min<sup>-1</sup> on the low physical activity group and 193 (165-221) mL · min<sup>-1</sup> on the high physical activity group with no significant difference between groups ( $t(28) = 0.74$ ,  $p = 0.463$ ). The relative contribution of the bilateral ICA to the global cerebral blood flow was similar between groups with 75 (71-79) % in the low physical activity group and 75 (71-78) % in the high physical activity group ( $t(28) = 0.23$ ,  $p = 0.820$ ). Table 4.3 shows the mean diameter and flow velocities from each measured artery.



**Figure 4.1.** Global and regional cerebral blood flow (CBF) in low and high physical activity groups.  
 Low, low physical activity group; High, high physical activity group; CBF, cerebral blood flow; ICA, internal carotid artery; VA, vertebral artery

**Table 4.3.** Arterial diameter and blood velocity measured in the internal carotid and vertebral arteries

	<b>Low activity</b>	<b>High activity</b>	<b><i>t</i> and <i>p</i>-value</b>
<b>R ICA diameter</b> (cm)	0.55 (0.09)	0.48 (0.09)	$t(28) = 1.96$ $p = 0.060$
<b>R ICA <math>V_{\text{mean}}</math></b> (cm · s <sup>-1</sup> )	25.7 (9.0)	26.7 (6.5)	$t(28) = -0.35$ $p = 0.728$
<b>R VA diameter</b> (cm)	0.36 (0.05)	0.33 (0.05)	$t(28) = 1.58$ $p = 0.124$
<b>R VA <math>V_{\text{mean}}</math></b> (cm · s <sup>-1</sup> )	15.2 (4.8)	16.1 (5.1)	$t(28) = -0.48$ $p = 0.637$
<b>L ICA diameter</b> (cm)	0.49 (0.09)	0.47 (0.06)	$t(28) = 0.104$ $p = 0.412$
<b>L ICA <math>V_{\text{mean}}</math></b> (cm · s <sup>-1</sup> )	24.0 (4.8)	28.5 (7.3)	$t(28) = -1.88$ $p = 0.070$
<b>L VA diameter</b> (cm)	0.38 (0.07)	0.37 (0.05)	$t(28) = 0.24$ $p = 0.811$
<b>L VA <math>V_{\text{mean}}</math></b> (cm · s <sup>-1</sup> )	16.9 (5.9)	16.5 (5.0)	$t(28) = 0.20$ $p = 0.842$

R, right; L, left; ICA, internal carotid artery; VA, vertebral artery;  $V_{\text{mean}}$ , mean blood velocity. Values are reported as mean (SD).

#### **4.4. Discussion**

This study aimed to assess the influence of objectively measured daily physical activity on cerebral blood flow using duplex Doppler ultrasound and cognition in a cohort of community dwelling healthy old individuals. Although the high physical activity group was more than twice as active as the low physical activity group, no difference in global cerebral blood flow or regional differences as measured at the ICAs and VAs was observed. Furthermore, a higher degree of daily physical activity did not significantly affect cognition in this population.

Regular physical activity and exercise are known to reduce the risk for cerebral vascular disease and dementia (Pearson *et al.*, 2002; Larson *et al.*, 2006). Current guidelines recommend that old individuals (men and women aged 65 years or more) perform at least 150 minutes per week of moderate intensity aerobic exercise in addition to strength-enhancing exercise (Nelson *et al.*, 2007; Garber *et al.*, 2011). The guidelines also suggest that greater amounts of activity may provide additional health benefits, reducing the risk of cardiovascular death, cerebrovascular disease, anxiety and depression, and should be recommended in all who are not limited by health conditions to perform more exercise (Nelson *et al.*, 2007). It has been shown that there is an inverse linear dose-response relation between physical activity and all-cause mortality (Lee & Skerrett, 2001), although the mechanisms underpinning exercise-related improvements in systemic and cerebral protection are not fully understood.

Sedentary behaviour leads to a pro-inflammatory state caused by accumulation of visceral adiposity, facilitating the atherosclerotic degeneration of the arterial



walls (Szostak & Laurant, 2011). In fact, animal studies show that after eleven generations of selective breeding in rats, a lower aerobic capacity increased blood pressure, fasting glucose, insulin, visceral adiposity and triglycerides while essential proteins for mitochondrial metabolism such as peroxisome proliferative activated receptor  $\gamma$  and ATP synthase  $H^+$ -transporting mitochondrial F1 complex, among others, were markedly reduced, supporting the idea that an impaired mitochondrial oxidation pathway may explain some of the metabolic risk profile of sedentary animals (Wisløff *et al.*, 2005). In addition, ageing vessels are known to generate excess reactive oxygen species, peroxides and superoxides mainly by mitochondrial respiratory chain production, which contribute to an impaired endothelial function (El Assar *et al.*, 2013). Exercise has been shown to contribute to a reduced oxidative stress (Miyazaki *et al.*, 2001), and improve systemic arterial function via mechanisms including NO (DeSouza *et al.*, 2000) and arterial compliance (Ashor *et al.*, 2014). Furthermore, it has been proposed that exercise improves the cerebral vasculature by stimulating the production of endothelial progenitor cells, optimizing the shear stress and NO vasodilatory pathways, enhanced sympathetic nervous system activation stimulating angiogenesis and reducing the mechanical load in arteries by a lower heart rate (reviewed in (Bolduc *et al.*, 2013). However, the mechanisms responsible for the improvement of cerebral vascular function with exercise are still unclear.

Maintaining a high cardiorespiratory fitness throughout life has been shown to offset the age-related decline in cerebral perfusion by approximately 17% (Ainslie *et al.*, 2008). In addition, studies showed that cerebral vasodilatory

reserve appears to be associated with cardiorespiratory fitness in old individuals (Bailey *et al.*, 2013b; Barnes *et al.*, 2013). However, other studies showed that cardiorespiratory fitness had no effect or even reduced  $\text{CVR}_{\text{CO}_2}$  in trained old individuals when compared to their sedentary peers (Thomas *et al.*, 2013; Zhu *et al.*, 2013). This highly controversial topic is the subject of the following thesis Chapter. Although several studies assessed the cerebral perfusion and its regulation comparing old trained and untrained individuals (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b; Thomas *et al.*, 2013; Flück *et al.*, 2014), the impact of daily physical activity on the cerebral blood flow of untrained healthy community-dwelling old individuals who are considered active or sedentary as per current guidelines is still unclear. Previous studies assessed cardiorespiratory fitness through  $\text{VO}_2\text{max}$  tests (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b; Flück *et al.*, 2014) or questionnaires (Rogers *et al.*, 1990; Weuve *et al.*, 2004). Although these techniques may be useful for assessing cardiovascular fitness or sedentary behaviour, the assessment of the optimal physical activity levels in late life as recommended by current guidelines is not possible. Questionnaires provide a low-cost easy solution to assess physical activity, but they may provide unreliable estimates of low-to-moderate intensity physical activities such as walking (Ainsworth *et al.*, 1993; Richardson *et al.*, 1994). On the other hand, the use of accelerometry provides not only the number of steps, counts and vectors, but also the time in moderate and vigorous activity that can be quantified and assessed comparing to current guideline recommendations. Although accelerometers are reported to correlate well with doubly labelled water or calorimetric methods (Westerterp, 1999), weaknesses of this method

include the cost and the fact that it is not sensitive to all kinds of activity such as swimming (due to lack of waterproofness in some models) or upper body exercise. Despite these weaknesses, accelerometers are considered a sound option for the assessment of physical activity in a population (Tudor-Locke & Myers, 2001). Only two participants in the low physical activity group from this study reached the minimum physical activity level as recommended, while all in the high physical activity group met the guideline recommendations. Thus, this study suggests that the difference in daily physical activity levels from community-dwelling healthy individuals in this cohort, albeit substantial, was not enough to impact resting global cerebral blood flow or the distribution of cerebral blood flow between the ICAs and VAs.

Previous studies have reported that cognition is lower in old sedentary individuals when compared to their physically active counterparts (Rogers *et al.*, 1990; Weuve *et al.*, 2004). In a study with 18766 women aged 70 to 81 years, women on the highest quintile of physical activity as measured by mailed questionnaires had a 20% lower risk of cognitive impairment when compared to the women on the lowest quintile (Weuve *et al.*, 2004). Cognition was assessed using telephone interview for cognitive status, which is based on the mini mental state exam, and the east Boston memory test. Furthermore, Rogers *et al.* (1990) have reported that in 83 old individuals (62 to 70 years), those who stayed physically active or kept working after reaching retirement age had higher cognitive capacity screening exam scores than their inactive retired counterparts after a 4-years follow-up. Working and physically active participants also had a stable cerebral blood flow, while their sedentary

counterparts significantly declined over time as measured by the [<sup>133</sup>Xe] technique. Serum cholesterol profile (Cheng *et al.*, 2014), arterial stiffness (Cooper *et al.*, 2016), endothelial function (Vendemiale *et al.*, 2013) and other are potential mechanisms contributing to cognitive decline in ageing, and exercise may be used as a means of counteracting these risk factors. High total cholesterol levels are known risk factors for dementia (Solomon *et al.*, 2009), and this association may be due to increased plasma homocysteine causing blood-brain barrier leakage and favouring inflammation (Cheng *et al.*, 2014). Stiffening of the large arteries may lead to microvascular damage due to excessive pulsatility, which increases the risk of cerebral damage and cognitive impairment (Cooper *et al.*, 2016). Furthermore, endothelial dysfunction may be present even in the early stages of cognitive decline, reducing the cerebral vasodilatory reserve, which in turn may lead to an inadequate response to cerebral demand for nutrients (Vendemiale *et al.*, 2013). Physical activity may help improve cerebral vascular function and reduce cognitive decline by improving blood lipids profile, reducing arterial stiffness and improving endothelial function, thus counteracting many of the proposed mechanisms for the cognitive decline (DeSouza *et al.*, 2000; Ashor *et al.*, 2014; Paillard, 2015). However, the results from this chapter did not show any difference in cognition between the high and low physical activity groups. One reason for this finding is that this study may be underpowered to assess cognition using the MoCA. Previous projects estimated that to detect a difference on a MoCA score of 1.5 between 2 groups would require 52 participants per group (Boss *et al.*, 2014). Another important limitation for this analysis is that this study is cross-sectional,

and results should be interpreted cautiously. Lifelong physical activity was not assessed, and the measured levels could be over or underestimating previous physical activity levels. This may limit the interpretation of the findings presented as participants might have long-term cerebral vascular adaptations of exercise even if they were allocated in the low physical activity group due to late-life change in physical activity habits. Furthermore, retirement was not assessed by this study, and interpretation of the findings in this Chapter should consider this limitation.

In conclusion, this study suggests that daily physical activity did not influence cerebral blood flow in this cohort of healthy old individuals. Although cognition was similar between groups, this study may have been underpowered to allow proper significance of the cognitive assessment findings. The use of instruments to assess specific areas of cognition affected by sedentary behaviour may be warranted in future studies. Higher levels of aerobic exercise might be required to promote substantial effects on the resting cerebral blood flow in healthy old individuals. Furthermore, this study assessed a very healthy sample which may not be representative of the majority of the ageing population, as the prevalence of disease is high. However, this study aimed to assess the effects of physical activity and age *per se* on the cerebral blood flow, which requires exclusion of confounding factors such as disease. Studies with people with chronic diseases are warranted to investigate the effect of daily physical activity in a more clinically diverse population.

**CHAPTER 5: IMPACT OF AEROBIC FITNESS ON CEREBRAL  
BLOOD FLOW AND CEREBRAL VASCULAR  
RESPONSIVENESS TO CO<sub>2</sub> IN YOUNG AND OLDER MEN**

This Chapter was published in the Scandinavian Journal of Medicine and Science in Sports (Braz *et al.*, 2016) and suffered minor modifications to adapt to the thesis format.

### **5.1. Abstract**

This study sought to test the hypothesis that brain blood flow and  $\text{CVR}_{\text{CO}_2}$  are greater in aerobically trained young and old individuals compared to their untrained counterparts. In eleven young trained (23 (20 to 26) yr [mean (95% confidence interval)]), ten young untrained (25 (22 to 28) yr, eight older trained (65 (61 to 69) yr, and nine older untrained (67 (64 to 71) yr healthy individuals, Doppler ultrasound of the internal carotid (ICA) and vertebral (VA) artery blood flow were determined, along with middle cerebral artery mean flow velocity (MCA  $V_{\text{mean}}$ ). Bilateral ICA blood flow was higher in trained individuals when compared to untrained ( $\approx 31\%$ ,  $p < 0.05$ ), but was not influenced by age. VA blood flow was not affected by age or cardiorespiratory fitness. MCA  $V_{\text{mean}}$  was reduced with age (59.5 (55.0 to 64.1)  $\text{cm}\cdot\text{s}^{-1}$  young vs. 43.6 (38.4 to 48.9)  $\text{cm}\cdot\text{s}^{-1}$  old,  $p < 0.05$ ) with no significant effect of training observed. MCA  $\text{CVR}_{\text{CO}_2}$  were not significantly affected by either age or training status, while ICA  $\text{CVR}_{\text{CO}_2}$  tended to be elevated in the old trained group. These findings indicate that endurance training enhances bilateral ICA but not VA blood flow in both young and older individuals.

## 5.2. Introduction

An unprecedented increase in life expectancy and global population ageing has occurred over the last few decades (Salomon et al., 2012). Age is one of the most important non-modifiable risk factors for cerebral vascular disease (Bos et al., 2012) and dementia (Lindsay et al., 2002), and associated with changes in brain structure (Scahill et al., 2003), chemical signaling and function (Burgmans et al., 2011). However, other modifiable risk factors such as diet (Gomez-Pinilla, 2008) and habitual physical activity (Hillman et al., 2006) may also have an impact on brain health.

Exercise training improves cognitive task performance and reduces the morphological and cellular decline of the brain in ageing animal models (van Praag et al., 2005). While in elderly humans, exercise training reduces the loss of brain volume (Colcombe et al., 2006), improves mental function (Erickson et al., 2011) and enhances cerebral vasomotor reactivity (Bailey et al., 2013b; Barnes et al., 2013) although the latter remains controversial (Thomas et al., 2013; Zhu et al., 2013).

The cerebral circulation is highly responsive to changes in the PaCO<sub>2</sub> (Kety & Schmidt, 1948a) and as a consequence CVR<sub>CO2</sub> is frequently used to provide an indication of general cerebrovascular health (Gupta et al., 2012). There is accumulating evidence to suggest that CVR<sub>CO2</sub> is impaired in neurodegenerative conditions such as Alzheimer's disease (Glodzik et al., 2013), Parkinson's disease (Zamani et al., 2011) and chronic traumatic brain injury (Bailey et al., 2013a) and may increase the risk of death by any cause (Portegies et al., 2014). The influence of healthy ageing on CVR<sub>CO2</sub> remains



equivocal, with evidence for (Bailey et al., 2013b; Barnes et al., 2013) and against (Kastrup et al., 1998; Oudegeest-Sander et al., 2014) the concept that age may impair  $CVR_{CO_2}$ . Furthermore, there is also contradictory evidence with respect to whether aerobic capacity influences  $CVR_{CO_2}$  in elderly individuals. While some studies have reported an enhanced  $CVR_{CO_2}$  in participants with higher aerobic capacity (Bailey et al. 2013; Barnes et al. 2013), other studies show that aerobic fitness has a negative or minimal impact on  $CVR_{CO_2}$  (Thomas et al. 2013; Zhu et al. 2013). One potential limitation of the majority of these studies is that the transcranial Doppler technique was employed to assess  $CVR_{CO_2}$  (Bailey et al. 2013; Barnes et al. 2013; Zhu et al. 2013). While this technique has a number of advantages, without an accurate measure of middle cerebral artery diameter it can only be assumed that increases in blood velocity are proportional to increases in cerebral blood flow. This is pertinent because recent studies have challenged this assumption and middle cerebral artery dilation has been reported in hypercapnic tests (Coverdale et al. 2014; Verbree et al. 2014). An alternative approach is to assess the  $CVR_{CO_2}$  of the extracranial vessels (ICA, internal carotid artery; VA, vertebral artery) as this permits the concurrent volumetric quantification of cerebral blood flow (Willie et al. 2012). In addition, anatomical and functional differences within the anterior and posterior cerebral circulations, such as neurotransmitter signaling, sympathetic innervation (Edvinsson et al. 1976; Hamel et al. 1988) and responsiveness to  $CO_2$  (Reinhard et al. 2008), may result in regional differences (i.e. ICA vs VA) in the vascular adaptations to exercise training. However, the influence of age and aerobic exercise training on the extracranial blood vessels remains unclear.

The aim of this study was to investigate if age and cardiorespiratory fitness influence resting cerebral blood flow and  $\text{CVR}_{\text{CO}_2}$ . To achieve this, in sedentary and aerobically trained young and old individuals bilateral ICA and VA blood flow were measured, and ICA blood flow was determined in response to a hypercapnic challenge (i.e.,  $\text{CVR}_{\text{CO}_2}$ ). This study tested the hypothesis that bilateral ICA and VA blood flow and  $\text{CVR}_{\text{CO}_2}$  would be greater in aerobically trained young and old individuals compared to their untrained counterparts.

### **5.3. Methods**

All experimental protocols and procedures were approved by the ethical committee of the Swiss Federal Institute of Technology Zurich (EK 2013-N-17) and conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants after they had received a detailed verbal and written explanation of the study. Twenty-one young and twenty-four older males were recruited to the study and underwent screening by a physician to identify any cardiovascular, pulmonary, metabolic or neurological disease or use of medications. Seven older volunteers were excluded after the health screening. Cardiorespiratory fitness was first determined via self-assessment and confirmed through measurement of  $\text{VO}_2\text{max}$  during an incremental exercise test to volitional exhaustion on a cycle ergometer while monitored by a physician (described below). In total twelve young trained, eleven young untrained, eight older trained and nine older untrained individuals took part in the study. Participants were requested to abstain from strenuous physical activity for 24 h, alcohol and caffeine for 12 h prior to experimental sessions. This study was conducted along with another investigation that tested a different hypothesis (Flück et al., 2014).

#### **5.3.1. Measurements**

HR was monitored using a lead II electrocardiogram and beat-by-beat MAP obtained from the middle finger of the left hand (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands).  $P_{\text{ETCO}_2}$  was monitored on a breath-by-breath basis through a face mask connected to a two-way valve (Hans Rudolph, Kansas City, USA) and respiratory analyzer (Cosmed Quark CPET,

Rome, Italy) and MCA  $V_{\text{mean}}$  by transcranial Doppler ultrasonography (Doppler Box, DWL, Sipplingen, Germany) using a 2 MHz probe fixed on the temporal window using an adjustable headband and ultrasound gel. Signals were sampled at 1 kHz and recorded for offline analysis using an analog-to-digital converter and data acquisition software (Powerlab and LabChart Pro, ADInstruments, Dunedin, New Zealand). Bilateral measures of arterial blood velocity and diameter were measured by a single experienced investigator (I.D.B.) at the ICA and VA using duplex Doppler ultrasound (Mindray M7, Shenzhen, GNG, China) with a 7.5 MHz linear array transducer and ultrasound gel, performed in brightness mode for the assessment of the diameter and the time-averaged mean blood flow velocity was acquired with the pulsed-wave mode. The sample volume was adjusted to capture the entire vessel lumen and the insonation angle was kept below 60°. Images were recorded and stored for offline analysis.

### **5.3.2. Protocol**

A preliminary visit to the laboratory consisted on a medical screening and a maximal incremental exercise test on a cycle ergometer. A protocol starting with a warm-up period of 5 min at 100 W (young untrained), 150 W (young trained), 50 W (old trained) and 20 W (old untrained) followed by 30 W increases per minute in young untrained and young and old trained individuals and 20 W increases per minute in old untrained individuals until exhaustion was used to determine  $\text{VO}_2\text{max}$  and maximal workload.

On the main study visit, participants were instrumented and then rested for 10 min in a comfortable semi-supine position followed by bilateral ICA and VA

diameter and velocity measurements performed for the calculation of global cerebral blood flow. MAP, HR,  $P_{ET}CO_2$  and MCA  $V_{mean}$  were then recorded for 3 min. After determination of basal  $P_{ET}CO_2$  levels, a hypercapnic test protocol was then conducted whereby  $CO_2$  from an external compressed gas cylinder was mixed and added to the inspired air (Altitrainer, SMTEC, Nyon, Switzerland) through the face mask to reach a stable plateau in  $P_{ET}CO_2$  at 1.5 mmHg (step 1) and 5.5 mmHg (step 2) above baseline measurements for 120 s in each step. This protocol was chosen because it has been previously reported that maintenance of  $P_{ET}CO_2$  at 1.5 mmHg above baseline allows for a better control of  $P_{ET}CO_2$  and consequently reduces the variability in MCA  $V_{mean}$  (Harris et al., 2006). MAP, HR,  $P_{ET}CO_2$  and MCA  $V_{mean}$  were recorded throughout, and ICA diameter and velocity measurements made at each step.

### **5.3.3. Data analysis**

Body mass index (BMI) was calculated dividing the participants' weight by the square of the height. MAP was calculated using the formula:  $\frac{SBP}{3} + 2\frac{DBP}{3}$ , where SBP is the systolic blood pressure and DBP is the diastolic blood pressure.

Analysis of the ultrasound data was made offline using the recorded images by a blinded investigator. Arterial diameter was measured using NIH ImageJ software (Schneider et al., 2012) and blood flow velocity was measured by the machine software. Internal carotid artery measurements were performed ~1.5 cm from the bifurcation of the common carotid artery. VA measurements were performed between the transverse process of the C3 vertebra and the subclavian artery. Mean systolic and diastolic diameters were averaged over at least 5 cardiac cycles to determine the mean artery diameter and the mean flow

velocity was obtained from the machine over 5 to 10 cardiac cycles. Blood flow in each artery was calculated as:

$$\pi \times \left( \frac{\text{mean diameter}}{2} \right)^2 \times \text{mean velocity} \times 60$$

CVCi was calculated dividing the MCA  $V_{\text{mean}}$  by the MAP and ICA conductance was calculated dividing the ICA flow by the MAP.

$\text{CVR}_{\text{CO}_2}$  was calculated as the change in ICA (flow) and MCA (mean velocity) per mmHg  $\text{P}_{\text{ETCO}_2}$  between the first +1.5 mmHg and the +5.5 mmHg  $\text{P}_{\text{ETCO}_2}$  steps (Harris et al., 2006) as shown by the formulae:  $\frac{\Delta\text{Flow}}{\Delta\text{P}_{\text{ETCO}_2}}$  or  $\frac{\% \text{Flow change}}{\Delta\text{P}_{\text{ETCO}_2}}$ ,

where  $\Delta\text{P}_{\text{ETCO}_2}$  is the absolute change in  $\text{P}_{\text{ETCO}_2}$ ,  $\Delta\text{Flow}$  is the absolute change in ICA blood flow or MCA  $V_{\text{mean}}$  and %Flow change is the relative (percent) change in either ICA blood flow or MCA  $V_{\text{mean}}$  between steps.

Due to insufficient image quality occasionally participants were omitted from aspects of the analysis and participant numbers are stated within the legend of each Table and Figure.

#### **5.3.4. Statistical analysis**

Values are reported as mean (95 % confidence interval) in text or SD in Tables and Figures. Two-way ANOVA with age (Young, Old) and training status (Trained, Untrained) as main factors was used, and the  $F$ -values are reported alongside the significance. Normality was assessed through Shapiro-Wilk test and non-normally distributed data underwent  $\log_{10}$  transformation prior to statistical analysis. Effect sizes ( $d$ ) were calculated as a quotient of the difference between means by the pooled SD. IBM SPSS Statistics 22 (IBM

Corp., Armonk, NY, USA) was used for analysis and statistical significance set at  $p < 0.05$ .

## 5.4. Results

### 5.4.1. Participant characteristics

The age difference between young and old participants was  $\approx 42$  years ( $F(1,31) = 454.5, p < 0.001$ ), however neither the trained and untrained young participants, nor the trained and untrained old participants differed in age (Table 5.1). BMI was higher in the old group ( $F(1,31) = 8.3, p = 0.007, d = 1.01$ ), while MAP was not different between groups and resting HR tended to be lower in the trained individuals ( $F(1,31) = 4.0, p = 0.053, d = -0.76$ ).  $P_{ET}CO_2$  was lower in the old ( $F(1,31) = 12.5, p = 0.001, d = -1.27$ ), and as expected  $VO_{2max}$  was higher in the trained ( $F(1,31) = 47.8, p < 0.001, d = 1.12$ ) and young individuals ( $F(1,31) = 168.6, p < 0.001, d = 2.86$ ).



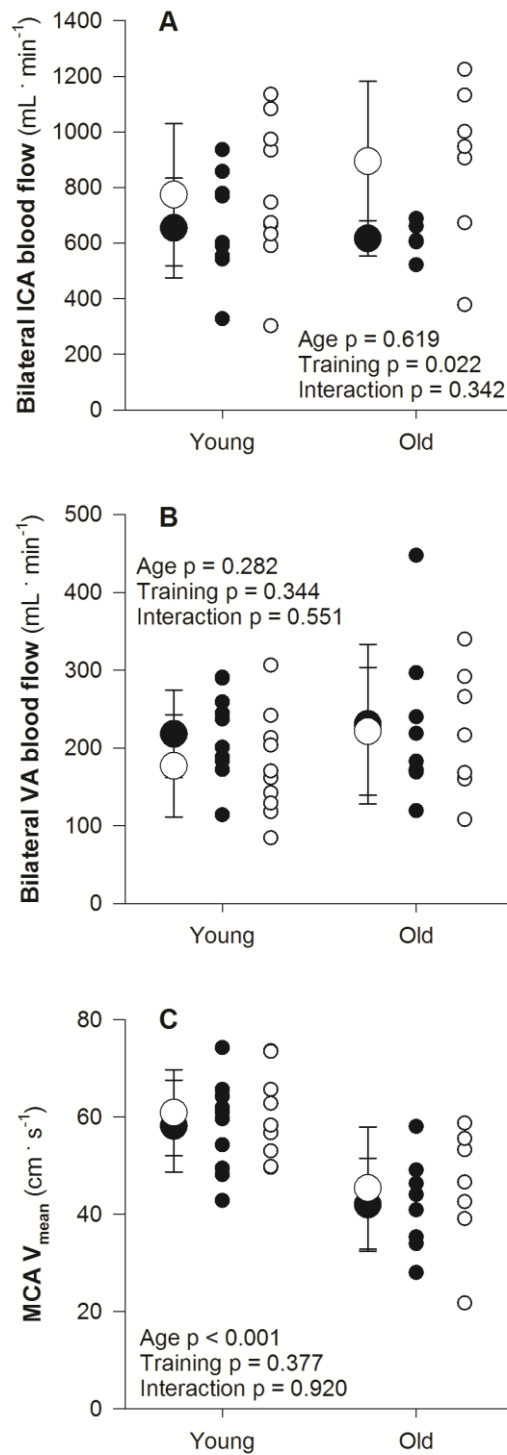
**Table 5.1.** Participants' characteristics

	Trained	Untrained	Age	<b>F and P value</b>	
				Training	Interaction
<b>Age (years)</b>					
Young	23 (4)	25 (5)	$F(1,31) = 454.5$	$F(1,31) = 0.8$	$F(1,31) = 0.1$
Old	65 (3)	67 (7)	$P < 0.001$	$P = 0.371$	$P = 0.824$
<b>BMI (kg·m<sup>-2</sup>)</b>					
Young	21.5 (1.5)	23.2 (1.9)	$F(1,31) = 8.3$	$F(1,31) = 1.6$	$F(1,31) = 0.8$
Old	24.5 (2.9)	24.8 (3.0)	$P = 0.007$	$P = 0.214$	$P = 0.383$
<b>VO<sub>2</sub> Max (mL·kg<sup>-1</sup>·min<sup>-1</sup>)</b>					
Young	65.0 (2.8)	50.3 (4.4)	$F(1,31) = 168.6$	$F(1,31) = 47.8$	$F(1,31) = 0.0$
Old	40.4 (8.3)	30.5 (3.3)	$P < 0.001$	$P < 0.001$	$P = 0.867$
<b>HR (bpm)</b>					
Young	72 (14)	86 (19)	$F(1,31) = 2.5$	$F(1,31) = 4.1$	$F(1,31) = 0.3$
Old	66 (13)	74 (12)	$P = 0.122$	$P = 0.053$	$P = 0.586$
<b>P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>					
Young	36.5 (2.5)	36.1 (3.4)	$F(1,31) = 12.5$	$F(1,31) = 0.0$	$F(1,31) = 0.2$
Old	32.5 (3.4)	33.0 (2.0)	$P = 0.001$	$P = 0.942$	$P = 0.651$
<b>MAP (mmHg)</b>					
Young	89.5 (8.8)	91.6 (8.0)	$F(1,31) = 0.0$	$F(1,31) = 0.5$	$F(1,31) = 0.0$
Old	89.7 (8.2)	92.2 (12.8)	$P = 0.911$	$P = 0.489$	$P = 0.954$

BMI, body mass index; MAP, mean arterial pressure; HR, heart rate; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of arterial carbon dioxide; VO<sub>2</sub>max, maximal oxygen consumption. Young untrained n = 10, young trained n = 10, old untrained n = 8, old trained n = 7. Values are reported as mean (SD).

#### **5.4.2. Resting cerebral blood flow**

Bilateral ICA and VA blood flow, and MCA  $V_{\text{mean}}$ , in young and old, trained and untrained groups are shown in Figure 5.1. Bilateral ICA blood flow was  $\approx 31\%$  higher in trained when compared to untrained individuals ( $F(1,28) = 5.8$ ,  $p = 0.022$ ;  $d = 0.85$ ). However, no significant main effect of age ( $F(1,28) = 0.3$ ,  $p = 0.619$ ,  $d = 0.28$ ) or interaction between training status and age ( $F(1,28) = 0.9$ ,  $p = 0.342$ ) was observed in bilateral ICA flow. No effect of age ( $F(1,31) = 1.2$ ,  $p = 0.282$ ,  $d = 0.39$ ), training status ( $F(1,31) = 0.9$ ,  $p = 0.344$ ,  $d = -0.38$ ) or interaction between age and training status ( $F(1,31) = 0.4$ ,  $p = 0.551$ ) was observed on the bilateral VA blood flow. However, MCA  $V_{\text{mean}}$  was lower in old individuals (Young:  $59.5$  ( $55.0$  to  $64.1$ )  $\text{cm}\cdot\text{s}^{-1}$  and Old:  $43.6$  ( $38.4$  to  $48.9$ )  $\text{cm}\cdot\text{s}^{-1}$ ,  $F(1,31) = 21.5$ ,  $p < 0.001$ ,  $d = -1.67$ ), with no significant effect of training status ( $F(1,31) = 0.8$ ,  $p = 0.377$ ,  $d = 0.29$ ) or interaction between age and training status ( $F(1,31) = 0.0$ ,  $p = 0.920$ ) observed.

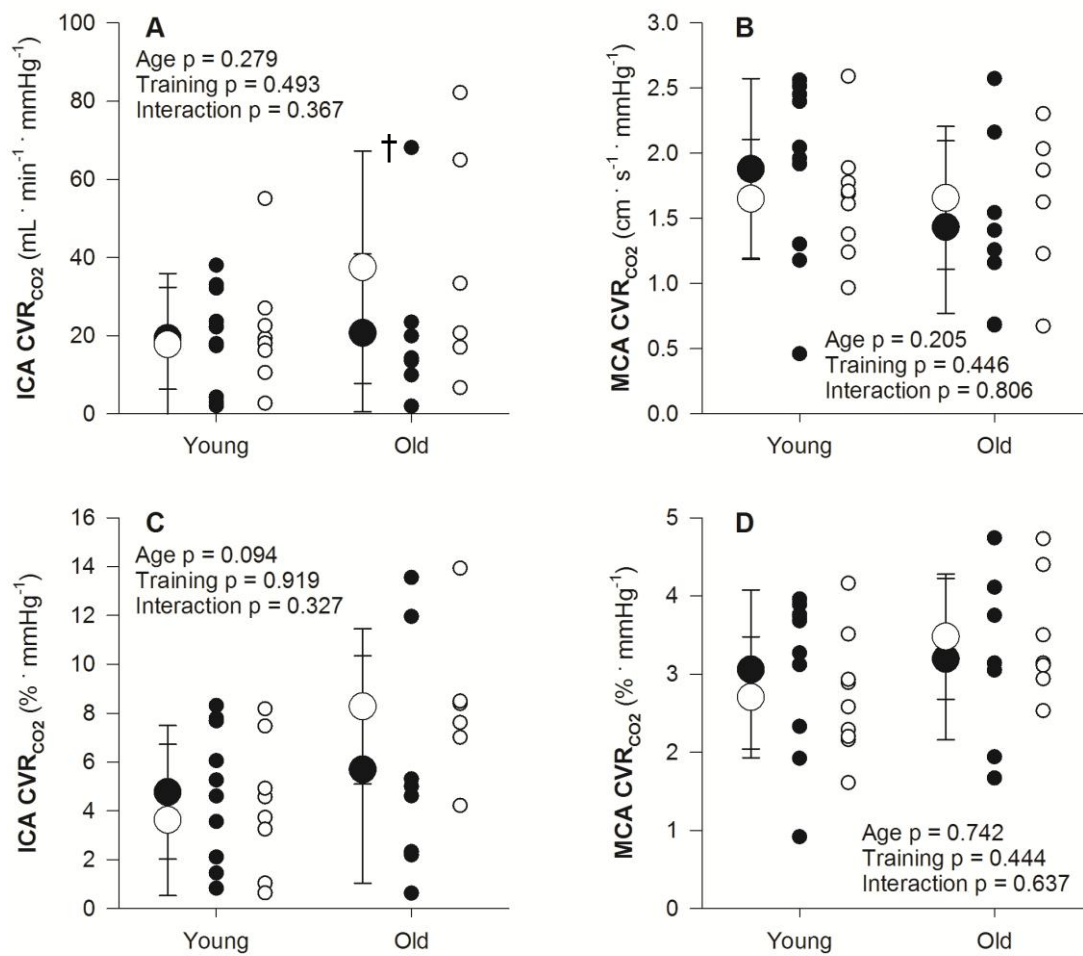


**Figure 5.1.** Bilateral internal carotid artery (ICA) blood flow (A), vertebral artery (VA) blood flow (B) and mean middle cerebral artery blood flow velocity (MCA  $V_{\text{mean}}$ , C) in young and old, trained and untrained subjects.

Young untrained  $n = 10$ , young trained  $n = 10$ , old untrained  $n = 5$  (A) or 8 (B and C), old trained  $n = 7$ . Filled circles show untrained and open circles show trained individuals. The small circles are individual values, while larger circles denote the mean for that fitness group.

### 5.4.3. Cerebral vascular reactivity

Table 5.2 shows changes in MAP,  $P_{ET}CO_2$ , blood flow, arterial diameter and blood velocity at the right ICA during hypercapnia in young and old, trained and untrained groups. All groups exhibited similar increases in  $P_{ET}CO_2$  ( $\approx 4$  mmHg) and MAP ( $\approx 4$  mmHg) with hypercapnia. Figure 5.2 shows the ICA and MCA  $CVR_{CO_2}$  in young and old, trained and untrained individuals. No main effect of age or training status was noted for the absolute ICA  $CVR_{CO_2}$  (Figure 5.2 A). However, one old untrained individual was classified as an outlier (i.e., an extreme). If removed the main effects of age ( $F(1,29) = 1.6$ ,  $p = 0.209$ ;  $d = 0.40$ ) and fitness ( $F(1,29) = 2.4$ ,  $p = 0.133$ ;  $d = 0.36$ ) remain, but a significant interaction is observed ( $F(1,29) = 4.6$ ,  $p = 0.041$ ). Post-hoc analysis using the Bonferroni adjustment shows a significant difference between old trained vs old untrained ( $t = 2.365$ ,  $p = 0.025$ ,  $d = 1.24$ ) and between old trained and young trained individuals ( $t = 2.368$ ,  $p = 0.025$ ,  $d = 1.01$ ). Relative ICA  $CVR_{CO_2}$  was not statistically different in the young and old, trained and untrained groups (Figure 5.2 C), but there was a tendency for a higher relative ICA  $CVR_{CO_2}$  in old when compared to young individuals ( $F(1,29) = 3.0$ ,  $p = 0.094$ ,  $d = 0.77$ ), being particularly marked in old trained individuals ( $d = 1.22$ ). No main effects of age or training status were noted in either the ICA flow, diameter or  $V_{mean}$  responses to hypercapnia, when expressed as either an absolute or percentage change. Although a tendency for a greater percentage ICA blood flow increase in old individuals was noted ( $F(1,29) = 3.2$ ,  $p = 0.083$ ,  $d = 0.63$ ). The MCA  $CVR_{CO_2}$  was not different in the young and old, trained and untrained groups (Figure 5.2 B and D).



**Figure 5.2.** Cerebral vascular reactivity to carbon dioxide ( $CVR_{CO_2}$ ) at the internal carotid artery (ICA, A and C) and middle cerebral artery (MCA, B and D) in young and old, trained and untrained subjects.

Young untrained  $n = 10$ , young trained  $n = 9$  (A and C) or  $n = 10$  (B and D), old untrained  $n = 8$ , old trained  $n = 6$  (A and C) or  $n = 7$  (B and D). Filled circles show untrained and open circles show trained individuals. The small circles are individual values, while larger circles denote the mean for that fitness group. † indicates the outlier (the data presented in the figure includes the outlier)

**Table 5.2.** Changes in P<sub>ET</sub>CO<sub>2</sub>, blood pressure, arterial diameter, blood flow velocity and blood flow at the right ICA in response to hypercapnia

	Trained	Untrained	Age	<b>F and P value</b>	
				Training	Interaction
<b>Δ P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>					
Young	4.1 (1.4)	4.2 (0.7)	<i>F</i> (1,29) = 0.1	<i>F</i> (1,29) = 0.5	<i>F</i> (1,29) = 0.2
Old	3.9 (1.3)	4.3 (1.2)	<i>P</i> = 0.823	<i>P</i> = 0.499	<i>P</i> = 0.655
<b>Δ MAP (mmHg)</b>					
Young	3.4 (4.8)	2.6 (3.6)	<i>F</i> (1,29) = 1.5	<i>F</i> (1,29) = 0.0	<i>F</i> (1,29) = 0.7
Old	3.9 (2.8)	5.2 (2.7)	<i>P</i> = 0.230	<i>P</i> = 0.837	<i>P</i> = 0.413
<b>Δ ICA blood flow (%)</b>					
Young	15.0 (14.2)	19.3 (10.1)	<i>F</i> (1,29) = 3.0	<i>F</i> (1,29) = 0.0	<i>F</i> (1,29) = 1.0
Old	29.8 (13.2)	23.6 (21.2)	<i>P</i> = 0.083	<i>P</i> = 0.854	<i>P</i> = 0.335
<b>Δ ICA V<sub>mean</sub> (%)</b>					
Young	6.3 (14.1)	6.8 (11.0)	<i>F</i> (1,29) = 2.3	<i>F</i> (1,29) = 0.0	<i>F</i> (1,29) = 0.0
Old	13.2 (11.0)	14.7 (18.5)	<i>P</i> = 0.142	<i>P</i> = 0.839	<i>P</i> = 0.921
<b>Baseline ICA diameter (mm)</b>					
Young	5.2 (0.6)	4.7 (0.9)	<i>F</i> (1,29) = 0.1	<i>F</i> (1,29) = 0.3	<i>F</i> (1,29) = 1.9
Old	4.9 (0.4)	5.1 (0.6)	<i>P</i> = 0.785	<i>P</i> = 0.571	<i>P</i> = 0.180
<b>Δ ICA diameter (%)</b>					
Young	2.7 (1.9)	5.8 (3.5)	<i>F</i> (1,29) = 0.7	<i>F</i> (1,29) = 0.0	<i>F</i> (1,29) = 4.0
Old	7.3 (7.5)	3.9 (5.3)	<i>P</i> = 0.419	<i>P</i> = 0.914	<i>P</i> = 0.052

ICA, internal carotid artery; V<sub>mean</sub>, mean blood velocity; MAP, mean arterial pressure. Young untrained n = 10, young trained n = 9, old untrained n = 8, old trained n = 6. Values are reported as mean (SD).

**Table 5.2 (continued).** Changes in  $P_{ET}CO_2$ , blood pressure, arterial diameter, blood flow velocity and blood flow at the right ICA in response to hypercapnia

	Trained	Untrained	Age	<b>F and P value</b>	
				Training	Interaction
<b>Δ ICA blood flow</b> (mL·min <sup>-1</sup> )					
Young	73.5 (86.6)	77.3 (47.6)	$F(1,29) = 0.9$	$F(1,29) = 0.1$	$F(1,29) = 0.3$
Old	114.8 (72.2)	89.8 (104.4)	$P = 0.347$	$P = 0.709$	$P = 0.614$
<b>Δ ICA <math>V_{mean}</math></b> (cm·s <sup>-1</sup> )					
Young	1.69 (2.87)	1.40 (2.06)	$F(1,29) = 0.3$	$F(1,29) = 0.0$	$F(1,29) = 0.0$
Old	2.02 (1.81)	2.00 (2.68)	$P = 0.592$	$P = 0.857$	$P = 0.879$
<b>Δ ICA diameter</b> (mm)					
Young	0.15 (0.11)	0.28 (0.15)	$F(1,29) = 0.9$	$F(1,29) = 0.1$	$F(1,29) = 3.5$
Old	0.39 (0.42)	0.21 (0.29)	$P = 0.349$	$P = 0.783$	$P = 0.072$

ICA, internal carotid artery;  $V_{mean}$ , mean blood velocity; MAP, mean arterial pressure. Young untrained n = 10, young trained n = 9, old untrained n = 8, old trained n = 6. Values are reported as mean (SD).

## 5.5. Discussion

This study sought to determine if cardiorespiratory fitness enhances cerebral blood flow and  $CVR_{CO_2}$  in healthy elderly individuals. The main findings of this study were that a higher cardiorespiratory fitness was associated with a  $\approx 31\%$  greater ICA flow whereas VA flow was not influenced by age or cardiorespiratory fitness. In addition, MCA  $V_{mean}$  was reduced with age, but did not appear to be affected by cardiorespiratory fitness. Finally, neither age nor cardiorespiratory fitness significantly influenced MCA  $CVR_{CO_2}$ , while there was a non-significant tendency for ICA  $CVR_{CO_2}$  to be enhanced in old trained individuals.

Reduced cerebral blood flow is a risk factor for cerebral vascular and neurodegenerative diseases, and regional cerebral hypoperfusion has been associated with cognitive decline and Alzheimer's disease (Thome et al., 1996). In key regions of the brain such as the hippocampus, aerobic exercise training can improve perfusion which is related to the hippocampal volume, indicative of a preserved vascular function in elderly individuals (Maass et al., 2014). It was found that cardiorespiratory fitness was associated with a greater global cerebral blood flow, in young and older individuals when compared to their untrained age-matched counterparts. This finding supports the concept that maintaining a high cardiorespiratory capacity through life reduces the risk of cognitive decline and other neurodegenerative diseases (Erickson et al., 2011). Although ICA blood flow was higher in trained participants when compared to untrained individuals, VA flow was not different. The irrigation of most of the cerebral cortex and deep forebrain structures originates from the ICA, whereas



the merged left and right VAs form the basilar artery, which is responsible for the blood supply to the cerebellum, pons and provides branches for some forebrain structures. Differences in the regulation of blood flow within the posterior and anterior circulations have been identified. For example, the carotid arteries possess a greater supply of autonomic nerve fibres than the vertebral arteries (Edvinsson et al., 1976), while resting CO<sub>2</sub> responsiveness is reportedly lower in the posterior inferior cerebellar artery than the contralateral middle cerebral artery (Reinhard et al., 2008). ICA and VA blood flow responses to physiological stressors also differ. During head-up tilt the blood flow in the ICA is reduced but preserved in the VA (Sato et al., 2012a). In addition, an acute bout of exercise promotes a blood flow increase in both ICA and VA, although at exercise intensities higher than 60%, the ICA flow returns to baseline and VA flow increases to reach a maximum at 100% VO<sub>2</sub>max (Sato et al., 2011). The findings from this chapter support the existence of a regional specificity in the cerebral blood flow adaptations to exercise training with a favouring of the areas of the brain that are responsible for the planning and execution of movement (Secher et al., 2008). However, whether the resting cerebral blood flow adaptations observed in this study influence the regional cerebral blood flow responses to an acute bout of exercise has not been investigated.

In rats, it has been shown that physical activity in early life can have long lasting positive effects in the brain such as increased neuron maturation and survival (Merkley et al., 2014), and aerobic exercise training also increases angiogenesis and recruitment of endothelial progenitor cells after MCA occlusion (Gertz et al., 2006). Accordingly, the reasons for greater cerebral

blood flow observed in aerobically fit individuals might range from increased microvasculogenesis and improved endothelial function to enhanced cardiac function (Bolduc et al., 2013). Additional studies are required to translate the mechanistic findings from animal models to humans. Cardiac output has been shown to independently influence cerebral perfusion in humans (Ogoh et al., 2005), but as resting cardiac output was not measured it is unclear whether it explains the greater cerebral blood flow observed in aerobically fit individuals. However, since resting cardiac output is reportedly unchanged with training, largely because an increase in stroke volume is counterbalanced by a decrease in HR (Scheuer & Tipton, 1977), it is likely that this would not explain the training-related cerebral blood flow differences observed.

When assessed by transcranial Doppler ultrasonography no effect of cardiorespiratory fitness on cerebral perfusion was detected. In contrast, in a study of 307 individuals aged between 18 and 79 years, higher MCA  $V_{\text{mean}}$  was observed in trained individuals (Ainslie et al., 2008). The discrepancy between previous studies may relate to the relatively small sample size of this study. Transcranial Doppler is a technique which can assess blood velocity with a high temporal resolution, but is reported not to correlate well with [ $^{133}\text{Xe}$ ] determined global cerebral blood flow at rest in some (Bishop et al., 1986; Hartmann et al., 1991) but not all studies (Sorteberg et al., 1989; Dahl et al., 1992), and the validity of transcranial Doppler as a surrogate marker of cerebral blood flow is subject of debate (Ainslie & Hoiland, 2014). On the other hand, duplex Doppler ultrasonography of the extracranial arteries correlates well with other techniques of global brain blood flow assessment (Dorfler et al., 2000), although duplex

ultrasound validation against a gold-standard technique is still lacking. The findings of studies investigating the impact of cardiorespiratory fitness on  $CVR_{CO_2}$  are conflicting. Zhu *et al* (2013) reported similar MCA  $CVR_{CO_2}$  in sedentary elderly individuals and masters athletes, suggesting that life-long exercise training has a minimal impact on cerebral vasomotor regulation. Whereas, using magnetic resonance imaging, Thomas *et al* (2013) showed that  $CVR_{CO_2}$  was in fact reduced in masters athletes when compared to sedentary elderly participants. In contrast, Barnes *et al* (2013) and Bailey *et al* (2013b) reported that MCA  $CVR_{CO_2}$  is better in those elderly individuals with higher aerobic capacity. Neither age nor cardiorespiratory fitness influenced MCA  $CVR_{CO_2}$  in this study. In addition, no significant main effect of age or training status was noted for the absolute ICA  $CVR_{CO_2}$ . However, upon close inspection of the data one old untrained individual was deemed to be an outlier (i.e., an extreme). With this individual removed a significant interaction between age and training was observed, with post hoc analysis a significantly greater ICA  $CVR_{CO_2}$  in old trained group compared to the old untrained and young trained groups was observed. As the outlier does not appear to have resulted from an artefact and the value is genuine, a consideration of the data both with and without them included was provided. Tendencies for a higher relative ICA  $CVR_{CO_2}$  and ICA blood flow during the hypercapnic challenge were noted in old trained individuals. Taken together these data suggest that the potential for an enhanced  $CVR_{CO_2}$  of the ICA cannot be completely discounted.

The effect of normal and healthy ageing on cerebral blood flow and its regulation is still debated. Although many studies show that cerebral perfusion

is reduced with age (Leenders et al., 1990; Ainslie et al., 2008), there is also evidence that global cerebral blood flow may not be reduced with age (Meltzer et al., 2000; van Es et al., 2010). It was observed in this study that cerebral blood flow was not influenced by age when assessed by Doppler ultrasound at the ICA and VA, but cerebral blood velocity assessed by transcranial Doppler ultrasonography at the MCA was reduced in older individuals. These unexpected findings may be explained by the increase in diameter of cerebral arteries during ageing (Ozdogmus et al., 2008) and discrepancies between transcranial Doppler with other measurement techniques such as the use of [ $^{133}\text{Xe}$ ] for resting absolute cerebral perfusion assessment (Bishop et al., 1986; Hartmann et al., 1991). However, only longitudinal studies with a long follow-up period using multiple methodologies could reduce the uncertainty over this age-related cerebral blood flow decline.

Duplex ultrasound has been extensively used clinically and for research purposes, and was shown to have a good correlation when compared to phase-contrast magnetic resonance imaging technique (Oktar et al., 2006). However, no account was made for cerebral volume and thus relative blood flow to cerebral mass. Post-hoc analysis showed that the sample size of this study was adequate to permit identification of a minimal detectable difference in bilateral ICA flow of  $200 \text{ mL}\cdot\text{min}^{-1}$  based on a two-way ANOVA model with an interaction term, at 80% power and 5% alpha. However, larger samples may be needed to assess the influence of cardiorespiratory fitness on the  $\text{CVR}_{\text{CO}_2}$ . Furthermore, the focus of this study on a small sample of healthy individuals restricts the possibility of extrapolation of these results to a wider population including

people with diseases. Finally, the absence of a gold-standard protocol to assess the  $CVR_{CO_2}$  greatly limits the comparison between studies.

### *Perspective*

Age is one of the most important non-modifiable risk factors for brain diseases such as dementia and stroke (Sacco et al., 1997; Lindsay et al., 2002). Alterations in brain structure and function occur even in 'healthy ageing' (Scahill et al., 2003; Burgmans et al., 2011). Regular physical activity has a number of beneficial effects on the brain, but the underlying mechanisms are incompletely understood. In the present study it was observed that aerobically trained individuals have higher bilateral ICA flow. Further studies are required to assess the impact of higher cerebral blood flow and cardiorespiratory fitness in healthy ageing and neurodegenerative conditions such as Alzheimer's and Parkinson's disease.

**CHAPTER 6: CEREBRAL BLOOD FLOW REGULATION IN  
ATRIAL FIBRILLATION**

## 6.1. Introduction

AF is the most common sustained cardiac rhythm abnormality, affecting  $\approx 2\%$  of the general population in Europe (Zoni-Berisso *et al.*, 2014). It is strongly associated with advanced age, with an incidence of 1.1/1000 person-years in people aged 55-59 rising to 20.7/1000 person-years in the 80-84 years group (Heeringa *et al.*, 2006). Although AF is known to be a major risk factor for stroke and dementia even without clinical history of cerebral vascular disease (Ott *et al.*, 1997), the mechanisms underlying the increased risk of cerebral thromboembolism and cognitive impairment are not fully understood.

The irregular blood flow through the heart in people with AF leads to left atrium stasis, and is associated with structural heart disease, endocardial dysfunction, and enhanced blood haemostatic properties. These factors contribute to a prothrombotic state in the heart that may result in cerebral thromboembolism by affecting all the components of the Virchow's triad (Watson *et al.*, 2009). The consequences of stroke in patients with AF are more severe when compared to patients without AF. It has been reported that AF patients have a higher 30-day mortality and poorer functional status as indicated by the Barthel scale when compared to sinus rhythm stroke patients (Lin *et al.*, 1996). Even in the absence of previous stroke, patients with AF have reduced hippocampal volume and perform worse in memory, learning, attention and execution tasks when compared to individuals without AF from the same community (Knecht *et al.*, 2008). The mechanisms promoting these poorer outcomes in people with AF have not been fully elucidated. An impaired cerebral vasomotor regulation is

one potential factor which may contribute to the worse stroke outcomes as well as poorer cognitive performance.

A reduced resting hemispheric cerebral blood flow of  $\approx 13\%$  has been documented in individuals with AF aged between 51 and 65 years, with no signs of heart failure or cerebral vascular disease (Lavy *et al.*, 1980). The authors suggested that low cardiac output and/or impaired cerebral autoregulation could be responsible for this cerebral blood flow reduction in AF patients. Poor cerebral blood flow in AF may lead to vasodilatation of cerebral arterioles which may impair cerebral autoregulation by reducing vasodilatory reserve (Aaslid *et al.*, 1989). However, another study has shown that cerebral perfusion improved with cardioversion even without significant changes in cardiac output (Petersen *et al.*, 1989b).

Endothelium-derived NO is an important local regulator of cerebral blood flow (Hainsworth *et al.*, 2015), and NO-dependent cerebral vessels dilatation is reportedly impaired in patients with hypertension and diabetes (Lavi *et al.*, 2006). Systemic endothelial function has been shown to be impaired in patients with AF when compared with healthy controls (Conway *et al.*, 2003; Freestone *et al.*, 2008). The concentration of plasma von Willebrand factor, a marker of endothelial damage that has been shown to be predictive of cerebral vascular events, is elevated in AF (Conway *et al.*, 2003). Furthermore, an impaired flow-mediated dilatation of the brachial artery has also been identified in patients with chronic AF, indicative of endothelial dysfunction and diminished NO signalling (Freestone *et al.*, 2008).



Although NO and the endothelium are known to modulate cerebral haemodynamics, it is still largely unknown whether the cerebral vascular function is chronically impaired in patients with AF. The purpose of this study was to determine whether cerebral vascular function is impaired in AF. To do that,  $CVR_{CO_2}$  and cerebral autoregulation were assessed in patients with AF, hypertension and healthy volunteers. Patients with hypertension were used as a 'disease control' group as AF is commonly associated with other cardiovascular risk factors and comorbidities. It was hypothesised that patients with AF would have a lower  $CVR_{CO_2}$  and diminished cerebral autoregulation when compared to healthy age-matched controls and patients with hypertension (i.e. disease control).

## **6.2. Methods**

### **6.2.1. Ethics**

All procedures performed were approved by the National Research Ethics Service Committee West Midlands - The Black Country (13/WM/0210) and conformed to the Declaration of Helsinki. Prospective participants were given an information sheet and all procedures were verbally explained in detail. After having the opportunity to ask questions and clarification over each procedure performed, written informed consent was obtained from willing volunteers.

### **6.2.2. Participants**

Fourteen AF patients, 8 hypertensive patients and 10 healthy control participants were recruited. Patients with AF and primary hypertension were recruited from dedicated clinics at the City Hospital, Birmingham while healthy age-matched controls were recruited from the surrounding communities. Seven of the fourteen AF patients also had hypertension and 5 were fibrillating during the examination. Patients with left ventricular dysfunction, valvular heart disease, previous myocardial infarction, respiratory, connective tissue, inflammatory or neurological diseases, malignancy or uncontrolled thyroid disorders upon screening were not recruited to participate in this study. Healthy participants were free from disease and not taking any prescription or over-the-counter medication. If a healthy participant was found to have high blood pressure (systolic >140 mmHg or diastolic >90 mmHg) during the test, they were recommended to have an appointment with the GP. Two healthy participants had systolic blood pressure readings higher than 140 mmHg and

were referred this way, but in a follow-up contact they were found to have normal blood pressure.

### **6.2.3. Measurements**

Cognitive function was assessed prior to the cardiovascular assessment using the MoCA. Beat-by-beat blood pressure was continuously obtained from the third or fourth finger from the left hand (Finometer MIDI, Finapres Medical Systems, Amsterdam, The Netherlands) and was verified by resting brachial arterial pressure (M2, Omron, Kyoto, Japan). MCA  $V_{\text{mean}}$  was also recorded continuously using transcranial Doppler (Doppler BoxX, DWL, Sipplingen, Germany) with the technique described in section 3.2.2. HR was monitored through lead II ECG (BioAmp, ADInstruments, Dunedin, New Zealand). Tidal volume and minute ventilation were measured using an oronasal mask connected to a heated pneumotach (Hans Rudolph, Kansas City, KS, USA). The mask was also connected to a capnograph which continuously sampled the gas and provided  $P_{\text{ETCO}_2}$  measures (RespSense, Nonin medical, Plymouth, MN, USA). Analogue signals were digitized at 1 kHz (Powerlab, ADInstruments, Dunedin, New Zealand) and recorded using a multi-channel data acquisition software (Labchart 7, ADInstruments, Dunedin, New Zealand).

### **6.2.4. Protocol**

Patients were asked to refrain from taking any medication (excluding anticoagulants) on the study day. On the study visit, a blood sample was taken and analysed for full blood count, glucose, lipids, kidney and liver function. Cognition was assessed using the MoCA. Then, participants were asked to lie

comfortably on a bed where the vascular assessment took place. Firstly, a ten-minute baseline was acquired while participants were breathing room air.

#### *CVR<sub>CO2</sub>*

CVR<sub>CO2</sub> was assessed by increasing or decreasing the inspired fraction of CO<sub>2</sub> through a two-way valve connected to either Douglas bags filled with enriched CO<sub>2</sub> or room air. Participants were given 4 % CO<sub>2</sub> ( $\approx 21$  % O<sub>2</sub>) for four minutes, followed by 7 % CO<sub>2</sub> ( $\approx 21$  % O<sub>2</sub>) for another four minutes, then switched back to room air. After P<sub>ET</sub>CO<sub>2</sub>, MCA V<sub>mean</sub>, haemodynamic and respiratory variables returned to baseline, participants were asked to increase the respiratory depth and rate in order to achieve the same magnitude but opposite change in P<sub>ET</sub>CO<sub>2</sub> during the hypercapnic states, with each step lasting 2 minutes.

#### *Cerebral autoregulation*

Cerebral autoregulation was assessed by asking the participants to stand after sitting comfortably in a chair for a period of 2 minutes. This manoeuvre generates a drop in MAP that is followed by a fall in MCA V<sub>mean</sub> and the magnitude of adaptations is an index of cerebral autoregulation (section 3.3.2, Figure 3.3). Cerebral autoregulation was also assessed in the frequency domain by the means of TFA, as described in section 3.3.2. Gain, phase and coherence were derived from the auto- and cross-spectrum between MAP and MCA V<sub>mean</sub>. As low coherence values may prevent the analysis of gain and phase, TFA was performed using spontaneous fluctuations of the MAP during baseline as well as during a period of five minutes of squat-to-stand manoeuvre

at 0.1 Hz. The repeated squat-to-stand aims to drive oscillations in MAP at the desired frequency range in order to improve TFA coherence.

#### **6.2.5. Data analysis**

BMI was calculated dividing the participants' weight by the square of the height. MAP was calculated as the mean blood pressure throughout each cardiac cycle by the acquisition software. CVCi was calculated as MCA  $V_{\text{mean}}$  divided by MAP. Baseline was calculated as the mean of the whole 10-minute period, and mean values were acquired over the last minute of each hypercapnic or hypocapnic step. Hypercapnic  $\text{CVR}_{\text{CO}_2}$  was calculated as the slope of the MCA  $V_{\text{mean}}$  change between the two hypercapnic steps, while the hypocapnic  $\text{CVR}_{\text{CO}_2}$  was calculated as the slope between the two hypocapnic steps (Peebles *et al.*, 2007). Sit-to-stand MAP, MCA  $V_{\text{mean}}$  and  $P_{\text{ETCO}_2}$  averages were obtained over the 20 s before standing for the baseline and over 5 cardiac cycles around the nadir of MAP after standing (den Abeelen *et al.*, 2014). TFA was performed with spontaneous fluctuation of MAP and during the repeated squat-to-stand manoeuvre as described in section 3.2.2 and following recommendations of current guidelines (Claassen *et al.*, 2016).

#### **6.2.6. Statistical analysis**

Normality was assessed by the Shapiro-Wilk test. Normally distributed data were analysed using one-way ANOVA, while non-normally distributed data were analysed using Kruskal-Wallis H tests. Given that the aim of the study was to determine whether cerebral vascular function is impaired in AF, one-way ANOVA was independently used to compare hypercapnic and hypocapnic  $\text{CVR}_{\text{CO}_2}$  in patients with AF, hypertension and healthy volunteers. Therefore,

interactions between group and CO<sub>2</sub> stimulus (i.e. hypocapnia and hypercapnia) were not considered. Significance was set at  $p < 0.05$ . Normally distributed data are presented as mean (SD) while non-normally distributed data are presented as median [interquartile range]. Multiple pairwise comparisons using the Dunn's method were performed when a significant difference between groups was found by the Kruskal-Wallis H test, and the Bonferroni method if a significant difference was found on the ANOVA. Effect sizes ( $d$ ) were calculated as the difference of means divided by the pooled SD. Statistical analysis was performed using Sigmaplot 12.5 (Systat Software Inc, London, UK).

### 6.3. Results

Participant characteristics and baseline data are presented on Table 6.1. There were no significant differences in age, BMI, HR, blood pressure parameters or cognition between the AF, hypertension and healthy control groups.

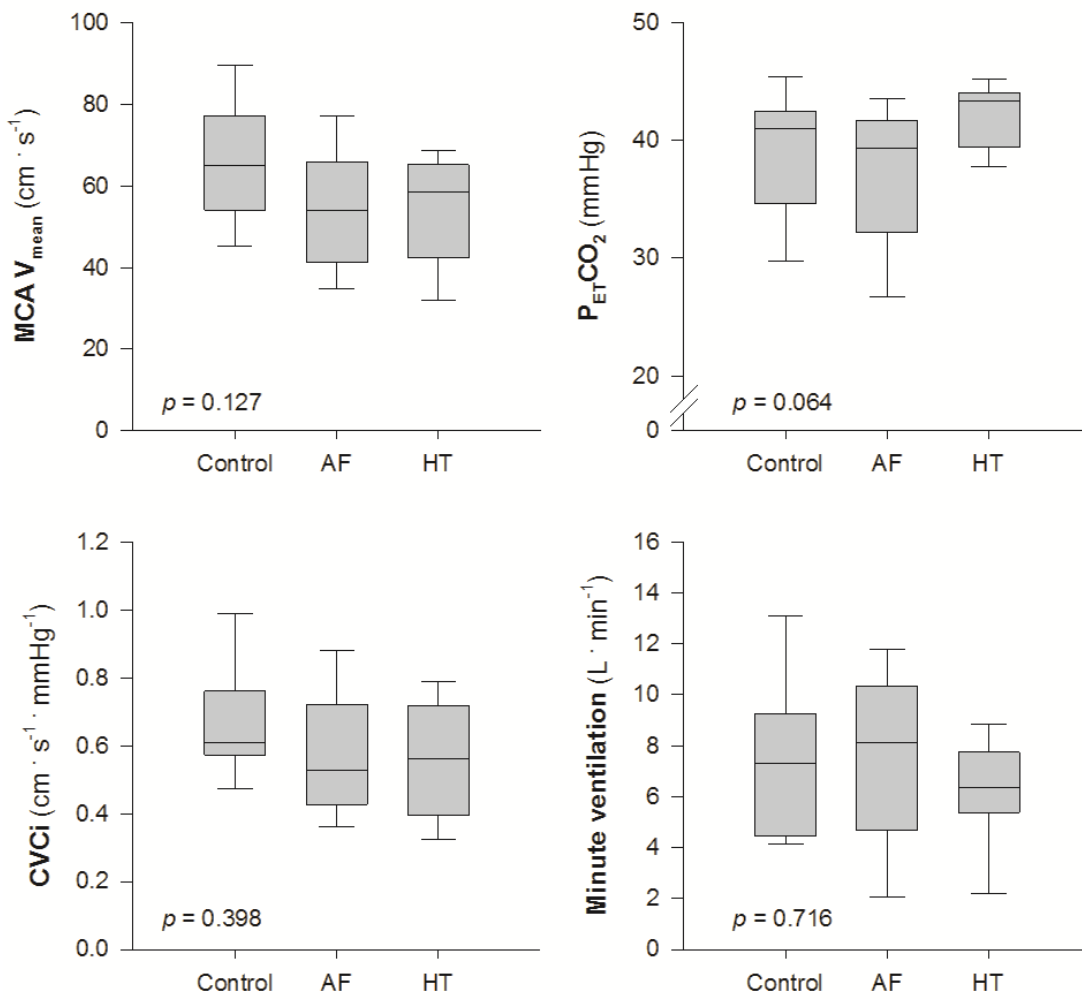
As shown in Figure 6.1, MCA  $V_{\text{mean}}$  tended to be higher in controls when compared to AF ( $d = 0.83$ ) and hypertensive patients ( $d = 0.82$ ), although they were not significantly different ( $p = 0.127$ ).  $P_{\text{ET}}\text{CO}_2$  tended to be higher in participants with hypertension, although this finding did not reach statistical significance ( $p = 0.064$ ). No differences in CVCi or minute ventilation were observed between groups.

**Table 6.1.** Participants characteristics and baseline data

	<b>Control (n=10)</b>	<b>AF (n=14)</b>	<b>HT (n=8)</b>	<b>p value</b>
<b>Age</b> (yr)	67.5 [66-70]	66.5 [59.5-69.25]	65.5 [63.5-71.5]	0.404
<b>Sex</b> (n female)	4	3	4	-
<b>BMI</b> (kg·m <sup>-2</sup> )	24.0 (3.1)	26.1 (4.7)	27.7 (4.5)	0.185
<b>MoCA</b> (score)	28.5 [26.75-29.25]	27 [25-28]	28 [26.25-30]	0.486
<b>Hypertension</b> (n)	-	7	8	-
<b>HR</b> (bpm)	58 [56-61]	60 [54-77]	57 [54-63]	0.589
<b>SBP</b> (mmHg)	143 (19)	134 (20)	138 (15)	0.525
<b>DBP</b> (mmHg)	78 (10)	76 (11)	78 (10)	0.800
<b>MAP</b> (mmHg)	100 (13)	95 (12)	98 (11)	0.666
<b>Medications</b> (n)				
• <b>β-blockers</b>	-	6	0	-
• <b>ACEi</b>	-	3	1	-
• <b>ARB</b>	-	1	1	-
• <b>Ca<sup>++</sup> channel blockers</b>	-	3	7	-
• <b>Diuretics</b>	-	2	4	-
• <b>OACs</b>	-	11	0	-

Values are displayed as mean (SD) when normally distributed or median [interquartile range] (non-normally distributed). BMI, body mass index; MoCA, Montreal cognitive assessment; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; OACs, oral anticoagulants. \**p* < 0.05 vs control.

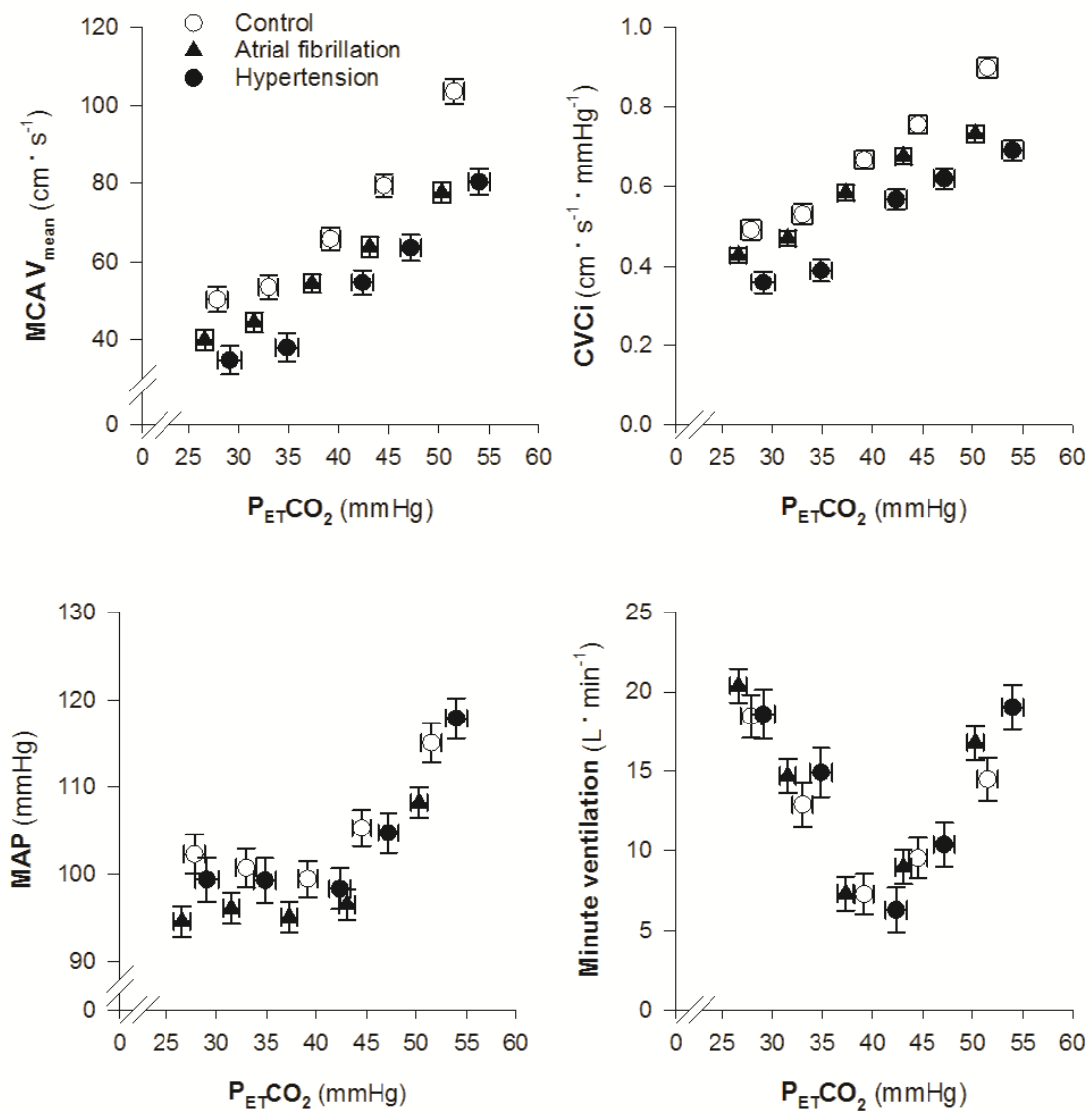




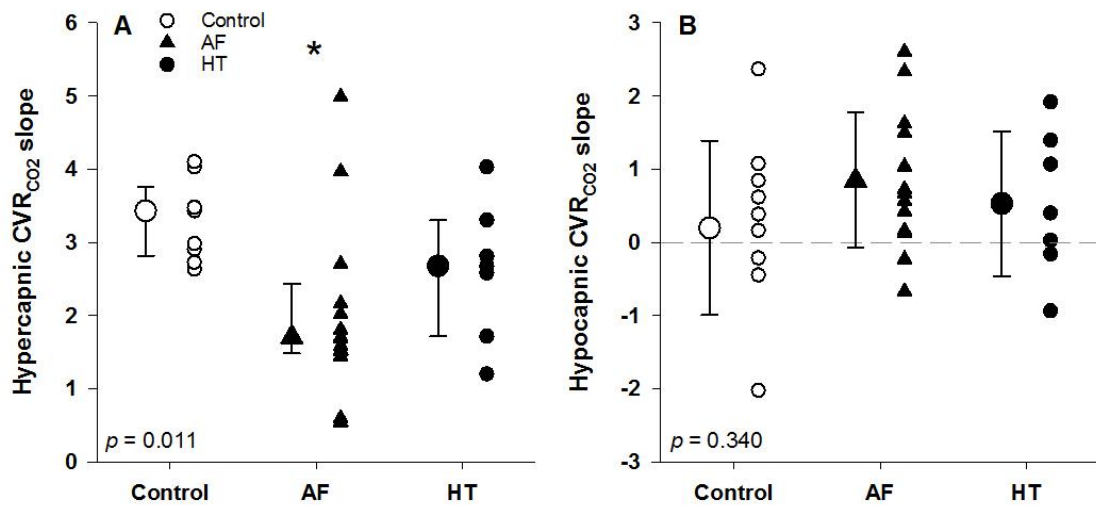
**Figure 6.1.** Resting cerebral vascular and respiratory characteristics in healthy controls, patients with atrial fibrillation (AF) and hypertension (HT). MCA  $V_{\text{mean}}$ , middle cerebral artery blood velocity;  $P_{\text{ET}} \text{CO}_2$ , partial pressure of end-tidal  $\text{CO}_2$ ; CVCi, cerebral vascular conductance index; AF, atrial fibrillation; HT, hypertension

### *CVR<sub>CO2</sub> assessment*

Figure 6.2 shows cerebral perfusion responses to the hypocapnic and hypercapnic steps in the AF, hypertension and control groups. The hypercapnic CVR<sub>CO2</sub> slope was lower in AF patients when compared to healthy controls ( $d = 1.29$ ), but not significantly different from hypertensive participants ( $d = 0.51$ ) (Figure 6.3 A). However, the hypocapnic CVR<sub>CO2</sub> slope was not significantly different between groups (Figure 6.3 B). One outlier (i.e.  $> 3^{\text{rd}}$  quartile + 3 \* interquartile range) was found in the AF group for the hypercapnic CVR<sub>CO2</sub> slope. As this value appeared not to be attributable to a methodological error with data collection or analysis, this individual was included in the statistical analysis. Haemodynamic and ventilatory responses to the hypercapnic and hypocapnic tests were not significantly different between groups at each step (Table 6.2).



**Figure 6.2.** Graphical representation of the cerebral and systemic haemodynamic and ventilatory responses to the hypocapnic and hypercapnic challenges. MCA  $V_{mean}$ , middle cerebral artery blood velocity;  $P_{ET}CO_2$ , partial pressure of end-tidal  $CO_2$ ; CVCi, cerebral vascular conductance index; MAP, mean arterial pressure.



**Figure 6.3.** Hypercapnic and hypocapnic cerebral vascular reactivity to CO<sub>2</sub> (CVR<sub>CO2</sub>) slopes in healthy controls, patients with atrial fibrillation (AF) and hypertension (HT). CVR<sub>CO2</sub>, cerebral vascular reactivity to CO<sub>2</sub>; AF, atrial fibrillation; HT, hypertension. The left column of each group represents median and interquartile range (A) or mean and standard deviation (B), while the right column of each group displays the individual values. \**p* < 0.05 vs Control. The dashed line in Panel B indicates the zero, with values below this line denoting an increase in MCA V<sub>mean</sub> between the two steps of hypocapnia.

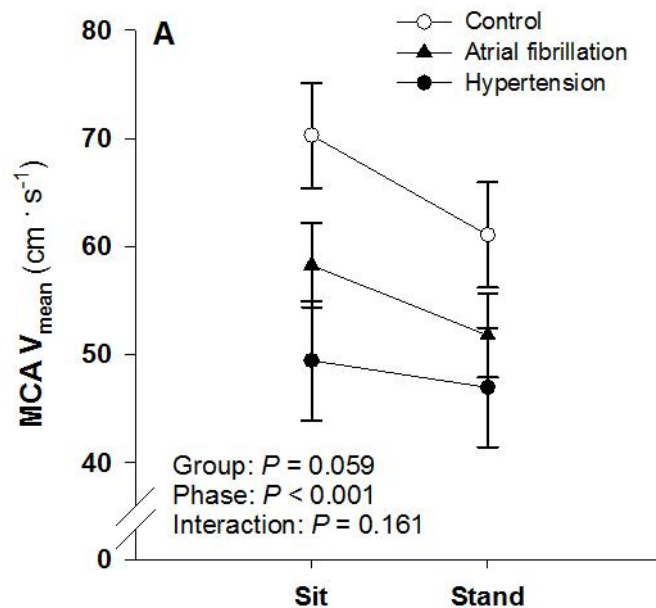
**Table 6.2.** Changes in haemodynamic and respiratory parameters during the hypercapnic and hypocapnic challenges

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
<b>Hypercapnic</b>				
$\Delta$ MAP (mmHg)	8.8 [4.4, 17.2]	9.6 [3.9, 18.9]	12.2 [7.1, 15.7]	0.686
$\Delta$ HR (bpm)	6.1 (4.6)	5.3 (4.8)	8.0 (5.4)	0.495
$\Delta$ P <sub>ET</sub> CO <sub>2</sub> (mmHg)	7.0 (3.8)	7.7 (4.5)	7.0 (1.9)	0.871
$\Delta$ V <sub>E</sub> (L·min <sup>-1</sup> )	5.3 (3.8)	8.3 (6.9)	8.1 (3.0)	0.394
<b>Hypocapnic</b>				
$\Delta$ MAP (mmHg)	1.6 (3.0)	-1.5 (3.7)	0.1 (4.7)	0.184
$\Delta$ HR (bpm)	4.2 (3.4)	3.3 (4.2)	1.2 (2.5)	0.279
$\Delta$ P <sub>ET</sub> CO <sub>2</sub> (mmHg)	-5.1 (2.1)	-4.9 (2.5)	-5.8 (1.5)	0.685
$\Delta$ V <sub>E</sub> (L·min <sup>-1</sup> )	5.5 (2.9)	5.7 (4.1)	3.7 (4.0)	0.490

Values are displayed as mean (SD) when normally distributed or median [interquartile range] (non-normally distributed). MAP, mean arterial pressure; HR, heart rate; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of end-tidal CO<sub>2</sub>; V<sub>E</sub>, minute ventilation. \**p* < 0.05 vs control.

### Cerebral autoregulation

As presented in Table 6.3 and Figure 6.4, there were no significant differences in changes in MCA  $V_{\text{mean}}$ , MAP, CVCi or  $P_{\text{ETCO}_2}$  evoked by a single sit-to-stand manoeuvre between groups.



**Figure 6.4.** Middle cerebral artery blood velocity responses to a single sit-to-stand manoeuvre in healthy controls, patients with atrial fibrillation and hypertension. MCA  $V_{\text{mean}}$ , middle cerebral artery mean blood velocity. \* $p < 0.05$  vs Control.

When assessed via TFA, the baseline MAP power in the very low frequency range and MCA  $V_{\text{mean}}$  power in the low frequency range were found to be lower in the hypertensive when compared to the control group (Table 6.4). Similarly, AF patients also had a significantly lower MAP power in the very low frequency range. Hypertensive patients had a lower coherence at the very low and low frequency range when compared to controls. Gain and phase in the frequency ranges studied were not different between groups. When the participants performed the repeated squat-to-stand manoeuvre, there were no significant differences in MAP or MCA  $V_{\text{mean}}$  power, gain, phase or coherence between

groups in the very low or low frequency range. MAP power in the high frequency range was found to be lower in the hypertensive when compared to the control group (Table 6.5). One AF and one HT participants were excluded from this analysis due to participants' time constraint on the testing day.

**Table 6.3.** Changes in haemodynamic, respiratory and cerebral vascular parameters during the single sit-to-stand manoeuvre

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
$\Delta$ <b>MCA V<sub>mean</sub></b> (cm·s <sup>-1</sup> )	-9.2 (9.5)	-6.5 (5.7)	-2.5 (3.9)	0.161
$\Delta$ <b>MAP</b> (mmHg)	-18.9 (9.9)	-16.7 (9.2)	-18.3 (6.1)	0.834
$\Delta$ <b>CVCi</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	-0.11 [-0.15, -0.01]	-0.07 [-0.14, 0.04]	-0.15 [-0.24, -0.03]	0.328
$\Delta$ <b>P<sub>ET</sub>CO<sub>2</sub></b> (mmHg)	-0.6 [-1.0, 0.0]	-0.5 [-2.3, 0.6]	-0.4 [-1.5, 0.1]	0.929

Values are displayed as the change ( $\Delta$ ) in the means (SD) when normally distributed or median [interquartile range] (non-normally distributed). MCA V<sub>mean</sub>, middle cerebral artery mean blood velocity; MAP, mean arterial pressure; CVCi, cerebral vascular conductance index; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of end-tidal CO<sub>2</sub>. \**p* < 0.05 vs control.



**Table 6.4.** Baseline dynamic cerebral autoregulation as quantified by transfer function analysis using signals of MAP and MCA  $V_{\text{mean}}$ 

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
<b>VLF (0.02–0.07 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	8.5 [6.9, 11.3]	4.4 [1.9, 6.7]*	2.6 [1.1, 4.9]*	0.003
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	3.8 [2.4, 6.2]	1.7 [1.0, 3.0]	3.0 [0.6, 4.5]	0.077
<b>Coherence</b>	0.68 [0.47, 0.76]	0.49 [0.26, 0.66]	0.32 [0.28, 0.39]*	0.006
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.59 (0.21)	0.60 (0.28)	0.71 (0.31)	0.611
<b>Gain</b> (%·mmHg <sup>-1</sup> )	0.89 (0.23)	1.08 (0.24)	1.22 (0.42)	0.079
<b>Phase</b> (radians)	55.8 [37.7, 74.2]	66.6 [41.3, 93.5]	32.0 [29.0, 56.2]	0.123
<b>LF (0.07–0.20 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	4.22 [2.42, 6.91]	3.83 [1.83, 9.97]	2.18 [1.20, 3.26]	0.106
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	3.85 (2.27)	2.92 (1.61)	1.60 (0.94)*	0.035
<b>Coherence</b>	0.87 [0.75, 0.89]	0.78 [0.67, 0.88]	0.69 [0.64, 0.78]*	0.046
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.86 (0.22)	0.79 (0.30)	0.76 (0.20)	0.664
<b>Gain</b> (%·mmHg <sup>-1</sup> )	1.41 [1.10, 1.52]	1.47 [1.21, 1.63]	1.40 [1.27, 1.60]	0.586
<b>Phase</b> (radians)	35.4 (12.0)	30.3 (11.9)	31.4 (14.6)	0.616

**Table 6.4 (continued).** Baseline dynamic cerebral autoregulation as quantified by transfer function analysis using signals of MAP and MCA  $V_{\text{mean}}$ 

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
<b>HF (0.20–0.50 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	1.02 [0.52, 1.85]	2.61 [0.35, 4.75]	0.52 [0.29, 1.03]	0.076
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	0.93 [0.65, 1.53]	1.66 [0.34, 4.66]	0.45 [0.21, 0.83]	0.108
<b>Coherence</b>	0.87 [0.81, 0.91]	0.88 [0.70, 0.94]	0.65 [0.42, 0.89]	0.174
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.91 (0.14)	0.81 (0.25)	0.81 (0.27)	0.531
<b>Gain</b> (%·mmHg <sup>-1</sup> )	1.42 (0.34)	1.50 (0.25)	1.48 (0.26)	0.775
<b>Phase</b> (radians)	-0.21 (5.24)	0.52 (4.05)	1.12 (7.89)	0.878

Values are displayed as mean (SD) when normally distributed or median [interquartile range] (non-normally distributed). AF, atrial fibrillation; HT, hypertensive; VLF, very low frequency; LF, low frequency; HF, high frequency; MAP, mean arterial pressure; MCA  $V_{\text{mean}}$ , middle cerebral artery mean blood velocity. \* $p < 0.05$  vs control.

**Table 6.5.** Dynamic cerebral autoregulation as quantified by transfer function analysis using signals of MAP and MCA  $V_{\text{mean}}$  during a repeated squat-to-stand manoeuvre

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
<b>VLF (0.02–0.07 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	8.29 [5.88, 11.96]	5.90 [4.44, 10.33]	7.35 [3.40, 13.89]	0.704
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	4.50 [1.60, 7.00]	3.87 [1.76, 5.48]	2.54 [0.56, 4.16]	0.420
<b>Coherence</b>	0.62 (0.14)	0.62 (0.15)	0.57 (0.09)	0.636
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.62 (0.23)	0.61 (0.18)	0.48 (0.19)	0.295
<b>Gain</b> (%·mmHg <sup>-1</sup> )	0.98 (0.42)	1.09 (0.29)	1.00 (0.23)	0.698
<b>Phase</b> (radians)	47.1 (16.5)	36.2 (12.4)	38.6 (7.8)	0.158
<b>LF (0.07–0.20 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	171.4 [131.1, 219.9]	179.3 [124.8, 301.3]	151.7 [107.0, 191.8]	0.844
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	110.8 (66.3)	118.0 (62.4)	74.1 (48.3)	0.300
<b>Coherence</b>	0.83 [0.63, 0.89]	0.88 [0.81, 0.91]	0.77 [0.75, 0.88]	0.145
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.76 (0.20)	0.79 (0.25)	0.63 (0.28)	0.349
<b>Gain</b> (%·mmHg <sup>-1</sup> )	1.02 [0.89, 1.39]	1.44 [1.12, 1.62]	1.25 [1.08, 1.38]	0.144
<b>Phase</b> (radians)	20.2 (5.0)	17.6 (7.9)	16.4 (7.1)	0.535

**Table 6.5 (continued).** Dynamic cerebral autoregulation as quantified by transfer function analysis using signals of MAP and MCA  $V_{\text{mean}}$  during a repeated squat-to-stand manoeuvre

	<b>Control</b>	<b>AF</b>	<b>HT</b>	<b>p value</b>
<b>HF (0.20–0.50 Hz)</b>				
<b>MAP power</b> (mmHg <sup>2</sup> )	11.1 [7.1, 16.2]	10.4 [4.9, 26.4]	5.4 [4.1, 16.7]	0.512
<b>MCA <math>V_{\text{mean}}</math> power</b> (cm <sup>2</sup> ·s <sup>-2</sup> )	8.11 (5.16)	7.63 (3.64)	3.20 (1.71)*	0.033
<b>Coherence</b>	0.71 [0.52, 0.88]	0.82 [0.72, 0.89]	0.64 [0.45, 0.92]	0.220
<b>Gain</b> (cm·s <sup>-1</sup> ·mmHg <sup>-1</sup> )	0.70 (0.16)	0.74 (0.22)	0.58 (0.29)	0.283
<b>Gain</b> (%·mmHg <sup>-1</sup> )	1.10 (0.31)	1.30 (0.27)	1.14 (0.32)	0.224
<b>Phase</b> (radians)	2.65 (9.47)	4.08 (7.54)	-0.25 (7.94)	0.534

Values are displayed as mean (SD) when normally distributed or median [interquartile range] (non-normally distributed). AF, atrial fibrillation; HT, hypertensive; VLF, very low frequency; LF, low frequency; HF, high frequency; MAP, mean arterial pressure; MCA  $V_{\text{mean}}$ , middle cerebral artery mean blood velocity. \* $p < 0.05$  vs control.

## 6.4. Discussion

This study sought to determine whether cerebral vascular function is impaired in AF compared to healthy age-matched individuals as well as hypertensive (disease) controls. The major novel finding of this study is that AF patients had a lower hypercapnic  $CVR_{CO_2}$  when compared to healthy age-matched controls, indicative of a lower cerebral vasodilatory reserve. In addition, it was observed that AF and hypertensive patients tended to have lower MCA  $V_{mean}$  than their healthy counterparts (non-significant), and that cerebral autoregulation via TFA showed a reduced coherence in very low and low frequency range in hypertensive, but not AF patients. The reduced cerebral vasodilatory reserve as characterized by the lower hypercapnic  $CVR_{CO_2}$  noted in AF indicates that an impaired cerebral endothelial function or neural-glial NO synthesis may contribute to the increased stroke risk and poorer cerebral vascular disease outcomes in these patients.

### *Resting cerebral perfusion*

Few studies have assessed cerebral perfusion in patients with AF. One study used the inhalation of [ $^{133}Xe$ ] to assess the cerebral blood flow in patients with AF in different age groups (Lavy *et al.*, 1980). They reported a 17.5% lower cerebral perfusion in the AF group aged 35-50 years, 13.4% lower in the 51-65 years group and a non-significant 5.5% reduction in the group >65 years when compared to age-matched healthy controls. Subgroup analysis revealed that the aetiology of AF (ischaemic heart disease, valvular AF or unknown origin) did not influence cerebral blood flow and it was suggested that cerebral perfusion would be reduced even with small changes in cardiac output. However, another

study assessed cerebral blood flow in patients undergoing electrical cardioversion and it was found that the cerebral perfusion was  $\approx 12\%$  higher after one day and  $\approx 30\%$  higher after 30 days of cardioversion with no significant changes in cardiac output (Petersen *et al.*, 1989b). Similarly, during a bout of AF patients had a  $\approx 12\%$  reduction in MCA  $V_{\text{mean}}$  when compared to measurements after pharmacological cardioversion, but no significant differences were found in the basilar artery or ICA (Totaro *et al.*, 1993). Furthermore, during acute stroke, individuals with AF have a lower MCA  $V_{\text{mean}}$  than people in sinus rhythm (Porebska *et al.*, 2008), although the mechanisms underlying this reduction is still unknown.

In accordance with previous studies, the results from this Chapter show that AF and hypertensive patients tended to have  $\approx 17\%$  lower baseline MCA  $V_{\text{mean}}$  with a moderate effect size, although this was not statistically significant. Furthermore, no significant differences were found in MAP or minute ventilation, but  $P_{\text{ETCO}_2}$  tended to be higher in hypertensive participants. A reduced cerebral perfusion particularly in the temporo-parieto-occipital region as assessed by SPECT has been found to correlate with lower scores in cognitive tests such as the mini mental state test (Thome *et al.*, 1996). Therefore, the lower cerebral perfusion in AF patients may have important implications for the risk of complications such as cognitive decline and cerebral vascular disease, although the mechanism underpinning this reduction is still unknown. In addition, exercise training has been shown to be effective in improving quality of life in patients with AF (Osbak *et al.*, 2011), and may be also used to improve cerebral haemodynamics in these patients.

## $CVR_{CO_2}$

This study was the first to assess the  $CVR_{CO_2}$  in AF patients.  $CVR_{CO_2}$  is the inherent ability of the cerebral vasculature to dilate in response to a hypercapnic stimulus or constrict in response to hypocapnia. It was found that AF patients had a lower hypercapnic but not hypocapnic  $CVR_{CO_2}$  slope when compared to healthy or hypertensive control participants.  $CVR_{CO_2}$  is reportedly impaired in conditions such as Alzheimer's disease (Glodzik *et al.*, 2013) and might increase the risk of complications in AF patients. One meta-analysis showed that an impaired  $CVR_{CO_2}$  was associated with an increased risk of stroke (pooled random effects odds ratio: 3.86) in patients with internal carotid artery stenosis (Gupta *et al.*, 2012). Furthermore, a study with 1695 participants and a follow-up time of 12 years revealed that there was an increase in hazard ratio for all-cause mortality of 1.1 per SD reduction in  $CVR_{CO_2}$  (Portegies *et al.*, 2014). This association was even stronger when cardiovascular mortality was considered, with an increase in hazard ratio of 1.15 per SD decrease in  $CVR_{CO_2}$ . Interestingly, these associations were independent from stroke or cardiovascular risk factors, suggesting that impaired  $CVR_{CO_2}$  may be associated with systemic vascular dysfunction. A low cerebral vasodilatory reserve may serve as an "early warning" as the cerebral vessels are not responding adequately to a physiological stressor. Future studies are needed to assess the impact of low  $CVR_{CO_2}$  on the incidence of cerebral vascular events and mortality in patients with AF, as well as associations with a reduction in cerebral perfusion and cognition.

Although the mechanisms of cerebral vessels dilatation stimulated by CO<sub>2</sub> are not fully known, it is accepted that extracellular pH, endothelial and smooth muscle potassium channels and NO are the major drivers of cerebral vascular tone (Kontos *et al.*, 1977; Faraci & Brian, 1994; Faraci & Sobey, 1996; Kinoshita & Katusic, 1997). Under normocapnic conditions, infusion of a NO blocker in rats caused a ≈9 to 32% decrease in cerebral blood flow, while MAP increased by ≈10 to 52% (Wang *et al.*, 1992). During a hypercapnic test, this effect was potentiated to a ≈32 to 52% reduction in cerebral blood flow when compared to control, indicating the importance of the NO pathway to the cerebral vasodilatory reserve. In humans, CVR<sub>CO<sub>2</sub></sub> has been shown to be impaired in patients with endothelial dysfunction (e.g. diabetes). Furthermore, administration of a NO donor such as sodium nitroprusside offsets this difference, indicating that the smooth muscle in these arteries appear to be intact (Lavi *et al.*, 2006). Previous studies suggest that AF patients may have systemic endothelial dysfunction characterised by an increase in plasma von Willebrand factor, reduced nitrite/nitrate product and impaired flow-mediated dilatation of the brachial artery, which may contribute to the increase in risk of cerebral vascular disease (Conway *et al.*, 2003; Freestone *et al.*, 2008). Furthermore, in cultured human umbilical endothelial cells, laminar or pulsatile flow upregulates NO production while this is not seen in conditions of turbulent flow (Noris *et al.*, 1995). This suggests that reduced shear stress may also play a role in the endothelium of AF patients due to the irregular heart rhythm. The data presented in this chapter is the first piece of evidence for a possible cerebral endothelial dysfunction in AF patients, possibly due to deranged NO



pathways. In addition, hypocapnic  $\text{CVR}_{\text{CO}_2}$  was not significantly different between groups. This may be explained by the different mechanisms of regulation, since the NO pathway may be less active during hypocapnia (Wang *et al.*, 1992). Alternatively, the lack of a difference between groups may be due to a “floor effect” of the  $\text{CVR}_{\text{CO}_2}$  curve, whereby hypocapnic changes in  $\text{PaCO}_2$  with the same magnitude of hypercapnic changes will generate a much smaller response in cerebral blood flow (Tominaga *et al.*, 1976).

### *Cerebral autoregulation*

Cerebral autoregulation is the ability of the cerebral blood vessels to adapt to changes in arterial blood pressure in order to protect the brain from hypo- and hyperperfusion (Aaslid *et al.*, 1989). It has been shown to be impaired in a myriad of brain diseases such as traumatic brain injury (Czosnyka *et al.*, 1997), cerebral vascular disease (Atkins *et al.*, 2010) and Alzheimer’s disease (den Abeelen *et al.*, 2014), as well as systemic diseases such as type 2 diabetes mellitus (Vianna *et al.*, 2015). It has been proposed that AF patients have lower cerebral blood flow due to autoregulatory mechanisms (Lavy *et al.*, 1980). However, it has also been proposed that a reduced cerebral blood flow could lead to cerebral vessels dilatation which may impair cerebral autoregulation (Aaslid *et al.*, 1989). In addition, short periods of ventricular arrhythmia have been shown to promote a paradoxical contraction of the cerebral vessels in people with AF, but the effects of chronic supraventricular arrhythmia on the cerebral autoregulation remain to be elucidated (Grubb *et al.*, 1997). However, in the present study no differences in TFA indices of cerebral autoregulation (gain or phase) were observed between AF patients and healthy controls. Thus,

this data suggests that cerebral autoregulation is preserved in AF patients, although a reduced TFA coherence at the very low and low frequencies was noted at baseline in hypertensive patients. Coherence has traditionally been used as an indication of the linearity between input (MAP) and output (MCA  $V_{\text{mean}}$ ) and therefore the effectiveness of the transfer function (Zhang *et al.*, 2002; Ogoh *et al.*, 2005). Many factors may cause low coherence values, including a nonlinear relationship between input and output, a poor signal-to-noise ratio and the presence of other factors influencing the MCA  $V_{\text{mean}}$ . Furthermore, a low power MAP and MCA  $V_{\text{mean}}$  may also contribute to the low coherence in the baseline very low and low frequency ranges found in this Chapter (van Beek *et al.*, 2008). Therefore, the results from baseline TFA could mean that there was a loss of linearity of the MCA  $V_{\text{mean}}$  responses to spontaneous fluctuations in MAP, with an increased influence of other factors and greater complexity of the responses in cerebral perfusion (Panerai *et al.*, 2006). However, the low coherence could also interfere with or even invalidate the interpretation of the TFA gain and phase, a reason that makes the use of manoeuvres to enhance coherence recommended (van Beek *et al.*, 2008). A strength of the present study is that a repeated squat-to-stand manoeuvre was employed to substantially enhance MAP and MCA  $V_{\text{mean}}$  power, and thus coherence. However, no significant differences in gain, phase or coherence were observed between groups in the frequency ranges assessed. The concept that cerebral autoregulation is preserved in AF patients is further supported by the observation that the cerebrovascular responses to the single sit-to-stand were not different between groups.

The proposed mechanisms involved in the autoregulation process include stretch-activated cation channels (Davis *et al.*, 1992), autonomic innervation (Hamner *et al.*, 2010; Hamner *et al.*, 2012) and even NO (White *et al.*, 2000), although the latter is still debated (Zhang *et al.*, 2004). As no differences in cerebral autoregulation were observed between groups, it is tempting to speculate that the underlying mechanisms are unaltered in AF patients, despite the  $CVR_{CO_2}$  responses being blunted. Thus, it may be further speculated that the reduced cerebral vasodilatory reserve in AF patients might be as a result of endothelial but not smooth muscle dysfunction. This study paves the way for further mechanistic assessment of cerebral vascular function in this population.

### *Cognition*

AF has been shown to be a risk factor for cognitive decline even without clinical history or radiologic signs of cerebral vascular disease (Ott *et al.*, 1997; Knecht *et al.*, 2008). Mild cognitive impairment is more likely to occur in AF patients with higher thromboembolic risk and lower education levels (Ball *et al.*, 2013). One proposed mechanism for the cognitive decline is a reduced cerebral blood flow that leads to white matter damage (Raiha *et al.*, 1993). However, other factors may influence such as the occurrence of cerebral microbleeds (Poels *et al.*, 2012) and increased inflammatory markers (Kuo *et al.*, 2005). No significant difference in cognition was found between the groups in this chapter. Some methodological considerations (explained below) must be taken under account when assessing the cognition findings from this chapter.

### *Limitations*

The use of changes in MCA  $V_{\text{mean}}$  measured by transcranial Doppler as a surrogate marker for cerebral blood flow is a subject of debate (Ainslie & Hoiland, 2014). There is contradictory evidence comparing this technique with absolute measures of cerebral blood flow (Bishop *et al.*, 1986; Sorteberg *et al.*, 1989), as discussed in Chapter 5. Although it is well known that AF is a risk factor for cognitive decline and dementia, the cognition was not different between groups in this study. Several reasons may account to this, but the small sample size and a relatively low risk profile (normal blood cholesterol, absence of heart failure and no signs or history of cerebral vascular disease) in the population studied may be the main reasons. Post-hoc power analysis using the results found in this study showed that 17 participants per group would be needed to assess statistical significance of cognitive function.

### *Perspective*

AF is strongly associated with age and is a risk factor for brain disease, although the mechanisms contributing to this increased risk are not fully understood. This study showed that AF patients had a lower hypercapnic  $\text{CVR}_{\text{CO}_2}$  when compared to healthy controls. It can be speculated that cerebral endothelial dysfunction or neural-glia NO synthesis may be a significant contributor to the cerebral vascular risk in these patients, as vascular smooth muscle function seems to be preserved in AF patients. Future research is needed to provide evidence for the possible cerebral endothelial dysfunction and NO pathways in the brain of AF patients. This could be evaluated by the

administration of NO donors and blockers and comparing with other groups of patients with endothelial dysfunction and healthy individuals. Furthermore, further research is needed to elucidate whether cerebral and systemic vasomotor regulation are modified following exercise training in AF patients.

**CHAPTER 7: GENERAL DISCUSSION AND FUTURE  
DIRECTIONS**

The brain requires a high nutritive flow of oxygen and glucose, as well as removal of by-products resulting from cerebral metabolism. Short interruptions in the cerebral blood supply leads to symptoms such as seizures and fainting within a few seconds and, if not reversed immediately, may lead to permanent neurologic damage, dysfunction and death. On the other hand, excessive cerebral perfusion can cause cerebral vascular damage, increased intracranial pressure, stroke, and may also lead to neurologic damage, dysfunction and death. To prevent such situations from happening, the cerebral circulation counts on many different mechanisms of blood flow regulation, including adaptations to changes in metabolic activity, arterial blood gases, blood pressure, cardiac output and autonomic nervous system activity. Ageing is associated with a reduction in cerebral perfusion and is a well-known risk factor for cardiovascular disease, cognitive decline, dementia and cerebral vascular disease. Given this, therapeutic strategies to enhance brain health, such as exercise are warranted. This thesis aimed to determine the impact of physical activity and cardiorespiratory fitness on the cerebral blood flow and its regulation. Heart disease may further compromise cerebral vascular function, and it has also been associated with an increased risk of cerebral vascular disease, cognitive decline and dementia. The final aim of this thesis was to determine whether cerebral vascular function is impaired in sedentary old individuals and patients with AF, and thus represents a potential mechanism for the increased risk of brain disease in these populations.

The study presented in chapter 4 aimed to determine if higher levels of objectively measured daily physical activity influence cerebral blood flow as

measured by duplex Doppler ultrasound and cognition in a cohort of healthy old individuals. It was found that: (i) cerebral blood flow was not different between groups despite a considerable difference ( $\approx 55\%$ ) in levels of daily physical activity; (ii) cognition was also not different between low and high physical activity groups. This was the first study to objectively measure daily physical activity using 7-day accelerometry while providing a volumetric assessment of cerebral blood flow using duplex Doppler ultrasound of the extracranial arteries in old individuals. Many previous studies showed that resting cerebral perfusion was associated with cardiorespiratory fitness (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b; Thomas *et al.*, 2013). However, the fact that the study presented in chapter 4 did not show a difference in cerebral blood flow between low and high daily physical activity groups suggests that a higher degree of physical activity (e.g. exercise training) might be needed to evoke a chronically increased cerebral blood flow. Furthermore, this study was underpowered to assess cognition in this cohort of healthy older individuals and care must be taken when interpreting the results presented.

Chapter 5 extended the findings of the previous chapter, with the aim of investigating if age and higher levels of cardiorespiratory fitness would influence cerebral blood flow at rest and during a hypercapnic challenge. This chapter also used duplex Doppler ultrasound of the extracranial vessels to determine cerebral blood flow and also assessed the blood flow responsiveness to increased levels of inspired  $\text{CO}_2$  at the ICA. It was found that: (i) bilateral ICA blood flow was  $\approx 31\%$  higher in trained individuals when compared to their untrained counterparts; (ii) bilateral VA flow was not influenced by either age or



cardiorespiratory fitness; (iii) MCA  $V_{\text{mean}}$  was lower in old individuals when compared to young, but was unaffected by cardiorespiratory fitness; (iv) there was a non-significant tendency for an enhanced ICA  $\text{CVR}_{\text{CO}_2}$  in old trained individuals ( $d = 1.22$ ), while MCA  $\text{CVR}_{\text{CO}_2}$  did not appear to be influenced by age or training status. Many important interpretations can be made from these results. Firstly, the fact that a high cardiorespiratory fitness was associated with an increased bilateral ICA blood flow is in agreement with the current literature (Ainslie *et al.*, 2008; Bailey *et al.*, 2013b), however with the added benefit of showing an objective measurement of flow instead of surrogate markers. Secondly, the absence of a difference in bilateral VA flow between groups provides a more comprehensive assessment of global perfusion, as the anterior circulation appears to be favoured by the chronic adaptations of the cerebral circulation caused by exercise. It has been previously shown that responses to acute stressors such as head-up tilt and exercise at the ICA and VA are different (Sato *et al.*, 2011; Sato *et al.*, 2012a). However, this is the first time differential chronic adaptations to exercise were assessed in the ICA and VA. The fact that the bilateral ICA flow but not the MCA  $V_{\text{mean}}$  was influenced by cardiorespiratory fitness may relate to the number of participants in the study, making it underpowered for the expected MCA  $V_{\text{mean}}$  difference between groups. Finally, the tendency for an increased cerebrovascular dilatory reserve in old individuals with high cardiorespiratory fitness levels may be a mechanism contributing to the improved brain health in old people who exercise.

The study presented in chapter 6 assessed the cerebral vascular function in patients with AF comparing with healthy controls or hypertensive individuals,

who served as a disease control. It was found that: (i) individuals with AF had lower  $CVR_{CO_2}$  when compared to healthy controls; (ii) AF and hypertensive patients tended to have a reduced MCA  $V_{mean}$  when compared to healthy controls; (iii) cerebral autoregulation was preserved in AF patients, but a lower coherence in very low and low frequency range was observed in hypertensive patients. This study provides novel evidence for the impact of AF on the cerebral vascular regulation, which contributes to the understanding of the pathophysiology of the cerebral vascular disease in this patient population. The reduced cerebral vasodilatory reserve found in AF patients may contribute to the poorer prognosis to cerebral vascular events in this group when compared to people in sinus rhythm as shown previously (Lin *et al.*, 1996). In fact, a lower  $CVR_{CO_2}$  is associated with increased incidence of cerebral vascular events and mortality (Markus & Cullinane, 2001; Portegies *et al.*, 2014). Potassium channels, NO and extracellular pH are proposed to be the major mechanisms underpinning the arterial dilatation promoted by  $CO_2$ . An impaired systemic endothelial function has been found on AF patients (Conway *et al.*, 2003; Freestone *et al.*, 2008). Considering the  $CVR_{CO_2}$  findings presented in chapter 6, together with a preserved cerebral autoregulation and the systemic endothelial dysfunction presented in the background literature, it can be speculated that there is a dysfunction in the synthesis of NO at the brain vessels, which should be further investigated.

### *Future directions*

Many new questions arise from the studies performed for this thesis. Firstly, due to the cross-sectional design of the studies presented in this work some questions would require randomized controlled trials in order to be answered. One question that remains is: are the physical activity recommendations proposed by the current guidelines enough to improve cerebral structure, function and cognition? It would require a randomized controlled trial where old individuals who are sedentary (determined objectively using accelerometry) would be subject to a physical activity intervention, with measures of cognition, cerebral imaging and cerebral vascular function before and after the intervention. Furthermore, the results presented in the Chapters 4 and 5 are from healthy older individuals and may not be representative of the general population, as there is a high prevalence of chronic diseases in the older segments of the population. However, these Chapters aimed to assess the effects of physical activity, cardiorespiratory fitness and age *per se* on the cerebral vascular function. It was beyond the scope of this thesis to provide scientific evidence in a clinically diverse population. It is possible that people who have diseases that are associated with increased vascular risk (e.g. AF) may benefit more from the effects of exercise on the brain vessels, as it is shown in the peripheral circulation in diseases such as diabetes (Mikus *et al.*, 2011). Taking this under account, high physical activity or cardiorespiratory fitness may have greater impact on a more clinically diverse population, which would be representative of the general population. The effects of exercise training, physical activity or cardiorespiratory fitness on the cerebral circulation

may be more pronounced in a “less healthy” sample which would be more representative of the general population, but an approach closer to a pragmatic trial is necessary to assess the impact of exercise, physical activity or cardiorespiratory fitness on the cerebral blood flow and its regulation on the general population. However, the positive effects of high physical activity and exercise on the cognitive function and cardiovascular system warrants that exercise promotion should be a population-wide intervention including healthy individuals to improve quality of life and reduce the impact of disease in the ageing population (Bijnen *et al.*, 1998; Garber *et al.*, 2011; United Nations, 2013).

It is not known whether the cerebral vasodilatory reserve in AF patients would be improved with exercise training. Exercise training has been shown to be effective in improving exercise capacity and quality of life in AF patients (Osbak *et al.*, 2011). In healthy old individuals, cardiorespiratory fitness seems to be associated with improved cerebral haemodynamics (Bailey *et al.*, 2013b; Barnes *et al.*, 2013; Braz *et al.*, 2016), although it is not an universal finding (Thomas *et al.*, 2013; Zhu *et al.*, 2013). It is possible that individuals with an impaired  $CVR_{CO_2}$  may benefit more from the exercise training than healthy individuals as exercise is known to improve endothelial function (Ross *et al.*, 2016), making this an attractive preventive and therapeutic intervention. Lastly, to confirm the role of NO on the impaired cerebral vasodilatory reserve in AF patients,  $CVR_{CO_2}$  should be assessed at a baseline condition and after administration of a NO donor, such as glyceryl trinitrate, nitroprusside or L-arginine. Animal studies may be required to assess if the dysfunction in NO

production has an endothelial, neuronal or glial origin with blockade of specific NO synthase enzymes.

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