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The Effects of Rapid and Prolonged Changes in Blood Pressure on Cerebral Blood Flow in Healthy Humans

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

The regulation of blood flow to the brain is complex and incompletely understood. Many local and systemic factors modulate cerebral perfusion, one of which is arterial blood pressure. The brain possesses intrinsic mechanisms which act to protect against rapid and also prolonged changes in perfusion pressure. However, recent evidence indicates that this supposedly powerful regulatory mechanism/s may not be as efficient as traditionally believed and that changes in arterial blood pressure have a profound effect on cerebral blood flow (CBF). This thesis explored different non-pharmacological means of perturbing mean arterial blood pressure (MAP) both rapidly (dynamic) and for prolonged steady-state periods (~5 min; static). Dynamic changes in blood pressure were induced via upright resistance exercise (**Chapter Five**) and standing Valsalva manoeuvres (VM; **Chapter Six**), while static changes were induced via lower body positive pressure (**Chapters Seven** and **Eight**). The effects of these changes in MAP on cerebral blood flow were assessed via transcranial Doppler ultrasound of the blood velocity in middle cerebral artery (MCAv). The findings of **Chapter Five** illustrated that *during* resistance exercise the peak mean MCAv ($MCAv_{mean}$) was unchanged between loads despite the increasing MAP with the increasing relative load. Following resistance exercise, however, the hypotension observed was matched by concomitant reductions in $MCAv_{mean}$, the magnitude of which was load dependent. **Chapter Six** investigated the role of the Valsalva manoeuvre (VM) alone in the stability of the MCAv response whilst standing. **Chapter Six** highlights that the VM protects the cerebral vessels during acute hypertension. In addition, more intense straining during a VM produced a similar response following the release of the manoeuvre to that seen following the resistance exercise. Thus, more pronounced decreases in blood pressure,

whilst upright, do result in concomitant decreases in $MCAv_{mean}$. The steady-state elevations in MAP examined in **Chapters Seven** and **Eight** increased $MCAv_{mean}$ with and without hypercapnia. Thus, illustrating that even when the regulatory mechanisms were functionally intact (normocapnia) the brain demonstrated a pressure passive relationship during relatively small increases in MAP. Overall, both abrupt and steady-state changes in perfusion pressure were coupled with alterations in $MCAv_{mean}$. This thesis contributes to the notion that the cerebral circulation is not independent of changes in MAP, and that sustained hypercapnia impairs the autoregulatory capacity of the cerebral circulation. Importantly, this thesis shows these effects without the use of pharmacological agents to confound the interpretation of the data.

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List of Abbreviations

	A
ABP	Arterial blood pressure
ACA	Anterior cerebral artery
ANOVA	Analysis of variance
AVM	Arteriovenous malformation
	B
BA	Basilar artery
beats·min^{-1}	Beats per minute
	C
CBF	Cerebral blood flow
cm	Centimetres
cm·s^{-1}	Centimetres per second
CO_2	Carbon dioxide
CPP	Cerebral perfusion pressure
CVC	Cerebrovascular conductance
CVP	Central venous pressure
CVR	Cerebrovascular resistance
	D
DBP	Diastolic blood pressure
DMCAv	Diastolic middle cerebral artery blood flow velocity
	E
ECG	Electrocardiogram
	H
HR	Heart rate
H_2O	Water

I

ICA	Internal carotid artery
ICP	Intracranial pressure

K

kg	Kilograms

L

LBNP	Lower body negative pressure
LBPP	Lower body positive pressure
$L \cdot min^{-1}$	Litres per minute
L-NMMA	N^G-monomethyl-L-arginine

M

m	Metre
MAP	Mean arterial blood pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery blood flow velocity
$MCAv_{mean}$	Mean middle cerebral artery blood flow velocity
min	Minute
mL	Millilitres
mm Hg	Millimetres of mercury
MVC	Maximal voluntary contraction
MSNA	Muscle sympathetic nerve activity

N

NTS	Nucleus tractus solitarius

O

O_2	Oxygen

P

P_aCO_2	Partial pressure of arterial carbon dioxide

P_aO_2	Partial pressure of arterial oxygen
PCA	Posterior cerebral artery
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end-tidal oxygen

Q

$\dot{Q}$	Cardiac output

R

RM	Repetition maximum

S

SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SMCAv	Systolic middle cerebral artery blood flow velocity
SV	Stroke volume

T

TCD	Transcranial doppler
TPR	Total peripheral resistance

V

VA	Vertebral artery
VM	Valsalva manoeuvre

Y

Y	Years

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Chapter One: Introduction

The brain, like any other organ, relies on a constant supply of blood for normal functioning. Despite the brain's obvious importance in homestasis, how blood flow is regulated to this vital organ is not clearly understood. It is clear, however, that the regulation of cerebral blood flow (CBF) is modulated by numerous factors. The myriad of factors that contribute to CBF regulation include the intrinsic autoregulatory mechanism/s that defend against changes in blood pressure, changes in arterial blood gases, cardiac output ($\dot{Q}$), the autonomic nervous system and local neuronal metabolism. When flow is compromised the resultant effects are obvious within seconds; for instance when CBF decreases acutely, consciousness is abruptly lost. Whilst the regulatory mechanisms of CBF are powerful, rudimentary tasks such as standing (Thomas *et al.* 2009b), lifting (Edwards *et al.* 2002) and coughing (Mattle *et al.* 1995) can challenge CBF. Even for healthy individuals simple tasks which challenge blood pressure may also challenge CBF.

The effectiveness of the brain's ability to defend against changes in blood pressure has recently been scrutinised with changes in blood pressure having a more pronounced effect than previously thought (Lucas *et al.* 2010). This includes both short term rapid (seconds) (Zhang *et al.* 1998a; Claassen *et al.* 2009) and prolonged (minutes) (Lucas *et al.* 2010) changes in blood pressure. An effective means of inducing acute (dynamic) perturbations in blood pressure is resistance exercise with pressures as high as 480/350 mm Hg having been reported (MacDougall *et al.* 1985) and blood pressure low enough following the effort to induce fainting (Compton *et al.* 1973). Also recruited during resistance exercise at high loads is the Valsalva manoeuvre (VM). Similarly to resistance exercise, when the VM is performed

in isolation large transient increases then decreases in blood pressure are experienced. Moreover, both resistance exercise and the VM provide a non-pharmacological means of acutely perturbing blood pressure that is capable of challenging CBF. Both resistance exercise and the VM are comparable to everyday tasks such as lifting a heavy object and coughing, respectively.

The brain is also challenged during prolonged (static) increases in blood pressure. Studies to date investigating this response have utilised pharmacologically-induced increases in mean arterial blood pressure (MAP) that may interfere with the measurement of CBF (Ogoh *et al.* 2011). These prolonged increases in blood pressure can also occur against a background of elevated arterial CO_2 (hypercapnia). The interaction between the increases in blood pressure and arterial blood gases is important as hypercapnia may impair the intrinsic regulatory mechanisms of the cerebral vasculature, although this has only been shown during dynamic changes in blood pressure (Aaslid *et al.* 1989). Understanding the effect of these changes in blood pressure in healthy humans may have implications for diseased populations that may be at greater risk of cerebral injury.

Against this background, the aim of this thesis was to investigate the cerebrovascular response to stimuli that induce rapid (dynamic) and prolonged (static) non-pharmacological changes in blood pressure. A further aim was to assess how altered arterial blood gases (i.e., hypercapnia) affected the cerebrovascular response to prolonged increases in blood pressure. This thesis aims to improve the understanding of CBF regulation in healthy individuals during changes in MAP induced by non-pharmacological means. The concepts briefly introduced above will be examined in depth in **Chapter Two,** which entails a thorough review of the literature to date. Specifically, intrinsic, extrinsic and systemic

factors that modulate CBF in specific reference to human data. **Chapter Three** critiques the techniques used in the collection of data for this thesis and provides general methodology for the experimental chapters. **Chapter Four** details the specific aims and hypotheses for the following experimental chapters (**Five** through **Eight**). The experimental chapters have been written as independent manuscripts and thus are written accordingly with an introduction, methods, results and discussion. The content of these manuscripts are intended to challenge both dynamic (**Chapters Five** and **Six**) and static (**Chapter Seven**) cerebral autoregulation and also when autoregulation is impaired (**Chapter Eight**). Finally, **Chapter Nine** provides a general discussion and highlights possible future avenues for research given the findings of this thesis.

Chapter Two: Literature Review

This chapter is devoted to a thorough review of the literature to date on the regulation of CBF, with a particular emphasis on human studies. This includes highlighting the anatomical vessels that supply the brain as well as how CBF is regulated. The regulation of CBF is complex and modulated by a myriad of both extrinsic and intrinsic physiological processes. The purpose of this chapter is to critically review data concerning these processes that govern CBF as they will be frequently addressed throughout the remainder of this thesis. Particular detail will be given to the modulators that will be perturbed in **Chapters Five** through **Eight.** As these processes include and/or are influenced by systemic factors (such as MAP) the regulation of these variables will also be reviewed. Thus, the scope of this review is broad and will discuss the factors that both directly and indirectly influence CBF. Nevertheless, areas that the thesis will focus on will be emphasised throughout. Most importantly, this review forms the foundation of the thesis from which specific aims, objectives and hypotheses will be formulated.

2.1 Cerebral Blood Flow

The human brain comprises ~2% of overall body mass, yet receives ~15% of cardiac output ($\dot{Q}$) and accounts for ~20% of the body's oxygen consumption (Willie *et al.* 2011). This large demand for flow is required to meet the brain's high metabolic demand. Further, the brain has a limited supply of energy substrates and thus requires precise blood flow control (Willie *et al.* 2011; Willie *et al.* 2014). The brain is perfused by two pairs of arteries: the internal carotid artery (ICA) and the vertebral artery (VA). The ICA bifurcates into the middle

cerebral artery (MCA) and the anterior cerebral artery (ACA), with the MCA being the largest branch of the ICA (Lee 1995). The MCA supplies ~80% of the blood to each hemisphere, and is believed to be merely a distributing artery translating flow to the downstream vessels (Olufsen *et al.* 2002). These arteries form the anterior circulation and perfuse the temporal, parietal and frontal regions of the brain (Alastruey *et al.* 2007). The two VAs anastomose to form the basilar artery (BA), which bifurcates to form the right and left posterior cerebral arteries (PCA) and supply regions of the brain stem and occipital lobe (i.e., posterior circulation; Alastruey *et al.* 2007). The anterior and posterior circulations are joined by pairs of anterior and posterior communicating arteries, respectively. These structures form the Circle of Willis (Figure 2.1) that in humans is mainly comprised of the ACAs, PCAs and posterior communicating arteries (Lee 1995). The Circle of Willis forms the main collateral pathway for the cerebral circulation, although many anatomical variations exist within the circle of Willis itself (Alastruey *et al.* 2007) and other major branches such as the MCA (Komiyama *et al.* 1998).

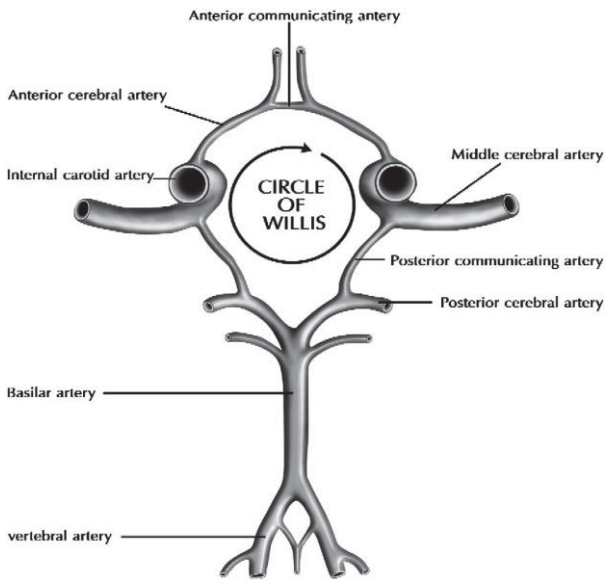

Figure 2.1 Diagrammatic representation of the arteries forming the circle of Willis (Franco Folino 2007).

The brain is exquisitely sensitive to numerous physiological stimuli including changes in neural metabolism, partial pressure of arterial carbon dioxide (P_aCO_2), blood pressure, $\dot{Q}$ and autonomic output (Ainslie & Duffin 2009) (Figure 2.2). Whilst the effects of these stimuli have pronounced effects on CBF, the underlying mechanisms in which these stimuli act remain unclear (Willie *et al.* 2014).

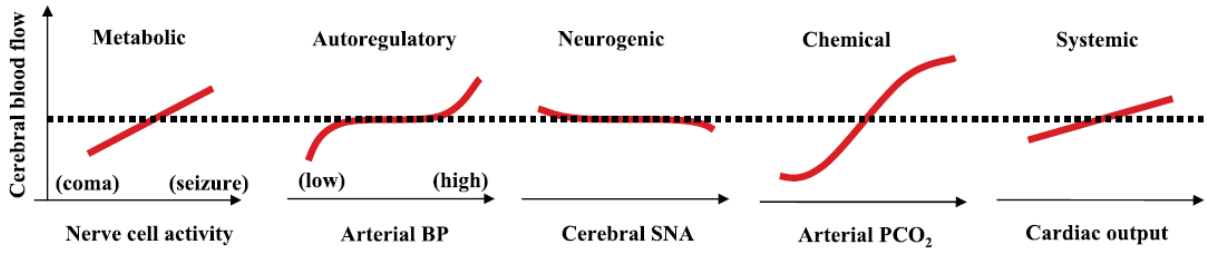

Figure 2.2 The effect of key physiological stimuli on CBF. BP, Blood pressure; PCO_2, partial pressure of carbon dioxide; SNA, sympathetic nerve activity (Ainslie & Duffin 2009).

However, like other circulations the brain is governed by the laws of haemodyanmics; namely, Darcy's law which describes that the steady-state flow is proportionate to the pressure difference between two points and the resistance of that circuit;

$$\text{Steady state flow} = \frac{P1-P2}{R}$$

Where P_1 is the inflow pressure and P_2 is the outflow pressure and R is the resistance. However, within the many circulations that comprise the human cardiovascular system, vessel diameter can be modified by both intrinsic and extrinsic signals. This gives rise to Poiseuille's law which relates vessel diameter, amongst other factors, to the resistance of a vessel. The resistance of a vessel is given by the following equation:

$$\text{Resistance} = \frac{8nL}{\pi r^4}$$

Where, *n,* is the fluid viscosity; L, tube length and r, vessel radius.

Therefore, flow through a vascular bed is dependent on a number of factors, the most important of which is the pressure difference across the vascular bed and the vessel radius. Given Poiseuille's law it is apparent that changes in vessel radius have a pronounced effect on resistance and subsequently flow through a given vascular bed. Further complicating the cerebral circulation is the relationship between transmural pressure and vessel radius. As the arteries that perfuse the brain parenchyma are enclosed within the skull, they are subject to changes in intracranial pressure and transmural pressure (Haykowsky *et al.* 2003). This in turn has an effect on the ability of the artery to dilate and therefore overall flow, as experienced during resistance exercise and the Valsalva manoeuvre (VM). Thus, cerebral perfusion pressure (CPP) can be defined as the difference between the arterial blood pressure and the intracranial pressure.

The brain, like other organs, is subject to changes in perfusion pressure. However, as detailed below (section 2.1.3), the brain displays an intrinsic mechanism to defend against changes in perfusion pressure by regulating vessel radius which is important given the cerebral intolerance to ischaemia (Van Lieshout *et al.* 2003). This mechanism is vital to the regulation of CBF as impaired or insufficient flow (i.e. stroke and syncope, respectively) may result in brain injury and/or damage. Despite this dependence on adequate perfusion several facets of CBF regulation are incompletely understood. In particular the CBF response to both rapid, and prolonged, changes in blood pressure. Despite the brain being pivotal to survival and homeostasis, somewhat rudimentary tasks such as lifting, breath holding and standing provide significant challenges to CBF and how these tasks effect CBF forms the basis of this thesis. Thus, how CBF is regulated is of upmost importance in order to

understand potential dangers that may compromise CBF and how these scenarios may be avoided in everyday activities.

The following section details the regulators of CBF depicted in Figure 2.2 with a particular focus on the modulators that will be manipulated in the experimental chapters.

2.1.1 Arterial Carbon Dioxide Content

In 1945 an accurate measurement of CBF was first described in conscious man (Kety & Schmidt 1945). It was not long before it was realised that P_aCO_2 had a pronounced effect on CBF. Hypercapnia induced a substantial increase in CBF (Kety & Schmidt 1948), whilst hypocapnia dramatically reduced CBF (Kety & Schmidt 1946). Both phenomena were described as being attributable to changes in vascular resistance. An increased P_aCO_2 results in cerebral vasodilation and an elevated CBF to increase the washout of CO_2 attenuating the rise in central PCO_2 and alterations in pH (Ainslie & Duffin 2009). Whilst the, vasoconstriction seen during hypocapnia reduces CO_2 washout. Changes in arterial pH at a constant P_aCO_2 result in no change in CBF (Lambertsen *et al.* 1961). However, direct manipulations in extracellular pH alter arteriolar tone (Kontos *et al.* 1977). Therefore, the changes in CBF induced by P_aCO_2 are independent of changes in arterial pH. A change in extracellular pH, via the movement of polar solutes (CO_2 rather than H^+) across the blood brain barrier, is required to induce changes in cerebrovascular resistance and in turn flow (Lambertsen *et al.* 1961). This inherent sensitivity of the brain's vasculature appears to be a vital homeostatic mechanism in regulating central pH (Chesler 2003). Thus, CBF is tightly linked with P_aCO_2 and therefore the central chemoreceptor control of respiration (see section 2.3.1).

The cerebrovascular response to P_aCO_2 does not appear to be homogenous, Ito *et al.* (2000) reported an elevated reactivity in the brainstem during both hypo- and hypercapnia in comparison to cortical areas using positron emission tomography. Similarly, Willie *et al.* (2012) reported an increase in VA CO_2 reactivity during hypocapnia in comparison to the ICA, MCA and PCA, with no difference reported during hypercapnia. In contrast, Sato *et al.* (2012b) reported a reduced CO_2 reactivity in the VA in comparison to the ICA. Further, the external carotid artery showed no change in flow in response to either hypo- or hypercapnia (Sato *et al.* 2012b). These disparate findings are contradictory as a reduced reactivity in the VA (posterior circulation supplying the brainstem) would support a maintained blood flow to the brainstem during hypocapnia and thus preserving homeostatic functions (Sato *et al.* 2012b).

Notwithstanding these potential regional differences, the cerebral circulation is exquisitely sensitive to changes in P_aCO_2, even more so than other circulations (Ainslie *et al.* 2005). Since the early studies of Kety and Schimdt in the 1940s, many subsequent experiments have confirmed these earlier results and indicated that there is a threshold for response, in that no vasodilation was observed until a >2.5% increase in inspired CO_2 (4.1 mm Hg P_aCO_2 increase) was administered (Patterson *et al.* 1955). Similar results were reported by Wasserman and Patterson (1961) with the vasodilation threshold in hypercapnia requiring a 4.5 mm Hg increase in P_aCO_2. A lower threshold (2 mm Hg decrease in P_aCO_2) was required for vasoconstriction during hypocapnia. Wasserman and Patterson (1961) also reported that the hypocapnic response was not linear and that at greater reductions in P_aCO_2 the CBF response was smaller per unit decrease in P_aCO_2. Further, the relationship between cerebral

blood volume and CBF during P_aCO_2 fluctuations follows a non-linear relationship (Grubb *et al.* 1974).

With the development of transcranial Doppler ultrasonography (TCD), the dynamic changes in CBF in response to fluctuations in P_aCO_2 can be observed (Markwalder *et al.* 1984). The response of the cerebral circulation to changes in P_aCO_2 has been termed CO_2 reactivity. Several investigators have reported that the MCAv response to P_aCO_2 is nonlinear and in fact sigmoidal (Figure 2.2) in shape (Ringelstein *et al.* 1988; Claassen *et al.* 2007; Battisti-Charbonney *et al.* 2011). Plateaus are seen at very low and high P_aCO_2 and support earlier observations using the steady state Kety-Schmidt technique (Wasserman & Patterson 1961). This response is dependent also on arterial blood pressure. Chemoreceptor activation has pronounced effects on MAP (see section 2.2.4). The relative fluctuations in cerebrovascular conductance during P_aCO_2 perturbations do not explain the CBF response seen during both hypo- and hypercapnia (Claassen *et al.* 2007). It appears that beyond a certain hypercapnic threshold of P_aCO_2, MAP increases. This threshold was also strongly correlated with the threshold at which MCAv increases (Battisti-Charbonney *et al.* 2011). Indicating that beyond this threshold the brain demonstrates a more pressure passive relationship, with both MAP and MCAv increasing linearly. This supports the idea that cerebral autoregulation is impaired during hypercapnia (further detailed in section 2.1.3.3.1). Below these MAP thresholds, changes in CBF during hypercapnia reflect changes in vasomotor tone induced by P_aCO_2 (Battisti-Charbonney *et al.* 2011). Interestingly there is no difference in CO_2 reactivity between normotensive and hypertensive patients (Tominaga *et al.* 1976). Importantly the midpoint of the linear portion of the CO_2 reactivity curve is close to resting

$P_{ET}CO_2$ such that the maximum cerebrovascular reactivity is observed during normocapnia (Battisti-Charbonney *et al.* 2011).

The mechanisms by which CO_2 modulates cerebrovascular tone remains unclear, but likely involves CO_2 -induced changes in pH activating potassium channels causing a hyperpolarising current that modulates vascular tone in both upstream and downstream vessels (Ainslie & Duffin 2009). Further, an increase in nitrate (a marker of the vasodilator nitric oxide) release from the brain has been reported during hyper- but not hypocapnia (Peebles *et al.* 2008). Although during nitric oxide synthase inhibition the cerebral hyperaemic response to hypercapnia was unaltered (White *et al.* 1998). In addition there was a net release of C-natriutetic peptide during hypocapnia (Peebles *et al.* 2008). Regardless of the mechanism/s it appears that the response to alteration in pH is fast acting. There is a short delay (~6 s) between the onset of hypercapnia and alterations in vascular tone, however the time constant for the "on" response is 7-times slower than the "off" response (Poulin *et al.* 1996). The opposite is true with hypocapnia. A faster "on" transient is seen during the onset of hypocapnia (~7 s) as opposed to the release of hypocapnia (~14 s) (Poulin *et al.* 1998). The delay in the drive to breathe during these scenarios may be due to the fact that CO_2 can diffuse across the blood brain barrier whereas H^+ cannot and thus central chemoreceptors are isolated from changes in arterial acid-base changes except for when changes in P_aCO_2 occur (Raichle & Plum 1972; Ainslie & Duffin 2009). High altitude can also alter the chemoreflex response due to an increase in arterial pH and reduction in bicarbonate. Resulting in a greater rise in central and arterial H^+ for a given change in P_aCO_2 (Fan *et al.* 2010). This change in buffering could be responsible for the increase in CO_2 reactivity seen at altitude as the vessels respond to pH rather than CO_2 directly (Fan *et al.* 2010). A reduction

in the CO_2 reactivity may result in a reduced cerebral CO_2 washout and an increased ventilatory stimulus (Peebles *et al.* 2007), again displaying the intricate balance between the central chemoreflex drive to breathe and the regulation of CBF by CO_2.

Given the above information it is clear that CBF is strongly modulated by P_aCO_2. As discussed later in this chapter this also has a pronounced effect on other physiological functions (i.e. ventilation). Although this mechanism does limit the change in central pH it appears that large changes in P_aCO_2 impair other regulatory mechanisms of CBF as discussed in section 2.1.3.3 of this chapter. Specifically, the ability to defend against changes in MAP although this effect has not been demonstrated during prolonged changes in blood pressure.

2.1.2 Arterial Oxygen Content

Similar to P_aCO_2 the partial pressure of arterial oxygen (P_aO_2) has a pronounced effect on CBF (Kety & Schmidt 1948; Cohen *et al.* 1967). Hypoxia *per se* induces cerebral vasodilation (Cohen *et al.* 1967) when arterial oxygen saturation ≤90% (Gupta *et al.* 1997). It appears that this dilation occurs via nitric oxide dependent mechanisms as the administration of the nitric oxide synthase inhibitor N^{G-}monomethyl-L-arginine (L-NMMA) abolished the CBF increase and returns cerebrovascular resistance (CVR) to baseline levels (Van Mil *et al.* 2002). Hypoxia induces a ventilatory response via activation of the peripheral chemoreceptors with a resultant hyperventilation and subsequent hypocapnia. Thus the hypocapnia, a potent vasoconstrictor is in opposition to the dilatory effects of hypoxia. When P_aCO_2 is unrestrained (i.e. Poikilocapnia) much of the CBF response is counterbalanced by the hypocapnia and no change (Van Osta *et al.* 2005; Jansen *et al.* 2007; Subudhi *et al.* 2009; Ogoh *et al.* 2013) or a decrease (Nishimura *et al.* 2010) in CBF is evident. In contrast during isocapnic hypoxia the increase in CBF is more marked (Ainslie &

Poulin 2004; Ogoh *et al.* 2013; Querido *et al.* 2013). Thus, lowering of P_aCO_2 can override the hypoxic vasodilation and the exact CBF response is dependent on the level of hypoxia and the level of hyperventilation (Verges *et al.* 2012) and ultimately the P_aO_2/P_aCO_2 ratio (Ainslie & Ogoh 2010; Lucas *et al.* 2011). This ratio is modified over time as ventilatory sensitivity adapts during chronic hypoxia, like that experienced at altitude. This hypoxic ventilatory response is highly variable between individuals and is tightly linked with the cerebrovascular response (Ainslie & Poulin 2004). There are several reports of an initial increase in CBF during hypoxia, despite a marked hypocapnia (Severinghaus *et al.* 1966; Jensen *et al.* 1990; Iwasaki *et al.* 2010; Subudhi *et al.* 2010; Lucas *et al.* 2011; Willie *et al.* 2012). This increase in CBF is evident during more severe hypoxia when P_aO_2 falls below 60 mm Hg (Kety & Schmidt 1948; Cohen *et al.* 1967). However, during prolonged hypoxia exposure, such as that seen upon ascent to high altitude, P_aO_2 increases (back toward baseline levels due to the acclimatisation process although is still decreased relative to sea level/baseline values) and the ventilatory stimulus induces a further reduction in P_aCO_2 and subsequently CBF decreases toward baseline levels (Severinghaus *et al.* 1966; Lucas *et al.* 2011).

The CBF response to hypoxia is also not uniform between the posterior and anterior cerebral circulations. During poikilocapnic hypoxia VA blood flow has been reported to increase whilst ICA flow is unchanged (Ogoh *et al.* 2013), indicating that in the territory supplied by the VA, hypocapnic constriction is outweighed by the hypoxia-induced vasodilation (Ogoh *et al.* 2013). Furthermore, the VA dilates during severe isocapnic hypoxia (P_aO_2 = 35 mm Hg) and yields a 50% greater increase in flow in comparison with the ICA and other major intracranial arteries (Willie *et al.* 2012). Therefore, the VA itself participates in

the hypoxic response rather than purely the intracranial vessels distal to it (traditional site considered to regulate conductance/resistance). This differential regulation of blood flow to the brainstem and cortex may act to maintain blood flow to the brainstem such that vital homeostatic mechanisms (i.e. ventilation and autonomic function) can be maintained during hypoxia (Willie *et al.* 2012; Ogoh *et al.* 2013).

2.1.3 Arterial Blood Pressure

The brain possess an intrinsic ability to maintain a constant CBF in the face of gradual and progressive changes in CPP that is termed static cerebral autoregulation (Paulson *et al.* 1990). The concept of cerebral autoregulation was first described in the classic review by Lassen (1959) who grouped data from a number of cohorts and calculated the classic autoregulatory curve (Figure 2.2). These data formed the basis for the traditional model of cerebral autoregulation in that CBF is held constant over a wide range of MAP (~60-150 mm Hg). The cerebral vasculature also displays a dynamic component of the autoregulatory response, which regulates CBF during rapid (e.g., 10 s) changes in arterial blood pressure (Aaslid *et al.* 1989). During changes in perfusion pressure the diameter of small arteries and arterioles respond rapidly (Symon *et al.* 1973; Kontos *et al.* 1978) such that perturbations in CPP are buffered by changes in cerebrovascular resistance (Aaslid *et al.* 1989). The terms static and dynamic cerebral autoregulation have arisen out of experimental necessity, rather than any physiological distinction (Willie *et al.* 2014). These two processes may be one in the same (Tan & Taylor 2014) and are used to purely describes the temporal nature of the MAP change used to perturb CBF. The regulation of CBF over a wide range of perfusion pressures occurring both transiently (dynamic) and over longer periods (steady state; i.e. several minutes) is pertinent to homeostasis as both hypo- and hyperperfusion can present

significant problems for the brain. A sustained hypoperfusion with concomitant reductions in oxygenation culminates in syncope (Van Lieshout *et al.* 2003; Franco Folino 2007) and severe hyperperfusion may result in breakdown of the blood brain barrier, cerebral oedema and possible stroke (van Mook *et al.* 2005; Pires *et al.* 2013).

The mechanism/s by which cerebral autoregulation operate have yet to be fully elucidated, but are believed to involve neurogenic, myogenic and endothelial derived factors (Carlson *et al.* 2008; Toda *et al.* 2009; Peterson *et al.* 2011; Tzeng & Ainslie 2013). These changes alter the ionic permeability which result in the contraction of the smooth muscle, altering vessel calibre (Panerai 2008). Due to the rapid changes in resistance this response is likely myogenic in origin (Symon *et al.* 1973) and independent of endothelial and neurogenic modulators as demonstrated in isolated vessel preparations (Jackson & Duling 1989). Recently, the dynamic autoregulatory response has been reported to be mediated via stretch-activated ion channels (Peterson *et al.* 2011), that alter intracellular calcium concentrations (Schubert & Mulvany 1999) and modulates smooth muscle tone. Blockade of these cerebral calcium channels in humans dilates cerebral vessels, decreasing absolute MCAv and increases pressure driven flow via the impairment of dynamic cerebral autoregulation (Tzeng *et al.* 2011).

A role of endothelial derived nitric oxide has also been implicated in the regulation of CBF (Toda *et al.* 2009). Yet the evidence for a role in the human circulation is equivocal. The nitric oxide synthase inhibitor L-NMMA was shown to impair the autoregulatory index to bilateral thigh-cuff deflation (White *et al.* 2000), although this study was underpowered (n = 6) and there was limited cross-over between treatments (cross over n = 4). Furthermore, global CBF, measured via positron emission tomography (White *et al.* 1999) and internal and

common carotid artery flow were decreased, indicative of an autoregulatory impairment (White *et al.* 1998) following L-NMMA administration; yet others report no change using magnetic resonance imaging (Van Mil *et al.* 2002). Zhang and colleagues (2004b) reported that nitric oxide synthase inhibition produced no change in CBF and transfer function metrics during spontaneous oscillations in MAP. However, similar to the systemic circulation up regulation of various flow mediators may occur when NO production is inhibited (Stoner *et al.* 2012). During L-NMMA and phenylephrine infusion the increase in MAP increased MCAv with concomitant increases in CVR, indicative of a myogenic response to the elevated perfusion pressure rather than the effect of the nitric oxide inhibitor (Zhang *et al.* 2004b). Whilst the cerebral circulation does display some peculiarities it is likely to be modulated by many factors such as acetycholine, lactate and adenosine that modulate vasodilation through various mechanisms (Attwell *et al.* 2010).

Regardless of the exact mechanism/s that regulate cerebral vessel tone; dilation maintains perfusion at low pressures and flow is restrained via a vasoconstriction at high perfusion pressures (Kontos *et al.* 1978). It has been shown that the pial arteriolar and arteries (vessels that run over the brain's surface) regulate CBF during changes in perfusion pressure and the lower limit of cerebral autoregulation is reached when the vasodilation of these vessels is inadequate to maintain flow as opposed to reaching a point of maximal dilation (Kontos *et al.* 1978; MacKenzie *et al.* 1979). However, it is now apparent that the larger intracranial arteries, originally thought to be merely conduit arteries, transferring flow to the cerebral circulation (Olufsen *et al.* 2002), may also participate in the regulation of CBF. This occurs via the mechanical properties of the arterial cerebral vessels that display a Windkessel effect, buffering CBF during MAP perturbations (Chan *et al.* 2011). Moreover,

this effect is dependent on the ΔMAP/Δtime rather than just the ΔMAP (Tzeng *et al.* 2011). Therefore, the regulatory response is dictated by the magnitude and the speed of the change in MAP. Resistance exercise and the VM provide excellent challenges for dynamic cerebral autoregulation due to the transient and abrupt nature of the changes in MAP they produce. These challenges mimic everyday tasks (heavy lifting and defecation for resistance training and the VM, respectively) that may be performed by a number of cohorts both with intact and impaired autoregulation. Improved understanding of how these challenges affect the regulation of CBF may improve guidelines (for instance during resistance training) in order to avoid cerebral injury during times of acutely elevated MAP, such as those discussed later in this review (see section 2.5).

2.1.3.1 Static Cerebral Autoregulation

The original autoregulatory curve (depicted in Figure 2.2) as purposed by Lassen (1959) was modelled using blood pressures and CBF values from 7 studies that included 11 patient groups with various pathologies and drugs that have been shown to directly influence CBF. It is now known that static cerebral autoregulation is sensitive to many physiological, pathological and experimental stimuli. During a wide range of cerebral pathologies including stroke, ischemia and trauma, static cerebral autoregulation can be impaired such that a rise in pressure is accompanied by an increase in CBF; i.e., a pressure-passive response (McHenry *et al.* 1974; Obrist *et al.* 1984; Strandgaard & Paulson 1984). This phenomenon is also seen in normotensive healthy patients with an induced hypertension beyond the upper autoregulatory limit (Strandgaard *et al.* 1973). The regulatory limits also show plasticity and can be modulated in diseased states with hypertension increasing the upper regulatory limit (Strandgaard *et al.* 1973). Patients treated for hypertension in both

the short- and long-term showed an adaptation of static cerebral autoregulation back toward control values, with no change in the lower limit (Strandgaard 1976; Zhang *et al.* 2007). The step wise increments induced by Strandgaard *et al.* (1973) were maintained for 5-10 minutes. As the static cerebral autoregulation takes ~90 s to rectify changes in perfusion pressure (Symon *et al.* 1973) it would be expected that any change would be accounted for in this time frame (5-10 mins). The anaesthetics Isoflurane (Tiecks *et al.* 1995a) and desflurane (Strebel *et al.* 1995) impair static cerebral autoregulation but propofol has no effect (Strebel *et al.* 1995). Thus, when the static autoregulatory mechanisms are impaired the brain shows a pressure passive response during sustained changes in CPP.

Lassen (1959) reported that the autoregulatory range in which CBF remains unchanged is between ~60-150 mm Hg and is represented by a plateau region between these arterial blood pressures (Figure 2.2). Revaluation of this original curve in healthy participants reveals a plateau region much narrower than that of Lassen's (1959) that may extend only 5 mm Hg each side of the baseline arterial blood pressure (Tan 2012). Furthermore, during pharmacological manipulation of MAP, MCAv was altered by 0.8% for every 1 mm Hg change in MAP, independent of $P_{ET}CO_2$ (partial pressure of end-tidal carbon dioxide; Lucas *et al.* 2010). Whilst no plateau region was evident in the study by Lucas *et al.* (2010), the stepwise changes in MAP induced may be greater than the plateau region (Tzeng & Ainslie 2013). Further, findings for Zhang *et al.* (2009) also question this wide range of stable CBF during prolonged changes in perfusion pressure, reporting that an increase in CBF was apparent following the highest steady-state infusion of phenylephrine. The use of phenylephrine and transcranial Doppler, as an index of CBF (see section 3.1.2), in the

experiments by Zhang *et al.* (2009) and Lucas *et al.* (2010) may be problematic as phenylephrine may directly constrict the MCA (Ogoh *et al.* 2011). CBF was also found to correlate strongly with perfusion pressure from 15-95mm Hg during cardiopulmonary bypass (Murkin *et al.* 1987). Therefore, it appears that the brain displays a pressure-passive component during steady-state changes in MAP, although a plateau may still exist where CBF is stable within a range of perfusion pressures, this region appears to be much narrower than originally purposed by Lassen in 1959 (Tan 2012). Research in healthy conscious humans where non-pharmacological induced changes in MAP is required to support these earlier studies that challenge the efficacy of static cerebral autoregulation.

2.1.3.2 Dynamic Cerebral Autoregulation

The process of dynamic cerebral autoregulation refers to the alterations in CVR to rapid and transient MAP perturbations (Symon *et al.* 1973). Direct observation of cerebral vessels in animal preparations have shown that the active response to abrupt changes in blood pressure occurs over a matter of seconds (Kontos *et al.* 1978). More importantly this response is dependent on the rate and direction of change (Kontos *et al.* 1978). With the development of TCD such dynamic changes in CBF can be traced in humans with high temporal resolution (Aaslid *et al.* 1982). TCD allows for the measurement of blood flow velocity in large intracranial arteries (such as the MCA). Further, changes in MCAv adequately reflect changes in flow during dynamic autoregulation (Newell *et al.* 1994; for further detail refer to section 3.1.2). Using TCD, dynamic regulation was first described in humans by Aaslid *et al.* (1989) following rapid thigh-cuff deflation. Subsequent investigations indicated an asymmetric dynamic cerebral autoregulatory response, that is, dynamic regulation is more effective in the hypertensive range (Tzeng *et al.* 2010b). Further

links between dynamic cerebral autoregulation and blood pressure can be drawn from data assessing baroreflex sensitivity. Individuals with an attenuated dynamic cerebral autoregulation appear to have a greater baroreceptor sensitivity and vice versa, indicative of a compensatory interaction for the regulation of CBF during rapid changes in blood pressure (Tzeng *et al.* 2010a).

This dynamic cerebrovascular response has been found to be highly dependent on the frequency of the arterial blood pressure oscillations. Using transfer function analysis the relationship between arterial blood pressure and cerebral blood flow velocity can be quantified, in terms of their dependence (coherence), relative changes (gain) and timing (phase) over a wide range of frequencies (Zhang *et al.* 1998a; Tzeng & Ainslie 2013). Using this approach the efficacy of dynamic cerebral autoregulation at different CPPs can be established. It has been found that low frequency fluctuations in arterial blood pressure (<0.07Hz) are adequately buffered (Zhang *et al.* 1998a) and within this frequency range autoregulation is strongest (Tan 2012). As the frequency of the arterial blood pressure fluctuations increases (>0.20 Hz) the effectiveness of dynamic cerebral autoregulation decreases and is characterised by increases in coherence, relatively large gain and reduced phase lead (Zhang *et al.* 1998a). Thus, the cerebral circulation can be characterised as a high pass filter, in that low frequency oscillations of blood pressure are effectively buffered yet high frequency perturbations are translated to the cerebral circulation culminating in CBF varying commensurate with arterial blood pressure (Diehl *et al.* 1998; Zhang *et al.* 1998a; Hamner *et al.* 2004). The delay between the onset of changes in arterial pressure and a cerebrovascular response is ~5 s (Zhang *et al.* 1998a). Subsequently, tasks such as standing (Sorond *et al.* 2009; Thomas *et al.* 2009b), the Valslava manoeuvre (Tiecks *et al.* 1995b; Pott

et al. 2000), rowing (Pott *et al.* 1997), body weight squats (Claassen *et al.* 2009) and resistance exercise (Edwards *et al.* 2002; Romero & Cooke 2007) produce blood pressure perturbations at a frequency too fast to be fully counteracted by changes in CVR (i.e., dynamic cerebral autoregulation) and are associated with concomitant changes in CBF.

2.1.3.3 Role of Arterial Gases in Cerebral Autoregulation

2.1.3.3.1 Carbon Dioxide

Carbon dioxide plays a significant role in the regulation of CBF (see section 2.1.1), yet it appears to have varying effects on cerebral autoregulation. Early investigations regarding CBF autoregulation by Aaslid *et al.* (1989) showed that dynamic autoregulation was impaired during hypercapnia and improved during hypocapnia. A number of studies have supported this original notion (Zhang *et al.* 1998a; Ainslie *et al.* 2005; Maggio *et al.* 2013) but it is commonly confounded by the concomitant chemoreceptor-mediated increase in MAP (Morgan *et al.* 1995). This elevation in perfusion pressure increases MCAv over and above that induced by the hypercapnic-induced vasodilation alone (Przybylowski *et al.* 2003; Ainslie *et al.* 2005). Przybylowski *et al.* (2003) ablated the chemoreceptor-induced MAP response to apnoea via ganglionic blockade and demonstrated that the increase in MCAv is partially attributed to the increase in CPP. As mentioned above, when the hypercapnic stimulus is sufficient to induce elevations in MAP the brain becomes pressure passive, with the chemoreceptor-induced increases in MAP resulting in concomitant elevations in MCAv (Battisti-Charbonney *et al.* 2011).

Dynamic cerebral autoregulation has been shown to be impaired during graded hypercapnia (Aaslid *et al.* 1989), and likewise for static autoregulation in patients under general

anasthesia (McCulloch *et al.* 2000). Conversely, hypocapnia appears to restore static cerebral autoregulation during isoflurane anaesthesia (McCulloch *et al.* 2005), which impairs static cerebral autoregulation (Strebel *et al.* 1995). Therefore, it appears that cerebral autoregulation is dependent on cerebral vasomotor tone (Aaslid *et al.* 1989), with the impairment due to the vasodilatory effect of CO_2 (McCulloch *et al.* 2000). Despite the obvious impairment of dynamic regulation by hypercapnia, no corresponding data for static regulation is currently available in conscious humans.

2.1.3.3.2 Oxygen

In a similar fashion to alterations in P_aCO_2, hypoxia has a pronounced effect on the efficacy of cerebral autoregulation. However, this effect is confounded by the ventilatory response that occurs in response to hypoxia (see section 2.3). The hypoxic ventilatory response induces a hypocapnia, which has been previously shown to improve indices of dynamic cerebral autoregulation (Aaslid *et al.* 1989). However, numerous studies have shown that poikilocapnic hypoxia (and the associated hypocapnia) results in an impairment of both dynamic (Jansen *et al.* 2007; Subudhi *et al.* 2009; Nishimura *et al.* 2010; Subudhi *et al.* 2010) and static autoregulation (Jansen *et al.* 2000) at rest and also during exercise (Ainslie *et al.* 2007). Although some reports indicate impairment only during hypobaric hypoxia, such as that experienced at altitude (Van Osta *et al.* 2005; Ainslie *et al.* 2008), with no impairment apparent at normobaric hypoxia. Research utilising transfer function analysis demonstrates that hypoxia impairs the ability for very low frequency (0.02 - 0.07 Hz) fluctuations in MAP to be buffered (Iwasaki *et al.* 2006; Iwasaki *et al.* 2010), despite dynamic autoregulation being most effective at these lower frequencies (Zhang *et al.* 2011).

Recently, Querido *et al.* (2013) assessed the effect of poikilo- and isocapnic hypoxia on dynamic cerebral autoregulation during both hypo-and hypertensive stimuli. It was found that the hypocapnia associated with the poikilocapnic hypoxia played a protective role and maintained the efficacy of dynamic regulation, however the isocapnic hypoxia, and the associated increase in CBF, impaired regulatory capacity. One could postulate that the dilatory effect of the hypoxia itself may mediate the impaired regulatory function (Ogoh *et al.* 2010a; Querido *et al.* 2013). As poikilocapnic hypoxia resulted in no change in CBF in previous studies (Jansen *et al.* 2007; Subudhi *et al.* 2009; Nishimura *et al.* 2010; Subudhi *et al.* 2010) the difference in vascular tone may explain the different results between the poikilocapnic and isocapnic conditions. Furthermore, this impairment can be rectified with hyperoxia (Jansen *et al.* 2007; Subudhi *et al.* 2010). Although hyperoxia alone does not modify cerebral dynamic regulatory efficacy (Ogoh *et al.* 2010a), hyperoxia decreases CBF (Bulte *et al.* 2006) via alterations in cerebrovascular resistance (Kety & Schmidt 1948) and also induces a ventilatory response in a dose dependent manner that results in hypocapnia (Becker *et al.* 1996). Although the resultant hypocapnia in response to 100% O_2 does not account for the ~20% reduction in CBF (Watson *et al.* 2000). It appears that alterations to dynamic regulation induced by P_aO_2 are similar to those of $PaCO_2$; i.e., that vasoconstrictors (hypocapnia, hyperoxia) improve regulatory capacity and vasodilators (hypercapnia, hypoxia) impair, which is consistent with the notion that regulatory capacity is dependent on resting vascular tone (Aaslid *et al.* 1989). In summary, cerebral autoregulatory capacity can be modified by the arterial concentrations of CO_2 and O_2, and the complex interaction between the two. This is further complicated by the highly variable ventilatory responses between individuals to, and level of, hypoxia and degree of the resultant hypocapnia (Ogoh *et al.* 2010a).

2.1.4 Cardiac Output

Evidence indicates that CBF may be modulated by $\dot{Q}$ independently of changes in MAP and P_aCO_2 (Ogoh *et al.* 2005a) despite $\dot{Q}$ not being an integral factor in the laws that govern flow i.e. Poiseuille's law (see also section 2.1) (Treib *et al.* 1996). The first line of evidence comes from atrial fibrillation patients who have an impaired ability to increase $\dot{Q}$ in comparison to healthy participants. During exercise these patients demonstrate a smaller increase in $\dot{Q}$ and therefore MCAv (Ide *et al.* 1999). Further evidence comes from healthy participants during exercise with beta blockade (metoprolol) where an impaired ability to elevate $\dot{Q}$ resulted in a smaller elevation in MCAv (Ide *et al.* 1998; Ide *et al.* 2000). Importantly these changes occurred without any variation in blood gases or CPP. This has been supported by Ogoh *et al.* (2005a), who showed that variations in $\dot{Q}$ during blood volume expansion (albumin) were paralleled by changes in CBF at rest and during exercise, despite the appearance of an intact dynamic cerebral autoregulation. However, Ogawa *et al.* (2007) reported an impairment in dynamic cerebral autoregulation during isovolumetric haemodilution (normal saline). As haemodilution dilates cerebral vessels (Tu & Liu 1996) the apparent impairment may have arisen from the volume infusion rather than the changes in $\dot{Q}$ *per se* as others have shown no effect of changes in $\dot{Q}$ on dynamic cerebral autoregulation during hypotension (Deegan *et al.* 2010). Furthermore, differences in infusion types (saline vs. albumin) may account for the inconsistent findings. During full cardiac blockade dynamic cerebral regulatory capacity is impaired (Ogoh *et al.* 2010c). Therefore, there is an important interaction between the cardiac-baroreflex and cerebral autoregulation (Ogoh *et al.* 2010c). Nevertheless, it is apparent that the variation in

systemic blood pressure has a greater influence on the regulation of CBF than $\dot{Q}$ (Ogoh *et al.* 2007).

2.1.5 Neurovascular Coupling

The regulation of local CBF is crucial in that local neuronal activity is closely matched by changes in perfusion. In the brain a functional hyperemia reflects local dilation of arterioles to increased neuronal activity that occurs within seconds and allows active neurons to be sufficiently supplied with O_2 and substrates (Carmignoto & Gómez-Gonzalo 2010). It was originally thought that local metabolites such as CO_2 mediate this response, however due to the extremely quick response time (matter of seconds) (Iadecola 2004; Takano *et al.* 2005) this is unlikely (Lou *et al.* 1987). It has been reported that astrocytes mediate the coupling between neuronal function and vascular responses via glutamanergic-mediated pathways (Zonta *et al.* 2002). Astrocytes are ideally positioned to mediate this response as they synapse with neighbouring neurons and their end feet are attached to the vessel wall (Simard *et al.* 2003). The pathways that mediate this process are calcium dependent (Filosa & Blanco 2007). Further detail on this topic can be found in a number of reviews (Iadecola 2004; Hamel 2006; Haydon & Carmignoto 2006; Filosa & Blanco 2007; Attwell *et al.* 2010; Filosa & Iddings 2013).

2.1.6 Autonomic Nervous System

Cerebral vessels are richly innervated by sympathetic nerves (Edvinsson & Owman 1976; Edvinsson *et al.* 1977) until the vessel enters the brain parenchyma, where, beyond the Virchow-Robin space this extrinsic nervous supply is lost and intrinsic innervation from neurons within the brain is apparent (Hamel 2006). However, the role of the sympathetic

nervous system in the regulation of the cerebral vasculature is controversial (Strandgaard & Sigurdsson 2008; Van Lieshout & Secher 2008). In humans, α_1 adrenergic blockade resulted in no change in absolute MCAv (Ogoh *et al.* 2008; Lewis *et al.* 2013) and CBF (Skinhoj 1972). Although, administration of the α_2 agonist clonidine decreased MCAv significantly, with the decrease persisting following restoration of MAP, indicating a direct sympathetic vasoconstrictive effect (Lee *et al.* 1997). Investigating the role of the sympathetic nervous system in cerebrovascular regulation in humans is difficult, although animal studies do give some potential insight. Animal models indicate that the activation of cerebral sympathetic nerves is a protective mechanism during acute hypertension (Bill & Linder 1976; Busija *et al.* 1980; Cassaglia *et al.* 2008), counteracting the forced dilation seen at high CPPs (Bill & Linder 1976). This effect may extend to humans as when cerebral sympathetic activity is attenuated via ganglionic blockade the phase IV response following a Valsalva manoeuvre (VM) is greatly accentuated (Zhang *et al.* 2004a). As this phase is associated with a transient hypertension it would appear consistent with the observations in animal models. It is therefore possible that during large increases in MAP, such as those seen during resistance exercise and the VM, activation of the sympathetic nervous system may protect the cerebral vasculature against hypertension.

Whilst sympathetic blockade may not alter absolute CBF (Ogoh *et al.* 2008; Lewis *et al.* 2013), data indicate that the autonomic nervous system may participate in beat-to-beat MCAv regulation (Zhang *et al.* 2002; Hamner *et al.* 2010). Transfer function analysis of spontaneous fluctuations in blood pressure during ganglion blockade revealed that the brain was unable to buffer even slow changes (very low frequency 0.02-0.07Hz (Zhang *et al.* 2002)), a frequency range in which cerebral autoregulation is most effective (Zhang *et al.*

1998a). Moreover, α_1 adrenergic blockade impaired dynamic regulation following bilateral cuff release (Ogoh *et al.* 2008) and during a supine-to-stand postural change (Lewis *et al.* 2013). This participation of cerebral sympathetics during transient perturbations in CPP may explain the asymmetric dynamic autoregulatory response between the hypo- and hypertensive ranges (Tzeng *et al.* 2010b). That is, sympathetic activation during hypertension may improve autoregulatory efficacy in this range and contributes to the notion that cerebral sympathetic activation is protective in nature.

Sympathetic regulation of the cerebral circulation by adrenergic vasoconstrictor (Ainslie *et al.* 2005; Hamner *et al.* 2010) and cholinergic vasodilatory (Hamner *et al.* 2012) fibres appears to differ greatly from peripheral circulations and even within the cerebral circulation (Gierthmühlen *et al.* 2010). The forearm and cerebral circulation showed nearly opposite effects during cholinergic blockade, and may act to balance the cerebral sympathetic vasoconstriction (Hamner *et al.* 2012). Likewise during sympathetic activation via handgrip exercise the cerebral vasculature response was less marked than that of the forearm (Ainslie *et al.* 2005). However, in the same study by Ainslie *et al.* (2005) a correlation between muscle sympathetic nervous activity (MSNA) and CVR was reported. In contrast to the systemic circulation the role of the sympathetic nervous system in the cerebral circulation is less pronounced, although a regulatory role is apparent during changes in blood pressure. Therefore, the sympathetic nervous system may participate in the active regulation, although the myogenic (autoregulatory) regulation is dominant (Ter Laan *et al.* 2013).

2.2 Cardiovascular Function

The cardiovascular system is of vital importance in the regulation of homeostasis. The primary role of the cardiovascular system is transport of oxygen, glucose, fatty acids, vitamins and water to tissues and also the removal of by-products (i.e., urea and CO_2) from tissues within the body (Levick 2010). The cardiovascular system is also involved in other processes vital to homeostasis, such as the transport of hormones and the secretion of bioactive agents. Furthermore the cardiovascular system is pivotal in thermoregulation due to its ability to finely regulate blood flow to the cutaneous circulation.

As previously stated (section 2.1), Darcy's law indicates that flow is linearly proportionate to the difference in pressure between two points. A simplified and rearranged version of this can be given by the following equation to determine mean arterial pressure:

$$MAP = \dot{Q} \times TPR$$

Where: MAP, mean arterial pressure (in mm Hg); $\dot{Q}$, Cardiac output ($L \cdot min^{-1}$); TPR, total peripheral resistance ($mm\ Hg \cdot L \cdot min^{-1}$).

$\dot{Q}$ is the product of the heart rate (HR) and stroke volume (SV) over one minute and represents flow for the entire circulatory system. HR is determined mainly by the balance of sympathetic and parasympathetic activity, regulated by the baroreflex. At rest SV is between ~70-80 mL and is determined by three main factors: preload, contractility and afterload (discussed in detail in section 2.2.6). TPR is the sum of vascular resistances throughout the systemic circulation, a major factor of which is vessel radius. Vessel radius can be modulated by extrinsic and intrinsic mechanisms, which ultimately modulate MAP. Therefore, MAP can be modulated by increases in the flow rate ($\dot{Q}$) and resistance within

the system (TPR). The maintenance of MAP is important to ensure adequate perfusion of vital organs such as the brain. As discussed below the baroreflex maintains a stable blood pressure to ensure adequate perfusion. When this reflex fails to adequately maintain MAP CBF is compromised (as detailed in section 2.4).

2.2.1 Baroreflex

The baroreceptor reflex is a crucial homeostatic mechanism that enables the maintenance of arterial pressure over a wide range of circumstances. This reflex is mediated by mechanoreceptors, also termed baroreceptors, located in the aortic arch and the carotid sinus bifurcations, which act to regulate short term maintenance of blood pressure. The baroreceptors are unencapsulated free nerve endings located in the medial-adventitial border of the carotid sinus (Sheehan *et al.* 1941). Afferent nerve fibres from aortic baroreceptors ascend in the sensory vagus (X). The afferent fibres from the carotid sinus travel in the carotid sinus nerve, which then joins the glossopharyngeal (IX) (Degtyarenko & Kaufman 2006). However, both sensory pathways converge on the nucleus tractus solitarius (NTS) within the brain stem. The majority of neurons within the NTS receive tonic inhibition from interneurons within the NTS and from other brain regions (Mifflin 2001). The balance of these excitatory and inhibitory inputs in the NTS dictates the response of the baroreflex. An elevated blood pressure would in turn increase carotid sinus nerve traffic (carotid distension). This raises NTS neuronal activity and reduces sympathetic outflow (firing rate), decreasing sympathetic drive to the heart (decreasing HR and contractility) and peripheral vasculature, reducing TPR and therefore MAP (Chen & Bonham 2010; Macefield & Henderson 2010). The reciprocal occurs during a reduction in MAP, that is an increase in

sympathetic drive to the heart and peripheral vasculature increasing HR and TPR, respectively (see Figure 2.3 below).

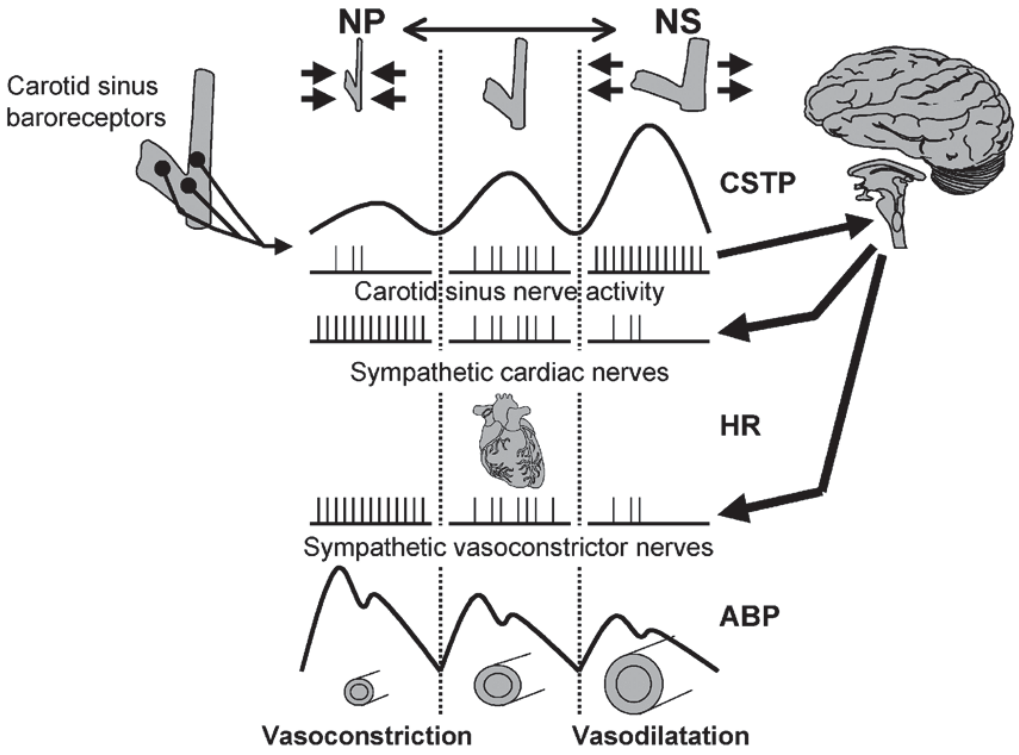

Figure 2.3 Schematic representation of the neural response to changes in carotid sinus transmural pressure (CSTP) (Fadel et al. 2003). Neck suction (NS) can produce an artificial hypertension, increasing carotid sinus nerve activity and reducing sympathetic drive to both the heart and peripheral vasoconstrictor nerves. Together, this results in a reduction in heart rate (HR) and a vasodilation that restores arterial blood pressure (ABP). The converse occurs during hypotension, as induced by neck pressure.

Pulsatile flow results in a grouped discharge in the carotid sinus nerve with bursts occurring at systole and early diastole of each cardiac cycle (Ead *et al.* 1952). Further, the pulsatile flow is more effective in reducing vasomotor discharge to the periphery than steady-state flow. Similar results have also been shown using sinusoidal neck suction. Sinusoidal neck suction prolonged the R-R interval and rhythmically modulated muscle sympathetic activity compared to static neck suction did not change sympathetic burst incidence (Båth *et al.* 1981). At a constant carotid pressure an increased pressure in the aortic arch can produce a

reduction in vascular resistance and HR (Hainsworth *et al.* 1970). Stimulation of aortic baroreceptors produce a similar response to the carotid baroreceptors (Levick 2010). Furthermore, the threshold for response in the aortic baroreceptors was greater than those seen in the carotid, however it is likely that changes in pressure occur concurrently in both carotid and aortic baroreceptors (Hainsworth *et al.* 1970).

Changes in arterial pressure can therefore produce changes in both HR and TPR although alterations in TPR are the primary means by which baroreceptor activation modulates MAP (Ogoh *et al.* 2002). It appears that SV does not play a major role in the maintenance of arterial pressure and any changes in $\dot{Q}$ are predominantly via changes in HR (Fadel *et al.* 2003). During the period of an increased arterial blood pressure there is a combined increased vagal tone and reduction in sympathetic output. Under periods of increased baroreceptor traffic (high carotid pressure), increased firing rates in the nucleus ambiguous elicits bradycardia with an associated negative inotropic effect (Levick 2010). However, during times of reduced arterial pressure, sympathetic outflow is increased and vagal activity inhibited (see Figure 2.3). A decreased carotid sinus pressure would result in an increased HR and TPR, which would in turn, increase arterial pressure (Levick 2010). Thus alterations in HR and vasomotor tone act in concert to maintain a stable arterial pressure via this reflex (Fadel *et al.* 2003). An example of the effectiveness of the baroreceptor in maintaining blood pressure is during changes in posture. From lying, sitting and then standing both HR and muscle sympathetic nerve activity increase such that only small fluctuations in blood pressure are seen (Burke *et al.* 1977), which is a vital homeostatic mechanism in maintaining cerebral perfusion.

Despite this reflex adequately accounting for short-term changes in blood pressure it is possible, even in healthy individuals, that the baroreflex fails to maintain MAP. An example of this is at syncope; where the baroreflex is unable to maintain CPP (see section 2.4.1). The baroreflex and cerebral autoregulatory mechanisms act to maintain adequate cerebral perfusion (Tzeng *et al.* 2010a) and in turn brain function although, large and abrupt changes in blood pressure may exceed the capability of both of these mechanisms (i.e., during resistance exercise or the VM)

2.2.2 Baroreflex During Exercise

Exercise causes both an increase in arterial blood pressure and HR. This is conflicting with the baroreflex response at rest, as an increase in blood pressure would produce a reflex drop in HR. As illustrated overleaf in Figure 2.4, exercise appears to reset the operating point of the baroreflex such that the arterial pressure that the reflex strives to maintain is elevated (Ogoh *et al.* 2005b). The operating point is the pre-stimulus MAP with the centring point being where there are equal pressor and depressor responses to a given change in blood pressure (Potts *et al.* 1993). The threshold is the point which no further increases in HR (Figure 2.4) are elicited by a decrease in arterial pressure (Norton *et al.* 1999). Saturation is defined where no further decreases in HR are elicited by increases in BP. The shift of the operating point away from the centring point and closer to the threshold on the curve with increasing exercise intensity positions the baroreflex in a better position to counteract hypertensive stimuli (Williamson *et al.* 2006). The upward and rightward shift allows a simultaneous increase in both HR and MAP (Norton *et al.* 1999).

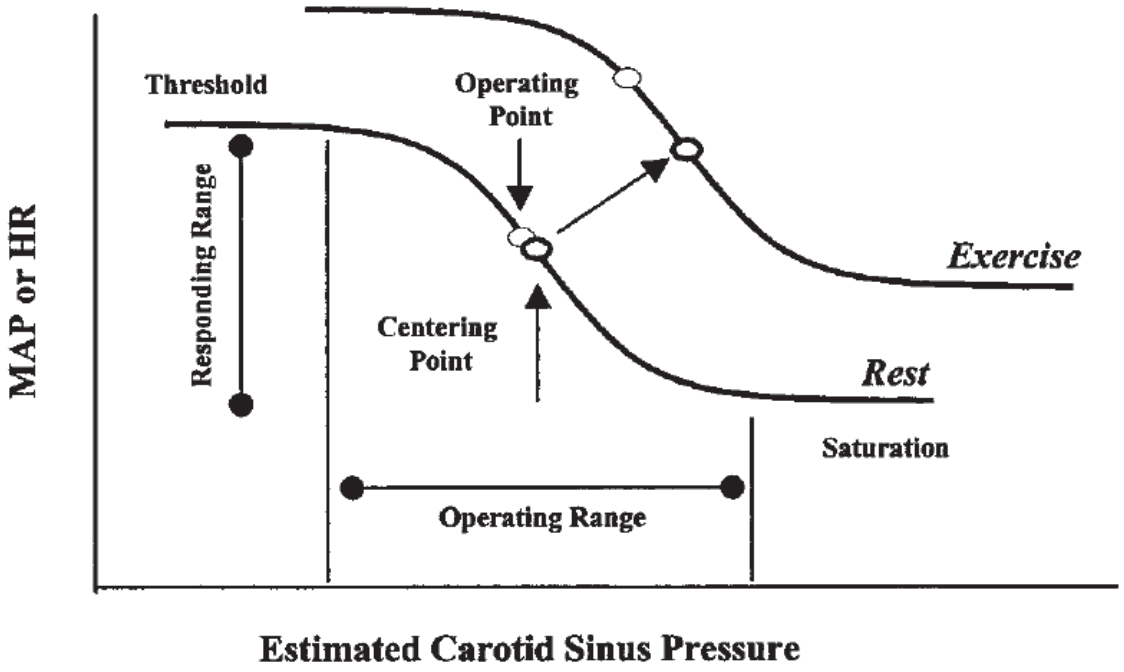

Figure 2.4 Stimulus response curves during exercise of varying intensity (Raven et al. 2002).

The magnitude of the resetting is directly proportionate to the intensity of the exercise (Fisher *et al.* 2007). Therefore, during maximal whole-body dynamic exercise the operating point is at threshold (see Figure 2.4) on the stimulus-response curve (Norton *et al.* 1999). At this point no further increases in HR or MAP are elicited by a decrease in blood pressure. Contrary to this, during cessation of exercise sympathetic drive is inhibited and parasympathetic tone is increased, decreasing blood pressure and HR returns toward baseline values.

2.2.3 Low Pressure Baroreceptors

As well as the high pressure baroreceptors of the aortic arch and carotid sinus there are also low pressure receptors that respond to changes in blood volume. These baroreceptors are located in the junctional tissues between the venae cavae and right atrium and the

pulmonary veins and left atrium in the right and left sides of the heart, respectively (Coleridge *et al.* 1957). Further, the afferent nerves are small myelinated fibres that increase in discharge with atrial systole (Coleridge *et al.* 1957). Like arterial baroreceptors (Ead *et al.* 1952) cardiopulmonary receptors are sensitive to both the mean pressure and pulse pressure (Coleridge & Kidd 1961), with the greatest discharge frequency occurring when systolic and pulse pressure are both elevated. The activation of these so called cardiopulmonary baroreceptors can be induced experimentally using techniques which reduce central venous pressure (CVP), and thus filling pressure for both the left and right ventricles, without a concomitant change in arterial pressure.

Moderate lower body negative pressure (LBNP) up to ~20 mm Hg can reduce CVP without any significant change in HR or arterial pressure (Zoller *et al.* 1972; Johnson *et al.* 1974; Vissing *et al.* 1989). The reduction of CVP results in an increase in muscle sympathetic outflow to the extremities (Victor & Leimbach Jr 1987; Vissing *et al.* 1989; Jacobsen *et al.* 1993). Elevated MSNA increases resistance in the forearm (Johnson *et al.* 1974) and calf (Roddie *et al.* 1957; Vissing *et al.* 1989) and to a lesser extent a reduction in splanchnic blood flow (Johnson *et al.* 1974).

During times of increased CVP resulting from lower body *positive* pressure (LBPP, up to + 20 mm Hg (Fu *et al.* 1998), head down tilt (Nagaya *et al.* 1995) and saline infusion (Vissing *et al.* 1989) MSNA is reduced. As a result of reduced MSNA, an increase in CVP was paralleled by reduction in calf (Vissing *et al.* 1989) and forearm (Roddie *et al.* 1957; Nagaya *et al.* 1995) vascular resistance. Passive leg raising also induced reductions in forearm blood flow, which is abolished by cuff inflation around the thighs (Roddie *et al.* 1957). Again, neither HR nor arterial pressure were altered during any of these experimental perturbations, indicating

that during fluctuations in CVP cardiopulmonary baroreceptors can modulate sympathetic outflow and limb vascular resistance, independent of alterations in arterial pressure (Charkoudian *et al.* 2004). There also appears to be intricate cross talk between carotid and cardiopulmonary baroreceptors. Loading of the cardiopulmonary baroreceptors using LBPP diminishes the influence of the carotid baroreceptors on HR and MAP once a threshold CVP is achieved (Shi *et al.* 1993b; Shi *et al.* 1997). Translocation of blood either away (LBNP) or towards (head down tilt/LBPP) the thorax can initiate changes in filling pressure as recognised by the stretch of the tissue at the veno-atrial junction. This is important as these low pressure baroreceptors are activated during LBPP, an effective non-pharmacological means to induce prolonged increases in MAP, as detailed in section 2.6.1. These prolonged increases in MAP may be used to challenge static cerebral autoregulation (see section 2.1.3.1).

2.2.4 Chemoreception and Arterial Blood Pressure

Specialised receptors in the cardiovascular system can sense changes in the partial pressure of CO_2 and O_2, the peripheral and central chemoreceptors. Whilst these receptors have pronounced effects on ventilation, which is covered later in this chapter (see section 2.3.1), their effects on blood pressure are addressed here. Peripheral receptors are located in well-vascularised nodules located in the aortic and carotid bodies. Like the baroreceptor afferents, the aortic and carotid afferents travel via the vagus and glossopharyngeal, respectively, and synapse initially in the NTS (Halliwill *et al.* 2003). Generally, the peripheral chemoreceptors respond to hypoxic stimuli while the central chemoreceptors respond mainly to PCO_2 (Kara *et al.* 2003). However, severe hypoxia can stimulate the central chemoreceptors directly (Guyenet 2000), dilate systemic resistance arterioles and constrict

pulmonary vessels and large systemic arteries (Levick 2010). Mild hypoxia stimulates an increase in muscle sympathetic activity (Somers *et al.* 1991; Halliwill & Minson 2002; Halliwill *et al.* 2003) as does hypercapnia (Claassen *et al.* 2007; Steinback *et al.* 2009), while the greatest sympathetic output is seen during combined hypoxia and hypercapnia (Somers *et al.* 1989). This increase in sympathetic outflow increases MAP (Halliwill & Minson 2002; Halliwill *et al.* 2003; Querido *et al.* 2011) and HR (Somers *et al.* 1991; Halliwill *et al.* 2003; Lusina *et al.* 2006) once a threshold hypercapnia is reached (Battisti-Charbonney *et al.* 2011). Despite the increase in MSNA, hypoxia induces a reduction in TPR (Steinback *et al.* 2009) possibly due to its direct vasodilatory effect on the systemic vasculature. This, however, is offset by the increase in HR and $\dot{Q}$ (Steinback *et al.* 2009). Collectively, the cardiovascular and ventilatory responses act to maintain oxygen delivery during periods of hypoxia.

It appears that hypoxia (Querido *et al.* 2011) and hypercapnia (Steinback *et al.* 2009) alter the baroreflex set point, which persists post hypoxia (Querido *et al.* 2011). This resetting allows the baroreflex to operate at a higher MAP and MSNA. This occurs with either a decrease in (Querido *et al.* 2011) or no change (Halliwill & Minson 2002; Halliwill *et al.* 2003) in either cardiovagal or MSNA gain during hypoxia and no change in cardiovagal gain during hypercapnia (Steinback *et al.* 2009). Baroreceptor stimulation, using vasoactive drugs, abolishes the sympathetic outflow response to hypoxia (Somers *et al.* 1991; Halliwill *et al.* 2003) but not hypercapnia (Somers *et al.* 1991). This response may be explained by the afferent neural circuitry as both afferents converge in the brainstem with peripheral chemoreceptors altering autonomic outflow via pathways that are both baroreflex dependent and independent (Halliwill *et al.* 2003). It appears that the peripheral

chemoreflex and the baroreflex do not act in isolation and integrate blood gas and arterial blood pressure homeostasis (Querido *et al.* 2011). This chemoreceptor mediated increase in MAP has a pronounced effect on the CBF response. As detailed in section 2.1.3.3.1 the increase in CBF seen during hypercapnia is partly attributed to the elevated MAP. Moreover, as hypercapnia impairs dynamic cerebral autoregulation (Aaslid *et al.* 1989) and any change in MAP, including those induced by chemoreceptor activation, are likely to have a greater effect on CBF (Battisti-Charbonney *et al.* 2011).

2.2.5 Blood Pressure Regulation and the Cutaneous Circulation

The skin, like muscle, has a large range of possible blood flow that is mediated via changes in conductance during certain physiological stressors. At rest the skin receives only ~5-10% of $\dot{Q}$, but can increase to 50-70% during severe heat stress, the range of which is only second to skeletal muscle (Rowell 1977). This large change in conductance and ability to receive large proportions of $\dot{Q}$ can have dramatic haemodynamic changes, namely on MAP. Whilst the contribution of muscle vasomotor tone to the baroreceptor regulation of blood pressure is well established (Beiser *et al.* 1970; Wallin *et al.* 1975; Burke *et al.* 1977), the role of the cutaneous circulation is less certain. The short-term regulator of blood pressure, the baroreflex, does appear to have some effect on the cutaneous circulation, although the extent of this control remains uncertain as does the exact mechanisms in which this response is mediated.

Some authors have reported that in perturbations of both high pressure, using neck suction (Beiser *et al.* 1970), and low pressure, using LBNP (Kellogg Jr *et al.* 1990; Crandall *et al.* 1996), baroreceptors illicit the appropriate baroreceptor-mediated changes in cutaneous vascular tone even with concomitant heat stress. That is, vasodilation during neck suction

and vasoconstriction during LBNP in order to maintain a stable blood pressure. The majority of this reduction in cutaneous vascular conductance has been attributed to unloading of the cardiopulmonary baroreceptors (Crandall *et al.* 1996). During moderate LBNP without concomitant changes in MAP, 73% of vasoconstriction occurred at -10 mm Hg of LBNP due to unloading of the cardiopulmonary baroreceptors, as evidenced by a reduction in CVP (Zoller *et al.* 1972). Recruitment of sino-aortic baroreceptors, via reduction in MAP with the prevailing LBNP, resulted in a relatively small further reduction in cutaneous vascular conductance (Zoller *et al.* 1972). However, selective activation of the carotid baroreceptors using neck suction produced increases in conductance (Beiser *et al.* 1970) with neck pressure reducing cutaneous conductance (Keller *et al.* 2006).

Whilst changes in conductance in response to baroreflex stimuli are well documented, the origin of this change in vascular tone is still uncertain. During baroreceptor activation it appears that skin sympathetic nerve activity is unchanged during a modified Oxford test with concomitant heat stress (Wilson *et al.* 2001) and direct carotid sinus nerve stimulation (Wallin *et al.* 1975). Further, axillary blockade had no effect on cutaneous vascular tone when upright or during changes in limb position (Vissing *et al.* 1997). During the same experiment by Vissing and associates (1997), no change in skin sympathetic nerve activity was seen during -50 mm Hg of LBNP. Therefore, an alternate hypothesis was proposed in that when upright the change in posture brought about changes in cutaneous vascular tone by the Veno-arteriolar reflex (Vissing *et al.* 1997). Despite the mechanisms not being entirely clear it appears that during haemodynamic challenges, that threaten circulatory homeostasis, changes in cutaneous conductance can be altered reflexively in an attempt to maintain MAP.

2.2.6 Stroke Volume

As discussed in section 2.1.4 of this chapter, CBF is modulated by changes in $\dot{Q}$, which is the product of HR and SV. As discussed above HR is determined by the baroreflex, with SV being modulated by local and systemic factors, which are discussed below in detail. These factors include preload (central venous pressure), afterload, contractility and HR. Although compliance may also affect SV it is unlikely to change within the time frame of the experiments conducted within this thesis.

2.2.6.1 The Frank-Starling Mechanism

Many of the basic laws describing the relationships between venous pressure/return, stroke volume and $\dot{Q}$ can be attributed to the early work of Frank, Patterson, Piper and Starling. This work with the length tension relationship extended past skeletal muscle and was described in the myocardium as reviewed by Katz (Katz 2002). A series of experiments by Starling and associates (Knowlton & Starling 1912; Patterson *et al.* 1914; Patterson & Starling 1914) built on the discoveries of Frank and others (Katz 2002). This series of papers demonstrated that venous inflow matched left ventricular output, independent of HR (Patterson *et al.* 1914; Patterson & Starling 1914). This elevated venous inflow and subsequent smaller rise in CVP results in a larger diastolic volume which generates a greater rise and maximum pressures in the preceding systole when arterial resistance is held constant (Patterson *et al.* 1914). The volume within the respective ventricles determines the length of the fibres whereas the tension of the muscle fibres will determine the pressure within the respective ventricle (Patterson *et al.* 1914). In summary, this collection of experiments demonstrated, in contrast to (Frank 1895), that it is the initial myocardial fibre

length that determines SV as opposed to the tension. Therefore, the CVP determines the fibre length and then the subsequent force of contraction in the next systole.

For each differing cardiovascular condition a Frank-Starling curve exists. In each curve a steep ascending limb exists where small changes in filling pressure result in large corresponding changes in SV. The plateau portion experienced at higher filling pressures result in small changes in ventricular output for a given change in filling pressure. The latter being the case in supine humans in which the central blood volume and pressure is higher and the normal ventricle operates on the plateau portion of the curve (Parker & Case 1979). The shift from supine to an upright posture results in the reduction in both left ventricular end diastolic pressure and SV (Poliner *et al.* 1980), blood shifts from the thorax into the veins of the legs and the operating point of the normal ventricle shifts to the steeper ascending portion of the curve (Parker & Case 1979). The reverse is true for times of increased central blood volume, for instance during head down tilt the translocation of blood back into the thorax results in an increased SV as a result of elevated CVP (Nagaya *et al.* 1995). Thus, SV is dependent on the filling pressure during diastole.

2.2.6.2 Arterial Blood Pressure

In the ventricle the afterload (systolic wall stress) is determined by Laplace's law (chamber radius and wall thickness) and arterial pressure (Levick 2010). The arterial load opposes ventricular ejection and has pronounced effects on ventricular volumes and performance. Early work showed that increasing arterial pressure results in a passive dilation within the next few beats as the heart does not empty as effectively (Von Anrep 1912; Patterson *et al.* 1914) with an associated reduction in shortening velocity (Sonnenblick & Downing 1963; Quinones *et al.* 1976). This culminates in a reduction in SV that varies directly with the

outflow resistance (Sarnoff *et al.* 1960; Imperial *et al.* 1961; MacGregor *et al.* 1974). Moreover, aortic pressure (Imperial *et al.* 1961) and end ventricular systolic pressure (Quinones *et al.* 1976), vary proportionately with the outflow resistance. Thus, the work performed by the heart at a given end diastolic volume and inotropic state is determined by the arterial blood pressure (Sonnenblick & Downing 1963). Therefore, it appears that the arterial load opposing the ejection of blood has profound effects on ventricular function. This has particular relevance for resistance trained individuals who are exposed to extremely high arterial pressures. As such these individuals demonstrate alterations in myocardial structure and arterial stiffness (see section 2.5.4).

2.2.6.3 Contractility

2.2.6.3.1 Autonomic Activation

Changes in contractile energy can occur without changes in initial fibre length. These non-Frank-Starling mechanism changes in contractility are caused by neurohumoral factors. The sympathetic nervous system richly innervates the atria and ventricular myocardium (Kawano *et al.* 2003). Parasympathetic nerves greatly outnumber sympathetic in the atria, however, the sympathetic nerves are more numerous in the ventricles (Kawano *et al.* 2003). Noradrenaline is released locally from sympathetic nerve varicosities and binds to β adrenergic receptors in the myocardium. Circulating adrenaline also binds to β adrenergic receptors, thus the two catecholamine's have similar cardiac effects (Levick 2010). The addition of noradrenaline increases myocardial force development and rate of shortening (Sonnenblick 1962) whilst time to maximum active state and overall contraction time are reduced (Goldberg *et al.* 1960; Sonnenblick & Downing 1963; Sonnenblick 1967). Identical doses of adrenaline and noradrenaline produced similar increases in contractile force and

both induced increases in heart rate (Goldberg *et al.* 1960) with the β agonists isoproterenol and dobutamine producing similar changes in contractile state (Quinones *et al.* 1976; Leier *et al.* 1977; Leier *et al.* 1978). Therefore, activation of cardiac β receptors by circulating or locally released catecholamines induce an increased ejection fraction, SV, rate of maximal shortening and rate of rise in systolic pressure culminating in an increased $\dot{Q}$.

2.2.6.3.2 Heart Rate

Alterations in the beat interval have significant effects on contractile state of the heart (Koch-Weser & Blinks 1963), with elevations in HR mediating increases in contractility (Maughan *et al.* 1985). Using atrial pacing, an increase in HR of 50 beats per minute increases both SV and the velocity of circumferential shortening, while afterload and preload are similar across beats (Ricci *et al.* 1979). The positive inotropic effect is strongly correlated with HR, with the augmentation of contractility during a lowering HR following an exponential relationship (Ricci *et al.* 1979). Whilst the vagus has significant effect on HR, its role in modulating contractility is less pronounced; with vagal stimulation producing a small effect on the ventricle compared to sympathetic activation (Randall *et al.* 1968). Whilst vagal stimulation may indirectly produce a reduction in contractility as a result of its effect on HR (Casadei 2001).

Collectively, the cardiovascular system plays a vital role in maintaining CBF by regulating MAP and therefore CPP. The cardiovascular reflexes and the brains intrinsic autoregulatory mechanisms act in concert to maintain adequate perfusion, oxygenation and, therefore, normal brain function when MAP is challenged.

2.3 Control of Ventilation

This section is devoted to the control of ventilation with a particular focus on how ventilation is modulated by the chemoreceptors in response to changes in arterial blood gases. As discussed in section 2.1, alterations in arterial blood gases have a pronounced effect on CBF. Thus, the arterial CO_2 (P_aCO_2) signal has a significant effect on the brain, in that it modulates flow but also provides a chemical feedback to the anatomical structures that govern ventilation.

2.3.1 The Chemoreflex Drive to Breathe

The regulation of breathing is a feedback loop with the sensing of arterial blood gases (chemoreflex) providing chemical feedback to control ventilation (Figure 2.5) (Ainslie & Duffin 2009; Duffin 2011). Other stimuli such as the wakefulness drive to breathe also control ventilation and maintain resting respiratory rhythm (Fink 1961). Humans have two sets of chemoreceptors: the peripheral and central chemoreceptors. The peripheral chemoreceptors are located in the aortic bodies and within the well vascularised carotid body (located at the carotid bifurcation), which has sensory innervation via the carotid sinus nerve (Gonzalez *et al.* 1994; Nurse 2010). These chemoreceptors, in particular the carotid receptors, 'taste' the blood entering the brain (Duffin & Mahamed 2003). The central chemoreceptors are located within the medulla and their response upon activation involves the complex interplay of many brainstem structures, reviewed by Guyenet *et al.* (2010) but it is beyond the scope of this current literature review. This process is vital in the maintenance of central pH and matching tissue oxygen requirements as well as the removal of CO_2 (Duffin & Mahamed 2003). The P_aCO_2 is the driving factor that determines the H^+

sensed by the chemoreceptors, with stimulation resulting in an increased ventilation, elimination of CO_2 at the lungs and subsequent lowering of P_aCO_2 (Ainslie & Duffin 2009).

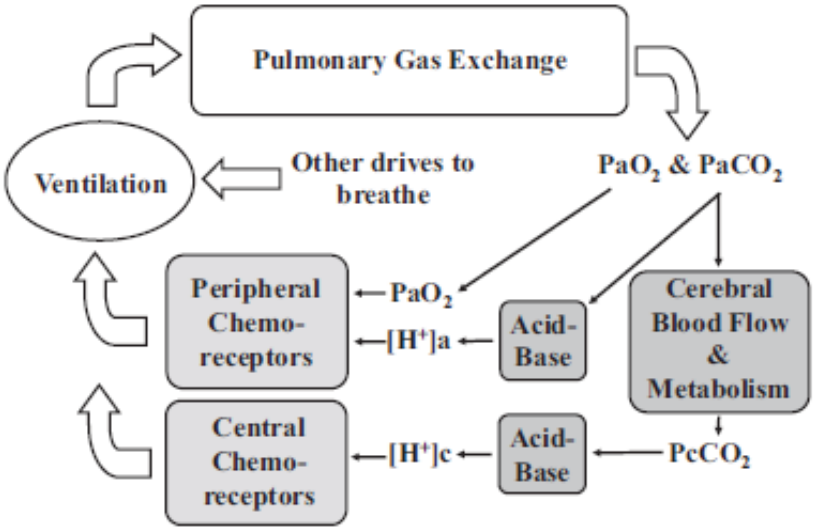

Figure 2.5 Simplified illustration of the control of ventilation by the peripheral and central chemoreflexes (Duffin 2011). $PcCO_2$, Partial pressure of central carbon dioxide; $[H^+]a$, arterial hydrogen ion concentration; $[H^+]c$, central hydrogen ion concentration.

The peripheral chemoreceptors are sensitive to changes in arterial CO_2 and O_2 (Figure 2.5 and 2.6) and in normoxia provide a tonic excitatory input (Forster *et al.* 2000). Central chemoreceptors respond to changes in medullary tissue CO_2 (central CO_2 and thus central H^+), which may differ from P_aCO_2 due to a number of factors. The presence of the blood brain barrier does not permit the passage of polar solutes, resulting in a potential difference between arterial H^+ and central H^+ (Duffin 2011). Also, the high cerebral vaso-reactivity to changes in CO_2, generates large variations in blood flow and finally an arterial and central CO_2 mismatch may occur due to the change in the diffusion gradient of CO_2 (Ainslie & Duffin 2009). Therefore, a delay between a change in P_aCO_2 and central H^+ is apparent with the central chemoreceptors taking approximately 5 minutes to equalise following a change in inspired CO_2 (Duffin 2010). Although the response is variable, it appears that the peripheral

chemoreceptors respond initially to rapid changes in P_aCO_2 such that the more responsive central chemoreceptors are prevented from contributing significantly to the ventilatory response (Smith *et al.* 2006). The drive to breathe arises from the central chemoreceptors which provides input to medullary respiratory centres and can be measured by pulmonary ventilation, the slope of this response to changes in P_aCO_2 determines the chemoreflex sensitivity (Duffin 2005). This slope can be modified by CBF in that pharmacological-induced reductions in cerebral perfusion is associated with increased ventilatory responsiveness to hypercapnia, despite reductions in the hyperaemic response to hypercapnia alone (Xie *et al.* 2006). Therefore, the cerebrovascular reactivity to hypercapnia has a role in chemoreflex ventilation.

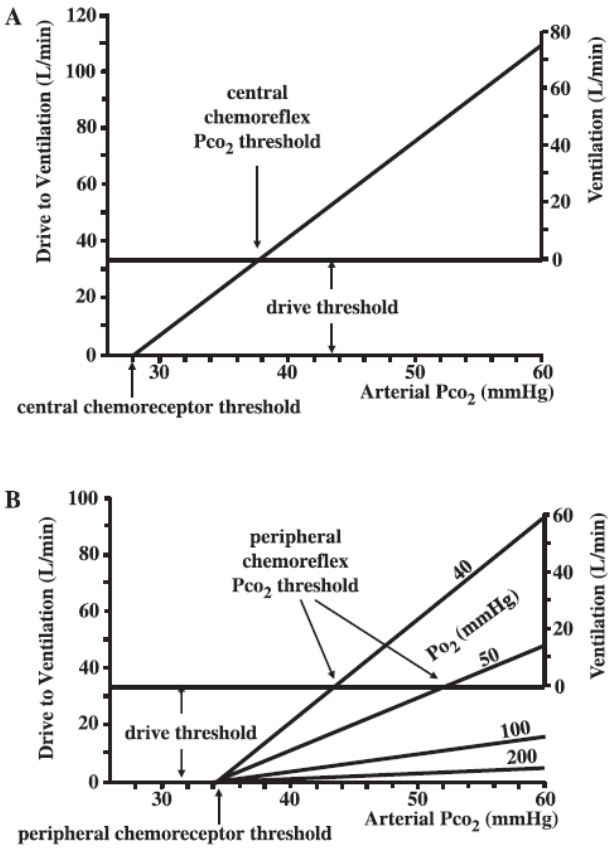

Figure 2.6 The central (A) and peripheral (B) chemoreflex response to changes in PCO_2 (Duffin & Mahamed 2003).

The sensitivity of the central chemoreflex (Figure 2.6 , A) is modulated by P_aO_2, as sensed by the peripheral chemoreceptors. During hypoxia, the chemoreflex ventilatory response to hypercapnia is enhanced (Cunningham 1987; Duffin 2007; Blain *et al.* 2010). However, hypoxia-mediated changes in ventilation only occur above the peripheral chemoreceptor P_aCO_2 threshold (Mohan & Duffin 1997) and the response is not associated with Hypoxia *per se*. Therefore, asphyxia which is associated with hypoxia and hypercapnia strongly activates the peripheral chemoreceptors (Cunningham 1987), whereas hyperoxia silences the peripheral chemoreceptors and reduces the hypercapnic ventilatory response (Dahan *et al.* 1990). Moreover, this ventilatory threshold can be lowered during repeated hypoxic exposures (Mahamed & Duffin 2001). Activation of the peripheral and central chemoreceptors produce a drive to breathe and transmit this signal to the medullary respiratory centres, although whether this combined response is hyperadditive (Blain *et al.* 2010; Teppema & Smith 2013), hypoadditive (Wilson & Day 2013) or additive (Duffin & Mateika 2013) remains unclear. Nevertheless increased ventilation stabilises arterial and therefore central CO_2 and pH.

2.4 Orthostasis

The simple act of a change in posture from siting to standing alters a myriad of systemic variables that have a profound effect on the cerebral circulation. The brain is at somewhat of a disadvantage being above heart level. For instance upon standing CPP decreases to ~20 mm Hg below brachial pressure (Hainsworth 2004), MCAv decreases by 15% (Pott *et al.* 2000; Van Lieshout *et al.* 2001) and cerebral oxygenation is reduced (Thomas *et al.* 2009b; Lin *et al.* 2011). Further, cerebral venous drainage changes upon the assumption of the

upright posture. When supine a large proportion of the blood is drained via the internal jugular veins (Doepp *et al.* 2004), however, when standing these veins collapse (Dawson *et al.* 2004; Gisolf *et al.* 2005) and blood is redirected through the vertebral venous plexus (Gisolf *et al.* 2004a). Furthermore, assumption of the upright posture has a gravitational effect on the cerebrospinal fluid and ultimately the critical closing pressure of the cerebral vessels (Zuj *et al.* 2013). Several cardiovascular (baroreflex) and cerebral adjustments (autoregulation) respond to the change in CPP to maintain an adequate CBF whilst upright. Despite these circulatory adjustments, the simple act of standing can produce a hypotension and cerebral hypoperfusion sufficient to produce syncope (i.e., fainting) even in healthy and disease-free individuals. Standing likely provides a further challenge to the regulation of CBF during tasks such as resistance exercise (see section 2.5.3) and the VM (section 2.5.3), although research to support this is lacking. In this section the cerebrovascular and cardiovascular responses during initial standing and during prolonged orthostatic stress will be discussed.

2.4.1 Syncope/Cerebral Hypoperfusion

Syncope can be defined as the transient loss of consciousness due to cerebral hypoperfusion, primarily due to an insufficient perfusion of the reticular activating system (Franco Folino 2007). Syncope has a rapid onset, short duration and subsequent complete recovery (Thijs *et al.* 2004; Moya *et al.* 2009). This may occur initially upon standing (initial orthostatic tolerance) in which a transient hypotension (systolic decrease of >40 mm Hg and/or diastolic 20 mm Hg) is evident within 15 s of standing that recovers in 30 – 60 s (Wieling *et al.* 2007). In addition, orthostatic intolerance can also occur following longer periods of orthostasis (see prolonged orthostasis, section 2.4.1.2 overleaf) typically after 3

minutes of standing and is characterised by a decrease in systolic blood pressure of >20 mm Hg and/or diastolic 10 mm Hg (Wieling *et al.* 2007). Orthostatic tolerance can also be tested experimentally via head up tilt (Immink *et al.* 2009) or simulated haemorrhage by LBNP (Levine *et al.* 1994), or a combination of the two. Whilst both initial orthostatic intolerance and general orthostatic intolerance have a common end point (cerebral hypoperfusion) (Thomas *et al.* 2009a), the origin of this hypotension differs. Moreover, the response to initial orthostatic hypotension does not reflect tolerance to more sustained orthostatic stress (Thomas *et al.* 2009b). Nevertheless, common symptoms other than loss of consciousness (i.e., syncope) at pre-syncope include; lethargy, fatigue, visual disturbances (loss of vision), hearing disturbances (Tinnitus), dizziness, sweating and palpitations (Moya *et al.* 2009).

2.4.1.1 Initial Orthostatic Hypotension

Upon standing, central blood volume decreases as in excess of 500 mL of blood is translocated into the veins of the pelvis and legs (Sjostrand 1952; Thijs *et al.* 2007; Stewart 2012). Although this relocation of blood does reduce central blood volume, the initial hypotension is produced via a reduction in peripheral resistance (Sheriff *et al.* 2007; Lewis *et al.* 2013) as $\dot{Q}$ is increased (Thomas *et al.* 2009b; Tschakovsky *et al.* 2011). This observed decrease in TPR may be due to a number of factors. For example, active standing as opposed to passive tilting induces a greater decrease in MAP (Sprangers *et al.* 1991). This has been attributed to the rapid leg vasodilation during activation of the leg muscles during active standing (Tschakovsky *et al.* 2011). Further, a brief rise in CVP, without a rise in oesophageal pressure (i.e., no Valsalva manoeuvre), may induce a cardiopulmonary reduction in vascular tone (Sprangers *et al.* 1991). During normal squatting vasodilation is

evident in the forearm as blood is displaced from the leg veins, elevating CVP. This dilation remains upon standing and an acute reduction in blood pressure is evident (Sharpey-Schafer 1956a). Lastly, an increase in the arterio-venous pressure gradient may play a minor role in the observed hypotension (Krediet *et al.* 2007; Tschakovsky *et al.* 2011). As discussed above, the baroreceptor-mediated vasoconstriction in response to the acute hypotension is the key mechanism that restores MAP (see section 2.2.1).

2.4.1.2 Prolonged Orthostasis

As mentioned, prolonged orthostatic stress can result in syncope in healthy individuals; in fact, elite endurance trained athletes are more at risk due to structural changes in the heart (Levine *et al.* 1991). The most common type of syncope is referred to as vagovagal syncope (Van Lieshout *et al.* 2003), and the process of this faint can be divided into four phases, as described by Julu *et al.* (2003) during head-up tilt: 1) The initial phase 1 response is a transient decrease in blood pressure that is compensated for usually within 30 s of the tilt and is associated with a rise in diastolic blood pressure (DBP) and an accompanied increase in HR, forearm vascular resistance and a reduction in cardiac vagal tone; 2) Phase 2 of the response consists of a progressive tachycardia and a reduced pulse pressure with little change in DBP. HR further increases from phase 1 and peaks during this phase; 3) Phase 3 is associated with blood pressure instability, demonstrating large oscillations; yet, the average systolic pressure (SBP) increases. HR also fluctuates during this phase, with the average HR being reduced from phase 2, and 4) Eventually phase 3 proceeds to phase 4, which is characterised by a sudden decrease in HR, associated with reductions in vagal baroreflex sensitivity (Morillo *et al.* 1997; Cooke *et al.* 1999; Kamiya *et al.* 2005) and blood pressure, with a concomitant intensification of symptoms, until consciousness is lost. At pre-syncope,

both SBP and DBP are decreased, however there is a selective reduction in diastolic middle cerebral artery blood flow velocity (DMCAv), which at times can reach zero and indicates a possible collapse of the vessels downstream of the MCA (Jørgensen *et al.* 1993). This occurs as the DBP drops below the critical closing pressure (Schondorf *et al.* 2001), which subsequently decreases immediately prior to syncope (Zuj *et al.* 2013).

2.4.2 Cerebro- and Cardiovascular Control at Vasovagal Syncope

The name vasovagal refers to the vasodilation (vaso) reported to occur at pre-syncope possibly via a withdrawal of MSNA (Convertino *et al.* 2004) and the bradycardia (Glick & Yu 1963) as a result of an increased vagal tone (Julu *et al.* 2003). What makes an apparent compensable system collapse is unknown (Stewart 2012). However, prolonged orthostatic stress decreases $\dot{Q}$ (in contrast to initial orthostatic stress), venous return and blood pressure as blood is trapped in the veins below heart level (Sprangers *et al.* 1991; Mosqueda-Garcia *et al.* 2000). Further, central blood volume decreases (Franco Folino 2007; Stewart 2012) as plasma shifts to the interstitial fluid due to an increase in microvascular pressure in the lower limbs (Levick & Michel 2010). Many counter measures aimed at increasing venous return and $\dot{Q}$ can restore circulatory stability; these include rhythmic cuff inflation (Niizeki *et al.* 2011), muscle tensing (Van Lieshout *et al.* 2001), LBPP (Fu *et al.* 2001), blood volume expansion (Keller *et al.* 2009), and leg crossing (Krediet *et al.* 2002).

Despite the exact origin of the vasodilation and bradycardia seen at pre-syncope being unknown, there are several hypotheses regarding this response. One theory suggests that activation of cardiac afferents in response to the contraction of an empty left ventricle plays a pivotal role of the development of syncope (Sharpey-Schafer 1956b). The large reductions in filling pressure activate these afferents and an inhibitory cardiac response (bradycardia) is

elicited with subsequent hypotension and syncope resulting (Sharpey-Schafer 1956b). This seems unlikely, however, as heart transplant patients, who have no nervous innervation of the myocardium, can still faint (Scherrer *et al.* 1990). Other theories indicate a role of central blood volume and of $\dot{Q}$ (Verheyden *et al.* 2008), which is feasible given that $\dot{Q}$ can modulate CBF independent of arterial blood pressure (as discussed in section 2.1.4). Hyperventilation, alterations in cerebral autoregulation and sympathetic outflow may also play a role in the development of syncope and are discussed below with specific reference to healthy individuals.

2.4.2.1 Cerebral Autoregulation

An adequate perfusion to the brain during orthostasis is critical in maintaining normal brain function. Despite the observed decrease in MCAv during standing of ~15% (Pott *et al.* 2000; Van Lieshout *et al.* 2001), an increase in O_2 extraction counteracts this reduction in flow (Zhang *et al.* 1998b). Interestingly this decrease in steady-state MCAv may occur without any concomitant decrease in MAP (Zhang *et al.* 1998b), indicating that the cerebral autoregulation curve may shift rightward resulting in an altered operational point (Levine *et al.* 1994). Following recovery from the initial orthostatic hypotension, MAP returns to baseline levels (Thomas *et al.* 2009b), however as SV (Harms *et al.* 1999; Murrell *et al.* 2009) begins to decline over time, along with $\dot{Q}$ (Sprangers *et al.* 1991; Thomas *et al.* 2009b), blood pressure begins to undergo large oscillations during phase 3 of head-up tilt (see section 2.4.1.2) (Julu *et al.* 2003). The high frequency oscillations in MAP are believed to be induced by respiration, while the lower frequency oscillations are possibly due to changes in sympathetic nerve activity (Rickards *et al.* 2007). Along with the fall in steady-state MCAv these blood pressure oscillations induce similar oscillations in MCAv during high levels of

LBNP, and would indicate an impaired dynamic cerebral autoregulatory capacity (Zhang *et al.* 1998b). However, others have demonstrated no change in dynamic cerebral autoregulation, as measured in the MCA, during head-up tilt in normal controls (Schondorf *et al.* 2001; Immink *et al.* 2009), postural tachycardia syndrome (Schondorf *et al.* 2005), frequent fainters (Schondorf *et al.* 2001), and healthy fainters (Novak *et al.* 1998a; Carey *et al.* 2001; Ocon *et al.* 2009). In contrast, regulation during head-up tilt was shown to be impaired in both the ICA and more so in the VA (Sato *et al.* 2012a). However, what is perplexing with this finding is that despite impaired regulation, VA flow was reported to be unchanged when tilted, whereas ICA flow and regulation were decreased (Sato *et al.* 2012a). Possible differences in results between these studies may arise from the vessel studied (MCA vs. VA and ICA) and methods used for flow assessment (TCD vs. duplex ultrasound).

Whilst it appears that autoregulation is intact during steady-state tilting, at pre-syncope in both patients and normal controls there is a sudden impairment of cerebral autoregulation (Carey *et al.* 2001; Ocon *et al.* 2009). However, in some patients this may be due to MAP dropping below the lower cerebral autoregulatory limit (Carey *et al.* 2001). However, the large oscillations immediately preceding syncope may be of benefit to the cerebral circulation. Once recognised as a sign of instability in the circulatory system, participants who demonstrate greater fluctuations in MAP and MCAv demonstrate a greater tolerance to orthostatic stress (Rickards *et al.* 2007; Rickards *et al.* 2011). Likewise, paced breathing (6 breaths per minute) increases orthostatic tolerance in comparison with spontaneous breathing via increased low frequency fluctuations in MAP, MCAv and improved dynamic autoregulation metrics (Lucas *et al.* 2013). Similar results have been shown during

inspiratory resistance, which increased both high and low frequency fluctuations in MCAv and increased time to syncope (Rickards *et al.* 2007). These fluctuations are associated with changes in pulsatility (Schondorf *et al.* 1997) and this pulsatile flow may maintain cerebral vasodilation during severe hypotension (Lewis *et al.* 1999; Zhang & Levine 2007) via shear stress-mediated mechanisms (Rubanyi *et al.* 1986). Once this flow-mediated dilation is exhausted, DMCAv decreases followed by a rapid reduction in mean CBF (Lewis *et al.* 1999). Therefore, this variability appears to be potentially protective during orthostatic stress and may act to maintain CBF during low perfusion pressures.

2.4.2.2 Sympathetic Nervous Activity

The reader is also referred to section 2.1.6 which details the regulation of cerebral blood flow by the sympathetic nervous system at rest. The role of MSNA during orthostatsis contributes to the regulation of blood pressure whilst the cerebral circulation may act only at extreme blood pressures to modulate flow. Therefore, cerebral sympathetic and muscle sympathetic activity are likely to show differential regulation (Ainslie *et al.* 2005). The following section details the role of cerebral and muscle sympathetic nerve activity during orthostasis.

2.4.2.2.1 Muscle Sympathetic Nervous Activity

The regulation of MSNA plays a pivotal role in the maintenance of arterial blood pressure via changes in TPR (see section 2.2.1). The regulation of MSNA has been implicated in the hypotension observed at pre-syncope. Although MSNA increases initially during steady-state orthostatic stress (Jardine *et al.* 1998; Cooke & Convertino 2002; Fu *et al.* 2004; Fu *et al.* 2012), reports have indicated a rapid withdrawal of MSNA at pre-syncope (Morillo *et al.*

1997; Mosqueda-Garcia *et al.* 1997; Jardine *et al.* 1998; Cooke & Convertino 2002; Convertino *et al.* 2004; Fu *et al.* 2012) with an associated reduction in TPR (Jardine *et al.* 2002; Goswami *et al.* 2009) and forearm vascular conductance (Julu *et al.* 2003). Interestingly, there is a disappearance of low frequency oscillations in MSNA (~0.1 Hz) before the onset of bradycardia and hypotension (Kamiya *et al.* 2005). The alteration in MSNA may arise via an impairment of venous return (Cooke *et al.* 2009), as fainters exhibit greater reductions in CVP (Mosqueda-Garcia *et al.* 1997) and increases in MSNA during orthostasis but TPR is actually reduced (Convertino *et al.* 2004). MSNA varies inversely with SV (Mosqueda-Garcia *et al.* 1997), which decreases during orthostasis (Levine *et al.* 2002). Terefore, it may not be the level of absolute MSNA, rather, individuals with greater vasoconstrictor reserve are more orthostatically tolerant (Fu *et al.* 2004). Reductions in sympathetic tone also mediate a venodilation, thereby limiting venous return and ultimately $\dot{Q}$ (Jardine *et al.* 2002).

Although sympathetic withdrawal is associated with a reduction in TPR, some research exists to support a maintenance of MSNA at pre-syncope (Vaddadi *et al.* 2010), with the withdrawal of MSNA not necessarily a prerequisite for syncope (Cooke *et al.* 2009). Further, the drastic hypotension observed at pre-syncope has been reported to occur before any change in MSNA (Fu *et al.* 2012). What induces a sudden reversal of baroreflex function is unknown; during normal homeostasis a decrease in MAP is reflected by elevations in both MSNA and HR, however during syncope MSNA, HR and MAP decrease concomitantly (Stewart 2012). This is further complicated in that the individual response is quite variable, with some individuals exhibiting a moderate reduction in TPR and $\dot{Q}$ with others showing predominantly large decreases in $\dot{Q}$ only, mainly via changes in HR (Verheyden *et al.* 2008;

Fu *et al.* 2012). In some individuals a decrease in HR occurs before the drop in blood pressure, whilst the opposite is also seen (Julu *et al.* 2003). Nevertheless, such results further support the notion that $\dot{Q}$ contributes to CBF regulation independently of perfusion pressure (Immink *et al.* 2009). Thus, individual responses to orthostasis vary and the exact role MSNA in the development of hypotension at pre-syncope have yet to be fully defined and a significant role of $\dot{Q}$ appears more likely.

2.4.2.2.2 Cerebral Sympathetic Nervous Activity

The role of the sympathetic nervous system in the regulation of CBF is controversial, but may play a role in cerebral autoregulation (as discussed in section 2.1.6). Normal cerebral autoregulatory responses to a hypotension would be a dilation to maintain cerebral perfusion (Aaslid *et al.* 1989), however at syncope a paradoxical increase in CVR has been reported (Grubb *et al.* 1991). Interestingly, this constriction occurs before the drastic hypotension synonymous with syncope (Levine *et al.* 1994; Grubb *et al.* 1998; Dan *et al.* 2002). It was hypothesised that this increase in cerebrovascular tone was sympathetically mediated and overrides the myogenic vasodilation (Levine *et al.* 1994). However, subsequent research using ganglionic blockade revealed that the observed vasoconstriction is unlikely to be caused by changes in sympathetic tone (Zhang & Levine 2007). Whilst a sympathetic regulation cannot be excluded (Zhang *et al.* 2002), the observed vasoconstriction is of an unknown origin.

2.4.2.3 Arterial Blood Gases

When standing ventilation increases and $P_{ET}CO_2$ decreases during spontaneous breathing (Thomas *et al.* 2009b; Lucas *et al.* 2013). The origin of this hyperventilatory response is not

clearly understood, although the increase in ventilation may be modulated by the decrease in MAP and baroreceptor unloading (Stewart *et al.* 2011). A decrease in MAP of 10-20% is associated with a 100% increase (~10 L·min^{-1}) in expiratory minute volume predominantly via increases in tidal volume (~500 mL) (Stewart *et al.* 2011). Regardless of the origin of the hyperventilation, the effect of hypocapnia on the cerebral circulation has been well documented (see section 2.1.1). Although the hyperventilation mediated hypocapnia may decrease CBF and could be viewed as primarily detrimental, an increased respiratory frequency may act as a respiratory muscle pump aiding venous return and cardiac filling (Lipsitz *et al.* 1998; Convertino *et al.* 2009).

Some reports indicate that the reduction in $P_{ET}CO_2$ accounts for the reduction in MCAv during orthostasis (Zuj *et al.* 2013), however this value may overestimate actual brain P_aCO_2 content (Immink *et al.* 2006). Moreover, this reduction is not simply due to changes in alveolar ventilation (Serrador *et al.* 2006). Upon standing pulmonary perfusion is altered with the basal alveoli gas diluting that of the under-perfused apical alveoli, and as such a ventilation perfusion mismatch occurs (Bjurstedt *et al.* 1962; Gisolf *et al.* 2004b; Serrador *et al.* 2006). This mismatch lowers $P_{ET}CO_2$ to a greater degree than the true P_aCO_2 content (Immink *et al.* 2006). So whilst some researchers believe that the reduction in MCAv is driven largely by a hyperventilatory reduction in $P_{ET}CO_2$ (Novak *et al.* 1998b; Zuj *et al.* 2013), when P_aCO_2 is measured directly the observed hypocapnia only explains approximately half of the reduction in MCAv (Serrador *et al.* 2006). Further evidence for a limited role of hypocapnia comes from head-up tilting protocols whilst isocapnia was maintained via end-tidal clamping; with the $P_{ET}CO_2$ clamping only alleviating the reduction in MCAv during the first minute of tilting (Immink *et al.* 2009). Although the reduction in $P_{ET}CO_2$ coincides with

the decrease in MCAv there appears to be no cause and affect (Immink *et al.* 2009) and hyperventilation-induced hypocapnia may play only a limited or partial role in the development of syncope (Folino 2006).

2.5 Resistance Exercise

Resistance exercise is a common form of exercise due to its positive effects on muscular strength, cardiovascular function, metabolism and psychological well-being (Williams *et al.* 2007; Garber *et al.* 2011). However, during resistance exercise blood pressure as high as 480/350 mm Hg (systolic/diastolic) have been reported (MacDougall *et al.* 1985) and therefore has the potential to induce cerebral vascular injury (Edwards *et al.* 2002) and challenge dynamic cerebral autoregulatory capacity. Paradoxically, following resistance exercise syncope can occur (Compton *et al.* 1973). Therefore, resistance exercise presents a unique challenge for the cerebral vasculature in that large, abrupt, non-pharmacological changes in MAP are likely to challenge cerebral autoregulation; yet these changes in MAP are bi-directional, with large increases in MAP during and large decreases following the effort. However, despite the large perturbations in blood pressure few studies have investigated the potential influence of these changes on CBF during such exercise. Moreover, the majority of the available literature investigates this response whilst semi-recumbent, despite the fact that syncope has been reported during upright exercise only (Compton *et al.* 1973). Thus, it is possible that when completed in the upright position the added stress of orthostasis (see section 2.4) may provide a further challenge in the maintenance of CBF, both during and following resistance exercise. The following section

discusses the current literature investigating the cerebro- and cardiovascular responses to resistance exercise.

2.5.1 Blood Pressure Response

2.5.1.1 During Resistance Exercise

Heavy resistance exercise can result in extreme blood pressures, with 480/350 mm Hg being reported in an individual and a group average of 320/250 mm Hg during a double leg press (MacDougall *et al.* 1985). Furthermore, the blood pressure response to weight lifting is dependent on the intensity of the exercise (MacDougall *et al.* 1992) and the muscle mass recruited (Mitchell *et al.* 1980; Lewis *et al.* 1985). The pressure response does not differ between static and dynamic exercises, although greater reductions in systemic resistance and increases in $\dot{Q}$ have been reported during dynamic exercise (Lewis *et al.* 1985). The largest increase in arterial blood pressure is seen at the greatest joint angle, corresponding with the weakest point of the strength curve (MacDougall *et al.* 1992; Lentini *et al.* 1993) and during repetitions latter in the set just prior to failure (MacDougall *et al.* 1985). As the angle decreases during the movement arterial pressure declines (MacDougall *et al.* 1992) or becomes equivalent to the top of the concentric phase during leg-press exercise (Lentini *et al.* 1993). During static contractions the intramuscular pressure in the *vastus medialis* is linearly related to the voluntary effort (Sejersted *et al.* 1984). This increase in intramuscular pressure compresses the artery such that above 70% of the maximal voluntary contraction (MVC) blood flow is completely occluded in the active musculature (Humphreys & Lind 1963). Along with the VM and exercise pressor response this mechanical compression of the arteries is responsible for the large increases in MAP (MacDougall *et al.* 1985).

2.5.1.2 Following Resistance Exercise

Reductions in blood pressure have been consistently reported immediately (within seconds) following resistance exercise (Compton *et al.* 1973; Romero & Cooke 2007; Moralez *et al.* 2012). As mentioned above during muscle contraction blood flow is inhibited, with the large increases in intramuscular pressures inhibiting arterial flow (Sejersted *et al.* 1984). Following exercise, however, there is an increase in blood flow mediated by metabolic dilation in the active vasculature, termed a functional hyperaemia (Barcroft & Millen 1939; Sjøgaard *et al.* 1988), that may be aided by a reduction in transmural pressure during relaxation (Rossberg & Peňaz 1988). This functional hyperaemia is exacerbated in the upright position (Folkow *et al.* 1971) and is dependent on contraction intensity (Walløe & Wesche 1988) and frequency (Corcondilas *et al.* 1964). When standing the translocation of blood into the limbs and the sharp reduction in MAP that follows, exceeds the baroreflex control of vascular tone (Thomas *et al.* 2009b). The reduction in blood pressure can be attributed to several mechanisms. Firstly, upon standing blood pools in the veins below the heart (Smit *et al.* 1999) reducing venous return. Secondly, this reduction in venous return impairs SV and $\dot{Q}$ (Harms *et al.* 1999), particularly during prolonged orthostasis (see section 2.4.1.1). Lastly, the available $\dot{Q}$ is pumped into a distended peripheral vasculature after exercise, evidenced by a reduced TPR (Wieling *et al.* 2007) with similar results having been reported during initial orthostatic intolerance (Thomas *et al.* 2009b; Tschakovsky *et al.* 2011).

2.5.2 Cerebrovascular Response

2.5.2.1 During Resistance Exercise

At rest, the MCAv response to MAP oscillations is dominated by the relationship between the change in MAP and the change in time (Tzeng *et al.* 2011), and appears to extend to resistance exercise. Resistance exercise has the ability to produce a fourfold increase in arterial blood pressure during the concentric phase of lifting (MacDougall *et al.* 1985). These increases in MAP greatly exceed the proposed autoregulatory limit (Lassen 1959). Due to the magnitude and speed of these fluctuations in blood pressure, the cerebral circulation is unable to fully counteract these changes in perfusion pressure such that alterations in MAP are tracked by MCAv accordingly (Edwards *et al.* 2002; Romero & Cooke 2007). Thus, the pattern of CBF during resistance exercise illustrates the high-pass filter characteristics of the cerebral circulation due to the inherent latency (~5 s) of the dynamic cerebral autoregulatory response (Zhang *et al.* 1998a).

Reports to date on the $MCAv_{mean}$ response during dynamic resistance exercise are equivocal, with some reporting no change in average MCAv during the entire exercise bout (Edwards *et al.* 2002) whilst others have reported a 15-30% increase (Koch *et al.* 2005), which is similar to the increase shown during static exercise (Imms *et al.* 1998; Pott *et al.* 2003). These differences are likely due to the type of resistance exercise (leg press versus leg extension) and repetition ranges. Dynamic exercise produces sinusoidal changes in MAP (MacDougall *et al.* 1985) and MCAv (Edwards *et al.* 2002) that are phase dependent. These changes may not be therefore best represented by a simple average over the exercise bout.

Despite the blood pressure response to resistance exercise being dependent on exercise intensity (MacDougall *et al.* 1992) and number of repetitions (MacDougall *et al.* 1985) no research exists investigating the effect of the load and number of repetitions on the MCAv response. As the cerebral vasculature acts as a high-pass filter (see section 2.1.3.2) it is possible these changes in MAP are translated to the cerebral circulation unbuffered during resistance exercise. Moreover, the load and number of repetitions determine training volume which is likely to be manipulated during any periodised training plan (Kraemer *et al.* 2002). Highlighting the physiological response to different loads and number of repetitions is vital in avoiding injury both during exercise and avoiding post-exercise syncope, as highlighted in the following section. Further, the application of this knowledge may also provide guidelines for compromised populations where blood pressure and/or CBF regulation may be impaired (e.g., stroke survivors).

The blood pressure and MCAv response to resistance exercise is exacerbated during the recruitment of the VM (Pott *et al.* 2003). Whilst the recruitment of a VM is discussed in detail below, many studies investigating the cerebrovascular response to resistance exercise aim to avoid the recruitment of a VM. Whilst this does decrease the pressor response to the exercise, the performance of the exercise even without the obvious appearance of the VM changes in intrathoracic pressure may still occur and have a pronounced effect on the venous drainage of the cerebral circulation (Bloomfield *et al.* 1997). The VM may also be involuntarily recruited when lifting heavy loads outside of a resistance exercise setting. It is therefore important to investigate the cerebrovascular response to lifting a heavy load when the VM is also recruited.

2.5.2.2 Following Resistance Exercise

The hypotension observed following resistance exercise also exceeds the autoregulatory capacity of the cerebral vessels (Romero & Cooke 2007). However, this phenomenon appears to be highly posture dependent. To date, studies investigating the haemodynamic response to resistance exercise have utilised a leg-press type movement (Edwards *et al.* 2002; Romero & Cooke 2007; Moralez *et al.* 2012), static/isometric type exercise (Mitchell *et al.* 1980; Ogoh *et al.* 2010b) or both (Lewis *et al.* 1985; Sale *et al.* 1993). The leg-press position facilitates venous return as the feet are at or above heart level, such that a hypotension is not observed when participants remain semi-recumbent (Edwards *et al.* 2002). However, upon standing following resistance exercise cerebral hypoperfusion sufficient to induce syncope has been reported (Compton *et al.* 1973). This effect is exacerbated by pre-exercise hyperventilation (Romero & Cooke 2007) and dehydration (Moralez *et al.* 2012). Research regarding upright resistance is lacking and is surprising given that body-weight squats have been found to significantly challenge dynamic cerebral autoregulation (Claassen *et al.* 2009) and given the larger MAP perturbations seen with additional load it would therefore be expected that this would further stress the cerebrovascular circulation. Similarly to the during exercise response discussed above, the effect of the load lifted and number of repetitions on the haemodynamic response to upright resistance exercise is unknown despite the potential for syncope to occur at high loads.

2.5.3 Role of the Valsalva Manoeuvre

Above ~80% MVC or during repetitions to failure lifters increase intrathoracic pressure via a VM (Fleck & Dean 1987; MacDougall *et al.* 1992). This increase in intrathoracic pressure is

directly related to the blood pressure rise (MacDougall *et al.* 1992) as the intrathoracic pressure is transmitted to the arterial tree (MacDougall *et al.* 1985). Furthermore, the level of straining, and therefore intrathoracic pressure, increases with the load lifted (Harman *et al.* 1988). Traditionally the VM is performed by forceful exhalation against a closed glottis, in resistance exercise this acts to stabilise the trunk and gives the lifter a mechanical advantage (MacDougall *et al.* 1992). There are four distinct phases of the VM, the first (phase I) consists of a brief spike in MAP and CBF due to translation of the intrathoracic pressure to the arterial tree (Pott *et al.* 2000). This occurs during both supine and standing VMs. A reduction in MAP, pulse pressure and SV then ensue as a result of a reduction in atrial filling (phase IIa) that then recovers partially, via the arterial baroreflex (phase IIb) until the strain is released. Upon release of the strain blood floods the distended pulmonary vessels and the reduction in intrathoracic pressure decompresses the thoracic arteries, reducing MAP (phase III). The now elevated $\dot{Q}$ is ejected against a constricted systemic circulation (phase IV) transiently increasing MAP (Goldberg *et al.* 1952; Tiecks *et al.* 1995b; Pott *et al.* 2000). This manoeuvre can be used to test dynamic cerebral autoregulation, as CBF tracks the abrupt changes in MAP throughout all phases (Pott *et al.* 2000) and although autoregulatory mechanisms may be active they appear to be unable to cope with the rapid changes in perfusion pressure induced by the VM (Greenfield *et al.* 1984; Tiecks *et al.* 1995b).

Although a portion of the MAP increase during resistance training is due to a VM, this increase in intrathoracic pressure may be of benefit in protecting the cerebral vasculature (MacDougall *et al.* 1985; Haykowsky *et al.* 2003). The increase in intrathoracic pressure as occurs during coughing, defection and the VM is translated to the cerebrospinal fluid, such

that increases in intracranial pressure (ICP) ensue (Hamilton *et al.* 1944; Greenfield *et al.* 1984) reducing the transmural pressure in the cerebral arteries (Haykowsky *et al.* 2003). Moreover, the increase in CVP associated with the execution of the VM may attenuate the pressure difference across the cerebral circulation as it is unlikely that the brain acts as a simple siphon (Dawson *et al.* 2004). In reference to Darcy's law (section 2.1) an increase in venous pressure (assuming arterial pressure and vessel tone are unchanged) would result in an attenuation of flow across a vascular bed. However, time and pressure are required to distend the collapsed outflow veins in the standing position (Pott *et al.* 2003; Gisolf *et al.* 2004a). These collapsed veins act as a Starling resistors that re-route blood through the vertebral venous plexus (Gisolf *et al.* 2004a). The mechanical effects of the elevated ICP and reduced venous outflow may restrain CBF during the VM (phase I).

Whilst the VM may protect the cerebral vasculature against rapid changes in perfusion pressure, following the release of the strain (phase III) rapid reductions in MAP and CBF to the point of syncope can occur (Duvoisin 1961). Due to the rapid decline in CPP, dynamic cerebral autoregulation is too slow to account for the observed hypotension, which may also be exacerbated by the lower efficacy of the regulatory response in the hypotensive range (Tzeng *et al.* 2010b). Following the nadir at phase III, MAP recovers rapidly and increases above pre-VM levels (phase IV). Despite similar increases in MAP between phase I and phase IV, the MCAv increase is greater during the latter (Tiecks *et al.* 1995b). This greater increase may be attributable to several factors: 1) The rapid reduction in CVP and ICP during phase III decreases the critical closing pressure (Dawson *et al.* 1999); 2) the increase in $\dot{Q}$ and associated rapid increase in MAP occurs too quickly for dynamic cerebral (myogenic) autoregulation and leads to a pressure-passive increase in MCAv and 3) the

dilation of cerebral vessels during phase II of the VM in response to the decreased CPP is maintained (Tiecks *et al.* 1995b). Non-mechanical factors act to control CBF during phase IV as ganglionic blockade results in an elevated MCAv response during phase IV of the VM (Zhang *et al.* 2004a), this would indicate an autonomic nervous system restraint (vasoconstriction) during the rapid increases in perfusion pressure during recovery of the VM.

Therefore, the mechanisms modulating MCAv may differ between phases of the VM and highlights the complex nature of the circulatory responses to the elevated intrathoracic pressure and alterations in CPP. Mechanical factors may control CBF during phase I of the VM with the autonomic nervous system predominating during phase IV and may explain the differential CBF response despite the similar changes in MAP. Similar to resistance exercise, the VM induces large, non-pharmacological perturbations in MAP that provide a significant challenge for the cerebral circulation given the magnitude and transient nature of these changes. These changes are likely to challenge dynamic cerebral autoregulation, when combined with resistance exercise or when performed in isolation. As the load increases the velocity of the lift decreases which would produce longer straining periods with greater intrathoracic (Harman *et al.* 1988) and intra-abdominal pressures (Cresswell & Thorstensson 1994). However, whether the MAP, and also MCAv, perturbations are length or intensity dependent are unknown. If indeed the VM is a protective mechanism the magnitude of the phase I MAP response would not be reflected in the MCAv change.

2.5.4 Circulatory Adaptations to Resistance Exercise

Resistance training induces many positive health benefits aside from increases in lean mass, and as such this type of training is now recommended during cardiac rehabilitation

(Williams *et al.* 2007). Chronic resistance training leads to many structural changes within the circulatory system, including the heart. In resistance trained individuals an increase in left ventricular mass (Fisman *et al.* 1997; Miyachi *et al.* 2003) and thickness (Miyachi *et al.* 2004) with no change in chamber size (concentric remodelling) (Longhurst & Stebbins 1997) has been reported. However, the proportional increase in lean muscle mass is much greater than the observed cardiac hypertrophy (Longhurst *et al.* 1980). This remodelling may occur in response to the large increases in blood pressure (section 2.2.6.2) and thus afterload (Longhurst & Stebbins 1997) seen during resistance exercise. Furthermore, the increased arterial stiffness associated with resistance training may also elevate afterload contributing to ventricular remodelling (Bertovic *et al.* 1999). Despite these structural changes, left ventricular function is maintained with no change in resting SV (Miyachi *et al.* 2004) or functional shortening (Bertovic *et al.* 1999).

Prior resistance training attenuates the pressor response to resistance-type exercise, characterised by lower blood pressure and HR responses (Fleck & Dean 1987; Fisman *et al.* 1997). Although studies regarding the effect of resistance training on resting blood pressure is equivocal; with prolonged resistance trained athletes (>2 years) showing elevated brachial SBP and DBP (Otsuki *et al.* 2007; Kawano *et al.* 2008) and pulse pressure (Bertovic *et al.* 1999), while others demonstrating no change (Miyachi *et al.* 2003) in comparison with controls. However, meta-analyses of resistance training studies have reported a lowering of both SBP and DBP post intervention (Kelley & Kelley 2000; Cornelissen *et al.* 2011), although the minimum training period was 1 month. Others have reported an increase in DBP following 3 months of training (Rakobowchuk *et al.* 2005a) and others no change in either MAP, SBP or DBP following 8 months (Miyachi *et al.* 2004) and 13 weeks (Cortez-Cooper *et*

al. 2008) of resistance training. Therefore, the blood pressure response may be dependent on the length and type of the training stimulus, whereas resting HR appears to be similar between resistance trained individuals and aged-matched sedentary controls (Fleck 1988), and following a period of resistance training (Cortez-Cooper *et al.* 2008).

Unlike endurance training (Vaitkevicius *et al.* 1993; Cameron & Dart 1994; Tanaka *et al.* 2000) resistance exercise appears to decrease central arterial compliance and stiffen the central elastic arteries (Bertovic *et al.* 1999; Miyachi *et al.* 2003; Miyachi *et al.* 2004; Otsuki *et al.* 2007). Despite this, endothelial function in response to the cold pressor test (Kawano *et al.* 2008) and as measured using flow-mediated dilatation (Rakobowchuk *et al.* 2005b) is unaffected by resistance training. This increased arterial stiffness may be restricted to the central arteries (i.e., aorta and carotid) (Kawano *et al.* 2008) as peripheral muscular arteries (i.e., femoral) remain unchanged (Miyachi *et al.* 2004). Whether this stiffness extends to the downstream vessels of the common carotid (i.e., the intracranial vessels such as the MCA) is unclear. The increased stiffness is likely due to the resistance training itself (Kawano *et al.* 2008) as changes in compliance are reversible upon detraining (Miyachi *et al.* 2004). The large transient increases in MAP observed during resistance training may alter the smooth muscle properties of the artery and the load bearing properties of collagen and elastin within the vessel wall (Bertovic *et al.* 1999).

Although, endothelial properties appear to be unaltered by resistance training (Rakobowchuk *et al.* 2005b; Kawano *et al.* 2008) it has also been speculated that alterations in endothelial production of nitric oxide and endothelin-1 may also alter the properties of the vessel wall (Otsuki *et al.* 2007). Further, the elevated blood adrenaline and noradrenaline may also have an effect (Kraemer *et al.* 1987; Kraemer & Ratamess 2005),

which may be fast acting as central arterial compliance is reduced following a single bout of resistance exercise that persist for up to an hour following (DeVan *et al.* 2005). Resistance training may induce significant structural modifications to the circulatory system some of which may be detrimental. Increased arterial stiffness has been associated with cardiovascular mortality in patients with hypertension (Laurent *et al.* 2001) and as an independent predictor of coronary heart disease (Weber *et al.* 2004) and stroke in healthy individuals (Mattace-Raso *et al.* 2006). However, when aerobic training and resistance training are combined the reductions in vascular function can be circumvented (Okamoto *et al.* 2007). Despite the potential maladaptations it appears these are outweighed by the many positive effects of resistance training (Garber *et al.* 2011).

The potential changes in both arterial conductance and myocardial structure in resistance trained individuals is important to this thesis as **Chapters Five** and **Six** investigate the effects of dynamic changes in MAP on cerebrovascular dynamics in this population. It is therefore possible that the results discussed in these chapters may differ for non-resistance trained individuals (i.e., endurance trained) as discussed in section 9.3.2.

2.6 Lower Body Positive Pressure

Lower body positive pressure (LBPP) has been used in medical emergencies (Wayne & Macdonald 1983) as well as for preventing g-force induced syncope in pilots (Wood 1987) by maintaining central blood volume. As detailed in the following section LBPP provides a means of increasing steady-state and prolonged increases in MAP that are non-pharmacological in nature. Although the haemodynamic response is marked the

cerebrovascular response to these changes in MAP has not been described in detail and the exact response whilst supine is unknown.

2.6.1 Blood Pressure Response

LBPP translocates blood from the limbs to the thorax and elevates CVP (Shi *et al.* 1993a). This increase in CVP is reflected by an increase in cardiac filling (Fu *et al.* 1998), although $\dot{Q}$ and HR remain unchanged, as the acute increase in afterload offsets the elevated filling pressure (Rubal *et al.* 1989; Williamson *et al.* 1994); although increases in $\dot{Q}$ have been reported during prolonged (30 min) periods of LBPP (Geelen *et al.* 1992). Below 20 mm Hg of LBPP moderate increases in CVP mediate a reduction in MSNA via activation of the low pressure baroreceptors (Fu *et al.* 1998). At pressure ≥30 mm Hg MSNA increases via activation of intramuscular pressure receptors, despite further increases in CVP (Shi *et al.* 1993a). During LBPP MAP increases proportionately to the applied pressure (Nishiyasu *et al.* 1998) as a result of reductions in both conductance and flow in the arm and leg (Nishiyasu *et al.* 2007). This is possibly due to the direct compression of the vessels (Nishiyasu *et al.* 1998) and increases in MSNA in the non-dependent limbs (Fu *et al.* 2001). LBPP reduces the orthostatic stress in response to head-up tilt as evidenced by a restoration of MAP and reductions in MSNA (Fu *et al.* 2001). However, the intramuscular mechanoreflexes may counteract the effect of the baroreflex at higher levels of LBPP (Fu *et al.* 2001). Further, loading of the cardiopulmonary baroreceptors diminishes the influence of the carotid baroreceptors on HR and MAP (Shi *et al.* 1993b). Thus, LBPP is an effective means of increasing MAP via mechanical compression and alterations in MSNA. More importantly LBPP provides a non-pharmacological means of producing prolonged and steady-state increases in MAP and provides a challenge for static cerebral autoregulation.

2.6.2 Cerebrovascular Response

Despite the pronounced haemodynamic effect of LBPP, data concerning the effects of LBPP on cerebral perfusion are limited. Short bouts (1 min) of LBPP applied to upright individuals are reported to have no effect on MCAv (Cutuk *et al.* 2006). Whilst not significant, Cutuk *et al.* (2006) reported an increase in $MCAv_{mean}$ from baseline during 20 mm Hg LBPP (from 75 to 81 $cm \cdot s^{-1}$), which then decreased at 40 mm Hg LBPP (78 $cm \cdot s^{-1}$). However, results from Shi *et al.* (1997) indicated the response to LBPP is not stable until several minutes after application. The initial response to LBPP produced a sharp increase in MAP that would be counteracted by dynamic autoregulation (Zhang *et al.* 1998a), and is therefore not a steady-state (i.e., static) response. The exact cerebrovascular response to prolonged LBPP induced increases in MAP has yet to be examined.

2.7 Summary

This literature review highlights the complexity of the cerebral circulation and the myriad of both local and systemic factors that contribute to its regulation. Whilst these modulators have been identified, their exact role and how their interactions influence CBF are yet to be fully understood. In particular the role of MAP and how the brain's intrinsic mechanisms act to defend against changes in perfusion pressure. Given the literature discussed within this chapter, it is apparent that the autoregulatory plateau first suggested by Lassen (1959) appears to be somewhat more linear and the efficacy of both static and dynamic autoregulation is not completely understood. Large and abrupt changes in MAP are associated with concomitant changes in CBF. Likewise, steady-state changes in MAP are accompanied by prolonged increases in CBF. If indeed changes in MAP are associated with

similar directional changes in CBF, then this has serious implications for a variety of movements, both in a recreation/sporting setting and during everyday life (heavy lifting, coughing and defecation). Furthermore, these mechanisms that may still be operative in healthy individuals can be impaired by the alteration of arterial blood gases. The impairment of these regulatory mechanisms in healthy individuals may mimic some pathological states. Thus, the primary focus of this thesis was to investigate the effect of changes in MAP on CBF during both rapid and prolonged perturbations.

Chapter Three: Review of Techniques and General Methodology

This thesis is centred around the effects of changes in MAP on CBF. Therefore, an accurate measure of both MAP and CBF is required to establish the relationship between these two variables during the experimental conditions detailed in the forthcoming chapters. This chapter is devoted to a thorough critique of the equipment and technology used for data collection of the two primary dependent measures obtained throughout this thesis; the non-invasive measurement of MCAv as an index of CBF and finger photoplethysmography as an index of intra-arterial blood pressure. The methods by which these two techniques function will be discussed along with the merits and limitations of each piece of equipment. Searching techniques used to insonate the M1 segment of the MCA using transcranial Doppler (TCD) and the correct cuff placement for the non-invasive measure of arterial blood pressure using finger photoplethysmography are also detailed.

3.1 Measurement of Cerebral Blood Flow

The technique first used to measure cerebral blood flow employed an inert tracer (nitrous oxide) and was described by Kety and Schmidt (1945). Briefly, N_2O was inhaled until a saturation point is reached at which point inhalation ceases and the concentration of the tracer declines. The direct Fick equation was applied via sampling concentrations of the tracer obtained from arterial and jugular samples. Subsequent tracers included Xe^{133} (Obrist *et al.* 1967; Thomas *et al.* 1979) and Kr^{85} (Ingvar & Lassen 1961; Lassen & Klee 1965) with the use of extracranial gamma detectors. Whilst this technique did provide an accurate

account of CBF, it was invasive, had poor temporal resolution with minutes to acquire a given flow that represented global increases in CBF, and required a steady-state condition (Willie *et al.* 2011). TCD offers excellent temporal resolution and can be used to track dynamic changes in CBF during a variety of perturbations.

3.1.1 Transcranial Doppler Ultrasound: Ultrasonography Principles

The use of sonography in measuring flow velocity in intracranial cerebral arteries is severely limited by the skull, in that bone attenuates the ultrasonic wave. However, at lower frequencies (1-2 MHz) this attenuation from bone, as well as soft tissue, is markedly less (Aaslid *et al.* 1982). Further the skull itself varies in thickness, some areas such as the temporal window, just above the zygomatic arch, provide further opportunity for ultrasonic waves to penetrate as the skull is thinner in this area. This window allows the insonation of the large basal cerebral arteries including the middle cerebral, the proximal anterior cerebral, and posterior cerebral arteries (Aaslid *et al.* 1982; DeWitt & Wechsler 1988). A technique using a lower emitted ultrasound frequency and these anatomical 'windows', coined TCD, was first described by Aaslid *et al*. (1982), and allows real time measurement of cerebral blood flow velocity.

TCD functions via traditional Doppler methods but differs in the fact that both the sound source and the observer (the transducer) are fixed in the same position. The transducer emits sound waves that are reflected by the moving erythrocytes within the insonated vessel, which are detected by the transducer. The Doppler shift resulting from the reflected waves is proportional to the velocity of blood (DeWitt & Wechsler 1988; Stroobant & Vingerhoets 2000; Willie *et al.* 2011). The following equation describes the Doppler shift (Moppett & Mahajan 2004):

Doppler frequency shift = $2 \times V \times Ft \times cos\theta / C$

where: *V* is the velocity of the reflector, which in this case is the erythrocytes within the insonated vessel; *Ft* is the transmission frequency of the Doppler transducer; *C* is the speed of sound in soft tissue and $cos\theta$ is a correction factor based on the insonation angle (θ). In pulsed Doppler the *Ft* is equivalent to 2MHz and *C* 1540 $m \cdot s^{-1}$, both of which remain constant. The remaining factors, the angle of insonation and flow velocity of blood, therefore have the largest influence on the resultant Doppler frequency shift (Moppett & Mahajan 2004). If the insonation angle varies from 0 to 30^{o} the resulting cosine will vary between 1 and 0.86, respectively, and the maximum error associated within this acute angle insonation range will be 15% (Aaslid *et al.* 1982).

As the TCD transducer emits a pulsed signal, the time interval between the emission and receiving of the signal will determine the depth of the Doppler frequency shift. Thus the depth of an insonated vessel can be manipulated by varying the time interval between the emitted and received signal (Moppett & Mahajan 2004). The obtained signal represents a distribution of velocities (Willie *et al.* 2011). This is due to the varying velocities of erythrocytes within the different lamina of a large artery with the fastest toward the middle of the artery and the slowest next to the vessel wall (Levick 2010). The TCD unit applies a spectral analysis to the mixed frequency shifts to enable three dimensional data to be displayed in two dimensions, with time on the x axis and the frequency shift (velocity) to be displayed on the y axis, and the signal intensity represented by the colour of the trace (Moppett & Mahajan 2004).

3.1.2 Validity

With the drastic improvements of temporal resolution in TCD, dynamic beat-to-beat fluctuations in CBF can now be investigated, however, as the measurement obtained is a velocity rather than an absolute flow measure, several problems can arise from this when trying to infer absolute flow. Using this type of technology, the artery diameter cannot be established and therefore is assumed to stay constant (Willie *et al.* 2011). As reviewed in **Chapter Two** (section 2.1), Poiseuille's law states resistance is inversely proportional to the radius of a vessel to the fourth power. Furthermore, as velocity can be defined as the flow in a vessel divided by its area (in cm^2) any change in vessel diameter will have profound effects on both flow and velocity of the blood within a given artery.

For the larger basal conduit arteries of the brain the vessel diameter has been found to be constant over varying physiological conditions. Direct observations of the large cerebral arteries of the brain (carotid, middle cerebral and vertebral arteries) revealed that during moderate pharmacological alterations in blood pressure and end-tidal PCO_2 the average change was less than 4% (Giller *et al.* 1993). The smaller arteries such as the anterior cerebral and more distal division of the middle cerebral arteries showed large diameter changes to the aforementioned stimuli (Giller *et al.* 1993). This was also found to be true in conscious humans using magnetic resonance imaging during alterations in arterial CO_2 (Valdueza *et al.* 1997) and during sympathetic nervous system activation, without a concomitant drop in blood pressure, induced via LBNP (Serrador *et al.* 2000). These results were also supported by a good correlation between the respective percentage changes when comparing TCD and Xenon (Xe^{133}) clearance technique during hypercapnia (Bishop *et al.* 1986). Further, using digital angiography it was shown that the cerebral arteries ≥0.57

mm in diameter (significantly smaller than the MCA) showed no alteration in calibre to standardised changes in arterial PCO_2 (Djurberg *et al.* 1998). It should be noted in the study of Djurberg *et al.* that these patients had arteriovenous malformations (AVM), despite diameter measurements being made on the ispilateral hemisphere to the AVM, the AVM was outside the vascular territory and therefore artery structure and function was assumed to be normal. Signal power measurements from transcranial Doppler also demonstrate no change in MCA diameter during carotid artery compression (Aaslid *et al.* 1991) and during blood pressure reduction via thigh-cuff deflation (Aaslid *et al.* 1989). Therefore, it appears that over a wide range of physiological stimuli both with and without anaesthesia, the MCA diameter does not appear to change.

TCD has been validated against the intravenous Xe^{133} technique at rest and during hypercapnia (Bishop *et al.* 1986). However, only the change in MCAv correlated suitably with changes in hemispheric blood flow, with absolute velocity correlating poorly with absolute flow. Further, changes in flow within the internal carotid artery were tracked similarly by the change in velocity within the MCA (Lindegaard *et al.* 1987; Newell *et al.* 1994). Similarly, during changes in MCAv as a result of pharmacological manipulations of MAP, MCAv tracked relative changes in global CBF (Fick, Xe^{133} clearance) adequately down to and including the lower limits of cerebral autoregulation (Larsen *et al.* 1994). Thus, whilst TCD cannot provide a measure of absolute flow the relative changes can be accurately measured and correlate well with absolute changes in CBF measured via more direct methods. *Accordingly, results from this thesis are presented as the absolute and relative change from baseline measures.*

3.2 Measurement of Arterial Blood Pressure

A non-invasive method of measuring arterial blood pressure was first described by Penaz (1973) using the volume-clamp method. The Finometer device (Finapres Medical Systems B.V, Amsterdam, The Netherlands) used in this thesis utilises the photo-plethysmography method that is based on the volume clamp method pioneered by Penaz (1973). This method has the benefit of being non-invasive and simple to operate, providing dynamic beat-to-beat measurements of arterial blood pressure. Further, using this non-invasive technique in combination with the Modelflow method, a dynamic account of SV and $\dot{Q}$ can be computed (Wesseling *et al.* 1993).

3.2.1 Beat–to-beat Arterial Blood Pressure

The volume clamp method maintains artery diameter constant inside the finger via manipulating the pressure inside an inflatable cuff wrapped around the mid-phalanx of the finger. This occurs despite fluctuations in arterial pressure during each cardiac cycle and is termed the 'set point'. Arterial diameter is kept constant via feedback from an infrared photo-plethysmograph, with increases in diameter being accounted for by rapid inflation using a rapid pressure servo-controller system (Bogert & van Lieshout 2005). During times of stable arterial diameter, the transmural pressure is zero across the arterial wall. During this time the artery is approximately a third of its original cross-sectional area and volume, and would require a cuff pressure greater than the finger intra-arterial pressure to collapse the artery (Bogert & van Lieshout 2005). When transmural pressure is zero the artery wall is said to be unloaded, which allows changes in arterial pressure throughout the cardiac cycle to be directly transferrable to the cuff pressure and subsequently represents finger arterial pressure (Imholz *et al.* 1998).

During initial cuff inflation the pressure is increased in a step-wise fashion, with each step being held constant until one beat is detected (Imholz *et al.* 1998). At the last pressure step the cuff pressure just exceeds systolic pressure, providing that cuff pressure exceeds 100 mm Hg. The plethysmogram pulsations at this maximum cuff pressure along with criteria to avoid venous pulsations represent the arteries in the unloaded state and reflect MAP and thus form the set point. However, the unloaded diameter of an artery is subject to change with haematocrit, smooth muscle tone and stress of the arterial wall (Bogert & van Lieshout 2005). Thus subsequent repeated calibration of the set point is required which is achieved via the Physiocal procedure. During the Physiocal calibration the blood pressure trace is interrupted after ~70 beats in which incremental stepwise changes in cuff pressure are applied again and the set point is adjusted accordingly, if required (Imholz *et al.* 1998).

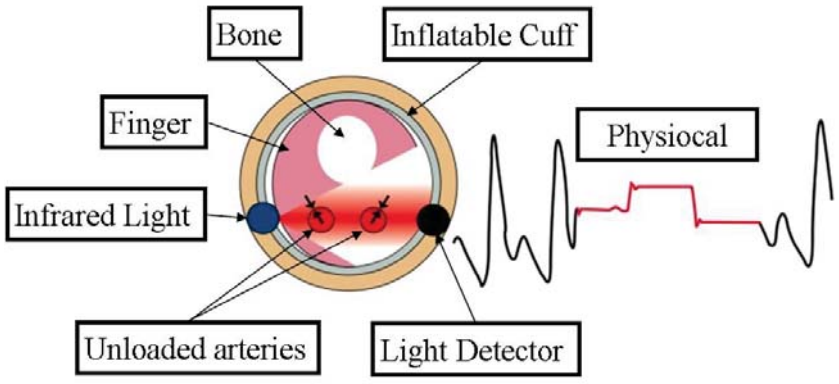

Figure 3.1 Cross sectional of finger and cuff with blood pressure output including calibration (Physiocal, Finapres medical systems website, http://www.finapres.com).

3.2.2 Validation of Non-Invasive Beat-to-beat Arterial Blood Pressure

As the arterial pulse travels down the vascular tree it is subject to distortion. This distortion is caused by reflected waves and pressure gradients produced by downstream resistance vessels (Bos *et al.* 1995; Bos *et al.* 1996; Gizdulich *et al.* 1997). This results in augmentation of the pressure wave as it travels along the arterial tree and peripherally measured pressures are greater than those measured centrally (Wilkinson *et al.* 2000). The degree of pulse-wave amplification has been extensively investigated, with the Finometer being referenced to the gold standard intra-brachial pressure. Such testing has shown that systemic factors such as heart rate and left ventricular ejection time have a larger effect than regional vascular tone (Parati *et al.* 1989; Bos *et al.* 1995). Further, during atrial pacing an increase in heart rate is accompanied by elevations in peripheral blood pressure most likely due to the timing of the reflected wave (Wilkinson *et al.* 2000). At rest, finger arterial pressure tracks brachial pressure closely (Imholz *et al.* 1990; Ganio *et al.* 2011). However, during heat stress with and without concomitant LBNP, finger pressure is reported to underestimate brachial pressure whilst mean and diastolic absolute finger values tracked brachial (Ganio *et al.* 2011). Further, relative changes in brachial pressure during concomitant heat stress and LBNP were accurately tracked. During normothermic LBNP (Imholz *et al.* 1990) and active standing (Imholz *et al.* 1990; Thomas *et al.* 2009b), finger pressure was also found to accurately track dynamic changes in intra-arterial pressure including at pre-syncope (Thomas *et al.* 2009b).

The discrepancy between finger and brachial waveforms and subsequent pressures can be partially alleviated by applying a digital filter. The transfer function from brachial to finger pressure has a maximum of ~8 Hz (Gizdulich *et al.* 1996). A general inverse model with an

anti-resonance at this frequency is applied to the finger waveform to dampen these oscillatory distortions, which improves accuracy (Gizdulich *et al.* 1996; Gizdulich *et al.* 1997). This replicates the brachial wave forms shape by accounting for pulse wave amplification, the values of which are corrected for by a multiple regression model (Gizdulich *et al.* 1997). With these advances in constructing brachial pressure and waveforms the Finometer gives and accurate, non-invasive and dynamic account of arterial blood pressure. Nevertheless, as the Finometer is an indirect measure and was not calibrated against the gold standard intra-arterial line during any of the studies contained within this thesis (manual blood pressures were used to spot check Finometer values where possible), the validity of the absolute values is uncertain. *Therefore, throughout this thesis the absolute and relative change in blood pressure from baseline is presented.*

3.2.3 Non-invasive Stroke Volume and Cardiac Output Estimates

The use of the Finometer allows a continuous measure of both $\dot{Q}$ and SV. This method termed the Modeflow method was developed by Wesseling and co-workers (1993), and can reconstruct the aortic flow pulsations from an arterial pressure recording. The Modelflow method incorporates the participant's demographics: gender, age, height, and weight (BeatScope v1.1, Finapres Medical Systems B.V., Amsterdam, The Netherlands). This model simulates a nonlinear, time varying three-element model of aortic input impedance which is summarised in Figure 3.2 (Wesseling *et al.* 1993). The three elements in this model are aortic characteristic impedance, arterial compliance (represented by Windkessel compliance in the model) and systemic vascular resistance. Aortic characteristic impedance and arterial compliance were predetermined during the study of the pressure-area relationship in

thoracic and abdominal aortas (Langewouters *et al.* 1984). This pressure-area relationship was found to be non-linear and also age dependent.

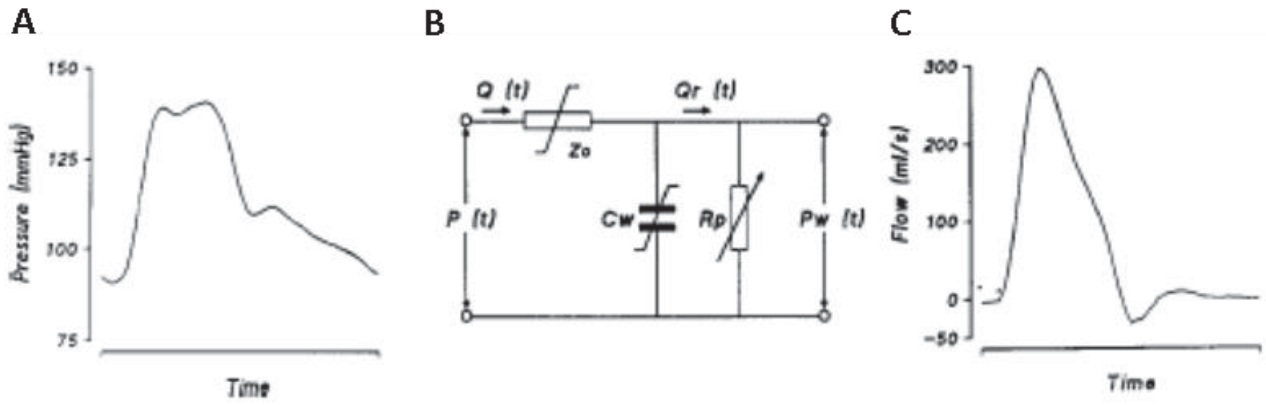

Figure 3.2 Computation of aortic flow from arterial pressure (Harms et al. 1999). (A) Non-invasive finger blood pressure input for one heartbeat. (B) The three elements of aortic impedance used to calculate aortic flow from the arterial pressure input: Z_0, characteristic impedance of the proximal aorta; C_W, Windkessel compliance of the arterial system (the ability of the arterial system to elastically store pulsatile flow from the left ventricle), and R_p, total peripheral resistance. Z_0 and C_W have non-linear pressure dependent properties (Langewouters *et al.* 1984) represented by the symbol ∫. R_p is time-dependent as indicated by the arrow. P (t), the arterial pressure waveform; Q (t), blood flow as a function of time; Pw (t), Windkessel pressure; (C) computed output displaying aortic flow as a function of time.

Stroke volume is determined by applying this Modelflow method during systole. The systolic duration and heart beat are also derived from this pressure waveform with $\dot{Q}$ being simply the product of SV and the instantaneous HR (Wesseling *et al.* 1993). Aortic characteristic impedance and arterial compliance are calculated from an in-built reference database based upon the findings of Langewouters *et al.* (1984). The peripheral resistance value used in the model is calculated from the previous beat as peripheral resistance changes occur slower than the elapsed time of one heartbeat (Sprangers *et al.* 1991). Total peripheral resistance is calculated as the average pressure to average flow (Wesseling *et al.* 1993). During initial cuff inflation peripheral resistance is assumed to be the ratio of 100 mm Hg mean pressure and a

$\dot{Q}$ of 3 $L \cdot min^{-1}$. Over the next few beats when the true arterial pressure and computed flow is applied to the model, the real (calculated) value is given within a few heartbeats (Wesseling *et al.* 1993).

3.2.4 Validation of Modelflow Estimates of Stroke Volume and Cardiac Output

The measurement of $\dot{Q}$ is of interest both in a clinical and experimental setting. However, despite its importance, technical difficulties have limited the ability for $\dot{Q}$ to be accurately and continuously measured. The development of a flow directed balloon-tipped catheter that allows catheterization of the right heart at the bedside (Swan *et al.* 1970) provides a measurement of $\dot{Q}$ (Forrester *et al.* 1972). This technique is referred to as the defacto clinical standard for measurement of $\dot{Q}$, using a modified indicator technique (Mathews & Singh 2008). However, whilst this measurement technique is accurate it is invasive and requires an operator to inject a fluid bolus, and more importantly not continuous (Mukkamala & Xu 2010). Other measures include Fick's measurement, CO_2 re-breathing, Doppler ultrasound and transoesophageal echocardiography amongst others (Mathews & Singh 2008). However, these techniques are either invasive and/or require an expert operator. Thus, throughout this thesis the Modelflow method employed by the Beatscope software has been used as a completely non-invasive and continuous account of $\dot{Q}$.

This system has been validated against the thermodilution technique during open heart surgery in which absolute values at rest and changes in $\dot{Q}$ were tracked with precision (Wesseling *et al.* 1993; Rödig *et al.* 1999; De Wilde *et al.* 2007). Further, at rest and without general anaesthesia, there was no difference between cardiac output derived from the finger arterial pressure wave, the radial arterial pressure wave and values from the

thermodilution technique (Shibasaki *et al.* 2011). In some instances the Modelflow SV values from the finger pressure waveform actually more accurately tracked thermodilution derived SV than did the intra-brachial pressure waveform (see Figure 3.3 overleaf (Harms *et al.* 1999)). During orthostasis (active standing, head down and heat-up tilt; Harms *et al.* 1999) and simulated haemorrhage using LBNP (Shibasaki *et al.* 2011) Modelflow derived SV values from finger arterial pressure waveforms accurately tracked thermodilution. However, during large changes in systemic vascular conductance (phenylephrine induced) (Rödig *et al.* 1999) and during heat stress with and without concomitant LBNP (Shibasaki *et al.* 2011), $\dot{Q}$ was significantly different from thermodilution derived values. Changes in sympathetic outflow may change in the elastic properties of the aorta (Van Lieshout & Wesseling 2001), which may have contributed to the possible differences in agreement between the Modelflow method and thermodilution techniques under the conditions of heat stress and LBNP. However, due to the relatively small amount of smooth muscle contained within the aortic wall (Van Lieshout & Wesseling 2001) any difference in smooth muscle diameter may be negligible. Further, as backflow is not factored into the Modelflow method, a competent aortic valve is required to prevent retrograde flow into the left ventricle during diastole (Van Lieshout & Wesseling 2001). Changes in body position may also have a varying effect on the transmural pressure of the abdominal aorta (Harms *et al.* 1999), which may be exacerbated with obesity (Van Lieshout & Wesseling 2001). Therefore, obese individuals and people with aortic valve insufficiency were excluded for the studies contained within this thesis. Due to these discrepancies between Modelflow and more accurate invasive techniques during physiological stressors, *both absolute and relative changes from baseline for SV and* $\dot{Q}$ *are presented throughout this thesis.*

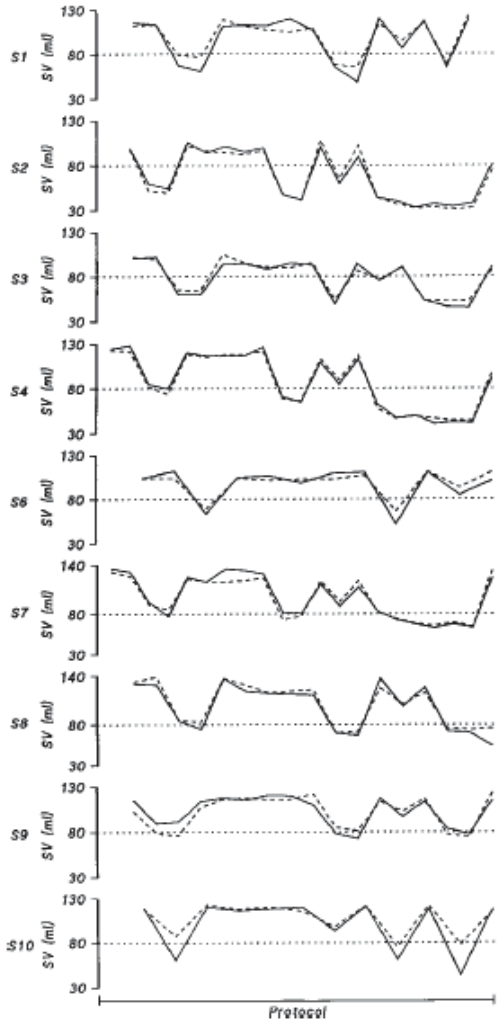

Figure 3.3 Thermodilution and Modelflow derived stroke volume (SV) values in 10 participants (Harms et al. 1999). Solid line, thermodilution derived SV; dashed line, Modelflow (from finger arterial pressure waveform) derived SV. Fluctuations in SV induced by standing and passive changes in tilt angle between 20 degrees head down and 70 degrees head up.

3.3 General Methodology

3.3.1 Insonation of the Middle Cerebral Artery (MCA) using Transcranial Doppler (TCD) Ultrasonography

For all studies in this thesis the TCD technique was used to measure cerebral blood flow, with the MCA insonated in each instance. As the MCA provides ~80% of each hemisphere

with blood (Willie *et al.* 2011), it would best represent any variation in perfusion or changes in vascular tone of the respective hemisphere. The following is a brief summary of the protocol used to insonate the MCA, which followed the recommendations of others (Aaslid *et al.* 1982; Stroobant & Vingerhoets 2000; Moppett & Mahajan 2004). The positioning of the probe varies between participants due to anatomical variations of the location and course of the MCA. Regardless, this position is generally in the temporal window above the zygomatic arch (see Figure 3.4 overleaf). Ultrasound gel is applied to both the transducer and the skin to improve conductance. The probe is then fixed to an adjustable headpiece. The probe is then positioned to what would be assumed to be perpendicular to the MCA, depth is then set to 50 mm (Aaslid *et al.* 1982). Once either a faint audible or visual trace is determined, small adjustments to the probe are made to improve signal intensity of the spectral envelope. To ensure the M1 segment of the MCA was insonated, the depth was increased until the terminal ICA was located. At this level the ICA bifurcates to form the MCA and anterior cerebral artery (ACA). Insonation of this bifurcation is evidenced by a negative envelope, as flow in the ACA is away from the probe. Flow in the MCA is demonstrated as a positive velocity and flow is toward the probe (Panerai 2009). Subsequent compression of the ispilateral internal carotid artery was used to further confirm the insonation of the MCA as a reduction in flow velocity is seen during ICA compression. Once this reference point has been located the depth of the probe is reduced to track the M1 segment, usually between 25-50 mm (Willie *et al.* 2011). The MCA produces a unique high-pitched audio signal and a greater velocity than other surrounding arteries. Once an adequate signal was found the probe was fixed in position to maintain a constant insonation angle. During all familiarisation sessions, the MCA was located. The depth, gain, position and angle of the probe were recorded for subsequent experimental trials. The day-

to-day coefficient of variation for the candidate is 4.4%, recorded from 8 participants over 4 days during supine rest.

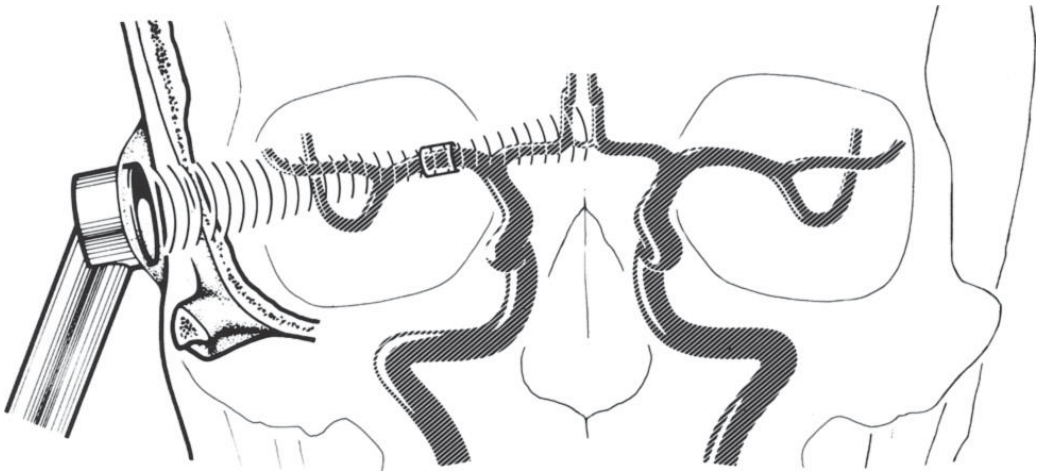

Figure 3.4 Frontal view depicting standard probe placement over the temporal window during MCA insonation (Aaslid et al. 1982). The cylinder around the MCA indicates the segment in which the TCD is insonating. The distance from this cylinder to the transducer is equal to the depth.

3.3.2 Calculation of Mean MCAv

The maximal frequency enveloped, calculated from the summated scatter signals from the moving erythrocytes, which is produced by the TCD system was exported into the recording software (LabChart Software (version 7.3.3), ADInstruments, Colorado Springs, USA). From this envelope the systolic (maximal, SMCAv) and diastolic (minimum, DMCAv) velocities for each cardiac cycle were determined. In all experiments the mean MCAv ($MCAv_{mean}$) was calculated as the integral of the velocity envelope divided by the corresponding pulse interval which accounts for any change in the flow velocity profile that may occur. For instance, at pre-syncope there is a selective reduction in DMCAv (Schondorf *et al.* 1997) that may skew the mean if a weighted calculation was used.

3.3.3 Calculation of Area Under the Curve

Following completion of resistance exercise (**Chapter Five**) and the VM (**Chapter Six**) the area under the curve for $MCAv_{mean}$ was calculated in accordance with the method described by Pruessner *et al.* (2003). This particular method incorporates the increase from the ground (i.e., the 0 on the x axis) and was calculated as follows:

$$AUC_G = \sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i)}{2}$$

Where; AUC_G, is the area under the curve in respect to the ground; m_i, is an individual measurement; n, the number of measurements.

3.3.4 Cerebrovascular Conductance/Resistance

Throughout this thesis an index of cerebral vascular resistance/conductance is required to estimate changes in cerebrovascular tone. Whilst it is recognised that neither conductance or resistance are ideal for representing changes in vascular tone (O'Leary 1991) both indices have their merits. In situations where changes in vascular tone result primarily in changes in flow, conductance gives a superior account of changes in vasomotor tone over resistance (Lautt 1989). Furthermore, during times of high flow rates cerebrovascular conductance has been found to be superior to resistance (Claassen *et al.* 2007). In the peripheral circulation the effect of a given change in resistance on MAP is dependent on the initial resistance, whereas when using conductance a given change in conductance will always produce the same change in MAP when $\dot{Q}$ is constant (O'Leary 1991). Therefore during times where changes in vascular tone result in changes in perfusion pressure, resistance is considered a superior index (Lautt 1989). Moreover, cerebrovascular resistance implies that flow will

cease when pressure equates to 0 mm Hg, however, vessel collapse may terminate flow at pressures above 0 mm Hg; i.e., at the critical closing pressure. Whether cerebrovascular conductance more adequately describes changes in vascular tone is uncertain. For consistency this thesis employed cerebrovascular conductance to reflect changes in vessel tone and was calculated as follows:

$$CVC = MCAv_{mean} / MAP$$

Where: CVC, is cerebrovascular conductance in $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$.

3.3.5 Technique of Photoplethysmography

Guidelines for correct cuff placement have been previously described (Imholz *et al.* 1998) and were followed for the data collection within this thesis. Briefly, the cuff was placed around the middle phalanx of the middle finger. The front unit was secured loosely, so not to occlude flow, around the wrist. The height correction unit was taped to the chest at the level of the right atrium, following the height nulling procedure. The participant's height, weight, gender and age were then entered into the BeatScope software. A manual sphygmomanometer blood pressure reading was then taken to confirm the accuracy of the Finometer. If an inaccurate recording persisted, heating of the hand by either water immersion or an electric blanket was performed until there was adequate agreement between the measurements. If the deficit was not abolished the cuff was changed to a different finger on the same hand. During experimental trials (when possible) manual blood pressure recordings were periodically taken on the contralateral arm to confirm accuracy.

3.3.6 Calculation of Mean, Systolic and Diastolic Arterial Blood Pressures

The accompanying software to the Finometer, BeatScope (v1.1, Finapres Medical Systems B.V., Amsterdam, The Netherlands), filters and corrects the pressure trace accordingly (as discussed in in section 3.2.1). During data collection the continuous blood pressure trace was exported and displayed with an interfaced computer via LabChart Software (ADInstruments) and stored for offline analysis. $\dot{Q}$ and SV data were exported into a text file and saved for offline analysis. From this output systolic and diastolic pressure were determined as the peak and lowest pressure during a cardiac cycle, respectively. MAP was calculated as the integral for each cardiac cycle divided by the corresponding pulse interval (as per the $MCAv_{mean}$ calculation).

From this MAP and the Modelflow method of calculating $\dot{Q}$, TPR was calculated (see Chapter 3.2.3). This measure is a sum of all the peripheral vascular resistance and was calculated by the equation:

$$TPR = MAP/\dot{Q}$$

Where: TPR, total peripheral resistance in mm $Hg \cdot L \cdot min^{-1}$; MAP, mean arterial pressure in mm Hg: $\dot{Q}$, cardiac output in $L \cdot min^{-1}$. As this value is derived from the Finometer's arterial pressure and Modelflow cardiac output, all the methodological considerations presented in the previous sections apply.

3.3.7 Electrocardiogram

For the measurement of heart rate a standard three-lead electrocardiogram (ECG) was used (model ML132, ADInstruments). Electrodes were placed on the left and right clavicles which

were connected to the positive and negative terminals, respectively. The earth was placed on the 7th intercostal space on the left torso. Lead III (earth to positive terminal) was used to register the R-R interval. Before application of ECG electrodes, the skin was cleaned using an abrasive cloth to clear loose skin. The skin was then cleaned with an alcohol swab to remove skin oils. This cleaning process improves the quality of the ECG signal. This signal was visually checked with the interfaced computer using LabChart software (ADInstruments). The software uses the R-R interval using the equation:

$$HR = 60 / \text{R-R interval}$$

Where; HR = Heart Rate in beats·min^{-1} and R-R interval in s.

3.4 Physiological Stressors

3.4.1 Valsalva Manoeuvre (VM)

The measurement of VM pressure was acquired during the studies presented in **Chapters Five** and **Six**. Mouth pressure served as an index of intrathoracic pressure, as used in previous studies (MacDougall *et al.* 1985; Morgan *et al.* 1993), as it reflects changes in oesophageal pressure in a variety of body positions (Goldberg *et al.* 1952; Flemale *et al.* 1988). In both studies the VM was performed in the standing position. Upon forceful exhalation the pressure was measured at the mouthpiece via a transducer that was manually calibrated against a column of mercury.

3.4.2 Lower Body Positive Pressure (LBPP)

The use of LBPP was used to increase MAP in the studies presented in **Chapters Seven** and **Eight**. Participants lay supine in a custom-made LBPP chamber, sealed distal to the iliac crest (Figure 3.5). Pressure was produced via two commercially available vacuum cleaners, measured (in mm Hg) via a pressure transducer mounted within the chamber and controlled via a manual bleed valve.

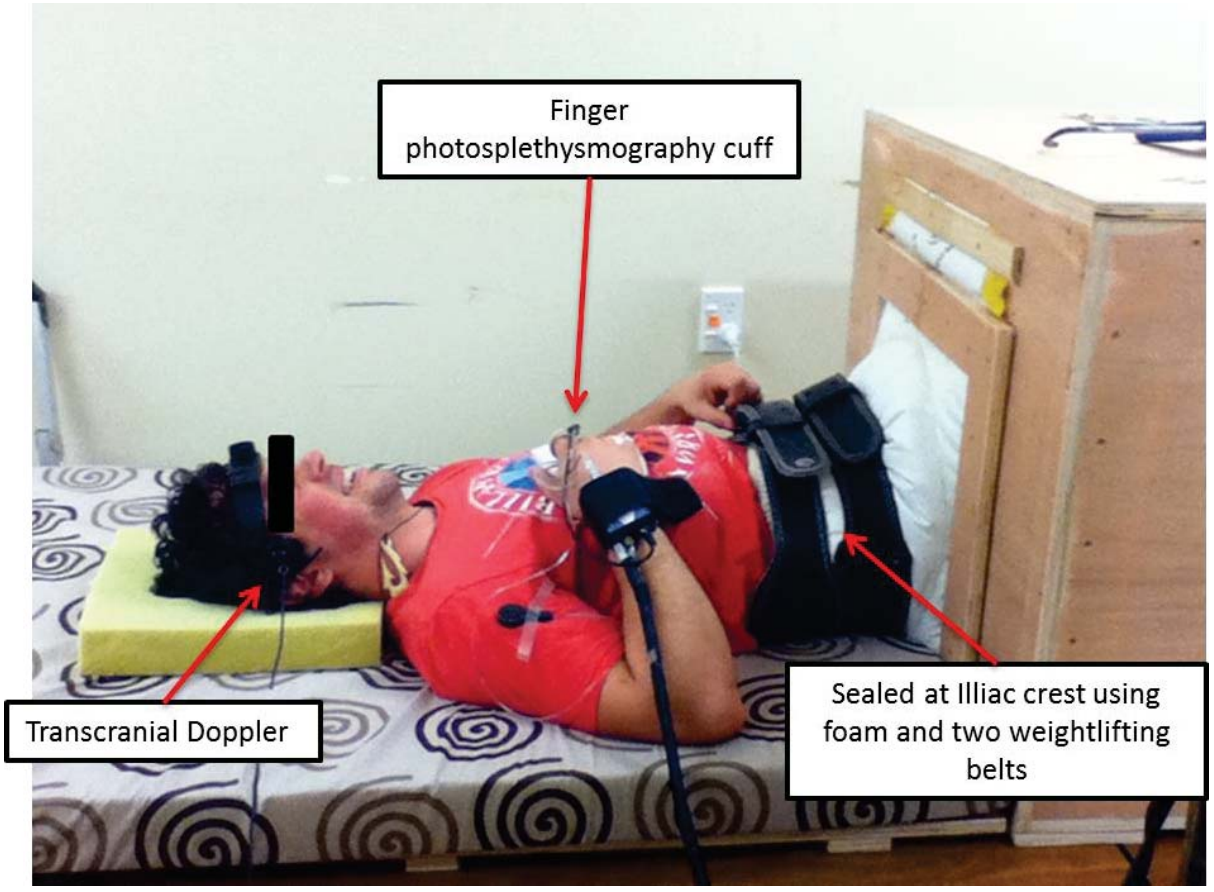

Figure 3.5 Participant laying supine in the custom-made LBPP chamber.

Chapter Four: Research Aims and Hypotheses

The research conducted in this thesis was based on the literature review (**Chapter Two**) which identified gaps in the current literature where further work was required to establish the cerebrovascular response to non-pharmacological changes in MAP in both the dynamic and static setting. For the purpose of this thesis several experimental hypotheses were formulated to examine the relation between BP and CBF and further contribute to the field of cerebrovascular regulation. Accordingly this thesis aimed to investigate how non-pharmacological-induced perturbations in MAP affect MCAv in healthy individuals. In particular these aims addressed 1) how large and dynamic fluctuations induced by simple exercise and everyday tasks can influence MCAv (**Chapters Five** and **Six**) 2) the influence of prolonged steady-state increases in MAP on MCAv when cerebrovascular regulatory mechanisms are intact (**Chapter Seven**) and 3) impaired (**Chapter Eight**). These aims formally tested the hypotheses that MCAv is indeed influenced by changes in MAP in both the dynamic and static setting. Subsequent hypotheses have been formulated for each Experimental chapter and are detailed below.

4.1 Chapter Five

From **Chapter Two** (section 2.5.1) it was clear resistance exercise is capable of producing large changes in MAP that can challenge cerebral perfusion. Moreover, completing resistance exercise in the standing position and the recruitment of the VM are likely to

provide a further challenge to CBF regulation. The purpose of this chapter was to determine the effect of the load and number of repetitions on the cerebro- and cardiovascular responses during upright resistance exercise. Specifically, the influence of the load lifted on the post-exercise arterial blood pressure response following upright resistance exercise and how this affects MCAv. The primary hypothesis for this study was that greater relative loads will induce a greater post-exercise hypotension that would result in a greater pressure passive reduction in CBF. Furthermore, the post-exercise hypotension and cerebral hypoperfusion would be exacerbated by performing more repetitions at any given relative intensity.

4.2 Chapter Six

Results from **Chapter Five** indicated that the VM may contribute to the regulation of CBF both during and immediately following resistance exercise. It appeared that the VM was protective during resistance exercise and limited the increase in MCAv during high MAPs. Whilst the VM contributes to the pressor response during resistance exercise the VM in isolation may also provide a significant challenge for the cerebral circulation especially when standing. For this chapter the purpose was to investigate the effect of graded VMs on the cerebro- and cardiovascular responses initially and during the phase III response following the strain. The hypothesis was that the VM will protect the brain against hyperperfusion initially during the manoeuvre; i.e., the increase in MAP during phase I will be intensity-dependent, however, no change in MCAv would be apparent. In contrast, following the release of the VM (phase III), an increased VM intensity would induce a greater hypotension and be matched by an intensity-dependent concomitant decrease in MCAv.

4.3 Chapter Seven

This thesis aimed to examine the effect of changes in both rapid and prolonged changes in MAP on MCAv. Chapters **Five** and **Six** examined the effect of rapid (dynamic) changes in MAP on MCAv whilst **Chapter Seven** and **Eight** examined the effects of prolonged (static) changes in MAP on the cerebral circulation. Previous evidence using pharmacological perturbations in MAP demonstrated a pressure passive cerebral circulation (**Chapter Two**, section 2.1.3.1); that is steady-state increases in MAP induced proportionate increases in CBF. The hypothesis for this chapter was to examine whether this pressure-passive relation could be replicated using non-pharmacological means. Moreover, LBPP has been shown to increase MAP sufficiently to challenge static cerebral autoregulation. The hypothesis for this chapter was that graded LBPP would induce graded increases in MAP that would be reflected by concomitant increases in MCAv.

4.4 Chapter Eight

Upon review of the literature in **Chapter Two** (section 2.1.3) it was apparent that CO_2 impairs dynamic cerebral autoregulation. However, research investigating whether this applies to static cerebral autoregulation during prolonged non-pharmacological increases in MAP is lacking. The purpose of this chapter was to investigate the effects of prolonged steady-state increases in MAP on CBF when cerebral autoregulatory mechanisms are potentially impaired. Prolonged steady-state increases in MAP were induced by graded LBPP. Impairment of cerebral autoregulation was induced by inhalation of hypercapnia (5% CO_2 in air). The hypothesis for this Chapter was that hypercapnia would impair static

cerebral autoregulation such that increases in MAP, over and above that induced by hypercapnia, will induce further increases in MCAv.

Chapter Five: Haemodynamic Response to Upright Resistance Exercise: Effect of Load and Repetition

Publication based on this Chapter;

Perry BG, Schlader ZJ, Barnes MJ, Cochrane DJ, Lucas SJE & Mündel T (2013). Hemodynamic response to upright resistance exercise: effect of load and repetition. *Med Sci Sports Exerc* DOI: 10.1249/MSS.0b013e3182a7980f

5.1 Introduction

As detailed in **Chapter Two** (section 2.5) resistance exercise has a pronounced effect on the cardiovascular system, although how this in turn affects the cerebral circulation has not been investigated in detail. Two key components of resistance training that determine training volume are the load lifted and the number of repetitions. However, the cerebro- and cardiovascular response to manipulation of these variables has not been described. This chapter investigated the effect of the load and number of repetitions on the haemodynamic response during and following upright resistance exercise.

Resistance training is a common mode of exercise due to its positive effects on muscular strength, cardiovascular function, metabolism and psychological well-being (Garber *et al.* 2011). Furthermore, many clinical populations including those with cardiovascular disease are now participating in resistance type training (Pollock *et al.* 2000). However, resistance exercise can produce extremely high blood pressures with systolic and diastolic values as

high as 480 and 350 mm Hg, respectively, being reported (MacDougall *et al.* 1985). Paradoxically, hypotension and cerebral hypoperfusion has also been observed following heavy, upright resistance exercise (Compton *et al.* 1973; Romero & Cooke 2007; Moralez *et al.* 2012). However, no research has examined the initial post-exercise blood pressure response to upright resistance exercise, and how this subsequently affects CBF.

Studies investigating the haemodynamic response during resistance exercise have utilised a leg-press type movement (Romero & Cooke 2007; Moralez *et al.* 2012), static/isometric type exercise (Mitchell *et al.* 1980; Ogoh *et al.* 2010b) or both (Lewis *et al.* 1985). Squatting exercise, with additional load, likely poses a further challenge via its orthostatic component, as, for example, body-weight squats have been found to significantly challenge dynamic cerebrovascular control (Claassen *et al.* 2009). In fact, standing alone causes a ~15% reduction in MCAv (Pott *et al.* 2000). Further, the post-exercise hypotension may be exacerbated by the execution of a Valsalva manoeuvre (VM) during exercise (Compton *et al.* 1973), which is recruited at higher relative loads (MacDougall *et al.* 1992). Thus, the upright posture and the recruitment of the VM may put individuals at greater risk of cerebral hypoperfusion following load-bearing squatting type movements due to large reductions in MAP. However, the effect of load lifted and/or the number of repetitions on the post-exercise hypotension and the subsequent influence of this reduction in CPP on MCAv have not been examined to date.

Therefore, the purpose of this chapter was to investigate the influence of the load and number of repetitions on the cardio- and cerebrovascular responses during repeated squatting exercise. Specifically, the influence of the load lifted on the post-exercise arterial blood pressure response following squatting exercise and how this subsequently affects

cerebral blood flow (indexed via velocity measures). The primary hypothesis for this chapter was that greater loads will induce a greater post-exercise hypotension that would result in a greater pressure-passive reduction in MCAv.

5.2 Methods

5.2.1 Participants

Twelve healthy, resistance trained, males were recruited for the study (mean ± SD: age, 26 ± 5 y; body mass, 94 ± 13 kg; height, 184 ± 8 cm; resistance training age 4.3 ± 3.0 y; 6 RM, 104 ± 19 kg). Each participant was fully informed of all potential risks and experimental procedures, after which informed written consent was obtained. All experimental procedures and protocols were approved by the Massey University Human Ethics Committee and performed in accordance with the *Declaration of Helsinki*. All participants were free from cardiovascular (including orthostatic hypotension and recurrent syncope) and cerebrovascular disease and were not taking medication. Participants arrived at the laboratory for the familiarisation and both experimental trials having abstained from strenuous exercise, alcohol, caffeine and nicotine for at least 24 hours.

5.2.2 Study Design

Participants visited the laboratory on three occasions, one familiarisation and two experimental trials at the same time of day. During the familiarisation session the participants were familiarised with all experimental procedures, including ideal squatting technique, and their 6 repetition maximum (RM) was determined. The squatting exercise was conducted on a fixed barbell path (Smith) machine (FitnessWorks, New Zealand) with

the participants wearing an adjustable front squat harness (Getstrength.com, New Zealand), which was fitted over the shoulders and rested on the chest and abdomen. This harness included two outward projecting metal pins that were located just inferior to each clavicle and were used to support the Smith machine barbell (see Figure 5.1). Collectively, the harness and Smith machine allowed for the squats to be executed without the participants holding the barbell (left hand free; refer to Figure 5.1), allowing for the measurement of finger blood pressure (see measurements). All participants were required to squat to a depth that was equivalent to the point at which the femur was parallel to the floor, which was confirmed via quantification of barbell displacement. Participants were instructed to breathe normally through a custom-made mouthpiece apparatus (detailed in Chapter 5), which allowed for the measurement of end-tidal expirate and facilitated a VM, if required. Participants were instructed to only perform this modified VM if they saw fit and to maintain normal ventilation otherwise. When a VM was required, participants exhaled forcefully through the mouth, which temporarily closed a valve in the mouthpiece apparatus to allow for the VM to be completed during the squat. Mouth pressure served as a surrogate for intrathoracic pressure (MacDougall *et al.* 1985; Morgan *et al.* 1993) and accurately reflects changes in oesophageal pressure (surrogate for intrathoracic pressure) in a variety of postures (Flemale *et al.* 1988). All participants were instructed to avoid hyperventilation immediately preceding each set.

Figure 5.1 Photo depicting the squatting movement used in this experiment at starting point of the movement (left) and deepest point of the squat (right).

5.2.3 Experimental Protocol

The experimental design and protocol is shown in Figure 5.2. The two experimental trials were randomised with one trial consisting of 2 repetitions and the other of 6 repetitions of 30, 60 and 90% of the 6 repetition maximum (RM) load. The order in which each load was lifted within each trial was also randomised. Both trials were conducted after 10 am in the morning as the efficacy of cerebrovascular regulation during orthostasis has been shown to be lower before this time of day (Lewis *et al.* 2010). Further, participants replicated the pre-trial meal to exclude any confounding dietary variables. First, participants stood for 2 minutes during which baseline measures were obtained. This was followed by body-weight squats being performed, the number of which was equal to the number of squats performed during the following work set. This allowed for the randomisation of all loads and excluded the possibility of participants having to complete the 90% set without a warm-up. A stable baseline period was established and recorded before each work set. During each work set verbal confirmation of adequate squat depth was given. Participants were instructed to maintain a 2 s down, 2 s up pace during all sets. After completing all sets,

participants were instructed to stand for 2 minutes, as still as possible and to avoid muscle-tensing, as this has been previously shown to restore circulatory stability (Van Lieshout *et al.* 2001; Krediet *et al.* 2002).

5.2.4 Measurements

Blood flow velocity in the right middle cerebral artery (MCAv) was measured using a 2-MHz pulsed Doppler ultrasound system (DWL, Compumedics Ltd, Germany, refer to **Chapter Three** section 3.3.1). Participants breathed through the aforementioned modified mouthpiece, that allowed both the measurement of mouth pressure (substitute for intrathoracic pressure) and the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$; gas analyser model ML206, ADInstruments, Colorado Springs, USA).

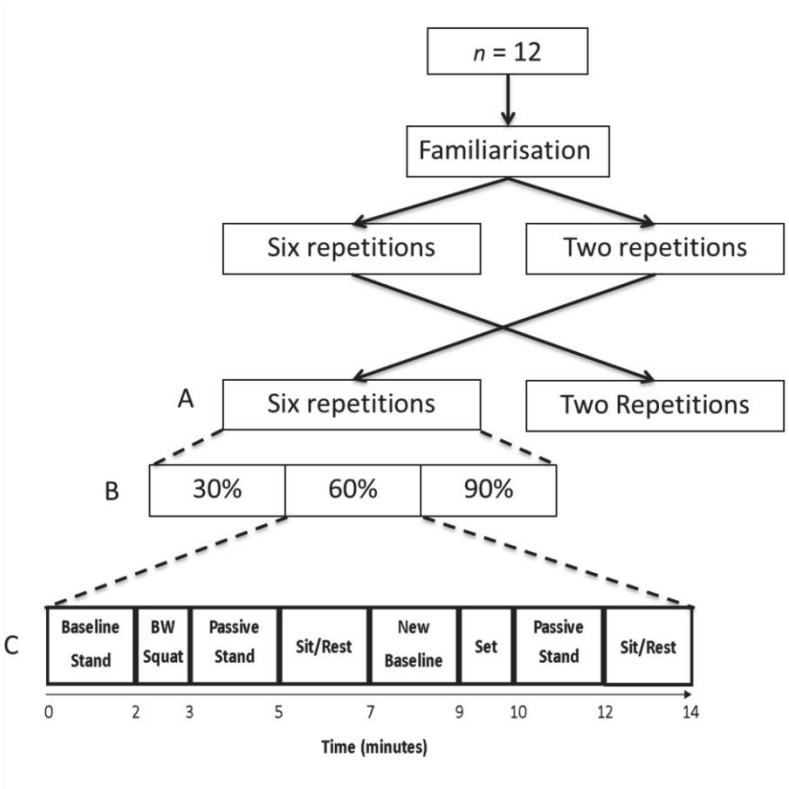

Figure 5.2 Experimental protocol. Each participant completed both six and two repetition trials (A); within each trial, each participant completed all relative loads (B) in a fully

counter-balanced order. For each relative load, each participant would complete the rotation C. BW, body weight; Set, work set equivalent to 30, 60 or 90% of the 6-RM load.

Blood pressure was measured non-invasively using finger photoplethysmography (see section 3.2, Finapres Medical Systems, Biomedical Instruments, Amsterdam, The Netherlands) and heart rate was measured via three-lead electrocardiogram (ADInstruments). Squatting depth, via barbell displacement, was recorded using a potentiometer (Model 533, Vishay, Malvern, USA) mounted to the upper cog of the Smith machine. All data were acquired continuously via an analogue-to-digital converter (PowerLab ML870; ADInstruments) at 1KHz. Data were displayed in real time and recorded for off-line analysis using commercially available Lab Chart software (v7.3.3, ADInstruments).

Chapter Three (section 3.3) details the methods for the calculation of $\dot{Q}$, MAP, $MCAv_{mean}$, TPR and CVC**.** Relative to baseline measures, the percentage decrease in $MCAv_{mean}$ was divided by the percentage reduction in MAP at $MCAv_{mean}$ nadir to assess differences in the MAP contribution to the $MCAv_{mean}$ reduction.

5.2.5 Data Analyses

Baseline data were acquired in the second minute of each passive stand between sets and presented as the mean across that minute. All variables upon the attainment of the highest $MCAv_{mean}$ (peak) during each repetition within a set were recorded and the average peak values were used in the analysis for exercise. Following each set, i.e., after racking the barbell (Time = 0, as in Thomas et al. (2009)), time to nadir, recovery and peak values for $MCAv_{mean}$ and MAP were used in the post-exercise analysis. Nadir was defined as the lowest

measured value immediately following the completion of the set, recovery as the point when the variable returned to baseline following the nadir, and peak as the maximum point following the racking of the barbell and before the subsequent seated rest. Additionally, the area under the curve (AUC, Refer to **Chapter Three**, section 3.3.2) for data following exercise (from time = 0 to 15 s post exercise) was calculated as previously described (Pruessner *et al.* 2003).

5.2.6 Statistical Analyses

All dependent variables were analysed using a two-way (repetition x load, 2 x 3) repeated measures ANOVA. Data within the 6-repetition trials were analysed using a load by time (2 x 6) repeated-measures ANOVA. Data for the 30-s period immediately post-exercise within each repetition trial was compared to the pre-exercise baseline using an ANOVA for repeated measures of time and load. Data were assessed for approximation to a normal distribution and sphericity with no corrections required. Main effects were isolated using *post-hoc* pairwise comparisons (Bonferroni corrected, where necessary). All data were analysed using SPSS statistical software (v20, IBM, New York, USA) with *a priori* statistical significance set at $P \leq 0.05$. All data are presented as the mean (± SD) absolute and/or relative change from the baseline preceding the respective work set, unless denoted otherwise.

5.3 Results

5.3.1 Haemodynamic Responses During Upright Resistance Exercise

Average peak $MCAv_{mean}$ values were elevated 31 ± 16% from baseline ($P < 0.001$), and this was consistent between loads ($P = 0.74$) and repetitions ($P = 0.89$). For the six repetition sets, peak $MCAv_{mean}$ remained unchanged across all repetitions ($P = 0.61$), despite MAP increasing as the set progressed in both the 30 and 60% sets ($P = 0.003$). Heart rate was higher at greater loads ($P = 0.001$) and repetitions ($P < 0.001$); specifically, 98 ± 4 vs. 108 ± 4 beats·min^{-1} for 2 and 6 repetitions at 30%, respectively; 106 ± 4 vs. 117 ± 4 beats·min^{-1} for 2 and 6 repetitions at 60%, respectively, and 112 ± 5 vs. 129 ± 3 beats·min^{-1} for 2 and 6 repetitions at 90%, respectively. Due to loss of the blood pressure trace only 30 and 60% loads were compared ($n = 6$), since participants could not refrain from placing pressure on the finger used for the measurement of blood pressure whilst lifting the 90% load, resulting in erroneous data at this workload in 9 participants. When peak values for a repetition were not suitable the data were excluded from the exercise (only) values. When applicable the physiocal (Finometer automatic calibration) procedure was then performed to allow the use of MAP data (and therefore $\dot{Q}$ and TPR) following exercise. Exercise-induced increases in MAP were greater during the 60% compared to the 30% set ($P = 0.015$) and during 6 repetitions ($P = 0.002$ vs. 2 repetitions); specifically, 122 ± 9 vs. 135 ± 11 mm Hg for 2 and 6 repetitions at 30%, respectively; and 128 ± 13 vs. 143 ± 14 mm Hg for 2 and 6 repetitions at 60%, respectively (Figure 5.3 and 5.4). In 3 participants a satisfactory blood pressure trace for both 90% sets was acquired for all repetitions with an average peak (± SD) of 150 ± 2 and 176 ± 6 mm Hg for 2 and 6 repetitions, respectively. Systolic blood pressure was dependent on the number of repetitions ($P = 0.001$) but not intensity ($P = 0.49$) with pressures during

the 60% set: 169 ± 19 and 194 ± 30 mm Hg, for 2 and 6 repetitions respectively, and during the 30% load: 168 ± 15 and 189 ± 18 mm Hg, for 2 and 6 repetitions, respectively. Diastolic blood pressures were dependent upon the number of repetitions (P = 0.012) and also on load (P = 0.033), with pressures during the 60% work load: 108 ± 13 and 118 ± 12 mm Hg, for 2 and 6 repetitions respectively, and during the 30% load: 99 ± 12 and 108 ± 13 mm Hg, for 2 and 6 repetitions respectively. During all sets $P_{ET}CO_2$ was reduced from baseline (32 ± 2 mm Hg) on average by 4 ± 4 mm Hg (all $P < 0.05$), whilst there were no significant differences between loads or repetitions (all $P > 0.10$). All participants performed a VM during both 90% repetition sets, with an average mouth pressure of 42.6 ± 6.2 and 43.6 ± 10.3 mm Hg for the 2 and 6 repetition sets, respectively. The VM was not recruited during the 30 and 60% 6-RM sets.

5.3.2 Cerebrovascular and Cardiorespiratory Variables at $MCAv_{mean}$ Nadir

Cerebrovascular and cardiorespiratory data at $MCAv_{mean}$ nadir during the passive stand are reported in Table 5.1. Briefly, the 90% load produced the largest reduction in $MCAv_{mean}$ (for P values see Table 5.1). These differences in $MCAv_{mean}$ were not mediated by differences in $P_{ET}CO_2$, but by a greater reduction in diastolic flow velocity at nadir. The 60% load tended to reduce $MCAv_{mean}$ to a greater degree than the 30% load (P = 0.069). The magnitude of the $MCAv_{mean}$ decrease was only significantly different between of the number of repetitions at the 30 and 60% relative loads. At $MCAv_{mean}$ nadir, the reductions in MAP were also load-dependent as were the reductions in both systolic and diastolic blood pressure (for P values see Table 5.1). The ratio of % change in MCAv versus % change in MAP was not significantly different between loads (P = 0.11) or repetitions (P = 0.48; grouped means: 1.1 ± 1.2, 2.6 ± 3.4 and 1.1 ± 0.6 for 30, 60 and 90% load respectively). Time to $MCAv_{mean}$ nadir was 6.7 ± 5.2

s without any significant difference between repetitions or loads (all $P > 0.10$). Due to the loss of data files, TPR and $\dot{Q}$ data for the 2 repetition trials were unable to be used for the analysis, therefore only comparisons of these variables between the loads in the 6 repetition trials were made ($n = 7$). TPR was reduced by 48 ± 17% at $MCAv_{mean}$ nadir, with no significant differences between loads ($P = 0.31$), whilst $\dot{Q}$ increased 51 ± 15% from baseline following the 90% load which was significantly lower than both the 60% (84 ± 32%; $P = 0.019$) and 30% loads (104 ± 38%; $P = 0.029$).

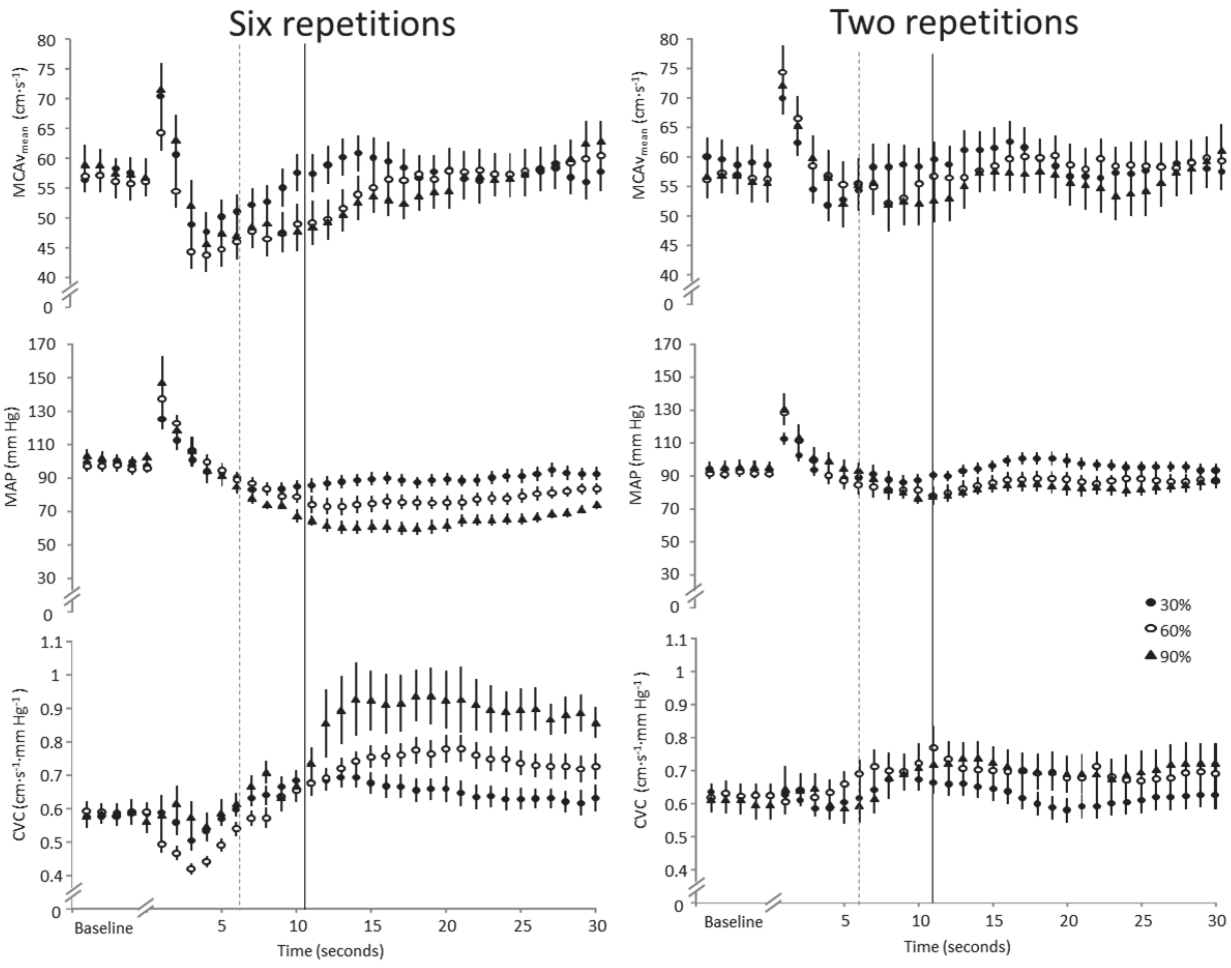

Figure 5.3 Average response of mean middle cerebral artery blood flow velocity ($MCAv_{mean}$), mean arterial pressure (MAP) and cerebrovascular conductance (CVC) following upright resistance exercise displayed every second. The peak values represent the last repetition of the respective bout of exercise. The vertical dashed and solid lines represent the grouped means for the time to $MCAv_{mean}$ and MAP nadir, respectively. These data illustrate the clear

distinction between the time of $MCAv_{mean}$ and MAP nadir, with $MCAv_{mean}$ returning towards baseline values before MAP nadir occurs. All values mean ±SE.

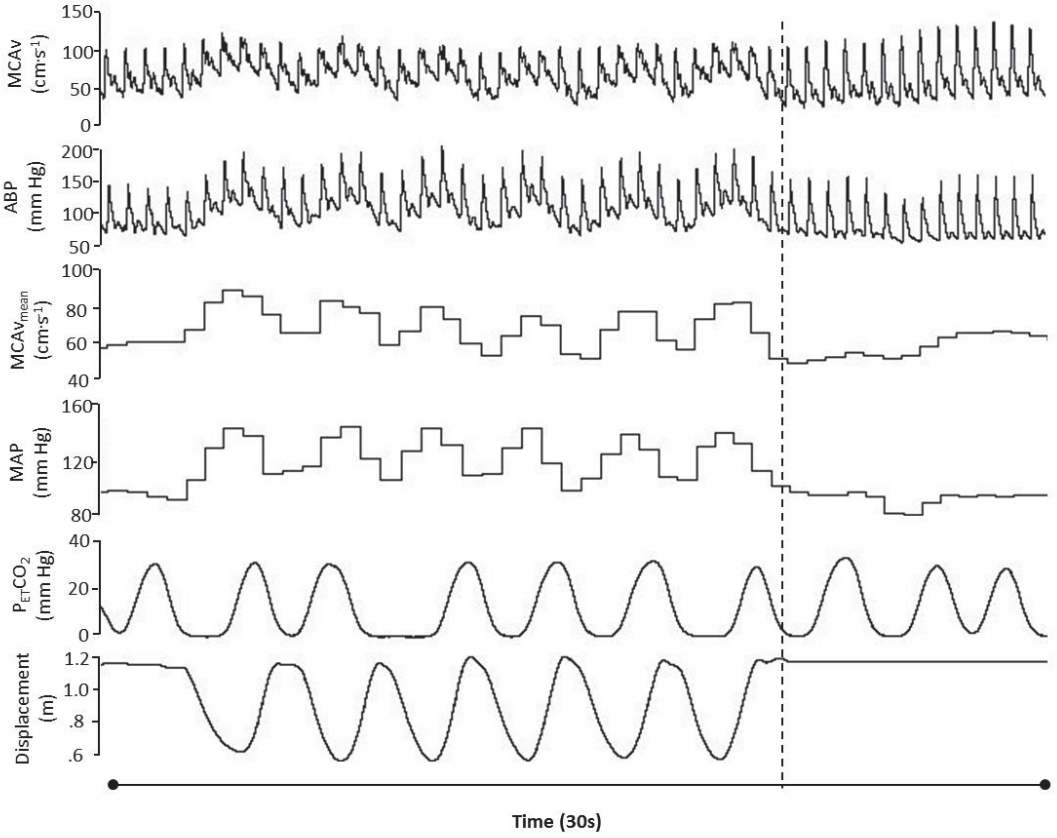

Figure 5.4 Raw and averaged haemodynamic responses to 6 repetitions at 30% 6RM in one participant. The dashed horizontal line represents the completion of the set and racking of the bar. MCAv, raw middle cerebral artery blood flow velocity; ABP, raw arterial blood pressure trace; $MCAv_{mean}$, mean middle cerebral artery blood flow velocity; MAP, mean arterial pressure; PET CO2; partial pressure of end tidal CO2.

5.3.3 Cerebrovascular and Cardiorespiratory Variables at MAP Nadir

Cerebrovascular and cardiorespiratory data at MAP nadir during the passive stand immediately following the exercise are reported in Table 5.2 (also see Figure 5.3). Briefly, the 90% load caused the greatest reduction in MAP following exercise. $MCAv_{mean}$ was significantly greater at MAP nadir than $MCAv_{mean}$ nadir for all loads and repetitions (all $P <$ 0.05). Overall, MAP nadir occurred later than $MCAv_{mean}$ nadir for all loads and repetitions (all $P \leq 0.05$). Specifically, whilst time to MCAv nadir was similar across the different loads

and repetitions (reported above), MAP nadir was influenced by load and repetitions with a higher percentage of 6 RM increasing the time to nadir ($P = 0.05$, see Figure 5.3).

5.3.4 Recovery Following Exercise

$MCAv_{mean}$ recovery was load-dependent ($P = 0.002$), with the greatest time to recovery taken following the 90% 2- and 6-repetition sets (17.5 ± 8.7 s and 14.8 ± 6.9 s, respectively); while the number of repetitions did not alter the time course of this response ($P = 0.572$). When data was pooled into 1-s bins (Figure 5.3) and analysed it was revealed for $MCAv_{mean}$ that there were significant main effects of time (both $P <0.001$), load ($P = 0.019$ and $P = 0.025$ for 2 and 6 repetitions, respectively) and time by load interactions (both $P < 0.001$). *Post hoc* analyses indicated that the time periods for which $MCAv_{mean}$ was below baseline levels following the 6 repetition set for 30% was 2 s (4-6s), for 60% was 6 s (3-9s) and for 90% was 10 s (4-14 s) (all $P < 0.01$). For the 2 repetition set, *post hoc* analyses indicated that $MCAv_{mean}$ was not reliably below baseline (all $P > 0.05$). In support of this, the AUC analysis for MCAv indicated a significant effect of load for 6 repetitions ($P = 0.05$, -26 ± 97, -96 ± 97, -118 ± 52 aU for 30, 60 and 90% loads, respectively); however, this effect was not significant for 2 repetitions ($P = 0.50$). Time to $MCAv_{mean}$ peak was significantly slower at greater loads ($P < 0.001$) and higher repetitions ($P < 0.001$).

Time to MAP recovery was delayed by both the load ($P < 0.001$) and number of repetitions ($P = 0.01$) with the slowest being the 90% 2- and 6-repetition sets (46.5 ± 20.7 and 49.9 ± 12.9 s, respectively). For MAP there were main effects of time (both $P < 0.001$) and time by load interactions ($P = 0.019$ and $P < 0.001$ for 2 and 6 repetitions, respectively). *Post hoc* analyses revealed the time period below baseline following 6 repetition sets for 30% was 16s (6-22s), for 60% was 22s (8-30s) and for 90% was 24s (6-30s) (all $P < 0.01$). The

hypotensive response following 2 repetitions was shorter and only significantly below baseline in the 30% set for 5s (9-14s) and in the 60% and 90% sets for 7s (8–15s) (all $P < 0.01$).

Table 5.1 Changes from baseline at MCAv nadir for 30, 60 and 90% 6RM loads.

Variable	Baseline	Repetitions	Δ From baseline			*P* values		
			30%	60%	90%	Load	Reps	L x R
$MCAv_{mean}$, $cm \cdot s^{-1}$	57 ± 10	2	-8±7 (-13±11)§	-11±8(-19±14)$^{+}$§	-18±6(-32±10)‡	<0.001	0.016	0.077
		6	-13±6(-23±12)	-19± 8(-32±12)$^{+}$	-18± 7(-31±8)‡			
Systolic MCAv, $cm \cdot s^{-1}$	94 ± 16	2	16±8(19±9)	19±12(22±14)	15±10(15±11)	0.84	0.10	0.40
		6	21±13(24±16)	19±11(22±12)	21±11(24±14)			
Diastolic MCAv, $cm \cdot s^{-1}$	42 ± 8	2	-20±10(-43±15)	-20±12(-46±25)§	-25±8(-61±21)‡	0.003	0.041	0.064
		6	-19±6(-47±16)	-28±9(-67±22)	-26± 9(-65±22)‡			
CVC, $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$	0.61 ± 0.11	2	0.04±0.07(6±11)	0.06±0.10(8±16)§	0.00±0.14(1±23)	0.26	0.05	0.017
		6	-0.06±0.12(-9±19)	-.013±0.10(-20±15)	0.02±0.10(-6±18)			
MAP, mm Hg	96 ± 11	2	-16±10(-16±11)	-17±11(-19±14)	-31±12(-30±10)‡†	<0.001	0.59	0.39
		6	-18±13(-17±14)	-17±11(-17±11)	-37±6(-37±8)‡†			
Systolic BP, mm Hg	137 ± 15	2	-14±21(-11±16)	-23±27(-17±22)	-43±25(-29±16)‡†	0.007	0.55	0.53
		6	-13±24(-9±18)	-11±27(-7±22)	-53±14(-31±14)‡†			
Diastolic BP, mm Hg	75 ± 11	2	-16±5(-20±7)	-19±11(-21±13)	-29±15(-31±10)‡†	<0.001	0.30	0.99
		6	-20±10(-24±12)	-20±6(-24±8)	-29±6(-33±7)‡†			
HR, $beats \cdot min^{-1}$	81 ± 14	2	22±5(29±11)§	28±8(36±15)*§	31±12(40±22)‡§	0.003	<0.001	0.21
		6	34±9(44±12)	38±13(46±17)*	48±16(61±26)‡			
$P_{ET}CO_2$, mm Hg	32 ±3	2	-4±6(-13±19)	-3±7(8±23)	-4±5(-12±17)	0.30	0.37	0.19
		6	-2±4(-6±16)	-4±4(-15±14)	-6±5(-19±15)			

Values are absolute mean difference from baseline ± SD and percentage change from baseline values (± SD) are denoted in parentheses. MCAv, middle cerebral artery velocity; CVC, cerebrovascular conductance; MAP, mean arterial pressure; BP, blood pressure; HR, heart rate; $P_{ET}CO_2$, partial pressure of end tidal carbon dioxide; Reps, Repetitions; R x L; Repetitions X load interaction; ‡ Statistically different from 30%, $P \leq 0.05$; † Statistically different from 60%, $P \leq 0.05$; § Statistically different from 6 repetitions, $P \leq 0.05$; *Trend for a difference between 90 and 60% $P = 0.076$; $^{+}$Trend for a difference between 30 and 60% $P = 0.069$. For all blood pressure data $n = 8$.

Table 5.2 Hemodynamic changes from baseline at MAP nadir for 30, 60 and 90% 6RM loads.

Variable	Baseline	Repetitions	Δ From baseline			*P* values		
			30%	60%	90%	Load	Reps	L x R
$MCAv_{mean}$, $cm \cdot s^{-1}$	57 ± 10	2	-5±8(-8±10)	-5±6(-8±10)	-9±5(-25±12)‡	0.047	0.46	0.071
		6	-9±9(-16±16)	-10±9(-16±14)	-16±8(-15±6)‡			
Systolic MCAv $cm \cdot s^{-1}$	94 ± 16	2	17±8(20±9)	25±8(28±9)	20±11(21±13)	0.38	0.97	0.002
		6	24±13(26±14)	28±11(30±10)	30±9(33±13)			
Diastolic MCAv, $cm \cdot s^{-1}$	42 ± 8	2	-18±11(-36±15)	-12±6(-28±12)	20±9(-45±15)	0.59	0.29	0.75
		6	-16±9(-38±23)	-20±20(-42±43)	19±7(-44±10)			
MAP, mm Hg	96 ± 11	2	-18±11(-18±10)	-21±12(-22±11)‡	-34±7(-34±7)‡§	0.005	0.001	<0.001
		6	-21±9(-21±9)	-27±9(-28±9)‡	-43±5(-43±5)‡			
CVC, $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$	0.61 ± 0.11	2	0.080±0.072(13±10)	0.10±0.091(16±12)‡	0.069±0.12(11±16)‡†§	0.027	0.029	0.063
		6	0.026±0.13(6±20)	0.069±0.11(12±16)‡	0.22±0.08(44±18)‡†§			

Values are absolute mean difference from baseline ± SD and percentage change from baseline values (± SD) are denoted in parentheses. MCAv, middle cerebral artery velocity; MAP, mean arterial pressure; CVC, cerebrovascular conductance; Reps, Repetitions; R x L; Repetitions X load interaction; ‡ Statistically different from 30%, $P \leq 0.05$; † Statistically different from 60%, $P \leq 0.05$; § Statistically different from 6 repetitions, $P \leq 0.05$.

5.4 Discussion

The main findings from this chapter were: 1) The $MCAv_{mean}$ response during upright squatting resistance exercise was similar regardless of load and number of repetitions; whereas 2) Blood pressure increased progressively with increased exercise load and repetition; 3) Following upright squatting exercise, the reduction in $MCAv_{mean}$ was mediated largely by reductions in diastolic MCAv, and was dependent on the load lifted and number of repetitions, and 4) The MAP response following such exercise was dependent upon the load lifted and number of repetitions. As $MCAv_{mean}$ was unchanged between exercise loads, despite the prevailing increasing MAP, regulatory mechanisms (e.g., myogenic and/or mechanical) appear to adequately account for these increases in perfusion pressure. However, immediately following repeated upright squatting exercise the cerebral vasculature alone was unable to actively counteract the rapid reduction in MAP such that proportionate reductions in MCAv ensued.

5.4.1 MAP Response During Upright Resistance Exercise.

At rest, the $MCAv_{mean}$ response to MAP oscillations is dominated by the relationship between the change in MAP and the change in time (Tzeng *et al.* 2011), and appears to extend to exercise. During resistance exercise (Edwards *et al.* 2002; Romero & Cooke 2007) and rowing (Pott *et al.* 1997) MAP perturbations are too rapid to be counteracted by dynamic cerebrovascular control, as the brain's vasculature is more successful at damping low frequency MAP oscillations (Zhang *et al.* 1998a). The observations here of increased $MCAv_{mean}$ from baseline during the exercise is consistent with these previous reports (Romero & Cooke 2007). Interestingly however, there was no difference in the magnitude of

that increase between loads or repetitions despite the load-dependent nature of the MAP response. Further, within the 6 repetition sets peak $MCAv_{mean}$ remained unchanged across the set, again, despite the increasing MAP as the set progressed. However, these observations assume that MCA diameter is unchanged despite the very high arterial pressures.

5.4.2 The Restraint of MCAv During Resistance Exercise

Animal models propose a graded sympathetic response to acute hypertension that may restrain CBF (Cassaglia *et al.* 2008). If this protective mechanism extends to humans, one would expect a constriction of vessels downstream of the MCA and a reduction (or a restricted increase) in cerebral perfusion at very high MAP. If applicable to humans, this may explain the restrained $MCAv_{mean}$ during higher blood pressures at the greater loads. Also, if extremely high blood pressures do indeed produce a sympathetic mediated change in vessel tone, the influence of this activation on the large conduit vessels is also unclear. Nevertheless, if MCA diameter were to decrease via a sympathetic activation, an increase in flow velocity would be expected; however, MCAv was similar across all the exercise conditions. Thus, this potential issue seems unlikely to influence the findings from this chapter.

The restraint of MCAv during exercise may also be attributable to recruitment of the VM during the highest load, since increases in thoracic pressure during a VM are translated to the cerebrospinal fluid such that an increase in intracranial pressure (ICP) ensues (Greenfield *et al.* 1984). A rise in ICP can minimise the change in transmural pressure and thus any increase in perfusion pressure will be reduced, reflected in only a modest increase in $MCAv_{mean}$ (Tiecks *et al.* 1995b). Moreover, the VM produces large increases in CVP (Pott *et*

al. 2000) that may reduce the pressure difference across the cerebral circulation. The internal jugular vein is responsible for venous drainage whilst supine, however upon standing the internal jugular vein collapses and blood is drained from the brain predominantly via the vertebral venous plexus (Gisolf *et al.* 2004a). The collapsed internal jugular vein may increase cerebral outflow resistance (i.e., a Starling resistor) that may be opened by an increase in CVP like that seen during a VM, however, this may require a prolonged increase in pressure to distend (Pott *et al.* 2003). The increase in CVP would reduce venous outflow and via Darcy's law reduce flow through the cerebral circulation. Another possible explanation is that the large transient increases in MAP exceed the maximal rate of cerebral vessel dilation and no further increase via changes in MAP were translated into further increases in $MCAv_{mean}$. The additional mechanical effect of the VM and possibility of sympathetic activation may also contribute to dynamic cerebrovascular control during such exercise, particularly at higher relative loads.

5.4.3 The Reduction in $MCAv_{mean}$ is Due to a Selective Decrease in Diastolic Flow Velocity.

Dynamic cerebral autoregulation (CA) is reported to be maintained during both static (Ogoh *et al.* 2010b) and dynamic exercise (Ogoh *et al.* 2007). Furthermore, while dynamic CA has been reported to be more effective at safeguarding against hypertension than hypotension during both rest (Tzeng *et al.* 2010b) and exercise (Ogoh *et al.* 2007), the blood pressure fluctuations and subsequent MCAv response following the type of activity examined in the present study has not been previously described. Data from this chapter indicates that this selective reduction in diastolic flow velocity following upright resistance exercise is the main

contributor to the reduction in MCAv. This may occur as a result of increased upstream resistance and this response is strikingly similar to that seen at syncope (Schondorf *et al.* 1997). Furthermore syncope has been reported following maximal Olympic style lifts (Compton *et al.* 1973). In addition, $MCAv_{mean}$ recovery was load-dependent and remained below baseline for the longest period (~15 s) at the highest relative load (90%). This was supported by all of the approaches used here to analyse the data i.e. time-to-recovery, time below baseline value and area under the curve. Thus, higher loads caused a greater and more sustained drop in $MCAv_{mean}$.

The magnitude of the hypotension observed in the present study is also likely exacerbated at higher loads via the recruitment of the VM. During static exercise the blood pressure response can be dominated by the VM (Pott *et al.* 2003), the results found here support this notion for dynamic upright resistance exercise. The additive effect of the increased load and recruitment of the VM produced a significantly greater hypotension, and the largest decrease in MCAv, following exercise at the 90% 6-RM load. The hypotension following the release of the VM (phase III) reported by Pott *et al.* (2000) was similar to that observed presently after the 90% sets, and at mouth pressures similar to those produced in the present study (~40 mm Hg). The release of the VM and cessation of exercise is likely to have coincided with lower limb hyperaemia and thus have an additive hypotensive effect.

5.4.4 The Role of Arterial CO_2 During and Following Heavy Resistance Exercise.

$P_{ET}CO_2$ was reduced following all trials but not significantly between trials (i.e., no effect of load or repetitions, see Table 5.1). However, during exercise and possibly for some time afterward (due to circulation time) $P_{ET}CO_2$ may not accurately reflect arterial PCO_2 (P_aCO_2) because of both the recruitment of the VM and the effect of thoracic loading-altered

ventilation and/or pulmonary perfusion patterns. For these reasons the accuracy of $P_{ET}CO_2$ as an index of P_aCO_2 during upright resistance exercise with concomitant thoracic loading may be limited. Although the MAP and $P_{ET}CO_2$ response was not altered by the number of repetitions, more repetitions at the 30 and 60% loads appeared to reduce $MCAv_{mean}$ following exercise (Table 5.1). The load-dependent reductions in MCAv reflect greater reductions in MAP as there was no significant difference in the ratio of the relative changes in MCAv and MAP at $MCAv_{mean}$ nadir. However as there was no difference in the reduction in MAP between repetitions following 30 and 60% loads (Table 5.1), the discrepancy in MCAv is likely due to P_aCO_2 differences that were not reflected by the $P_{ET}CO_2$ values. This may reflect the greater metabolic stress during 6 repetitions, which is supported by the higher heart rates during and after these sets.

5.5 Conclusion

Immediately following upright resistance squatting exercise changes in MAP and $MCAv_{mean}$ are dependent on the load lifted and the number of repetitions. $MCAv_{mean}$ was elevated similarly across all exercise loads regardless of the differential increase in MAP during exercise. The mechanically induced changes in ICP as a result of the VM may contribute to the regulation of CBF during resistance exercise and explain this restraint despite the greater MAPs. The large oscillations in MAP induced by squatting exercise and the large decrease immediately following exercise reflect the 'high pass filter' characteristics of the cerebral circulation. This was most apparent at higher loads, where the VM was recruited, which produced the greatest reductions in both MAP and MCAv. The decrease in $MCAv_{mean}$ during the post-exercise hypotension was mediated via a selective reduction in diastolic

flow velocity and was consistent with the hypothesis tested in this study. Finally, dynamic cerebral autoregulatory processes rectified MCAv within the same time frame regardless of the magnitude of the drop in MCAv, and the magnitude *and* time course of the reduction in MAP. The results of this chapter indicated a potential protective effect of the VM during resistance exercise. The proceeding chapter investigated the role of the VM in the regulation of CBF. In particular the cerebrovascular response to graded VMs performed in isolation.

Chapter Six: The Cerebrovascular Response to Graded Valsalva Manoeuvres Whilst Standing

Publication based on this Chapter;

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6.1 Introduction

In **Chapter Five** it was established that a greater load produced a more substantial decrease in both MAP and $MCAv_{mean}$ following the effort. However, during the effort there was no difference in the average peak $MCAv_{mean}$ response. At the highest load lifted (90% of 6RM) this response may be attributed to the VM. That is, the VM may be protective during the effort limiting the increase in MCAv despite the pronounced changes in MAP but exacerbating the decrease in both MAP and MCAv following the effort. Like resistance exercise the VM is capable of inducing large non-pharmacological increases and decreases in MAP that are capable of challenging cerebral perfusion when performed in isolation (as detailed in **Chapter Two**, section 2.5.3). Like **Chapter Five** this chapter investigated the effects of dynamic changes in blood pressure on the MCAv response although this chapter utilised graded VMs to provide varying perturbations in MAP. The purpose of this chapter was to examine the haemodynamic response to graded VMs whilst standing and extend the findings detailed in **Chapter Five**. Many studies investigating the haemodynamic response to

the VM have adopted a supine position which alleviates the orthostatic component and abolishes the phase III response (Pott *et al.* 2000). As the VM is commonly recruited in the standing position during resistance exercise and everyday tasks (lifting), documentation of the haemodynamic response would be advantageous for the identification of the risk factors that may challenge cerebral perfusion.

The VM is defined as a forced exhalation against a closed glottis (Hamilton *et al.* 1936) and is executed during cough (Hamilton *et al.* 1944), defecation and also during resistance exercise (MacDougall *et al.* 1992). The VM can be separated into four distinct phases: Phase I, an increase in MAP at the onset of the strain as the elevated intrathoracic pressure is translated to the arterial circulation; phase IIa, a reduction in stroke volume as atrial filling pressure is reduced; phase IIb, an increase in heart rate mediated by the arterial baroreflex to offset the reduction in stroke volume; phase III, a rapid decline in MAP as the strain is released; phase IV, rapid recovery and overshoot of MAP as the now restored $\dot{Q}$ is ejected into a constricted arterial tree (Goldberg *et al.* 1952; Tiecks *et al.* 1995b; Pott *et al.* 2000). Further, when standing the orthostatic component contributes further to the observed post-strain hypotension as the Phase III response is greatly reduced when supine (Pott *et al.* 2000).

The VM may be viewed as eliciting undesirable cardiovascular and cerebrovascular responses, but there is also evidence that it may indeed protect the cerebral circulation during phase I of the manoeuvre (Tiecks *et al.* 1995b; Niewiadomski *et al.* 2012). Specifically, increases in intrathoracic pressure are translated to the cerebrospinal fluid (Hamilton *et al.* 1944) such that increases in intracranial pressure (ICP) ensue (Greenfield *et al.* 1984), reducing transmural pressure in the cerebral arteries (Haykowsky *et al.* 2003). This

alteration in transmural pressure may mechanically restrain the passive dilation of vessels downstream of the MCA in response to the greatly elevated cerebral perfusion pressure. Via a maintained resistance, flow would be limited and would culminate in no change in MCAv. Further, the increased central venous pressure (CVP) experienced during a VM may reduce the pressure difference across the cerebral vascular bed (Pott *et al.* 2000). Nevertheless, whilst these mechanical mechanisms may limit the increase in MCAv, an elevation is observed during phase I (Tiecks *et al.* 1995b) and reflects the high pass filter characteristics of the cerebral circulation (Zhang *et al.* 1998a).

Many studies investigating the haemodynamic response to the VM have adopted a supine position which alleviates the orthostatic component and reduces the phase III response (Pott *et al.* 2000). Tiecks *et al.* (1995) assessed the effects of two mouth pressures, 20 and 40 mm Hg, on the cerebrovascular response whilst supine and reported no differences in the phase III decrease between pressures. However, syncope is apparent following orthostasis (Julu *et al.* 2003) and a vigorous VM whilst sitting upright (Duvoisin 1961). Therefore, the hypothesis was that the VM will protect the brain against hyperperfusion during phase I of the manoeuvre whilst standing; i.e., the increase in MAP during phase I will be intensity-dependent, however no change in MCAv will be observed. In addition, following the release of the VM (phase III), an increased VM intensity will induce a greater hypotension and be matched by an intensity-dependent concomitant decrease in MCAv.

6.2 Methods

6.2.1 Participants

Ten healthy non-smoking males were recruited for the study (mean ± SD: age, 26 ± 4 y; body mass, 93 ± 12 kg; height, 181 ± 8 cm). All participants were resistance trained and had a training age of 4.5 ± 3.0 y. Participants were informed of the potential risks and experimental procedures, and informed written consent was obtained. All procedures and protocols were approved by the University of Otago Human Ethics Committee and performed in accordance with the *Declaration of Helsinki*. All participants were free from disease and were not taking any medication. Participants abstained from strenuous exercise, alcohol and caffeine for at least 24 hours before the experimental trial.

6.2.2 Study Design

Participants visited the laboratory on two occasions; one familiarisation and one experimental trial. During the familiarisation session the participants were familiarised with all experimental procedures and equipment, including practicing VMs at end-inspiration following a quiet period of spontaneous breathing. This enabled pre-VM hyperventilation to be minimised during experimental trials. Mouth pressure served as a surrogate for intrathoracic pressure (MacDougall *et al.* 1985; Morgan *et al.* 1993) and reportedly reflects changes in oesophageal pressure (Goldberg *et al.* 1952; Flemale *et al.* 1988). All VMs were performed in the standing position.

6.2.3 Experimental Protocol

During the experimental trial each participant would first stand for 5 min during which baseline measures were obtained. Participants upon instruction would then complete a maximal VM for 10 s. Following recovery (i.e., when all values returned to baseline), relative VMs of 30, 60 and 90% of the maximal Valsalva pressure were then performed for both 5 and 10 s, the order of which (both intensity and duration) was randomised. Visual feedback of the absolute mouth pressure was given in real time in order to aid the participant. Each VM was separated by 5 minutes or until values had returned to baseline. Participants were verbally instructed what pressure and duration to obtain immediately before the performance.

6.2.4 Measurements

Blood flow velocity in the right middle cerebral artery (MCAv) was measured, as described in **Chapter Three** (section 3.3.1). Participants breathed through an adjustable mouthpiece (see **Chapter Three**, section 3.4.1), which allowed for the measurement of mouth pressure and the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$; gas analyser model ML206, ADInstruments, Australia). Mouth pressure was measured via a transducer attached to the mouthpiece and was used to measure the pressure during all VMs. Blood pressure was measured non-invasively and continuously using finger photoplethysmography (see section 3.2, Finapres Medical Systems, Biomedical Instruments, Amsterdam, The Netherlands), and heart rate was measured via three-lead electrocardiogram (ADInstruments); as detailed in **Chapter Three** (section 3.3). All data were acquired continuously via an analogue-to-digital converter (PowerLab ML870; ADInstruments) at 1 kHz. Data were displayed in real time and recorded for off-line analysis using commercially-available software (v7.3.3 LabChart, ADInstruments).

6.2.5 Data Analyses

Calculation of MAP, $MCAv_{mean}$ and CVC are detailed in **Chapter Three** (section 3.3). Relative to baseline measures, the percentage decrease in $MCAv_{mean}$ was divided by the percentage reduction in MAP at $MCAv_{mean}$ nadir to assess differences in the MAP contribution to the reduction in $MCAv_{mean}$. The Gosling pulsatility index for MCAv was calculated as SMCAv - DMCAv/$MCAv_{mean}$ (Gosling & King 1974). Baseline data were acquired in the last minute of each baseline period between VMs, and presented as the mean across that minute. All variables at the attainment of the 'peak' for both the $MCAv_{mean}$ and MAP phase I responses were used in the analysis (i.e., a data set for each individual peak). Time to peak was taken from the start of the VM to the peak of phase I response for both $MCAv_{mean}$ and MAP. Each VM was analysed for $MCAv_{mean}$ and MAP times to nadir, magnitude of nadir (phase III), and time to recovery from the end of the strain (similar to **Chapter Five** and Stolz *et al.* (2010)). Nadir was defined as the lowest measured value immediately following the VM, and recovery as the point when the variable was equal to the baseline value during the ascent from nadir. Additionally, the area under the curve (AUC) was calculated as described in **Chapter Three** (section 3.3.2).

6.2.6 Statistical Analyses

Inferential statistical analyses of dependent variables were analysed using a three-way ANOVA (intensity x duration x phase) for change from baseline and time to peak (during phase I), nadir (Phase III) and recovery (early phase IV). Intensity refers to the relative intensity of the VM performed and duration the length the strain was held for (either 5 or 10 s). Phase refers to the comparison of a specific time point to the baseline reference value (i.e., peak and nadir). Data during and 15 s post the VM was compared to the pre-VM

baseline using an ANOVA for repeated measures of time and intensity. The AUC was analysed within each duration using a one-way ANOVA. Data were assessed for approximation to a normal distribution and sphericity, with no corrections required. Main effects were isolated using *post-hoc* pairwise comparisons (Bonferroni corrected, where necessary). All data were analysed using SPSS statistical software (v20, IBM, New York, USA) with *a priori* statistical significance set at $P \leq 0.05$.

6.3 Results

Absolute and relative changes from baseline during the VM (phase I) are displayed in Table 6.1. Briefly, both MAP and $MCAv_{mean}$ increased from baseline although only MAP was intensity dependent (see Table 6.1 for P values). Changes from baseline following the VM at the nadir in phase III are shown in Table 6.2. In contrast to Phase I of the VM, once the strain was released larger reductions in MAP were associated with greater reductions in systolic, diastolic and $MCAv_{mean}$ flow velocities (see Table 6.2 for P values). In support of this, the AUC analysis for $MCAv_{mean}$ indicated a significant effect of intensity for both 10 ($P = 0.002$, -6 ± 58, -51 ± 92, -114 ± 129 aU for 30, 60 and 90% intensities, respectively) and 5 s ($P = 0.004$, 3 ± 36, -38 ± 43, -48 ± 62 aU for 30, 60 and 90% intensities, respectively). In regards to pulsatility, this index was significantly different between VMs driven by the intensity-by-phase interaction (P = 0.002). The greatest change between the phase I and II responses was observed during the 90% VM with 0.74 ± 0.27 and 0.74 ± 0.15 during phase I for 10 and 5s respectively versus 1.29 ± 0.34 and 1.52 ± 0.61 during phase III.

When data were pooled into 1-s bins (Figure 6.1 and 6.3) and analysed it was revealed for $MCAv_{mean}$ that there were significant main effects of time (P <0.001) and intensity (P =

0.002) for the 10-s VM. *Post hoc* analyses indicated that the periods for which $MCAv_{mean}$ was below baseline levels following the 10 s, 60% VM was 3 s (seconds 3-6 following the onset of the VM) and 6 s (3-9s) for the 90% VM (all $P < 0.01$) with the 30% VM producing no consistent reduction in $MCAv_{mean}$. No main effect of intensity ($P = 0.967$) was apparent for the 5 s VM, although there was a significant effect of time ($P <0.001$) and an intensity-by-time interaction ($P = 0.006$). This cerebral hypoperfusion was sufficient to produce syncope in two participants but only following the maximal and 90% 10-s VMs. Interestingly, similar reductions in MAP during phase III were observed for both the 5 and 10 s 90% VM, however $MCAv_{mean}$ was lower following the 5 s VM (Table 6.2). Baseline data including $P_{ET}CO_2$ (grouped mean 32 ± 5 mm Hg) was unchanged between baseline periods. These data were then grouped, the means of which are displayed in Tables 6.1 and 6.2 (all $P > 0.05$). Mouth pressures of 22 ± 6, 45 ± 12 and 67 ± 19 mm Hg were measured during 30, 60 and 90% VM intensities, respectively.

Time to peak $MCAv_{mean}$ (pooled mean 0.7 ± 0.5 s) and peak MAP (pooled mean 1.2 ± 0.6 s) during phase I of the VM was unaffected by Valsalva pressure ($P = 0.40$ and 0.50, respectively) or duration ($P = 0.69$ and 0.74, respectively). However, time to peak $MCAv_{mean}$ was significantly shorter ($P = 0.004$) than time to peak MAP. Time to nadir following the VM for $MCAv_{mean}$ (pooled mean 0.5 ± 0.5 s) and MAP (pooled mean 0.8 ± 0.4 s) showed no significant difference between intensities ($P = 0.40$ and 0.39, respectively) or durations ($P = 0.58$ and 0.47, respectively). However, similar to the time to peak responses, the nadir occurred earlier for $MCAv_{mean}$ than MAP ($P = 0.017$). Finally, time to recovery following the VM showed no effect of VM intensity ($P = 0.30$ and 0.62) or duration ($P = 0.73$ and 0.33,

respectively) for $MCAv_{mean}$ (pooled mean 1.6 ± 1.0 s) or MAP (pooled mean 3.6 ± 4.3 s); however, $MCAv_{mean}$ recovered before MAP (P = 0.006).

Table 6.1 Changes from baseline at Peak (phase I) for 30, 60 and 90% VM intensities.

Variable	Baseline	Time (s)	Δ From baseline			*P* values						
			30% VM	60% VM	90% VM	Intensity	Duration	Time	I x D	I x T	T x D	I x D x T
$MCAv_{mean}$, $cm \cdot s^{-1}$	57 ±9	5	15±7(27±12)	14±5(23±6)	16±8(29±13)	0.11	0.78	<0.0001	0.63	0.11	0.78	0.62
		10	12±5(22±9)	14±5(24±7)	17± 9(32±13)							
Systolic MCAv, $cm \cdot s^{-1}$	95 ±15	5	11±9(12±9)	12±6(12±6)	12±12(13±13)	0.24	0.87	<0.0001	0.50	0.24	0.87	0.50
		10	10±8(12±9)	10±6(11±6)	16±7(17±8)							
Diastolic MCAv, $cm \cdot s^{-1}$	40 ±6	5	15±7(38±18)	13±5(31±10)	16±8 (40±20)	0.41	0.50	<0.0001	0.85	0.51	0.50	0.85
		10	13±6(32±13)	13±6(32±14)	16±9(41±25)							
CVC, $cm \cdot s^{-1} \cdot mm$ Hg	0.69 ±0.16	5	0.050±0.12(10±16)	-0.050±0.079(-6±11)	-0.11±0.11(-15±15)‡	0.002	0.79	0.25	0.037	0.002	0.79	0.037
		10	-0.0040±0.063(1±11)	-0.027±0.12(-3±17)	-0.058±0.12(-6±17)‡							
MAP, mm Hg	85 ±13	5	12±9(14±10)	24±14(29±17)‡	35±14(41±19)‡*	<0.001	0.96	<0.001	0.23	<0.001	0.96	0.23
		10	17±9(21±11)	21± 14(25±16)‡	30±22(36±24)‡*							
Systolic BP, mm Hg	131 ±17	5	13±11(10±8)	31±22(24±18)	38±19(30±16)‡	0.020	0.60	<0.001	0.05	0.020	0.60	0.046
		10	29±19(23±14)	28±25(21±19)	35±27(27±19)‡							
Diastolic BP, mm Hg	70 ±12	5	11±8(16±12)	22±15(31±19)‡	32±15(49±26)‡	<0.001	0.77	0.29	0.29	<0.001	0.77	0.29
		10	14±7(22±11)	19±13(27±18)‡	28± 21 (41 ±28)‡							
HR, $beats \cdot min^{-1}$	80 ±10	5	6±12(7±15)	7±11(10±15)	11±11(14±13)	0.083	0.90	0.021	0.66	0.083	0.90	0.66
		10	4±14(6±19)	10±9(13±13)	11±17(14±22)							

Values are absolute mean difference from baseline ± SD, with percentage change shown in parentheses. VM, Valsalva manoeuvre; MCAv, middle cerebral artery velocity; CVC, cerebrovascular conductance; MAP, mean arterial pressure; BP, blood pressure; HR, heart rate; Interactions are shown for Intensity (I) Duration (D) and Time (T); ‡ Statistically different from 30%, $P \leq 0.05$; *Trend for a difference between 90 and 60% $P = 0.07$.

Table 6.2 Changes from baseline at Nadir (phase III) for 30, 60 and 90% VM intensities.

Variable	Baseline	Time (s)	Δ From baseline			*P* values						
			30% VM	60% VM	90% VM	Intensity	Duration	Time	I x D	I x T	T x D	I x D x T
$MCAv_{mean}$, $cm \cdot s^{-1}$	57 ± 9	5	-6 ± 9 (-9 ± 15)	-16 ± 11 (-25 ± 18)*	-19 ± 13 (-31 ± 22)‡†	<0.001	0.045	0.002	0.90	<0.001	0.045	0.90
		10	-3 ± 7 (-5 ± 13)	-11 ± 12 (-19 ± 19)*	-15 ± 11 (-26 ± 20)‡†							
Systolic MCAv, $cm \cdot s^{-1}$	95 ± 15	5	-9 ± 14 (-8 ± 14)	-15 ± 19 (-14 ± 20)	-24 ± 16 (24 ± 14)‡	0.004	0.84	0.004	0.83	0.004	0.84	0.83
		10	-10 ± 10 (-10 ± 10)	-18 ± 20 (-17 ± 20)	-22 ± 18 (-22 ± 19)‡							
Diastolic MCAv, $cm \cdot s^{-1}$	40 ± 6	5	-5 ± 11 (-9 ± 26)	-17 ± 13 (-38 ± 29)	-20 ± 15 (-47 ± 35)‡†	<0.001	0.077	0.004	0.40	<0.001	0.077	0.40
		10	-1 ± 9 (-4 ± 21)	-9 ± 13 (22 ± 32)	-18 ± 12 (-44 ± 28)‡†							
CVC, $cm \cdot s^{-1} \cdot mm$ Hg	0.69 ± 0.16	5	0.081 ± 0.11 (15 ± 19)	0.026 ± 0.12 (3 ± 18)	0.044 ± 0.15 (7 ± 22)	0.58	0.33	0.017	0.76	0.58	0.33	0.76
		10	0.085 ± 0.097 (10 ± 12)	0.059 ± 0.16 (6 ± 22)	0.10 ± 0.055 (15 ± 10)							
MAP, mm Hg	85 ± 13	5	-16 ± 7 (-19 ± 8)	-18 ± 16 (-21 ± 20)	-23 ± 11 (-28 ± 13)‡	0.006	0.21	0.70	0.70	0.006	0.21	0.70
		10	-12 ± 7 (-14 ± 9)	-14 ± 12 (-18 ± 16)	-23 ± 9 (-28 ± 11)‡							
Systolic BP, mm Hg	131 ± 17	5	-17 ± 16 (-19 ± 10)	-24 ± 29 (-19 ± 22)	-40 ± 14 (-32 ± 11)‡	0.003	0.29	0.001	0.84	0.003	0.29	0.84
		10	-25 ± 14 (-14 ± 13)	-23 ± 29 (-18 ± 19)	-37 ± 24 (-29 ± 17)‡							
Diastolic BP, mm Hg	70 ± 12	5	-13 ± 7 (-18 ± 10)	-14 ± 12 (-20 ± 19)	-17 ± 10 (-25 ± 14)	0.040	0.31	0.65	0.65	0.040	0.31	0.65
		10	-10 ± 7 (-14 ± 10)	-11 ± 10 (-17 ± 14)	-17 ± 6 (-26 ± 9)							
HR, $beats \cdot min^{-1}$	80 ± 10	5	13 ± 7 (17 ± 10)	15 ± 11 (19 ± 14)‡	22 ± 14 (28 ± 18)‡	<0.001	0.038	<0.001	0.015	.038	0.038	0.015
		10	14 ± 8 (17 ± 10)	24 ± 9 (31 ± 13)‡§	35 ± 13 (43 ± 17)‡§							

Values are absolute mean difference from baseline ± SD and percentage change from baseline values (± SD) are denoted in parentheses. VM, Valsalva manoeuvre; MCAv, middle cerebral artery velocity; CVC, cerebrovascular conductance; MAP, mean arterial pressure; BP, blood pressure; HR, heart rate. Interactions are shown for Intensity (I) Duration (D) and Time (T); ‡, Statistically different from 30%; †, Statistically different from 60%, $P \leq 0.05$; §, Statistically different from 5 s, $P \leq 0.05$; * Trend for a difference between 60 and 30% $P = 0.084$; §, Statistically different from 5 s, $P \leq 0.05$.

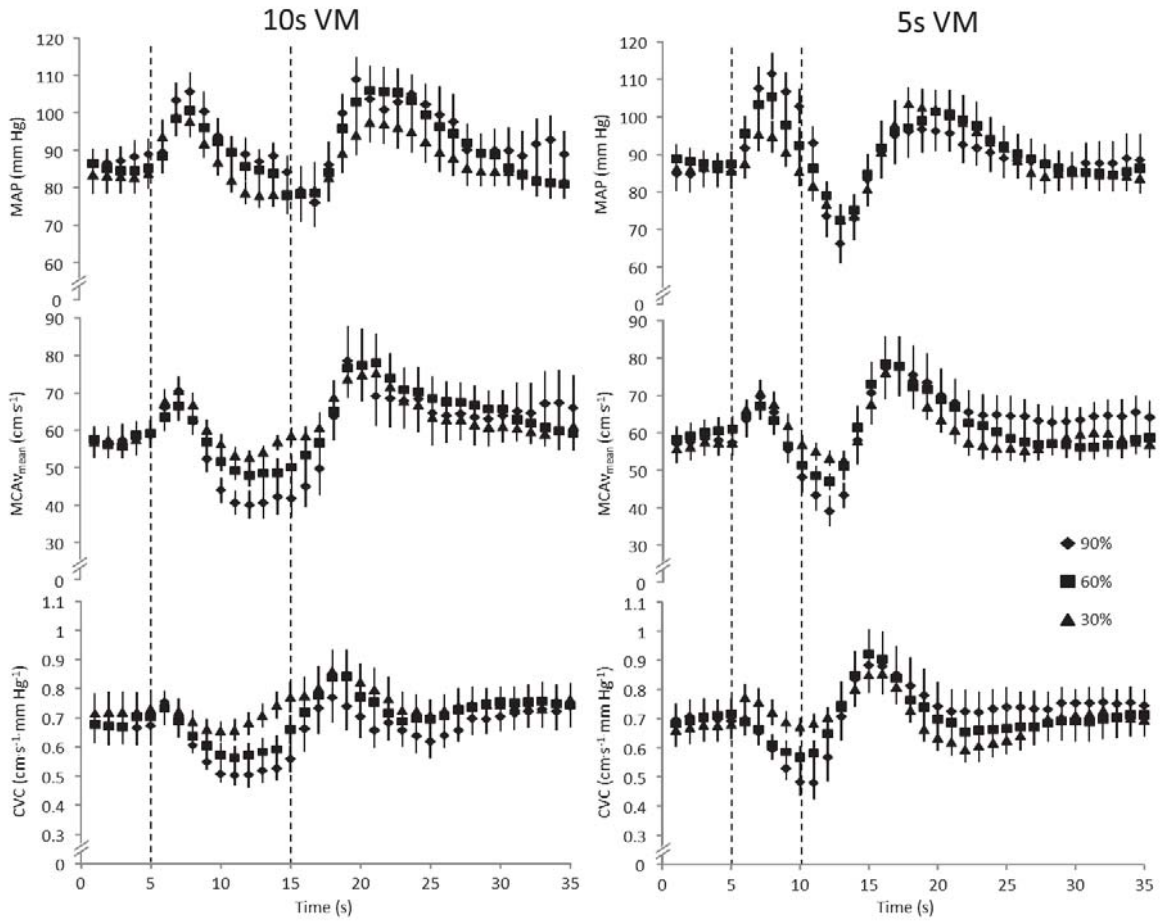

Figure 6.1 The response of mean middle cerebral artery blood flow velocity ($MCAv_{mean}$), mean arterial pressure (MAP) and cerebrovascular conductance (CVC) during and following a Valsalva manoeuvre (VM) at 30, 60 and 90% of maximal VM pressure, displayed every second. The vertical dashed lines represent the initiation and completion of the VM. All values are means ± SE.

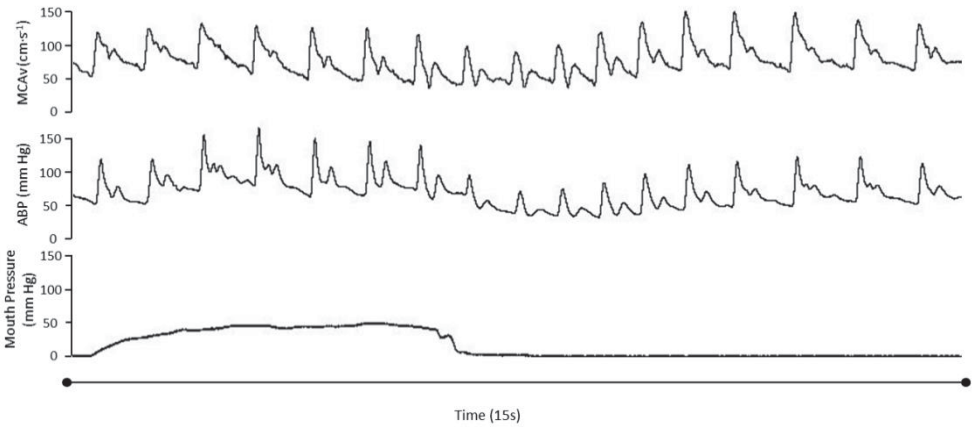

Figure 6.2 Representative trace of middle cerebral artery blood flow velocity (MCAv), arterial blood pressure (ABP) and mouth pressure during a 90% 5s VM in one participant.

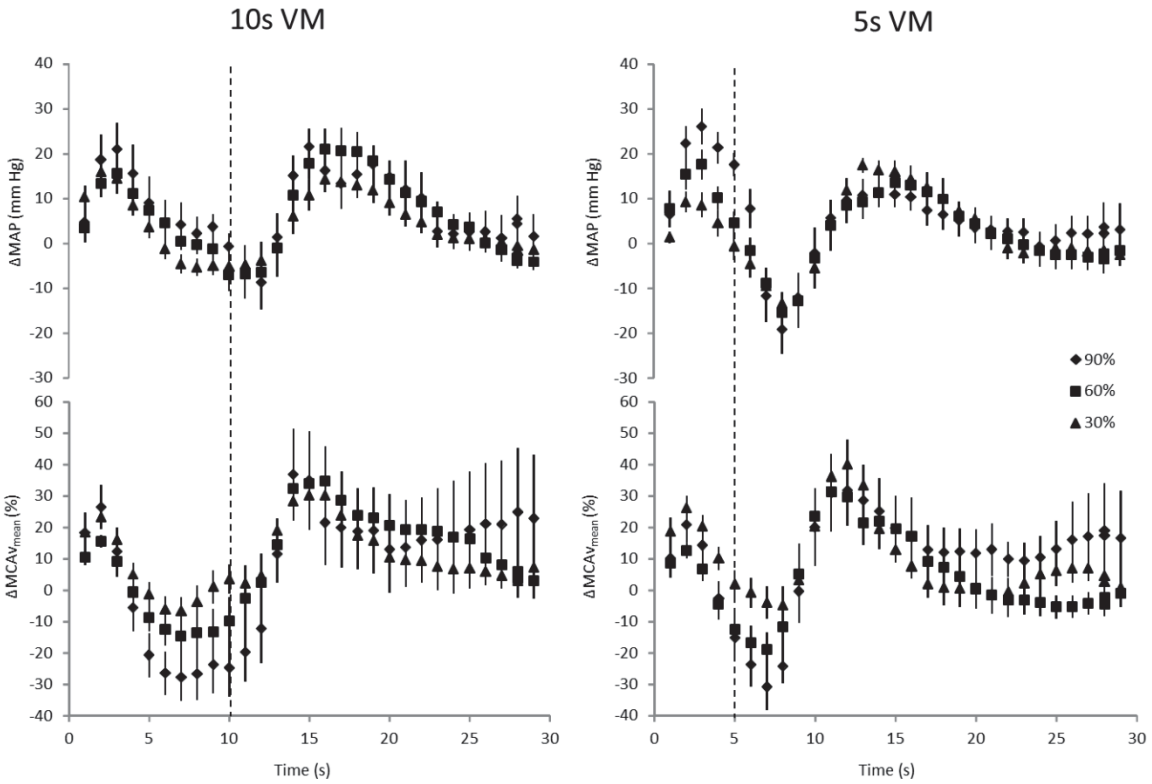

Figure 6.3 The percentage change from baseline for mean middle cerebral artery blood flow velocity ($MCAv_{mean}$) and the absolute change in mean arterial pressure (MAP) during and following a Valsalva manoeuvre (VM) at 30, 60 and 90% of maximal VM pressure, displayed every second. The zero time point represents the initiation of the VM with the vertical dashed lines representing the completion. All values are means ± SE.

6.4 Discussion

The main findings of this chapter were that: 1) Across the range of intensity and duration of VM tested, increases in MCAv during phase I were comparable despite the progressive increase in MAP with VM intensity; 2) higher VM intensities resulted in a greater reduction in both MCAv and MAP upon release of the VM (phase III), and 3) time to peak, nadir and recovery occurred earlier for MCAv than MAP. Consistent with the hypothesis, an asymmetrical response in cerebral blood flow control during rapid increases and decreases in perfusion pressure was observed. Specifically, while the MAP response to VM was found to be intensity-dependent for both phases I and III, the MCAv response was not coupled to

MAP during phase I but was during phase III. These findings illustrate how the VM both challenges and contributes to the regulation of CBF. The following discussion outlines the evidence supporting this conclusion.

6.4.1 The Haemodynamic Response During Phase I of the VM

The rapid time course of the blood pressure response during a VM (particularly Phase I) occurs too quickly (~1 s) to be counteracted by cerebral myogenic autoregulatory processes (Greenfield *et al.* 1984). Therefore, one might expect that the greater increase in MAP associated with a more intense VM would cause a proportionately larger increase in MCAv. Whilst there was an increase in MCAv during phase I relative to baseline, consistent with the high-pass filter characteristics of the cerebral circulation (Zhang *et al.* 1998a), it was not reliably different between intensities despite the prevailing MAP response and is in agreement with the results of **Chapter Five**. In addition, when we included the maximum VM data in the analysis and compared it with the 30, 60 and 90% 10-s VMs (using a one-way ANOVA), the VM intensity still had no distinguishable effect on $MCAv_{mean}$ (P=0.09), yet MAP was further elevated.

The maintenance of MCAv despite the greater MAP response (Table 6.1) may be attributable to the mechanical effects of the increased intrathoracic pressure during a VM. The intrathoracic pressure is rapidly translated to the cerebrospinal fluid at the onset of the VM such that intracranial pressure (ICP) rises (Hamilton *et al.* 1944; Greenfield *et al.* 1984) and reduces the transmural pressure within the cerebral arteries (Haykowsky *et al.* 2003). The reduction in transmural pressure may restrain the passive dilation in response to the acute increases in CPP during phase I of the VM and limiting the increases in MCAv. Further, right atrial pressure increases linearly with expiratory pressure (Korner *et al.* 1976) and may

attenuate the pressure difference across the cerebral circulation. However, blood flow velocity in the straight sinus has been reported to increase during phase I, although this may be due to the partial collapse of walls of the sinus due to an increased ICP, and therefore increased velocity may be due to changes in vessel diameter rather than flow *per se* (Stolz *et al.* 2010). This is further complicated in the standing position as the jugular vein is collapsed (Dawson *et al.* 2004; Gisolf *et al.* 2005), which may increase cerebral venous resistance (thus acting as a Starling resistor) and redirect blood flow through the vertebral venous plexus (Gisolf *et al.* 2004a). Although not measured here, CVP may rise proportionately with VM intensity and provide graded venous outflow resistance that would combat the graded increases in MAP during phase I. Whilst the cerebral vasculature has multiple draining vessels a rise in CVP for a given arterial pressure would limit venous outflow and overall flow through the vascular bed (Darcy's law, see section 2.1).

Whilst an autoregulatory myogenic response cannot be excluded during phase I, the rapid transfer of intrathoracic pressure to the cerebrospinal fluid (Hamilton *et al.* 1944) likely occurs before dynamic autoregulation has fully counteracted the increase in MAP. Dynamic cerebral autoregulation has an inherent latency of ~5 s (Zhang *et al.* 1998a) and the observed peak MCAv observed here was achieved 0.7 s following the onset of the VM. Therefore, a likely explanation for the restrained MCAv (i.e., despite the increasing MAP with greater VM intensity) would seem to be a graded resistance due to VM intensity-dependent rises in ICP and possibly CVP, protecting the brain from possible hyperperfusion injury during phase I. This may also explain the differences in pulsatility observed between the Phase I and III responses, where during phase I a mechanical restraint of dilation (elevated ICP) would reduce the pulsatility index and later the waveform profile between

the phases (Figure 6.2). The increase in pulsatility may maintain CBF via pulsatile flow when perfusion pressures are low, as observed during phase III of the VM. This may be a compensatory mechanisms that promotes flow at the lower limits of cerebral autoregulation as beyond this point CBF declines rapidly (Lewis *et al.* 1999). Further, the effect of this resistance to flow is apparent when comparing the haemodynamic response between phase I and the overshoot during phase IV. Despite similar increases in MAP during phase I and IV, the MCAv increase is greater during phase IV (Tiecks *et al.* 1995b) where ICP would be expected to be declining to near baseline levels (Greenfield *et al.* 1984). Other mechanisms of regulation, such as the autonomic nervous system, may operate during these latter phases (phase IV) of the VM to regulate cerebral blood flow (Zhang *et al.* 2004a), albeit less effectively than the mechanical regulatory processes apparent during phase I.

6.4.2 The Haemodynamic Response During Phase III of the VM

Unlike during phase I of the VM, the reduction in MAP and MCAv in phase III was dependent on VM intensity, such that the more intense VMs produced a greater reduction in both MAP and MCAv (Table 6.2). This was further evidenced by the occurrence of syncope in two participants whilst performing their maximal and 90% (10 s) VMs, with another three participants showing symptoms of (pre-) syncope (light-headedness). This rapid reduction in MAP is likely attributable to the passive effect of intrathoracic pressure on the arteries (Tiecks *et al.* 1995b; Dawson *et al.* 1999) and the refilling of the distended pulmonary vessels (Pott *et al.* 2000). Similar to phase I of the VM, the cerebral autoregulation myogenic response is too slow to counteract this acute hypotension, such that MCAv matches the drop in MAP, at least initially (Figure 6.1). Given that the nadir of MCAv occurred before that

of MAP, it would appear that the dynamic myogenic response was functional and played a role in limiting the hypoperfusion of the brain.

To the best of the candidate's knowledge this is the first study to investigate the duration of the VM on the haemodynamic response. Interestingly, MAP and MCAv appear to fall more following the 5-s than 10-s VM during phase III (Table 6.2). This may be attributable to the release of the manoeuvre coinciding with the reduction in cardiac output during phase IIa (Pott *et al.* 2000) (no plateau in MAP trace, Figure 6.1) and subsequently a larger decrease in MAP (Table 6.2). Although CBF and $\dot{Q}$ are causally linked (Ogoh *et al.* 2005a), it is unlikely that a simple relation exists during the VM (Pott *et al.* 2003). Higher heart rates following the 10-s VM indicate a baroreflex-mediated contribution to the circulatory stability (Table 6.2), presumably in an attempt to offset the reduction in stroke volume (Pott *et al.* 2000); interestingly this effect was not apparent in the shorter 5 s VMs.

6.4.3 The Effect of Posture on the VM

The phase III response is exacerbated in the standing position, as the reduction in MCAv during this phase is not apparent whilst supine (Pott *et al.* 2000). Further, Tiecks *et al.* (Tiecks *et al.* 1995b) found that graded VMs do not produce a greater hypotension during phase III whilst semi-recumbent with no associated change in MCAv from baseline (Tiecks *et al.* 1995b). In the same study, MCAv was increased from baseline during phase I of the VM whilst supine but unchanged between graded pressures of 20 and 40 mm Hg (mouth pressure), which is consistent with the results of this chapter. Therefore, the haemodynamic response during phase III of the VM appears highly posture-dependent whereas the phase I appears to be unaltered. When standing, the severe and rapid reduction in MAP during

phase III ultimately challenges cerebral oxygenation sufficiently to induce syncope (Van Lieshout *et al.* 2003).

6.4.4 Contribution of $P_{ET}CO_2$

Alterations in arterial CO_2 alter the efficacy of cerebral autoregulation (Aaslid *et al.* 1989). The measurement of $P_{ET}CO_2$ served as a substitute for arterial PCO_2. It was observed that $P_{ET}CO_2$ was unchanged between baselines and thus cerebral tone would have been similar at the onset of the VM. The time course of the vascular response to changes in arterial CO_2 is asymmetric with the 'on' constant much slower than the 'off' (Poulin *et al.* 1996). The time constant of the increase in MCAv during a step change in $P_{ET}CO_2$ is ~6 s (Poulin *et al.* 1996; Poulin *et al.* 1998). Pott *et al.* (Pott *et al.* 2000) reported that the reduction in arterial PCO_2 contributed to 10-15% of the reduction in MCAv during a 15-s VM. As the maximal duration of the VM in this experiment was 10 s the influence of changes in arterial PCO_2 would be expected to be less, although the exact effect of possible changes in arterial CO_2 tension during the VM performed here is unknown. However, due to the delay in the vascular response and moderate changes in arterial PCO_2 reported during longer VMs (Pott *et al.* 2000), the main driving factor during and initially following the VM appears to be the rapid changes in perfusion pressure.

6.4.5 Implications for Resistance Exercise

This rapid and large increase in MAP has the potential for cerebrovascular injury (Edwards *et al.* 2002). Data from **Chapter Five** indicated that the average peak MCAv velocity is unchanged by the load lifted during upright squatting exercise, despite the peak MAP response being load-dependent. Furthermore, the VM was recruited only during the highest

squatting intensity (90% of 6 repetition maximum), although an increase in intrathoracic pressure and CVP may still be apparent at lighter loads (Pott *et al.* 2003). Earlier work by Pott *et al.* (2003) investigating the role of the VM during leg extension resistance exercise reported that MCAv increased from baseline (59 ± 9 to 77 ± 7 $cm \cdot s^{-1}$) during phase I when a VM was recruited, yet with continued ventilation MCAv increased to 70 ± 6 (statistics not reported). However, during the combined VM and exercise MAP was 21 mm Hg greater (absolute MAP: 141 ± 6) than during continued ventilation and was approaching the purported upper limits of cerebral autoregulation (~150 mm Hg).

Ultimately it is difficult to compare the cerebrovascular effect of a VM in isolation and in combination with resistance exercise (whether seated or standing) as the MAP profile is difficult to match between conditions. Likewise, as a VM is recruited at higher loads (MacDougall *et al.* 1992) and induces additional increases in MAP (Narloch & Brandstater 1995; Pott *et al.* 2003), the potential protective effect may only be evident at these extreme MAPs that would be difficult to reproduce experimentally without straining. Therefore this study design in this chapter provided a condition where graded increases in MAP are concomitant with a VM; revealing that despite increases in MAP with increasing relative intensity MCAv remains unchanged.

The force-velocity curve dictates that higher relative loads will be lifted at slower velocities (Hill 1938). These slower lifting velocities produce longer straining periods, greater intrathoracic (Harman *et al.* 1988) and intra-abdominal pressures (Cresswell & Thorstensson 1994). Thus, heavy loads associated with long straining periods and more forceful VMs may culminate in syncope, which has been previously shown during maximal upright resistance exercise (Compton *et al.* 1973). Although the phase I increase in ICP appears to be

protective during exercise and straining in healthy resistance trained participants, the drastic changes in MAP during phase III is detrimental and is capable of producing syncope. Thus simple strategies (VM avoidance, reducing load) to reduce the risk of post-exercise syncope are important for reducing the risk of injury, but then may elevate the risk of hyperperfusion injury if not recruited during large strains. Although the VM may induce syncope, this acute cerebral ischemia is associated with rapid recovery, whereas hyperperfusion injury (i.e. stroke) is severe in nature with chronic impairments and death possible. As such, further research is needed to determine the best practice of when to use or avoid the VM, especially in clinical populations.

6.5 Conclusion

The MAP response to phase I of the VM was intensity-dependent, while the MCAv response was similar across the range of intensities tested here. At the end of the straining, the reduction in both MAP and MCAv was intensity dependent, resulting in marked transient reductions in MCAv, which were sufficient to induce syncope in some instances. The results presented in this chapter were consistent with those from **Chapter Five** and the hypothesis, and indicate that the VM may protect the brain from hypertension at the onset the VM, presumably as a result mostly of the mechanical effects of the elevated intrathoracic pressure. Although this response is protective during the manoeuvre, more intense VMs produce a greater post-strain hypotension and cerebral hypoperfusion. Again this is consistent with Chapter Five in that greater reductions in MAP are accompanied by changes in MCAv of similar relative magnitude. The time to peak (phase I) and nadir (phase III) for

MCAv occurred before those of MAP. Therefore, the regulation of cerebral perfusion during the distinct phases of the VM are likely different and are not simply pressure passive.

The aim of this thesis was to investigate the effects of both rapid (dynamic) and prolonged (static) changes in MAP on CBF. **Chapters Five** and **Six** have documented the cerebrovascular response to dynamic, non-pharmacological, changes in MAP. The forthcoming chapters investigated the cerebrovascular response to more prolonged changes in MAP and the efficacy of static cerebral autoregulation.

Chapter Seven: Middle Cerebral Artery Blood Flow Velocity in Response to Lower Body Positive Pressure

Publication based on this chapter:

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7.1 Introduction

As detailed in **Chapter Two** the efficacy of static cerebral autoregulation has recently been challenged. The studies investigating static cerebral autoregulatory efficacy have utilised pharmacological-induced increases in MAP that may have a direct effect on the measurement of CBF using TCD (section 2.1.3.1). Lower body positive pressure (LBPP) provides a non-pharmacological means to provide significant increases in MAP as highlighted below. Whereas, **Chapter Five** and **Six** have investigated the effects of dynamic changes in MAP on CBF the aim of this chapter and **Chapter Eight** was to investigate the effects of static changes in MAP on CBF. Given the results of the previous chapters (**Five** and **Six)** and that there may be no difference in the underlying physiological processes between dynamic and static autoregulation (Willie *et al.* 2014), it is possible that slower changes in MAP may produce similarly prolonged increases in MCAv.

Lower body positive pressure (LBPP) has been used for the treatment of hypotensive and hypovolemic patients (Wayne & Macdonald 1983) as well as for preventing g-force induced syncope in pilots (Wood 1987). Graded LBPP produces incremental increases in CVP (Shi *et al.* 1997) and elevates arterial blood pressure (Fu *et al.* 1998). Despite the pronounced haemodynamic effect of LBPP, data concerning the effects of LBPP on cerebral perfusion are unclear. Short bouts (1 min) of LBPP applied to upright individuals are reported to have no effect on middle cerebral artery blood flow velocity (MCAv) (Cutuk *et al.* 2006). However, the applicability of these findings to situations involving a 'steady state' is not clear given that physiological stability is reported to be achieved only after several minutes of LBPP (Shi *et al.* 1997). Thus, the effects of LBPP on steady-state MCAv and whether the MCAv response to LBPP is similar between the supine and upright posture is not known.

As mentioned in previous chapters of this thesis, the brain possesses an intrinsic autoregulatory mechanism that acts to maintain adequate blood flow in the face of changes in perfusion pressure. However, recent evidence indicates that static cerebral autoregulation is more pressure passive than traditionally accepted in the Lassen (1959) model, and that the baroreflex response to such gradual changes in arterial blood pressure is an important contributor to stable cerebral perfusion (Lucas *et al.* 2010). Notably, the increases in arterial pressure in the experiment by Lucas *et al.* (2010) were induced via phenylephrine administration, which may directly constrict the middle cerebral artery and subsequently increase MCAv, but not CBF *per se*. Thus, LBPP represents a non-pharmacological means of increasing arterial blood pressure and thus, the effect of this increase in perfusion pressure on MCAv can be investigated.

Therefore, the purpose of this chapter was to investigate the effect of LBPP on MCAv in humans; i.e., examine static changes in MAP as opposed to the dynamic effects examined in **Chapters Five** and **Six**. The hypothesis for this chapter was that graded LBPP would produce proportionate increases in MAP with concomitant increases in MCAv.

7.2 Methods

7.2.1 Participants

Fifteen healthy participants were recruited for this study (10 males, 5 females, mean ± SD: age, 26 ± 5 y; body mass, 79 ± 10 kg; height, 174 ± 9 cm). Each participant was fully informed of all potential risks and experimental procedures, after which informed written consent was obtained. All experimental procedures and protocols were approved by the University's Human Ethics Committee and performed in accordance with the *Declaration of Helsinki*. All participants were free from cardiovascular and cerebrovascular disease, were non-smokers and were not taking medication. Participants arrived at the laboratory for the familiarisation and experimental trials having abstained from strenuous exercise, alcohol and caffeine for at least 24 hours.

7.2.2 Study Design

Participants visited the laboratory on two occasions, one familiarisation and one experimental trial. During the familiarisation session the participants were familiarised with all experimental procedures. Experimental trials were conducted in the supine position in a LBPP chamber (see **Chapter Three**, section 3.4.2) at an ambient temperature of 20-23^{o}C, relative humidity of 40-50% and a barometric pressure of 759 ± 5 mm Hg.

7.2.3 Experimental Protocol

Following instrumentation and 20 minutes of supine rest, baseline values of all measures were recorded. This was followed by the first stage (either 20 or 40 mm Hg) of LBPP, which was applied in a randomised order. Baseline values were again recorded for 5 minutes or until all measures were stable and matched those of the preceding baseline, followed by the second LBPP stage. Participants were sealed in a custom made LBPP chamber (For details on LBPP refer to **Chapter Three** section 3.4.2). By design, participants maintained a stable $P_{ET}CO_2$ throughout both LBPP stages. This was achieved by the PhD candidate giving the participant verbal breathing instructions during testing to maintain $P_{ET}CO_2$ at baseline values.

7.2.4 Measurements

Blood flow velocity in the right middle cerebral artery (MCAv) was measured using a 2-MHz pulsed Doppler ultrasound system (DWL, Compumedics Ltd, Germany, refer to **Chapter Three** for details). The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was sampled at the mouth via a gas analyser (gas analyser model ML206, ADInstruments, Colorado Springs, USA). Blood pressure was measured non-invasively using finger photoplethysmography (see section 3.2 Finapres Medical Systems, Biomedical Instruments, The Netherlands) and heart rate via three lead electrocardiogram (ADInstruments). All data were acquired continuously via an analogue-to-digital converter (PowerLab ML870; ADInstruments) at 200 Hz. Data were displayed in real time using commercially available LabChart software (v7.3.3, ADInstruments) and recorded for subsequent off-line analysis.

7.2.5 Data Analyses

The methods of calculating $\dot{Q}$, MAP, $MCAv_{mean}$, TPR and CVC are detailed in **Chapter Three** (section 3.3). For each stage of LBPP a $\Delta MCAv_{mean}$-to-ΔMAP ratio from baseline values was calculated. Baseline data were acquired during the fifth minute of each baseline and averaged across that minute. Steady-state data for each LBPP stage were also averaged during the fifth minute, as previous reports indicate that 4 min is required to achieve a steady-state [(Shi *et al.* 1997), also see Figure 7.1]. As there were no difference between both baseline periods preceding each LBPP stage (see results), baseline data were collapsed (averaged) for analysis.

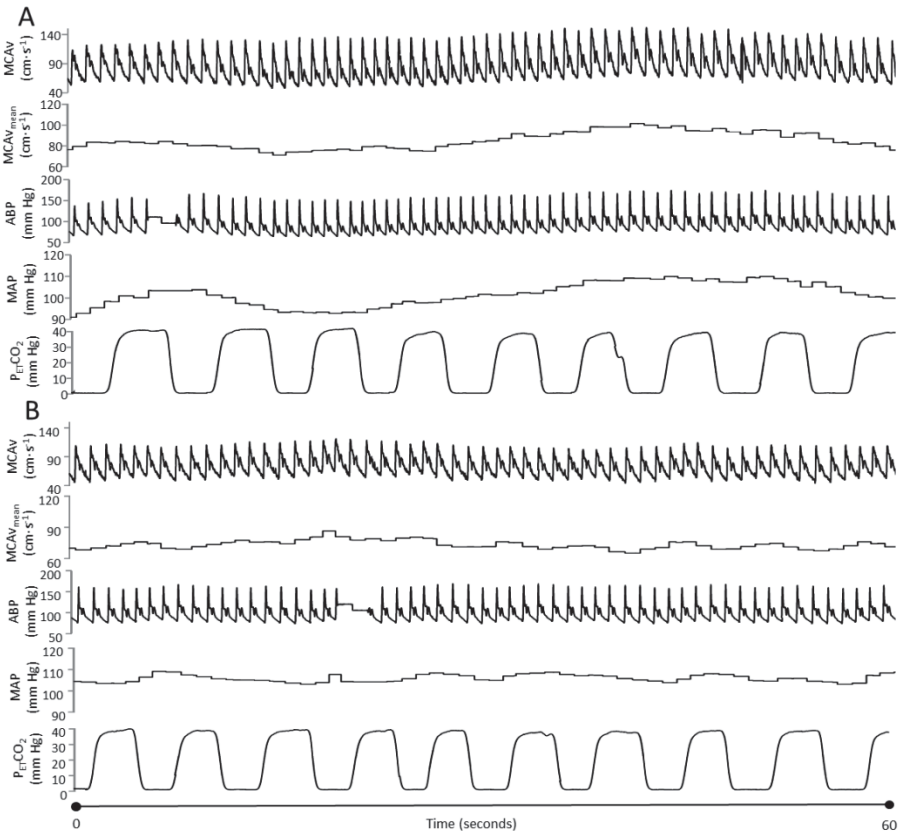

Figure 7.1 Cerebrovascular, cardiovascular and respiratory responses in the first minute (A) and in the fifth minute (B) to 40 mm Hg of lower body positive pressure (LBPP) in one individual. In part A of this figure, LBPP was applied at time point 0 and a resultant increase

in MAP and MCAv are seen that demonstrate the initial dynamic response. In the fifth minute (B) haemodynamic variables exhibit a far more stable profile when compared to the initial response. For these reasons, steady-state data were recorded in the 5th minute of each stage. MCAv, middle cerebral artery flow velocity; ABP, arterial blood pressure; MAP, mean arterial blood pressure; $P_{ET}CO_2$, partial pressure of end tidal carbon dioxide.

7.2.6 Statistical Analyses

All dependent variables were analysed using a one-way ANOVA. When a significant *F*-value was observed (*a priori* set at $P \leq 0.05$), *post-hoc* pairwise comparisons (paired *t*-tests with a Bonferroni correction, $P \leq 0.02$) were performed. All data were analysed using SPSS statistical software (v20, IBM, New York, USA) and presented as the mean (± SD) absolute and/or relative change from the preceding baseline.

7.3 Results

There were no differences between all baseline variables and no effects of LBPP order were observed (all $P > 0.30$). Haemodynamic changes from baseline are reported in Table 7.1 and the individual MAP and MCAv responses in Figure 7.2. Briefly, $MCAv_{mean}$ was elevated from baseline ($P = 0.003$) during the 20 mm Hg LBPP stage, which was mediated by increases in both systolic ($P < 0.001$) and diastolic MCAv ($P < 0.01$, for values see table 7.1). In contrast, $MCAv_{mean}$ was unchanged from baseline ($P = 0.18$) during the 40 mm Hg stage. There was no difference in the $\Delta MCAv_{mean}/\Delta MAP$ ratio between 20 mm Hg (-0.1 ± 3.7) and 40 mm Hg (0.8 ± 3.7, $P = 0.63$). As expected, $P_{ET}CO_2$ was unchanged from baseline values (1 ± 1 and 0 ± 2 mm Hg for 20 and 40 mm Hg respectively, $P > 0.05$).

Table 7.1 Haemodynamic changes from baseline during 20 and 40 mm Hg of lower body positive pressure.

Variable	Baseline	Δ From baseline	
		20 mm Hg	40 mm Hg
Mean MCAv, $cm \cdot s^{-1}$	74 ± 12	3 ± 4 (5 ± 5%)*†	-2 ± 6 (-2 ± 7%)
Systolic MCAv, $cm \cdot s^{-1}$	110 ± 14	5 ± 4 (4 ± 4%)*†	-3 ± 6 (-2 ± 5%)
Diastolic MCAv, $cm \cdot s^{-1}$	53 ± 10	2 ± 3 (5 ± 6%)*†	-2 ± 5 (-3 ± 10%)
CVC, $cm \cdot s^{-1} \cdot mm\ Hg^{-1}$	1.0 ± 0.2	0.0 ± 0.1 (-2.3 ± 8.0%)*†	-0.2 ± 0.1 (-17.0 ± 11.3%)*
MAP, mm Hg	81 ± 15	7 ± 6 (8 ± 7%)*†	13 ± 7 (19 ± 11%)*
Systolic BP, mm Hg	124 ± 20	8 ± 11 (7 ± 9%)*†	16 ± 9 (15 ± 8%)*
Diastolic BP, mm Hg	59 ± 13	5 ± 4 (9 ± 6%)*†	12 ± 7 (24 ± 20%)*
HR, $beats \cdot min^{-1}$	64 ± 15	-3 ± 6 (-5 ± 8%)	-2 ± 6 (-3 ± 9%)
TPR, mm Hg/ $L \cdot min^{-1}$	13 ± 3	1 ± 2 (11 ± 12%)*†	3 ± 2 (26 ± 17%)*

Values are absolute mean difference from baseline ± SD with percentage change from baseline values (± SD) denoted in parentheses. MCAv, middle cerebral artery velocity; CVC, cerebrovascular conductance; MAP, mean arterial pressure; BP, blood pressure; HR, heart rate; TPR, total peripheral resistance. *Statistically different from baseline, $P \leq 0.02$; † Statistically different from 40 mm Hg LBPP, $P \leq 0.02$.

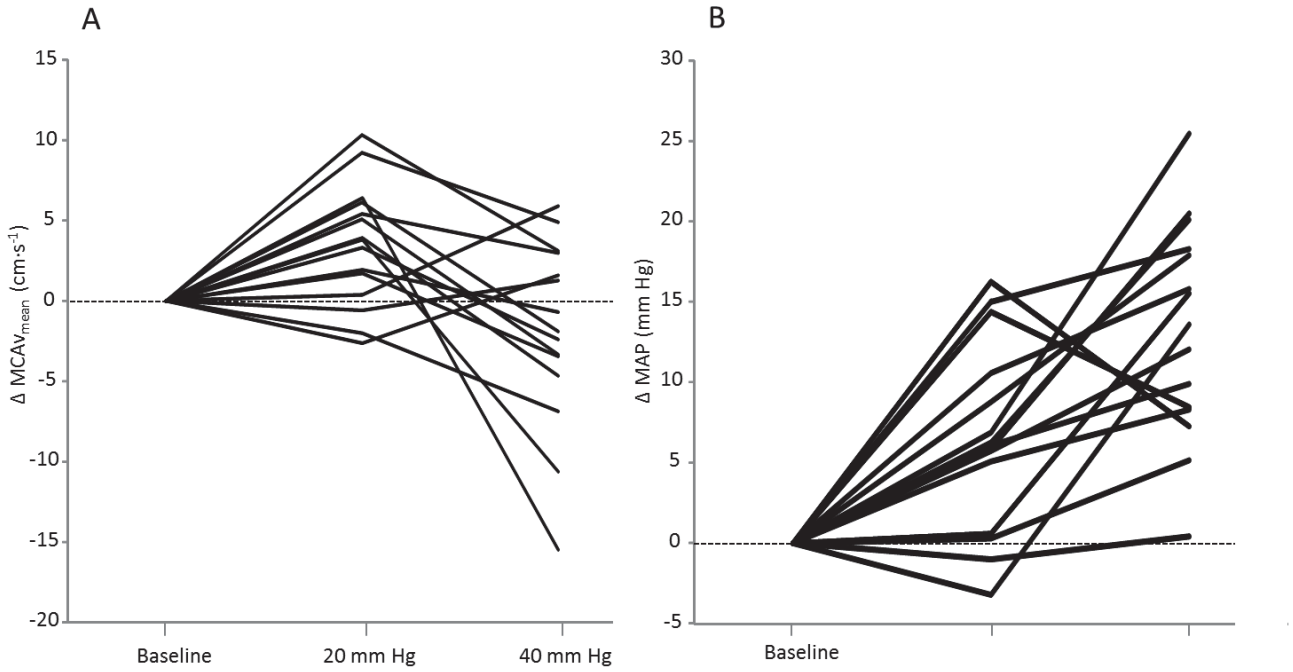

Figure 7.2 Individual changes from baseline for mean middle cerebral artery blood velocity ($MCAv_{mean}$, A) and mean arterial pressure (MAP, B).

7.4 Discussion

The main finding from this chapter was that MCAv was elevated from baseline during 20 mm Hg LBPP but not during 40 mm Hg LBPP, which occurred despite the greater elevation in MAP at 40 mm Hg LBPP. Given that $P_{ET}CO_2$ and $\dot{Q}$ were not different between baselines or LBPP stages, a regulatory role of cerebral sympathetic nerve activity in response to the observed hypertension may explain this observation. The following discussion details the possible mechanism/s regulating CBF during LBPP with an emphasis on a potential role of the autonomic nervous system.

7.4.1 LBPP Induced Changes in MAP and Static Cerebral Autoregulation

Earlier reports by Cutuk *et al.* (2006), who applied short bouts (1 minute) of LBPP in the upright position, are consistent with the data presented here. Whilst not significant, Cutuk *et al.* (2006) reported an increase in $MCAv_{mean}$ from baseline during 20 mm Hg LBPP (from 75 to 81 $cm \cdot s^{-1}$), which then decreased at 40 mm Hg LBPP (78 $cm \cdot s^{-1}$). However, data in this chapter (Figure 7.1) and from others (Shi *et al.* 1997) indicate the response to LBPP is not stable until several minutes after application. The initial response to LBPP produces a sharp increase in MAP that would be counteracted by dynamic autoregulation (Zhang *et al.* 1998a), and is therefore not a steady-state (i.e., static) response. Once circulatory stability is reached, moderate levels of LBPP increase $MCAv_{mean}$, while during greater levels $MCAv_{mean}$ is maintained at resting baseline values.

Although pharmacologically-induced changes in perfusion pressure have been reported to vary linearly with $MCAv_{mean}$ (Lucas *et al.* 2010), the MCAv response to LBPP indicates that the changes in $MCAv_{mean}$ are not purely a result of changes in perfusion pressure (Table 7.1). Similar results have been found during lower body negative pressure with a reduction in MCAv during decreases in MAP, despite the mean pressure still being within the reported autoregulatory range (Rickards *et al.* 2011; Jeong *et al.* 2012). Individuals that displayed the tightest coupling between fluctuations in MAP and MCAv displayed the greatest tolerance to central hypervolemia which may be protective during orthostatic stress (Rickards *et al.* 2011). Although, if this were the case during LBPP it would be expected that MCAv would have increased further at 40 mm Hg LBPP, and contrary to the hypothesis for this chapter, it did not. In fact, while mild increases in MAP did increase MCAv, when MAP was further increased MCAv was unchanged from resting baseline values. This is a novel finding in

healthy participants. Interestingly, the grouped data from Lucas *et al.* (2010), showing the MCAv response to changes in MAP lends support to the observations of this chapter, with less of a pressure-passive effect taking place in the hypertensive range compared to the hypotensive range.

7.4.2 Role of the Sympathetic Nervous System

The underlying mechanism(s) for the observations in this chapter are not inherently clear. Although a linear relationship exists between $MCAv_{mean}$ and $\dot{Q}$ (Ogoh *et al.* 2005a), LBPP produced no change in $\dot{Q}$ at the pressures examined here, which is consistent with other studies in which $\dot{Q}$ was measured via echocardiography (Fu *et al.* 1998). By design, $P_{ET}CO_2$, a strong modulator of cerebral perfusion, was similarly unchanged throughout the protocol. While a direct effect of the sympathetic nervous system on cerebral vessels remains controversial, one potential mechanism driving the changes in $MCAv_{mean}$ observed here may be that of sympathetically-induced vasoconstriction of the cerebral vessels. As such, one could speculate that 40 mm Hg LBPP increases cerebral sympathetic nerve activity that constricts vessels downstream of the MCAv. The increased downstream resistance restrains $MCAv_{mean}$, which is not evident during the mild hypertension that occurred at 20 mm Hg LBPP (7 mm Hg increase).

This conclusion is supported by the data of Tzeng *et al.* (2010b) who showed a differential regulatory response during hyper- and hypotension, with cerebral sympathetic nerve activity speculated as being responsible for the improved regulation within the hypertensive range. While it is likely that a myogenic response is operating within this hypertensive range, it may be that the sympathetic activation during acute (moderate to severe)

hypertension is an important mediator of this asymmetrical cerebral blood flow control. This concept is reinforced by animal data, reporting increased cerebral sympathetic nerve activity during hypertension, but not hypotension (Cassaglia *et al.* 2008). Further, individuals with autonomic failure also display impaired cerebral regulation during increases in MAP (Ogawa *et al.* 1998). This sympathetic control of the cerebral circulation during hypertension has been suggested to restrain cerebral blood flow, and thus protect the brain from hyperperfusion injury (Cassaglia *et al.* 2008). Although not directly assessed here, as all the participants were free from cerebrovascular disease, the cohort recruited for the present study would be expected to have intact cerebral autoregulatory mechanisms. Many disease states have been linked to altered cerebral autoregulatory capacity, including ischaemic stroke (Dawson *et al.* 2000) and carotid artery stenosis (White & Markus 1997). Therefore individuals with a compromised autoregulatory capacity may display a greater pressure-passive response to LBPP as the regulatory mechanism defending against such increases in MAP (such as a myogenic response and/or cerebral sympathetic nerve activity) may be impaired.

In humans, pharmacologically-induced increases in MAP reduces MSNA (Rudas *et al.* 1999). In the LBPP paradigm used here, it is likely that at lower LBPPs (and mild increases in MAP), MSNA is also reduced (Fu *et al.* 1998). However, at higher LBPPs (40 mm Hg) a concomitant increase in both cerebral sympathetic nerve activity and MSNA may occur, with the latter increased due to the activation of intramuscular mechanoreceptors as a result of an increased external pressure (Fu *et al.* 1998); while the possible increase in cerebral sympathetic nerve activity may arise from the hypertension-related changes in intramural pressure within the artery (Tzeng *et al.* 2010b), highlighting the differential regulation

between these two circulations by the sympathetic nervous system (Hamner *et al.* 2010). Moreover, increases in CVP modulate MSNA independently of changes in heart rate via the cardiopulmonary receptors (Charkoudian *et al.* 2004). This cardiopulmonary baroreceptor loading reduces carotid baroreflex sensitivity once a threshold CVP is reached (Shi *et al.* 1993b; Shi *et al.* 1997). Lucas *et al.* (2010) used prolonged and sustained infusions of phenylephrine to increase MAP, which would not produce the same concomitant increase in CVP that would be expected during LBPP (Shi *et al.* 1993b), and would potentially load the high pressure carotid and aortic baroreceptors rather than low pressure cardiopulmonary receptors. The role (if any) of low pressure baroreceptors in the regulation of cerebral sympathetic tone is unknown, however, the differential baroreceptor loading between the present study and that of Lucas *et al.* (2010) may have contributed to the difference in the MAP-MCAv relationship observed.

7.5 Conclusion

The results of this chapter support those of **Chapters Five** and **Six** in that changes in MAP do have an effect on CBF. At 20 mm Hg LBPP increases in both MCAv and MAP were evident. However, despite a greater MAP response at 40 mm Hg LBPP, $MCAv_{mean}$ was unchanged from baseline values. These results indicate a divergent response to graded LBPP. Given this separation in the relationship between MAP and $MCAv_{mean}$, and that $P_{ET}CO_2$ and $\dot{Q}$ were unchanged, modulation of cerebral sympathetic nerve activity may provide a plausible explanation. This chapter induced increases in MAP in healthy individuals that would be expected to have intact static cerebral autoregulation. As detailed in **Chapter Two** (section 2.1.3) hypercapnia impairs dynamic cerebral autoregulation. However, no research has been

conducted on the role of hypercapnia during non-pharmacological induced perturbations in MAP. As demonstrated in this chapter LBPP is capable of stressing the intrinsic autoregulatory mechanisms of the cerebral circulation. Therefore, the proceeding chapter investigated the role of hypercapnia on static cerebral autoregulation during LBPP induced increases in MAP in healthy individuals.

Chapter Eight: The Effect of Hypercapnia on Static Cerebral Autoregulation.

8.1 Introduction

The study that comprised **Chapter Seven** of this thesis illustrated that steady-state increases in MAP appear to challenge 'static' cerebral autoregulation during moderate hypertension. Importantly, the methodology of lower body positive pressure (LBPP) used avoids possible pharmacologically-induced changes in MCA diameter (Ogoh *et al.* 2011). **Chapter Eight** explores this methodological approach further, under conditions of impaired cerebral autoregulation by hypercapnia as studies have only tested this during dynamic changes in MAP (see section 2.1.3.3).

The control of the cerebral circulation is complex and is modulated by many factors (see section 2.1), the most potent of which is the partial pressure of arterial carbon dioxide (P_aCO_2) (Ogoh & Ainslie 2009). Alterations in P_aCO_2 result in pronounced cerebrovascular responses, with increased P_aCO_2 (hypercapnia) dilating cerebral resistance vessels leading to an increase in cerebral blood flow (CBF) and reductions in P_aCO_2 (hypocapnia) constricting vessels and reducing CBF (Kety & Schmidt 1948). This mechanism acts to maintain central pH with the resultant changes in CBF altering CO_2, and thus $[H^+]$, washout from the brain (Ainslie & Duffin 2009). Another key modulator is mean arterial pressure (MAP), which in part determines CPP (CPP = MAP – intracranial pressure). Whilst the cerebral vasculature

does possess an intrinsic ability to defend against changes in blood pressure (Lassen 1959), both steady-state (static) (Zhang *et al.* 2000; Lucas *et al.* 2010; Liu *et al.* 2013) and transient (dynamic) (Tiecks *et al.* 1995b; Edwards *et al.* 2002; Claassen *et al.* 2009) changes in CPP result in concomitant perturbations in CBF.

The regulation of the cerebral circulation is further complicated by the interaction between MAP and $PaCO_2$. Hypercapnia increases sympathetic discharge and elevates blood pressure via the chemoreflex (Morgan *et al.* 1995). Whilst the majority of the observed increase in CBF is due to the direct vaso-active effect of CO_2, the hypercapnic-induced vasodilation reduces the efficacy of dynamic cerebral autoregulation and the ability to defend against dynamic changes in CPP (Aaslid *et al.* 1989). Thus, the chemoreceptor-mediated elevation in CPP increases middle cerebral artery blood flow velocity (MCAv) over and above that induced by the hypercapnic-induced vasodilation alone (Przybylowski *et al.* 2003; Ainslie *et al.* 2005) via a pressure-passive effect (Battisti-Charbonney *et al.* 2011). This effect has been established during dynamic response testing, however, whether hypercapnia alters the efficacy of the cerebral vasculature to respond to steady-state increases in MAP has not been investigated to date. If further increases in MAP are superimposed onto the chemoreflex-induced elevation, it would be expected that MCAv would increase via a pressure-passive response.

Therefore, the purpose of this chapter was to investigate the effect of hypercapnia on cerebrovascular regulation during steady-state increases in MAP induced by LBPP. The hypothesis for this experiment was that hypercapnia would impair static cerebral autoregulation; i.e., hypercapnia will render the brain pressure passive such that additional

increases in MAP, over and above those induced by the chemoreflex alone, will result in further increases in MCAv during higher levels of LBPP.

8.2 Methods

8.2.1 Participants

Fifteen healthy participants were recruited for this study (11 males, 4 females, mean ± SD: age, 28 ± 6 y; body mass, 77 ± 12 kg; height, 175 ± 7 cm). Each participant was fully informed of all potential risks and experimental procedures, after which written consent was obtained. All experimental procedures and protocols were approved by the University's Human Ethics Committee and performed in accordance with the *Declaration of Helsinki*. All participants were free from cardiovascular and cerebrovascular disease, were non-smokers and were not taking medication (apart from oral contraceptive). Participants arrived at the laboratory for the familiarisation and experimental trials hydrated (urine specific gravity 1.008 ± 0.006) and having abstained from strenuous exercise, alcohol and caffeine for at least 24 hours.

8.2.2 Experimental Protocol

Participants visited the laboratory on two occasions, one familiarisation and one experimental trial. During the familiarisation session the participants were familiarised with all experimental procedures. Experimental trials were conducted in the supine position in a LBPP chamber at an ambient temperature of 19-22^{o}C, relative humidity of 40-50% and barometric pressure of 758 ± 8 mm Hg. The experimental protocol is outlined in Figure 8.1. Following instrumentation and 20 minutes of supine rest, baseline values of all measures

were recorded. All LBPP and hypercapnia (5% CO_2 in air) stages were 5 minutes in duration with the order of the LBPP stages randomised. Baseline and washout periods lasted until all variables had returned to initial baseline levels (≥5 min).

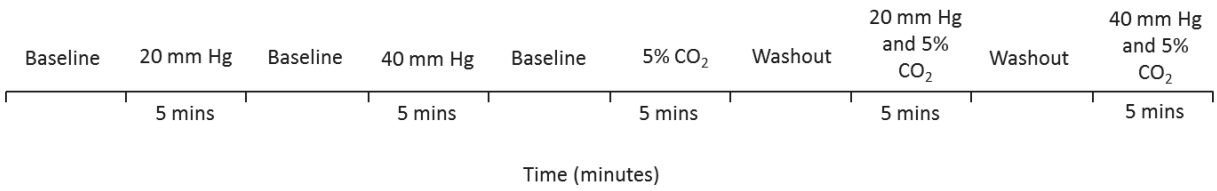

Figure 8.1 Experimental protocol. All experiments were conducted in the above order, however, the lower body positive pressure stages were randomised. Baseline and washout periods lasted until all variables had returned to initial baseline levels.

Participants lay supine in a custom-made LBPP chamber, sealed distal to the iliac crest (see Figure 3.5). Pressure was produced via two commercially available vacuum cleaners, measured (in mm Hg) via a calibrated pressure transducer mounted within the chamber and controlled via a manual bleed valve. By design, participants' $P_{ET}CO_2$ was matched between each respective stage; i.e., $P_{ET}CO_2$ was matched within eucapnic and hypercapnic stages. Eucapnia was achieved by the PhD candidate giving the participant verbal breathing instructions during testing to maintain $P_{ET}CO_2$ at baseline values.

8.2.3 Measurements

Blood flow velocity in the right middle cerebral artery (MCAv) was measured using a 2-MHz pulsed Doppler ultrasound system (DWL, Compumedics Ltd, Germany, refer to **Chapter Three** section 3.3.1 for details). The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was sampled at the mouth using a gas analyser (ML206, ADInstruments). Blood pressure was measured non-invasively using finger photoplethysmography (see section 3.3, Finapres

Medical Systems, Biomedical Instruments, The Netherlands) and heart rate via three-lead electrocardiogram (ADInstruments). All data were acquired continuously via an analogue-to-digital converter (PowerLab ML870; ADInstruments) at 1000 Hz. Data were displayed in real time using commercially available LabChart software (v7.3.3, ADInstruments) and recorded for subsequent off-line analysis.

In a subset of five participants, the internal carotid artery (ICA) diameter was measured during the last minute of the baseline and each steady-state stage. A B-mode image of the ICA was obtained in longitudinal section and the diameter was measured approximately 2 cm distal to the carotid bifurcation. All ultrasound examinations were performed by the same experienced vascular technologist on an ultrasound machine (Terason 3000, Teratech, Burlington, MA, USA) with a 10 MHz linear array transducer. Ultrasound settings (depth, focus position, gain and compression) were optimised for each participant and these were kept consistent throughout each examination. Care was taken to ensure the transducer was stable. At least 15 cardiac cycles were used to obtain average data for diameter and velocity. Cine loops were recorded as AVI files for offline analysis using an edge-detection software program, Cardiovascular Suite UE v 2.5 (Quipu, Pisa, Italy).

8.2.4 Data analysis

For the calculation of $\dot{Q}$, MAP, $MCAv_{mean}$, TPR and CVC refer to **Chapter Three** (section 3.3). In addition cerebrovascular reactivity to CO_2 was calculated as the absolute $\Delta MCAv/\Delta P_{ET}CO_2$.

Baseline data were acquired in the last minute of the baseline period preceding each stage, and presented as the mean across that minute. Similarly, data were averaged in the last

minute of each stage [hypercapnia alone, LBPP (20 & 40 mm Hg LBPP) and hypercapnia + LBPP (5% CO_2 + 20 mm Hg LBPP & 5% CO_2 + 40 mm Hg LBPP)].

8.2.5 Statistical Analysis

Inferential statistical analyses of dependent variables were analysed using a two-way ANOVA (pressure (0, 20, 40 mm Hg LBPP) x CO_2 (eupcapnia, 5% CO_2)) for change from the preceding baseline period. The cerebrovascular reactivity to CO_2 was determined between hypercapnic stages using a one-way ANOVA. Data were assessed for approximation to a normal distribution and sphericity, with no corrections required. When a significant *F*-value was observed (*a priori* set at $P \leq 0.05$), *post-hoc* pairwise comparisons (Bonferroni corrected) were performed. All data were analysed using SPSS statistical software (v20, IBM, New York, USA) and presented as the mean ± SD, unless otherwise denoted.

8.3 Results

Absolute changes from eucapnic baseline for $MCAv_{mean}$, MAP and CVC are displayed in Figure 8.2. In summary, a differential effect of LBPP-induced increases in MAP on $MCAv_{mean}$ with and without hypercapnia was observed (interaction: $P < 0.001$). Specifically, MCAv was not altered from baseline during both LBPP stages in eucapnic conditions, despite the increased MAP with LBPP (Δ6 ± 5 and Δ8 ± 3 mm Hg for 20 and 40 mm Hg LBPP respectively, both $P<0.001$ vs. baseline; Figure 8.2). In contrast, the hypercapnic-induced increases in $MCAv_{mean}$ ($P <0.001$) were greater during the 40 mm Hg LBPP stage (Δ31 ± 13 $cm \cdot s^{-1}$) compared to hypercapnia alone (Δ25 ± 11 $cm \cdot s^{-1}$; $P = 0.026$, Figure 8.2A), which was consistent with the greater elevation in MAP during the 40 mm Hg stage (Δ14 ± 7 mm Hg)

compared to 20 mm Hg LBPP (Δ10 ± 4 mm Hg, P = 0.026) and hypercapnia alone (Δ5 ± 6 mm Hg, P <0.001). Consequently, CO_2 reactivity was greatest for the 40 mm Hg LBPP stage (3.8 ± 1.3 cm·s^{-1}·mm Hg^{-1}) compared to hypercapnia alone (3.0 ± 1.0 cm·s^{-1}·mm Hg^{-1}; P = 0.029) and the 20 mm Hg LBPP stage (3.2 ± 1.0 cm·s^{-1}·mm Hg^{-1}; P = 0.070). Furthermore, CVC was unchanged between hypercapnic stages (P = 0.65; Figure 8.2C). Individual responses are displayed in Figure 8.3.

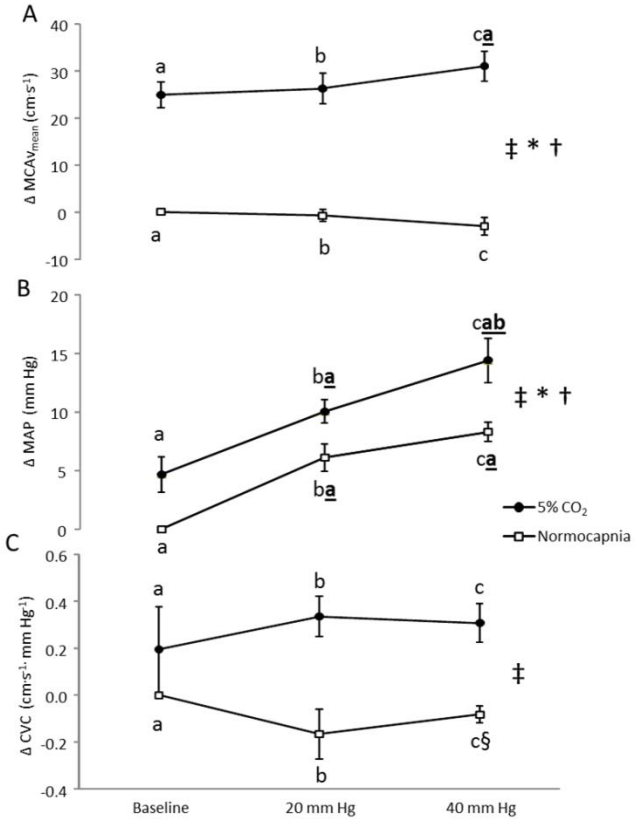

Figure 8.2 Absolute changes from baseline for mean middle cerebral artery blood flow velocity ($MCAv_{mean}$, A), mean arterial blood pressure (MAP, B) and cerebrovascular conductance (CVC, C). The 0 reference on the y axis represents the eucapnic baseline values for each variable. The letters a, b and c represent the LBPP levels baseline (no pressure), 20 and 40 mm Hg, respectively. Bolded and underlined letters represent differences between these pressure stages within each CO_2 trial (P < 0.05). ‡, Significant main effect of pressure, P ≤ 0.05; * Significant main effect of CO_2, P ≤ 0.05; † Pressure-by-CO_2 interaction, P ≤ 0.05, §, trend to be different from baseline P = 0.06. Values are means ± SE.

Finally, the diameter of the ICA demonstrated small changes during each condition. Changes from baseline for eucapnic 20 mm Hg and 40 mm Hg LBPP were -0.9 ± 5.7 and +4.9 ± 4.5%, respectively. ICA diameter increased +3.9 ± 3.5% for hypercapnia alone and +3.4 ± 4.0 and +3.2 ± 3.6% for hypercapnia 20 mm Hg and hypercapnia 40 mm Hg LBPP, respectively.

Table 8.1 Changes from baseline during hypercapnia and lower body positive pressure.

Variable	Condition	Δ From baseline			*P* values		
		Baseline	20 mm Hg	40 mm Hg	Pressure	CO_2	P x CO_2
Systolic MCAv, $cm \cdot s^{-1}$	Eucapnia	98 ±17[a]	0 ±5(0±5)[b]	-3 ±8(-3±7)[c]	0.48	<0.001	0.014
	5%	28 ±13(28±13)[a]	31 ±14(33±14)[b]	36 ±17(39±18)[ca]			
Diastolic MCAv, $cm \cdot s^{-1}$	Eucapnia	44 ±8[a]	-1 ±4(-1±10)[b]	-2 ±6(-5±13)[c]	0.25	<0.001	0.016
	5%	20 ±9(44±21)[a]	20 ±10(48±25)[b]	27 ±13(68±41)[ca]			
Systolic BP, mm Hg	Eucapnia	129 ±14[a]	6 ±8(5±7)[ba]	12 ±7(10 ±6)[cab]	<0.001	0.356	0.011
	5%	8 ±9(6±7)[a]	13 ±7(10±6)[b]	16 ±12(14±7)[ca]			
Diastolic BP, mm Hg	Eucapnia	61 ±8[a]	6 ±4(10±8)[ba]	8 ±3(14±7)[cab]	<0.001	0.049	<0.001
	5%	3 ±6(5±8)[a]	9 ±5(15±7)[ba]	13 ±6(22±11)[cab]			
HR, beats·min^{-1}	Eucapnia	59 ±9[a]	-3 ±3(-4±5)[b]	-1 ±4(0±6)[c]	0.14	<0.001	0.047
	5%	0 ±5 (0 ±7)[a]	1 ±4(3±6)[b]	5 ±5(10±10)[c]			
$\dot{Q}$, L·min^{-1}	Eucapnia	6 ±1.4[a]	-0.2 ±0.6(-2±11)[b]	0.0 ±0.5(0±8)[c]	0.14	0.071	0.92
	5%	0.5 ±0.8(9±14)[a]	-0.1 ±0.8(-1±14)[b]	0.5 ±1.8(1±14)[c]			
TPR	Eucapnia	14 ±4[a]	1.6 ±1.7(13±11)[ba]	1.7 ±1.4(12±10)[ca]	0.018	0.67	<0.001
	5%	-0.3 ±2.3(-1±14)[a]	2.4 ±3.7(17±22)[b]	2.6 ±2.4(17±13)[ca]			
$P_{ET}CO_2$, mm Hg	Eucapnia	40 ±3[a]	1 ±2(2±5)[b]	-1 ±1(-1±4)[cb]	<0.001	<0.001	<0.001
	5%	8 ±3(22±10)[a]	8 ±2(20±6)[b]	8 ±2(21±5)[c]			

Values are absolute mean difference from baseline ± SD and percentage change from baseline values (± SD) are denoted in parentheses. The letters a, b and c represent the LBPP levels baseline (no pressure), 20 and 40 mm Hg, respectively. Multiple letters represent differences between these pressure stages within each CO_2 intervention ($P < 0.05$). MCAv, middle cerebral artery velocity; BP, blood pressure; HR, heart rate; $\dot{Q}$,cardiac output; TPR, total peripheral resistance; $P_{ET}CO_2$, Partial pressure of end-tidal carbon dioxide are shown for Pressure (P) and carbon dioxide.

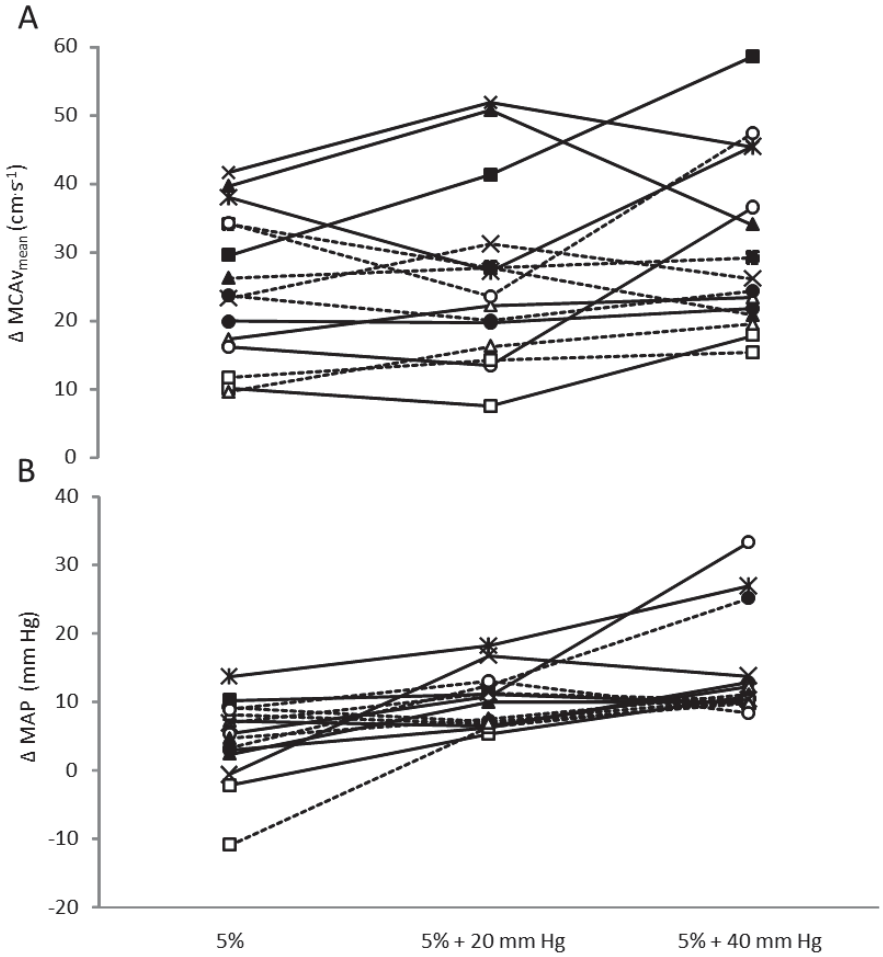

Figure 8.3 Individual mean middle cerebral artery blood flow velocity ($MCAv_{mean}$, A) and mean arterial blood pressure (MAP, B) responses to 5% CO2 (5%) alone and in combination with 20 and 40 mm Hg of lower body positive pressure. The 0 reference on the y axis represents the eucapnic baseline values. Individuals are represented by the same symbol in both graphs.

8.4 Discussion

The main finding was that hypercapnia impaired cerebrovascular control of blood flow (velocity) during steady-state increases in MAP such that MCAv increased concomitantly with blood pressure over and above that mediated by chemoreceptor activation. The increase in $MCAv_{mean}$ was mediated via elevations in both systolic and diastolic flow velocities and despite the elevated MAP, CVC remained unchanged. Thus, consistent with

the hypothesis, hypercapnia impaired steady-state CBF regulation and rendered the brain pressure passive.

8.4.1 The Efficacy of Static Cerebral Autoregulation

In **Chapter Seven** evidence was given to question the efficacy of static cerebral autoregulation. Indeed this question has been asked before of both static (Lucas *et al.* 2010) and dynamic autoregulation (Edwards *et al.* 2002; Claassen *et al.* 2009). This evidence indicates the cerebral circulation is much more pressure passive than originally described (Lassen 1959). Furthermore, there is evidence to indicate that hypercapnia impairs dynamic cerebral autoregulation (Aaslid *et al.* 1989; Zhang *et al.* 1998a; Ainslie *et al.* 2005; Maggio *et al.* 2013). Przybylowski *et al.* (2003) ablated the chemoreceptor-induced MAP response to apnoea via ganglionic blockade and demonstrated that the increase in MCAv is partially attributed to the increase in CPP rather than CO_2 *per se*. Thus, when the hypercapnic stimulus is sufficient to induce elevations in MAP the brain becomes pressure passive, with the chemoreceptor-induced increases in MAP resulting in concomitant elevations in MCAv (Battisti-Charbonney *et al.* 2011). Whilst these studies demonstrate a decreased efficacy for dynamic regulation during hypercapnia, the findings of this chapter demonstrates a similar result for static cerebral autoregulation. Moreover, these earlier studies did not elevate MAP above that induced by hypercapnia. As $\dot{Q}$, another modulator of MCAv (Ogoh *et al.* 2005a), and $P_{ET}CO_2$ were unchanged across the hypercapnic stages (Table 9.1), it appears that the moderate hypertension elevated $MCAv_{mean}$ independently of CO_2 and $\dot{Q}$. Thus, these data demonstrate that when MAP is consistently elevated over and above that induced by the chemoreceptor response, MCAv continues to increase via a pressure-passive response, ultimately indicating an impairment of static cerebral autoregulation.

In **Chapter Seven** a divergent response for MAP and MCAv during incremental LBPP was demonstrated, with $MCAv_{mean}$ decreasing at 40 mm Hg LBPP relative to 20 mm Hg despite further elevations in MAP. Accordingly, the eucapnic results presented here are consistent with those of the previous chapter that showed a decreased MCAv despite the increasing MAP (Figure 8.2). However, a group effect for an increase in $MCAv_{mean}$ during mild hypertension (+20 mm Hg LBPP) in this cohort was not observed, although some individual's $MCAv_{mean}$ did increase in concert with MAP (Figure 8.3). The reasons for these differences between the chapters are not immediately clear, but may be possibly due to the large individual variance in cerebral autoregulatory processes (Zhang *et al.* 2000) and therefore differences between the recruited cohorts. Nevertheless, the decrease in $MCAv_{mean}$ at 40 mm Hg LBPP observed here may be confounded by the small (2 mm Hg) yet significant decreases in $P_{ET}CO_2$ between the eucapnic 20 mm Hg and 40 mm Hg LBPP stages. Given the reactivity (MCAv-$P_{ET}CO_2$ sensitivity = ~2.5%) in the hypocapnic range (Ide *et al.* 2003; LeMarbre *et al.* 2003) this small decrease in $P_{ET}CO_2$ could account for the observed decrease in MCAv (-5%). During the eucapnic positive pressures, the trend for CVC to be lower than baseline would indicate an active regulation (vasoconstriction) against the hypertension. However, despite the greater hypertension observed with the combination of hypercapnia and positive pressures, CVC remained unchanged from hypercapnia alone (Figure 8.2C). Thus, the increased MCAv responsiveness during the hypercapnic 40 mm Hg stage supports the notion that the regulatory mechanisms that would otherwise defend against the moderate hypertension observed during the eucapnic LBPPs are impaired during hypercapnia.

Aaslid et al. (1989) demonstrated that the efficacy of dynamic autoregulation is dependent on resting vascular tone and the present results support this notion for static autoregulation. Autoregulation is complex and thought to involve several mechanisms of action including endothelium-dependent, myogenic and neurogenic mechanisms (Tzeng & Ainslie 2013). Although it is clear that the hypercapnia-associated changes in pH relaxes cerebral vascular smooth muscle (Ainslie & Duffin 2009), the dilatory mechanisms are not entirely clear, although a role for nitric oxide has been suggested (Iadecola & Zhang 1996; Peebles *et al.* 2008). Regardless of the mechanisms responsible for the static regulation of cerebral blood flow and how these mechanisms are impaired or modulated by hypercapnia, it is clear that hypercapnia has a profound effect on the regulatory mechanisms that are otherwise intact during eucapnia.

8.4.2 Role of the Sympathetic Nervous System

In **Chapter Seven** it was speculated that the moderate hypertension associated with 40 mm Hg of LBPP may induce a cerebral sympathetic response that would restrain MCAv. As already mentioned elsewhere in this thesis, the role of the sympathetic nervous system in the regulation of the cerebral vasculature is controversial (Van Lieshout & Secher 2008). Animal models indicate a protective mechanism during acute hypertension (Bill & Linder 1976; Busija *et al.* 1980; Cassaglia *et al.* 2008). In humans the sympathetic nervous system may be tonically active and participate in beat-to-beat MCAv regulation (Zhang *et al.* 2002; Hamner *et al.* 2010), and may explain the asymmetric dynamic autoregulatory response between the hypo- and hypertensive ranges (Tzeng *et al.* 2010b). LBPP activates intramuscular pressure receptors (Fu *et al.* 1998) and maintains muscle sympathetic nerve activity despite cardiopulmonary baroreceptor loading at positive pressures ≥30 mm Hg (Shi

et al. 1997). Ainslie *et al.* (2005) reported a correlation between MSNA and cerebral resistance and it is therefore possible, yet unsubstantiated, that sympathetic modulation by LBPP may influence the cerebral vasculature.

8.4.3 Interaction Between Sympathetic Activation and Arterial CO_2

The cerebral circulation is further complicated by the interaction of neural inputs and arterial blood gases (see **Chapter Two** for discussion on this). However, data detailing this interaction is equivocal with some studies reporting a reduced CO_2 reactivity during sympathoexcitation (Zhang *et al.* 2011) and increased reactivity during ganglionic blockade (Jordan *et al.* 2000), whilst others have shown no influence (LeMarbre *et al.* 2003; Przybylowski *et al.* 2003). As sympathetic regulation of the cerebral circulation was not assessed here it is not possible to define a regulatory role of the sympathetic nervous system under the current conditions. The complex regulatory interaction between arterial blood gases and the neural innervation of cerebral vessels requires further research, and in particular relevance to this experiment, the role, if any, of hypertensive modulation of cerebral sympathetic tone.

8.5 Conclusion

Hypercapnia has been previously demonstrated to impair dynamic cerebral autoregulation. The findings of the present chapter support this notion for static autoregulation during non-pharmacological increases in MAP induced by LBPP. It is apparent that when MAP is elevated, over and above those induced by the chemoreceptor response alone, further increases in MCAv ensue. Despite the elevated CPP, CVC remained unchanged and showed a

differential response to eucapnic LBPP where the moderate hypertension was restrained by an active regulation of the cerebral vasculature consistent with 40 mm Hg of LBPP in **Chapter Seven**. Therefore, hypercapnia impairs static cerebral autoregulation when MAP is consistently elevated.

Chapter Nine: General Discussion

The purpose of this thesis was to determine the effects of non-pharmacological changes in blood pressure on CBF. The perturbations used in the experimental chapters presented here aimed to test the MCAv response to both dynamic and static changes in MAP induced by non-pharmacological means. Static cerebral autoregulation describes the cerebrovascular response to slow and prolonged changes in MAP whilst dynamic autoregulation describes the response to rapid changes in MAP. The underlying physiological processes of these responses may actually be one in the same (Tan & Taylor 2014) and the difference merely reflecting the speed of which MAP is perturbed in a particular experimental approach. Nevertheless, these terms are useful in describing data in the time domain and have been adopted throughout this thesis and the following discussion follows this trend. The results that have been presented in this thesis indicate that both rapid (**Chapters Five** and **Six**) and prolonged (**Chapter Seven**) changes in MAP are accompanied by concomitant changes in MCAv. However, the VM may limit the extent of these dynamic changes via the mechanical effects of elevated intracranial and central venous pressures. Further, it was found that the normal operative autoregulatory mechanisms that would defend against prolonged changes in arterial blood pressure are impaired by hypercapnia (**Chapter Eight**).

9.1 Dynamic Changes in MAP

During heavy resistance exercise extremely high MAPs are possible (MacDougall *et al.* 1985) with these pressures exceeding the proposed autoregulatory upper limit (Lassen 1959; Tan

2012). Despite the progressive increases in MAP experienced at greater loads *during* the effort, MCAv remained unchanged. Whilst MCAv did increase from baseline with all loads, which reflects the high-pass filter characteristics of the cerebral circulation (Zhang *et al.* 1998a), there was no difference between loads. If indeed the cerebral circulation is a high pass filter then a larger transient MAP increase would expected to be translated un-buffered with a large MCAv increase apparent. Given the magnitude of the MAP increases, elevated sympathetic tone in the cerebral vessels cannot be excluded (Cassaglia *et al.* 2008). However, due to the MAP profile and the speed of the increases a more mechanical effect may be apparent. The results from **Chapter Five** indicated that the VM may be responsible for this potential restraint of MCAv at the highest load (90% 6RM). The rapid translation of intrathoracic pressure to the cerebral circulation and subsequently elevations in ICP could explain this restraint. This was tested experimentally in **Chapter Six,** and again, despite the increasing MAP with the relative intensity of the VM, MCAv remained unchanged.

Recruitment of the VM is obligatory above 80% of the MVC (MacDougall *et al.* 1992) and is in agreement with the data presented in **Chapter Five** as the VM was only recruited at the greatest load. Recruitment of the VM does exacerbate the MAP response during resistance exercise (Pott *et al.* 2003), although despite this dramatic increase in MAP, MCAv is restrained. The reduction in transmural pressure of the cerebral arteries (Haykowsky *et al.* 2003) in response to changes in ICP (Greenfield *et al.* 1984) may limit the dilation of the cerebral vessels in response to increases in perfusion pressure. Although CPP is also dependent on venous outflow pressure, the internal jugular veins collapse in the standing position (Gisolf *et al.* 2004a) which take some time to distend during a VM (Pott *et al.* 2000) and is therefore only likely to have an effect after a significant increase in venous pressure

has been established for some time. At least initially the restraint occurs possibly through the reduction in transmural pressure, however as central venous and/or cerebrospinal fluid/Intracranial pressure was not measured this remains speculative. The maintenance of a high venous outflow pressure and ICP will have a pronounced effect on cerebral blood flow in the face of a declining MAP as seen during phase II of the VM. So whilst the elevated ICP may restrain MCAv during phase I and be potentially positive, as the VM progresses this maintained resistance to flow as MAP declines may contribute to cerebral hypoperfusion/syncope. Throughout all the experiments presented in this thesis MAP is reported as a reflection of CPP, in reality in the standing position CPP is likely to be lower because of the physical distance between the cerebral circulation and the heart. Thus, some caution must be made when comparing the reported absolute MAP and the pressure at the cerebral circulation. Regardless of this it appears that irrespective of the MAP response to either the VM in isolation or in combination with resistance exercise, the VM appears to be protective in combating large increases in MAP, despite actually contributing to this increase.

Immediately following resistance exercise and during phase III of a VM a large hypotension was seen with subsequent cerebral hypoperfusion. In both instances the hypotension appears to be posture dependent. Previous experiments utilising a leg-press movement, after which the participants remained seated (Edwards *et al.* 2002) or stood following exercise (Romero & Cooke 2007), resulted in smaller reductions in MCAv (both absolute change and values) than reported in **Chapter Five**. However it should be noted that VMs were not performed in these previous studies which would contribute to the hypotension and subsequent reduction in MCAv. Further, previous work has shown that when a VM is

performed whilst supine (i.e., similar to the leg-press movement) the phase III reduction in MCAv is minimal (Tiecks *et al.* 1995b; Pott *et al.* 2000). Thus, the effects of the phase III response to the VM is highly posture dependent as syncope was observed in several participants during standing VMs in **Chapter Six**. The added circulatory stress from orthostasis results in a greater hypotension and cerebral hypoperfusion following resistance exercise and during phase III of the VM. Although this was not formally addressed in this thesis, when comparing the current data with that previously published the effect of posture is evident. Therefore, the results of **Chapters Five** and **Six** demonstrate that in healthy resistance trained males the VM is protective against acute dynamic changes in MAP during upright resistance exercise and during the strain of a VM. However, dynamic changes in MAP are reflected proportionately by changes in MCAv following resistance exercise and release of the VM (phase III). This demonstrates that the VM both contributes and challenges cerebral regulation.

A potential limitation of the experiments presented in **Chapters Five** and **Six** was that dynamic cerebral autoregulation was not formally assessed. Although the primary aim of this thesis was not to assess dynamic cerebral autoregulatory efficacy, its quantification does have implications for the results presented within the experimental chapters and therefore requires comment. Although there are several ways of defining the dynamic autoregulatory relationship between changes in MAP and MCAv, many are problematic and assume a simplistic relationship (Tzeng & Ainslie 2013). Indeed, transfer function analysis does describe the dynamic relationship between the arterial blood pressure (input) and MCAv (output) and their linear dependence upon each other (coherence) (Zhang *et al.* 1998a). Also, using this analysis technique the relative magnitude (gain) and timing (phase)

of changes between the input and output can be determined (Zhang *et al.* 1998a). However, the relationship between these variables does not appear to be linear (Tan 2012; Tan *et al.* 2013; Tan & Taylor 2014), with differential efficacies between the hypo- and hypertensive ranges (Tzeng *et al.* 2010b). Furthermore, the efficacy of dynamic regulation is dependent on P_aCO_2 (Aaslid *et al.* 1989). Transfer function analysis, along with non-linear analyses such as project pursuit regression (Tan 2012), require oscillatory changes in MAP at varying frequencies that are larger than that at resting as these analyses determine efficacy within the frequency domain. Accordingly, the results of such analyses are difficult to determine and often present conflicting results (Tzeng & Ainslie 2013). Also, a large recording of data is required to conduct this analysis (>30 s up to 5 min). As the perturbations utilised in **Chapters Five** and **Six** were of such a short duration this method of analysis would not have been appropriate. Whilst a disturbance in cerebral autoregulation has been reported following semi-recumbent resistance exercise these data were collected during 30 s following the effort (Koch *et al.* 2005). More importantly the changes in cerebrovascular resistance and MCAv pulsatility were conflicting and mimicked those at syncope (Koch *et al.* 2005), and thus are in agreement with the results in **Chapter Five**. However, as the data were taken over the 30 s post exercise, and the participants remained semi-recumbent, no reduction in MAP or MCAv was reported and thus the results are difficult to compare.

The VM can also be used to assess the efficacy of dynamic cerebral autoregulation in patients (Tiecks *et al.* 1996) and in healthy individuals (Tiecks *et al.* 1995b) by comparing the changes in MAP and MCAv between the distinct phases within phase II. Whilst MAP and subsequently MCAv are reduced during phase II when performed in isolation, syncope occurred in **Chapter Six** upon the release of the manoeuvre (phase III). Accordingly, phase II

was not the focus in this thesis. Due to the nature of the resistance exercise utilised in **Chapter Five** (short dynamic efforts), length of VMs (short ~2 s) performed and the elevated MAPs, phase II (either a or b) of the VM was not discernible. Therefore, these types of autoregulatory quantification were not conducive to the main aims of this thesis. Moreover, syncope during phase III ultimately highlights the inadequacies of cerebral autoregulation, presumably as arterial blood pressure has decreased below the lower limit of autoregulation. As all participants were health screened before taking part and none presented with cerebral or cardiovascular pathologies normal operative cerebral autoregulatory processes were assumed. This appears to be the case in both **Chapter Six** and **Seven** as immediately following resistance exercise and during phase III of the VM, respectively, MCAv reached nadir before MAP and also recovered earlier. Therefore, although not formally assessed dynamic cerebral autoregulation still appears to be operative in the healthy individuals investigated in this thesis.

9.2 Static Changes in MAP

An additional aim of this thesis was to challenge the other arm of cerebral autoregulation, the response to static, prolonged increases in MAP. The classic static autoregulatory curve purposed by Lassen (1959) was based on steady-state CBF flow during changes in MAP in a varied cohort that included patients with various pathologies and that were taking medications. More recently Lucas *et al.* (2010) revisited this concept and used transracial Doppler to assess MCAv during steady state pharmacologically induced perturbations in MAP. The results presented by Lucas *et al.* (2010) indicate that the brain is much more pressure passive than traditionally accepted. Although, an autoregulatory curve similar to

that presented by Lassen (1959) has been shown during oscillatory lower body negative pressure. Only at slow frequencies of 0.03 Hz was a plateau apparent, with the plateau only extending 5 mm Hg each side of baseline pressure with changes beyond this resulting in a pressure passive region (Tan 2012; Tan & Taylor 2014). This plateau may have gone undetected in the study by Lucas *et al.* (2010) due to the magnitude of the step changes of MAP. As these MAP oscillations increase in frequency autoregulatory gain increased such that these fluctuations were un-buffered and a pressure-passive relationship was apparent (Tan 2012). Whilst dynamic cerebral autoregulation takes ~5 s to compensate for changes in MAP (Zhang *et al.* 1998a), the variance of MCAv is dependent on the ΔMAP/Δtime (Tzeng *et al.* 2011). Therefore, to assess the effectiveness of static cerebral autoregulation perturbations in MAP outside the autoregulatory plateau (>5 mm Hg increase in MAP from baseline) were used, as purposed by Tan *et al.* (2012). Moreover, slow steady state prolonged (5 min) increases in MAP were used as this is where static cerebral autoregulation appears to be more effective (Zhang *et al.* 1998a).

There is evidence to indicate that phenylephrine may constrict the MCA resulting in an increase in MCAv but not CBF *per se* (Ogoh *et al.* 2011). Therefore, the results of Lucas *et al.* (2010) may have been confounded by the use of pharmaceuticals. Therefore, a non-pharmacological means of increasing MAP was utilised in this thesis in the form of LBPP, which increases MAP in a dose dependent manner (Nishiyasu *et al.* 2007). In **Chapter Seven** it was demonstrated that LBPP induced static increases in MAP elevated MCAv, consistent with the results from **Chapters Five** and **Six** in that dynamic changes in MAP can induce similar changes in MCAv. Contrary to the stated hypotheses this phenomenon was only apparent at 20 mm Hg. At higher pressures (40 mm Hg) MCAv demonstrated a small

decrease from baseline although this was not statistically significant. It was speculated that this restraint of MCAv despite the elevated MAP at 40 mm Hg LBPP is due to sympathetic modulation of the cerebral circulation. Indeed, the brain was pressure passive but only at lower pressures.

Interestingly, this increase at 20 mm Hg of LBPP was not replicated in **Chapter Eight** although the same slight decrease from baseline at +40 mm Hg was apparent. The difference in results between these two experiments may be explained by the difference in cohort recruited. As autoregulatory efficacy differs greatly between individuals (Zhang *et al.* 2000), physiological variance (i.e., sensitivities) between the cohorts may underpin these differences. Moreover, the efficacy of cerebral autoregulation is inversely related to baroreflex sensitivity, indicative of a compensatory mechanism between the systemic and cerebral circulations (Tzeng *et al.* 2010a). In these individuals with poor autoregulatory efficacy an un-compensable forced increase in MAP is possibly unable to be counteracted by the cerebral resistance vessels. Therefore differential regulation between the systemic and cerebral circulations in the two cohorts may explain this variation.

Hypercapnia has been shown to impair dynamic cerebral autoregulatory processes (Aaslid *et al.* 1989; Zhang *et al.* 1998a; Ainslie *et al.* 2005; Maggio *et al.* 2013), however whether this extends to static cerebral autoregulation is unclear. Previous work has demonstrated during dynamic rebreathing that a break point is achieved during hypercapnia in that chemoreceptor-mediated increases in MAP result in subsequent elevations in MCAv. In **Chapter Eight** the efficacy of static cerebral autoregulation during concomitant hypercapnia was tested using LBPP-mediated increases in MAP. At 40 mm Hg LBPP the brain became pressure passive; i.e., the control mechanisms that would otherwise defend against the

elevated arterial blood pressure are impaired by hypercapnia and confirms the notion that autoregulatory efficacy is reliant on resting vascular tone (Aaslid *et al.* 1989). The superimposed 40 mm Hg of LBPP during hypercapnia (5% CO_2) increased MAP by 14 ± 7 mm Hg from baseline versus an increase 5 ± 6 mm Hg due to hypercapnia alone. This increase during 5% CO_2 + 40 mm Hg LBPP elevated MCAv by 31 ± 13 $cm \cdot s^{-1}$ with hypercapnia alone increasing MCAv 25 ± 11 $cm \cdot s^{-1}$. Thus, a 9 mm Hg increase in MAP resulted in a 6 $cm \cdot s^{-1}$ increase in MCAv. Despite the relatively small increase in MAP a significant change in MCAv was observed. Ideally larger increases in MAP would be used to demonstrate this autoregulatory impairment. However, given the assessment of static rather than dynamic autoregulation producing large and prolonged non-pharmacological increases in MAP is difficult. In order to produce such changes in MAP, a pharmacological intervention would be required (i.e., phenylephrine infusion). Despite this method's potential influence on the MCA, further exploration in this area would require such interventions. Regardless, **Chapter Eight** demonstrates impairment of static autoregulatory processes during hypercapnia and confirmed the hypothesis.

9.3 Limitations

9.3.1 Transcranial Doppler

Transcranial Doppler ultrasound provides a measure of flow velocity rather than blood flow *per se*. Under various stimuli (Valdueza *et al.* 1997) including simulated orthostasis (Serrador *et al.* 2000), MCAv accurately reflects the magnitude of changes in flow as the diameter of the MCA remains unchanged (also see **Chapter Three**, section 3.1.2). In this thesis the assumption has been that MCA has remained unchanged. During modest changes in MAP

(30 ± 16 mm Hg) the MCA diameter changes <4% (Giller *et al.* 1993). Consistent with this, a change in ICA diameter of <5% across all conditions in **Chapter Eight** was observed during changes in MAP much lower than that reported by Giller *et al.* (1993). Furthermore, the results here are in agreement with previous data demonstrating that hypercapnia driven increases in ICA flow are mediated by changes in velocity rather than arterial diameter (Sato *et al.* 2012b; Willie *et al.* 2012). ICA flow has also been found to correspond well with changes in MCAv during pharmacological stepwise perturbations in MAP (Liu *et al.* 2013). One possible caveat of this type of measure is that although the MCA is a branch of the ICA, which is an extracranial vessel and thus not subject to changes in intracranial pressure. As participants were supine throughout the LBPP experiments (**Chapters Eight** and **Nine**), both venous and intracranial pressures would be expected to be unchanged throughout, however, as these measures were not recorded any potential change in transmural pressure cannot be quantified.

In contrast to LBPP, resistance exercise and the VM produce much higher MAPs and the subsequent effect of this large increase in arterial pressure on conduit vessel diameter remains unknown. Despite this uncertainty, transcranial Doppler measurements of MCAv have been used extensively in the research of the cerebrovascular response to resistance exercise (Edwards *et al.* 2002; Pott *et al.* 2003; Romero & Cooke 2007; Ogoh *et al.* 2010b; Moralez *et al.* 2012), squatting (Claassen *et al.* 2009) and the VM (Tiecks *et al.* 1995b; Pott *et al.* 2000) because of its ability to track dynamic changes in blood flow velocity. Given the possible protective role of the cerebral sympathetics at high perfusion pressures (Cassaglia *et al.* 2008) and the extremely high blood pressures experienced during resistance exercise, it is possible that the MCA may change diameter. Likewise, cerebral sympathetic activation

has been shown to possibly influence the phase IV response of the VM (Zhang *et al.* 2004a). Although, the retest reliability has been shown to be strong during repeated VMs using transcranial Doppler (Wallasch & Kropp 2012). Thereby, during resistance exercise it is possible that there is a passive dilation of the MCA during the large increase in perfusion pressure that may exceed the autoregulatory threshold (MacDougall *et al.* 1985). To oppose this increase in perfusion pressure it is possible that a protective, sympathetically mediated, vasoconstriction may occur.

In an attempt to clarify the effect of the VM on conduit artery dimensions the measurement of the carotid artery diameter was piloted. However, due to the large increases in CVP, jugular pressure rises and causes the carotid to shift from the initial position. Acquisition of an adequate image within the VM time frame was not possible and the data was unable to be recorded. Therefore, the response of the conduit arteries, including the middle cerebral artery, to the VM remains unknown. Similarly, due to the dynamic nature of the exercise performed in **Chapter Six,** measurement of flow and/or diameter in any upstream vessels of the MCA is highly unlikely. Therefore, static and/or single joint (i.e., isometric leg extension) exercise without a concomitant VM may be required to gauge the vascular response to resistance exercise. However the pressor response to such exercise will likely be lower than that produced here as the VM will not be recruited (MacDougall *et al.* 1992).

Nevertheless, caution should be taken with any interpretation of transcranial data under conditions where the diameter of the measured vessel may change. If MCA diameter did increase, absolute blood flow may have increased without an evident increase in $MCAv_{mean}$. Further research is warranted to investigate the effects of large MAP perturbations on conduit vessel diameter (including the MCA). However, given current temporal restrictions

in alternative technology (e.g., MRI, Xenon clearance) and the experimental perturbations used in **Chapters Six** and **Seven**, transcranial Doppler measurement of MCAv remains the most appropriate option for the questions addressed in this thesis.

9.3.2 Participants

The participants recruited to participate in the experiments detailed in **Chapters Six and Seven** were resistance trained males. This cohort was chosen to avoid any potential learning effect or strength gains across the study which would render the established relative loads inaccurate. All participants were familiar with the exercise utilised in **Chapter Six** and thus minimal familiarisation was required. More importantly this enables comparison between studies as a similar cohort was recruited. Whilst endothelial function appears to be well maintained (Rakobowchuk *et al.* 2005b; Otsuki *et al.* 2007) resistance training alters central vascular compliance that includes the carotid artery (**Chapter Two,** section 2.5.4). Whether these changes in vascular compliance extend to the intracranial vessels is unknown. As the cerebral conduit arteries are no longer viewed as primarily feed arteries and actively contribute to the cerebral regulatory processes during large changes in MAP (Chan *et al.* 2011; Liu *et al.* 2013), a potentially reduced arterial compliance in resistance trained individuals cannot be ignored. Thus, the application of these results to non-resistance trained individuals must be made with caution. The differences, if any, in cerebral autoregulatory processes between resistance trained and non-resistance trained individuals therefore requires further research. Indeed, data of this nature comparing athletes with reduced arterial compliance (resistance trained) and increased compliance (endurance athletes) has been collected in collaboration with the University of Otago and is currently being analysed.

9.3.3 Implications for Special Populations.

The participants who completed the experimental protocols in **Chapters Five** and **Six** were healthy and relatively young, disease-free individuals; however, a number of different populations with varying physiological states participate in resistance exercise due to its many positive physiological outcomes (Garber *et al.* 2011). The participation in such exercise increases the possibility of recruiting the VM. Older healthy individuals, despite having a maintained dynamic cerebral autoregulatory capacity have an increased resting baseline cerebrovascular resistance (Lipsitz *et al.* 2000), which may actually defend against acute increases in MAP like those experienced during resistance exercise and during the VM. However, for disease states where dynamic cerebrovascular control is impaired (e.g., acute ischaemic stroke (Dawson *et al.* 2000)) the rapid increases in blood pressure, that have been reported during heavy resistance exercise (MacDougall *et al.* 1985; MacDougall *et al.* 1992) may exclude these impaired populations from performing such exercises. Moreover, everyday heavy lifting is likely to produce a similar response to strict resistance exercise. Similarly, the VM is also recruited in everyday tasks (defecation, lifting and during coughing). Thus, further research is required to identify the haemodynamic response and associated risks (and/or benefits) of performing resistance exercise and a VM when standing in compromised, sedentary individuals and also endurance athletes.

9.4 Conclusions

The purpose of this thesis was to investigate the effects of changes in blood pressure on cerebral perfusion in healthy humans. This included non-pharmacological rapid and transient changes (dynamic) and also steady-state prolonged changes (static) in blood

pressure. Further, static changes in blood pressure were induced when the normal operative autoregulatory mechanisms of the cerebral circulation were impaired by hypercapnia. In specific regards to the aims and hypotheses outlined in **Chapter Four** it can be concluded that:

- Regardless of the number of repetitions performed during upright dynamic resistance exercise, MCAv is unchanged
- Immediately following upright resistance exercise in the upright position both the hypotension and cerebral hypoperfusion are dependent on the relative load lifted
- MCAv is unchanged by VM intensity during Phase I regardless of the progressive increases in MAP
- Increases in VM relative intensity results in a greater phase III hypotension and cerebral hypoperfusion, which can be severe enough to induce syncope
- Prolonged steady-state increases in MAP induced by LBPP can challenge static cerebral autoregulation
- Hypercapnia impairs static cerebral autoregulation such that increases in MAP are associated with increases in MCAv

9.5 Future Directions

9.5.1 Lower Body Positive Pressure

Further work is required to explain the differential response to LBPP between **Chapter Seven and Eight.** Volumetric flow using duplex ultrasound in all participants during incremental LBPP may help resolve this issue. Further, exploration is required to establish

the large variation observed between participants both during normo- and hypercapnia. This may be related to differences between sensitivities of the baroreflex and autoregulation between individuals.

Previous work has shown that skin cooling induces similar changes in CVP to that of LBPP during normothermia (Shi *et al.* 1993a; Cui *et al.* 2005). While skin cooling has been used to restore CVP during heat stress (Wilson *et al.* 2002), to the best of the candidates knowledge, whether moderate levels of LBPP (e.g., ~20 mm Hg) could also be used to restore central blood volume and rectify reductions in MAP and MCAv during heat stress is unknown and warrants further study. Ethical approval for this study has been granted. Graded LBPP will be applied during incremental heat stress in an attempt to restore central blood volume.

As discussed in **Chapter Two** (section 2.1.3.3.2) hypoxia impairs cerebral autoregulation. However, whether hypoxia renders the brain pressure passive in a similar fashion to hypercapnia **(Chapter Eight)** is unknown. This question could be answered by using the protocol utilised in **Chapter Eight** to non-pharmacologically increase in MAP via lower body positive pressure.

9.5.2 The Valsalva Manoeuvre

As shown in **Chapter Six**, the Valsalva manoeuvre produces drastic changes in cerebral perfusion with large reductions evident during phases II and III. These reductions are sufficient to produce syncope in some instances. These reductions are followed by large increases in MCAv during phase IV. In one participant who became syncopal, the peak phase IV response was associated with a near tripling of MCAv in comparison with baseline measures. Thus, this phase IV response may be hyperaemic in nature and offset the

reduction in perfusion and oxygenation that occurs during the manoeuvre. Data using near infrared spectroscopy is currently being analysed and will reveal if this phase IV is indeed a reactive response to the reductions in oxygenation.

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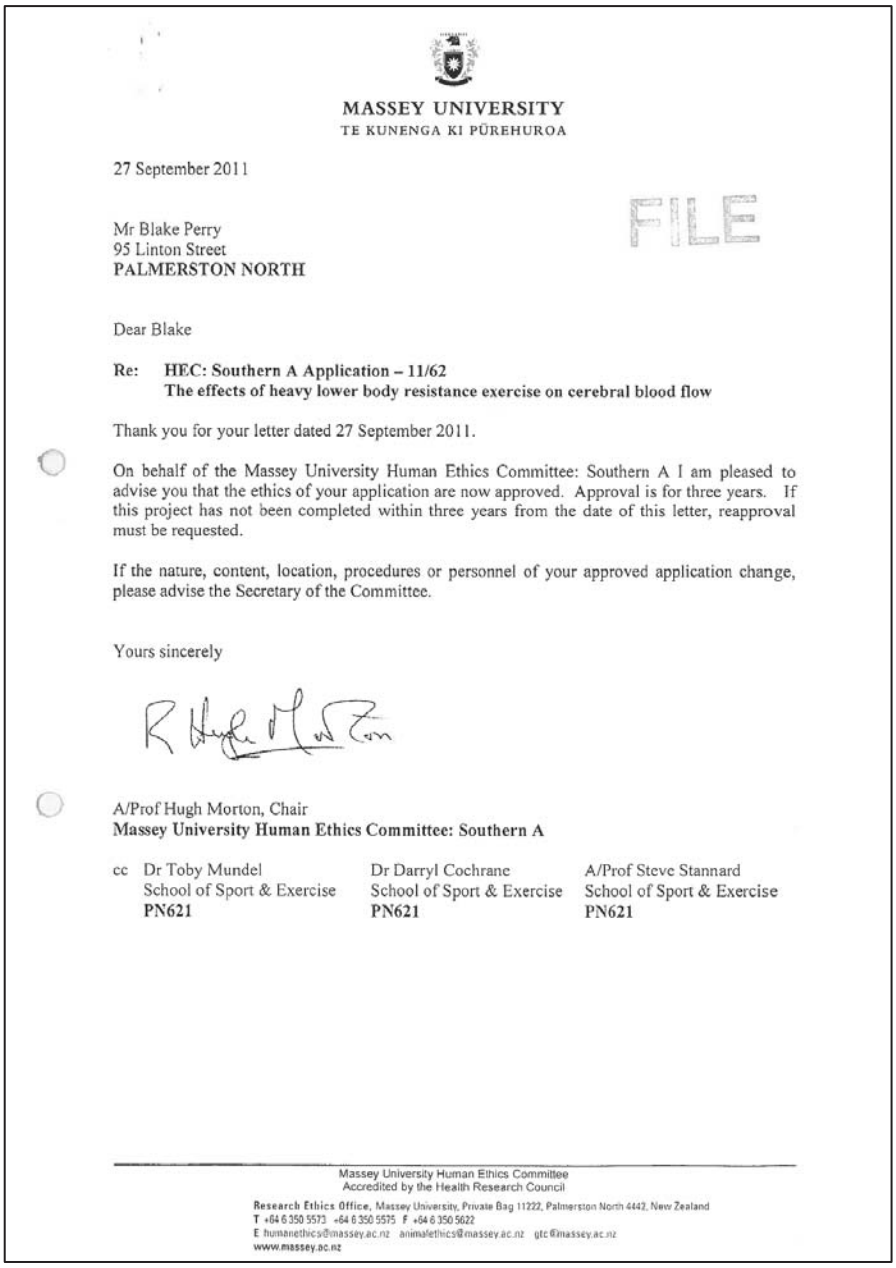
Appendix A

A: Ethical Approval Documentation

Chapter Five: Haemodynamic Response to Upright Resistance Exercise: Effect of Load and Repetition



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

27 September 2011

FILE

Mr Blake Perry
95 Linton Street
PALMERSTON NORTH

Dear Blake

Re: HEC: Southern A Application – 11/62
The effects of heavy lower body resistance exercise on cerebral blood flow

Thank you for your letter dated 27 September 2011.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

A/Prof Hugh Morton, Chair
Massey University Human Ethics Committee: Southern A

cc Dr Toby Mundel, School of Sport & Exercise, **PN621**
Dr Darryl Cochrane, School of Sport & Exercise, **PN621**
A/Prof Steve Stannard, School of Sport & Exercise, **PN621**

Massey University Human Ethics Committee
Accredited by the Health Research Council

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Chapter Six: The Cerebrovascular response to Graded Valsalva Manoeuvres Whilst Standing

12/153

Academic Services
Manager, Academic Committees, Mr Gary Witte

21 August 2012

Dr J Cotter
School of Physical Education
Division of Sciences
46 Union Street West

Dear Dr Cotter,

I am again writing to you concerning your proposal entitled "**Do strength-trained athletes have adaptive protection of brain blood flow**", Ethics Committee reference number **12/153**.

Thank you for providing your amended documentation, which show that you had amended the wording in the Information Sheet relating to ACC compensation, and had obtained approval from the Ngai Tahu Research Consultation Committee.

We also note your request for an amendment to including additional researchers onto your team, being PhD students Mr Blake Perry and Ms Kate Thomas, as their expertise will enhance your study and benefit their own research. This amendment is approved.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

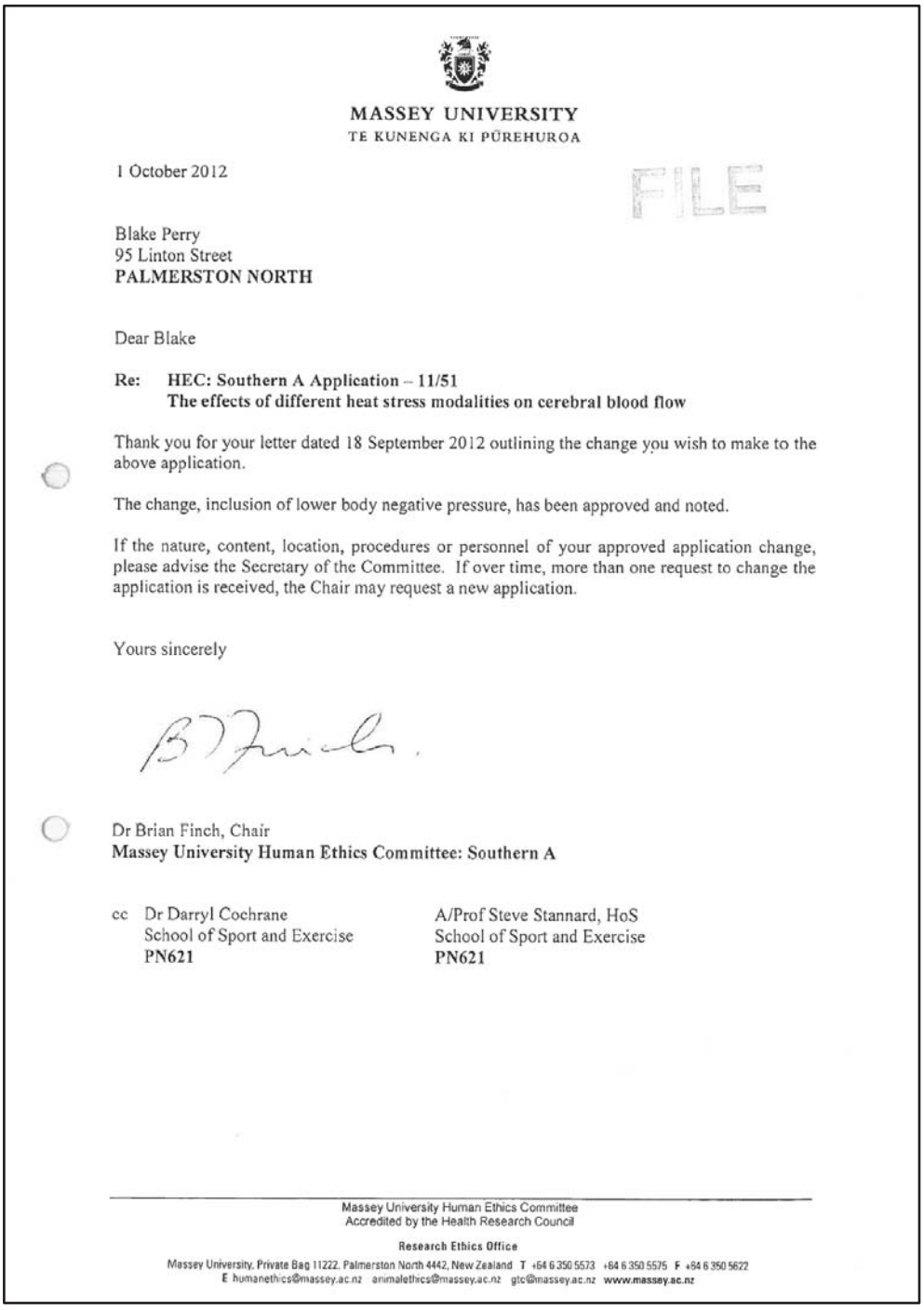
Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor D G Booth Dean School of Physical Education

Chapter Seven: Middle Cerebral Artery Blood Flow Velocity in Response to Lower Body Positive Pressure

*Note amendments were made to this Ethical application to include lower body positive pressure.



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

1 October 2012

FILE

Blake Perry
95 Linton Street
PALMERSTON NORTH

Dear Blake

Re: HEC: Southern A Application – 11/51
The effects of different heat stress modalities on cerebral blood flow

Thank you for your letter dated 18 September 2012 outlining the change you wish to make to the above application.

The change, inclusion of lower body negative pressure, has been approved and noted.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee. If over time, more than one request to change the application is received, the Chair may request a new application.

Yours sincerely

Dr Brian Finch, Chair
Massey University Human Ethics Committee: Southern A

cc Dr Darryl Cochrane
School of Sport and Exercise
PN621

A/Prof Steve Stannard, HoS
School of Sport and Exercise
PN621

Massey University Human Ethics Committee
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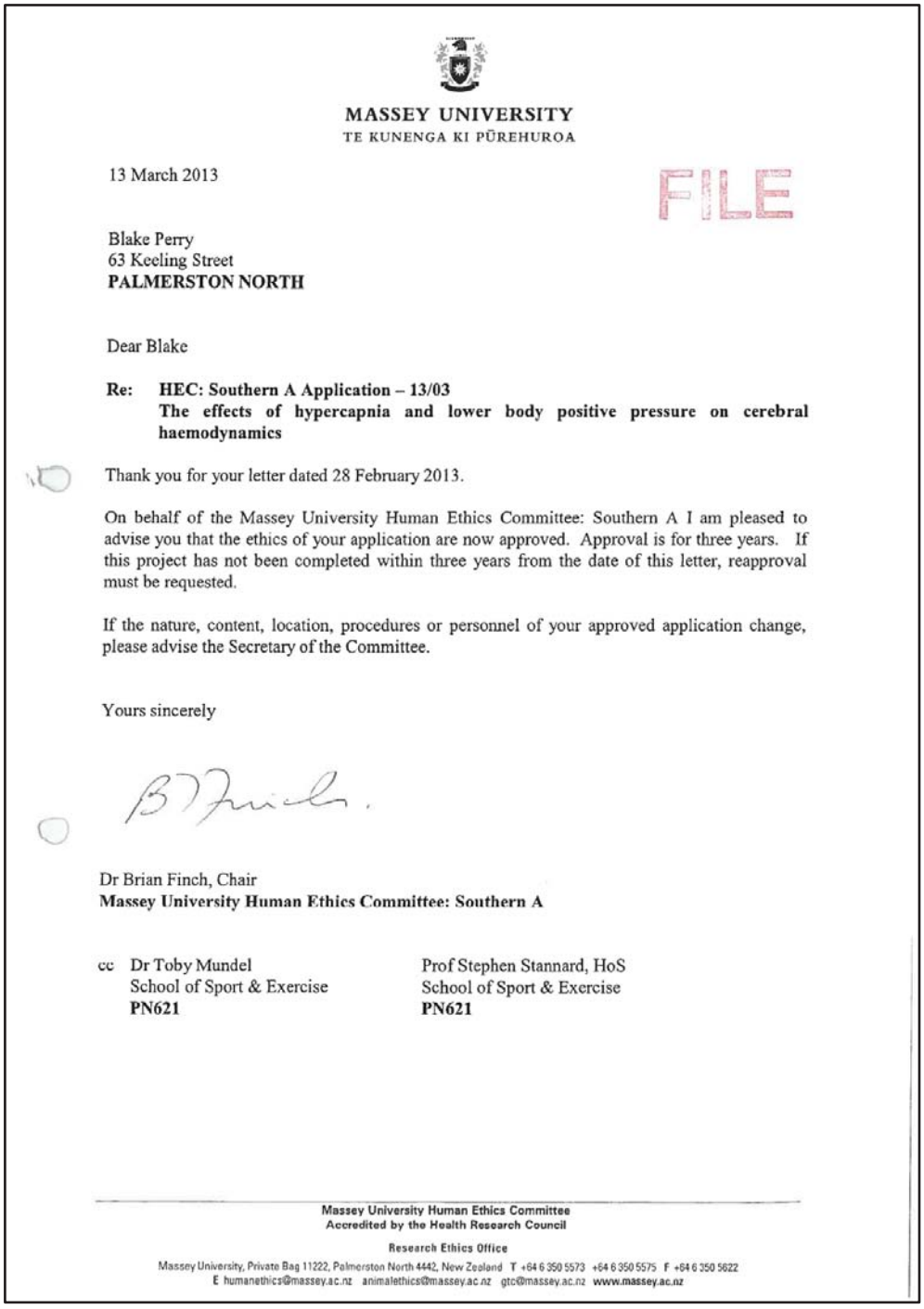
Chapter Eight: The Effect of Hypercapnia on Static Cerebral Autoregulation



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

13 March 2013

FILE

Blake Perry
63 Keeling Street
PALMERSTON NORTH

Dear Blake

Re: HEC: Southern A Application – 13/03
The effects of hypercapnia and lower body positive pressure on cerebral haemodynamics

Thank you for your letter dated 28 February 2013.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

B J Finch.

Dr Brian Finch, Chair
Massey University Human Ethics Committee: Southern A

cc Dr Toby Mundel
School of Sport & Exercise
PN621

Prof Stephen Stannard, HoS
School of Sport & Exercise
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Appendix B

B: Statements of Contribution

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Blake Graeme Perry

Name/Title of Principal Supervisor: Dr. Toby Mundel

Name of Published Research Output and full reference:

Perry BG, Schlader ZJ, Barnes MJ, Cochrane DJ, Lucas S & Mündel T (2013). Hemodynamic Response to Upright Resistance Exercise: Effect of Load and Repetition. Med Sci Sports Exerc DOI: 10.1249/MSS.1240b1013e3182a7980f.

In which Chapter is the Published Work: Chapter Five

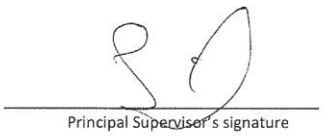
Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 85%

  and / or
- Describe the contribution that the candidate has made to the Published Work:

Candidate's Signature

21/3/2014
Date



Principal Supervisor's signature

21/3/2014
Date

GRS Version 3– 16 September 2011

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

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Name/Title of Principal Supervisor: Dr. Toby Mundel

Name of Published Research Output and full reference:

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Name of Candidate: Blake Graeme Perry

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Perry BG, Schlader ZJ, Raman A, Cochrane DJ, Lucas SJ & Mündel T (2013). Middle cerebral artery blood flow velocity in response to lower body positive pressure. Clin Physiol Funct Imaging DOI: 10.1111/cpf.12046.

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Appendix C

C: Published Papers

Medicine & Science IN Sports & Exercise

The Official Journal of the American College of Sports Medicine

. . . Published ahead of Print

Hemodynamic Response to Upright Resistance Exercise: Effect of Load and Repetition

Blake G. Perry[1], Zachary J. Schlader[1], Matthew J. Barnes[1], Darryl J. Cochrane[1], Samuel J.E. Lucas[2,3], and Toby Mündel[1]

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ORIGINAL RESEARCH

The cerebrovascular response to graded Valsalva maneuvers while standing

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Keywords
Cerebral blood flow, orthostasis, syncope, Valsalva maneuver.

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Abstract

The Valsalva maneuver (VM) produces large and abrupt increases in mean arterial pressure (MAP) at the onset of strain (Phase I), however, hypotension, sufficient to induce syncope, occurs upon VM release (phase III). We examined the effect of VM intensity and duration on middle cerebral artery blood velocity (MCAv) responses. Healthy men (n = 10; mean ± SD: 26 ± 4 years) completed 30%, 60%, and 90% of their maximal VM mouth pressure, for 5 and 10 sec (order randomized) while standing. Beat-to-beat MCAv and MAP during phase I (peak), at nadir (phase III), and recovery are reported as the change from standing baseline. During phase I, MCAv rose 15 ± 6 cm·s^{-1} (P < 0.001), which was not reliably different between intensities (P = 0.11), despite graded increases in MAP (P < 0.001; e.g., +12 ± 9 mmHg vs. +35 ± 14 for 5 sec 30% and 90% VM, respectively). During Phase III, the MCAv response was duration- (P = 0.045) and intensity dependent (P < 0.001), with the largest decrease observed following the 90% VM (e.g., −19 ± 13 and −15 ± 11 cm·s^{-1} for 5 and 10 sec VM, respectively) with a concomitant decrease in MAP (P < 0.001, −23 ± 11 and −23 ± 9 mmHg). This asymmetric response may be attributable to the differential modulators of MCAv throughout the VM. The mechanical effects of the elevated intrathoracic pressure during phase I may restrain increases in cerebral perfusion via related increases in intracranial pressure; however, during phase III the decrease in MCAv arises from an abrupt hypotension, the extent of which is dependent upon both the duration and intensity of the VM.

Introduction

The Valsalva maneuver (VM) is defined as a forced exhalation against a closed glottis (Hamilton et al. 1936) and is executed during coughing (Hamilton et al. 1944), defecation, and also during resistance exercise (MacDougall et al. 1992). The VM can be separated into four distinct phases: Phase I, an increase in mean arterial pressure (MAP) at the onset of the strain as the elevated intrathoracic pressure is translated to the arterial circulation; phase IIa, a reduction in stroke volume as atrial filling pressure is reduced; phase IIb, an increase in heart rate mediated by the arterial baroreflex to offset the reduction in stroke volume; phase III, a rapid decline in MAP as the strain is released; phase IV, rapid recovery and overshoot of MAP as the now restored cardiac output is ejected into a constricted arterial tree (Goldberg et al. 1952; Tiecks et al. 1995; Pott et al. 2000).

The VM may be viewed as eliciting undesirable cardiovascular and cerebrovascular responses, but there is also evidence that it may indeed protect the cerebral circulation during phase I of the maneuver (Tiecks et al. 1995; Niewiadomski et al. 2012). Specifically, increases in intrathoracic pressure are translated to the cerebrospinal fluid (Hamilton et al. 1944) such that increases in intracranial pressure (ICP) ensue (Greenfield et al. 1984),

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Clin Physiol Funct Imaging (2013) doi: 10.1111/cpf.12046

SHORT COMMUNICATION

Middle cerebral artery blood flow velocity in response to lower body positive pressure

Blake G. Perry[1], Zachary J. Schlader[1,*], Aaron Raman[1,†], Darryl J. Cochrane[1], Samuel J. E. Lucas[2,3,4] and Toby Mündel[1]

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Summary

Lower body positive pressure (LBPP) has been used in the treatment of haemorrhagic shock and in offsetting g-force induced fluid shifts. However, the middle cerebral artery blood flow velocity (MCAv) response to supine LBPP is unknown. Fifteen healthy volunteers (mean ± SD: age, 26 ± 5 year; body mass, 79 ± 10 kg; height, 174 ± 9 cm) completed 5 minutes of 20 and 40 mm Hg LBPP, in a randomized order, separated by 5 minutes rest (baseline). Beat-to-beat MCAv and blood pressure, partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) and heart rate were recorded and presented as the change from the preceding baseline. All measures were similar between baseline periods (all $P>0.30$). Mean arterial pressure (MAP) increased by 7 ± 6 (8 ± 7%) and 13 ± 7 mm Hg (19 ± 11%) from baseline during 20 and 40 mm Hg ($P<0.01$), respectively. The greater MAP increase at 40 mm Hg ($P<0.01$ versus 20 mm Hg) was mediated via a greater increase in total peripheral resistance ($P<0.01$), with heart rate, cardiac output (Model flow) and $P_{ET}CO_2$ remaining unchanged (all $P>0.05$) throughout. MCAv increased from baseline by 3 ± 4 cm s^{-1} (5 ± 5%) during 20 mm Hg ($P = 0.003$), whilst no change ($P = 0.18$) was observed during 40 mm Hg. Our results indicate a divergent response, in that 20 mm Hg LBPP-induced modest increases in both MCAv and MAP, yet no change in MCAv was observed at the higher LBPP of 40 mm Hg despite a further increase in MAP.

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arterial blood pressure; cerebral autoregulation; cerebral blood flow; cerebral haemodynamics; sympathetic nervous system

Introduction

Lower body positive pressure (LBPP) has been used for the treatment of hypotensive and hypovolemic patients (Wayne & Macdonald, 1983) and in preventing g-force induced syncope in pilots (Wood, 1987). Graded LBPP produces incremental increases in central venous pressure (Shi et al., 1997) and elevates arterial blood pressure (Fu et al., 1998). Despite the pronounced haemodynamic effect of LBPP, data concerning the effects of LBPP on cerebral perfusion are unclear. Short bouts (1 min) of LBPP applied to upright individuals are reported to have no effect on middle cerebral artery blood flow velocity (MCAv, an index of cerebral blood flow) (Cutuk et al., 2006). However, the applicability of these findings to situations involving a 'steady-state' is not clear given that physiological stability is reported to be achieved only after several minutes of LBPP (Shi et al., 1997). Thus, the effects of LBPP on steady-state MCAv and whether the MCAv response to LBPP are similar between the supine and upright posture is not known.

The brain possesses an intrinsic autoregulatory mechanism that is capable of maintaining adequate blood flow in the face of changes in perfusion pressure. This autoregulatory mechanism has both a dynamic and a static component. Dynamic cerebral autoregulation (CA) refers to the regulation of blood flow during rapid blood pressure perturbations (Zhang et al., 1998). Static CA describes the myogenic response of cerebral vessel smooth muscle to constant and relatively prolonged (e.g. >1 min) changes in intramural pressure such that cerebral blood flow is maintained relatively constant across a wide range of arterial blood pressures (Lassen, 1959). However, recent

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Appendix D

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