CEREBRAL BLOOD FLOW IN MAN: REGULATION BY ARTERIAL BLOOD GASES

by

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Abstract

Due to the high metabolic rate of brain tissue and nominal substrate storage, brain perfusion must be precisely regulated to ensure continuous delivery of oxygen and substrates. Cerebral blood flow (CBF) is principally regulated by tissue metabolism, perfusion pressure, autonomic nervous activity, and the partial pressures of arterial oxygen (PaO$_2$) and carbon dioxide (PaCO$_2$) – an integrative process thus involving the marked influence of pulmonary gas exchange and cardiovascular function, in addition to intracranial mediators of cerebrovascular resistance. This thesis explicates the roles of PaO$_2$ and PaCO$_2$ in human regulation of regional CBF. In study 1, to elucidate their discrete roles, PaO$_2$ and PaCO$_2$ were independently manipulated at sea level through the widest range tolerated in humans. Flow reactivity to hypocapnia (low PaCO$_2$) and hypoxia (low PaO$_2$) was greater in the vertebral (VA) than internal carotid (ICA) artery, whereas similar reactivity was observed during hypercapnia (high PaCO$_2$) and hyperoxia (high PaO$_2$). Cerebral oxygen delivery was well protected except in cases of extreme hypocapnia. The ventilatory response to hypoxia mitigates falling PaO$_2$ and reduces PaCO$_2$, particularly during initial exposure to high altitude. Study 2 assessed regional CBF during ascent to 5050m and every 12 hours during the first 3 days of acclimatization. Although total CBF increased by ~50% and was modestly related to reductions in oxygen saturation of hemoglobin, no regional CBF differences were observed. To extend these findings, Study 3 aimed to determine if cerebrovascular responses to changes in PaO$_2$ and PaCO$_2$ differed at 5050m compared to sea level. Despite respiratory alkalosis and partial metabolic compensation at 5050m restoration of PaO$_2$ to sea level values decreased CBF, and CBF sensitivity to acutely altered PaCO$_2$ remained similar to sea level. To elucidate the interactive effect on CBF of profound hypoxemia and hypercapnia, study 4 examined the temporal changes in elite breath-hold divers during maximum apneas. Despite 40-50% reductions in arterial oxygen content, CBF elevations were regionally similar (up to +100%) thereby facilitating maintenance of brain oxygen delivery throughout apnea. Although the regulation of CBF is multifaceted, the cerebrovasculature prioritizes oxygen delivery and adjusts to chronic changes in arterial blood gases.
Preface

A version of Chapter 2 has been published: Willie CK, Tzeng YC, Fisher JA, & Ainslie PN (2014). Integrative regulation of human brain blood flow. J Physiol 592(5), 841–859. I drafted the manuscript, worked with a medical illustrator through multiple iterations of figure 2.1; worked on figure 2.4 in cooperation with Prof. Ainslie, and created all other figures in the chapter. The co-authors each edited the initial version once before submission to The Journal of Physiology. I completed all revisions following the reviewers’ comments, and in cooperation with Prof. Ainslie completed the final version that was accepted for publication. Copyright approval was obtained from John Wiley and Sons for reproduction of figures and text.

Aspects of Chapter 3 have been published: Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK & Ainslie PN (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. J Neurosci Methods 196, 221–237. I drafted and revised the manuscript. Figure 3.1 was provided by Prof. Damian M. Bailey. I created all other figures. The other co-authors edited the manuscript and provided feedback on content and style. I completed all revisions following the reviewers’ comments, and in cooperation with Prof. Ainslie completed the final version that was accepted for publication. Copyright approval was obtained from Elsevier for reproduction of figures and text.

Chapter 4 has been published: Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood flow in man during acute changes in arterial blood gases. J Physiol 590(14), 3261–3275. Prof. Ainslie and myself envisaged the study. Part A (CO₂ investigations) was completed at the University of British Columbia – Okanagan where Kurt J. Smith, Dr. Nia C. Lewis, and Prof. Philip N. Ainslie assisted with data collection. Part B (O₂ investigations) was completed in the laboratory of Dr. David B. Macleod, Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA. Dr. Macleod, Dr. Andrew D. Shaw, Kurt J. Smith, Dr. Keita Ikeda, Dr. Joseph Graham, and Prof. Philip N. Ainslie assisted with data collection. I completed all data analysis and wrote the manuscript. Drs. Macleod, Shaw
and Ainslie provided critical feedback on the manuscript. Drs. Tzeng and Day edited the manuscript. I revised the manuscript based on the reviewers’ comments in cooperation with Prof. Ainslie. I wrote the ethics application with assistance from Prof. Ainslie which was approved by the University of British Columbia Clinical Research Ethics Board (ID: H11-01105). Copyright approval was obtained from John Wiley and Sons for reproduction of figures and text.

Chapter 5 has been published: Willie CK, Smith KJ, Day TA, Ray LA, Lewis NC, Bakker A, Macleod DB & Ainslie PN (2013). Regional cerebral blood flow in humans at high altitude: Gradual ascent and two weeks at 5050 m. J Appl Physiol (1985). doi: 10.1152/japplphysiol.00594.2013. Kurt J. Smith, Lauren A. Ray, Dr. Nia C. Lewis, Akke Bakker, Dr. David B. Macleod, and Prof. Philip N. Ainslie assisted with data collection. Akke Bakker conducted all transcranial color-coded Doppler measures and analyzed those data only. I analyzed all other data, wrote and revised the manuscript in cooperation with Prof. Ainslie. I further revised the manuscript based on the reviewers’ comments. In cooperation with Prof. Ainslie I wrote the application for ethical approval for all studies taking place on the Nepal 2012 research expedition which were approved by the UBC clinical research ethics board (ID# H11-03287). Copyright approval was obtained from the American Physiological Society for reproduction of figures and text.

Chapter 6 will be submitted for publication to The Journal of Cerebral Blood Flow and Metabolism. Willie CK, Macleod DB, Smith KJ, Lewis NC, Foster GE, Ikeda K, Hoiland RL & Ainslie PN (2014). Cerebral blood flow and fuel utilization in man at high altitude. All co-authors assisted with data collection. I wrote the chapter with feedback from Prof Ainslie. This study was approved by the UBC clinical research ethics board (ID# H11-03287).

Chapter 7 will be submitted for publication to The Journal of Cerebral Blood Flow and Metabolism. Willie CK, Ainslie PN, Drvis I, MacLeod DB, Bain AR, Madden D, Maslov PZ & Dujic Z (2014). Brain blood flow in elite breath-hold divers during changes in arterial blood gases. All co-authors assisted with data collection. I wrote the chapter with feedback from Prof. Ainslie. This study was approved by the UBC clinical research ethics board (ID#H13-02620).
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<tr>
<td>∆</td>
<td>Delta, change in the suffixed parameter</td>
</tr>
<tr>
<td>[Ca(^{2+})]</td>
<td>Calcium ion concentration (mM)</td>
</tr>
<tr>
<td>[Cl(^-)]</td>
<td>Chloride ion (mM)</td>
</tr>
<tr>
<td>[H(^+)]</td>
<td>Hydrogen ion concentration (nmol/L)</td>
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<td>[HCO(_3^-)]</td>
<td>Bicarbonate ion concentration (mEq/L)</td>
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<tr>
<td>[K(^+)]</td>
<td>Potassium ion concentration (mM)</td>
</tr>
<tr>
<td>[Na(^+)]</td>
<td>Sodium ion concentration (mM)</td>
</tr>
<tr>
<td>BE</td>
<td>Base excess (mEq/L)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index (kg/m(^2))</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
</tr>
<tr>
<td>CA</td>
<td>Cerebral autoregulation</td>
</tr>
<tr>
<td>CaO(_2)</td>
<td>Arterial content of oxygen (mL O(_2)/dL)</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow (mL/min)</td>
</tr>
<tr>
<td>CDO(_2)</td>
<td>Cerebral delivery of oxygen (mL O(_2)/min)</td>
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<td>Cerebral delivery of glucose (µmol/100g/min)</td>
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<tr>
<td>CO(_2)</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CVC</td>
<td>Cerebrovascular conductance (mL/min / mmHg)</td>
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<tr>
<td>CvO(_2)</td>
<td>Jugular venous content of oxygen (mL O(_2)/dL)</td>
</tr>
<tr>
<td>CVR</td>
<td>Cerebrovascular resistance</td>
</tr>
<tr>
<td>dCA</td>
<td>Dynamic cerebral autoregulation</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>H(^+)</td>
<td>Hydrogen ion (proton)</td>
</tr>
<tr>
<td>HA</td>
<td>High altitude</td>
</tr>
<tr>
<td>HCO(_3^-)</td>
<td>Bicarbonate ion</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>tHb</td>
<td>Hemoglobin concentration (g/dL)</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>1-NMMA</td>
<td>N\textsuperscript{G}-monomethyl-L-arginine (nitric oxide synthase inhibitor)</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>MCAv</td>
<td>Middle cerebral artery blood velocity (cm/s)</td>
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<tr>
<td>MRI</td>
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<tr>
<td>NVC</td>
<td>Neurovascular coupling</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>Oxygen</td>
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<td>PaCO\textsubscript{2}</td>
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<tr>
<td>Q\textsubscript{ICA}</td>
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<tr>
<td>SNA</td>
<td>Sympathetic nervous system activity</td>
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<td>Vertebral artery</td>
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For my Mom

“Wisdom does not inspect, but behold. We must look a long time before we can see.”

-Henry David Thoreau
Chapter 1: Introduction

1.1 Background

Maintenance of adequate cerebral blood flow (CBF) is necessary for normal brain function and survival. That the brain receives ~15% of total cardiac output and accounts for ~20% of the body’s oxygen consumption, despite being 2-3% of total body weight, is testament to its high energetic requirements. Unconsciousness follows even transiently compromised CBF (in less than 10 seconds) (Smith et al., 2011) due to the need for constant oxygen supply, which, when combined with a very limited ability to store energy, requires effective mechanisms to prioritize and maintain blood flow. These mechanisms are generally very effective, permitting some humans to survive extreme decreases in arterial blood oxygen content (hypoxaemia) such as with ascents to very high altitudes (West, 2006) or during prolonged apnea during free-diving (Dujic et al., 2013) and are an integral component in the maintenance of homeostasis.

Despite over a century of study (Mosso, 1880; Roy & Sherrington, 1890), establishing an integrative understanding of the mechanisms regulating CBF in humans has been difficult (if not impossible) to achieve for a number of reasons. First, over the past 50 years a reductionist approach to cerebrovascular physiology has dominated the field, largely because of the difficulty of in vivo brain vascular assessment. Secondly, the prevailing use of transcranial Doppler ultrasound in human CBF research since the late 1980s introduced assumptions of intracerebral vessel characteristics that are increasingly being shown incorrect. And finally there has been limited study of CBF regulatory mechanisms in humans in the literature of the past decade.

The cerebral vasculature rapidly adapts to changes in perfusion pressure (termed cerebral autoregulation; CA), to regional metabolic requirements of the brain (neurovascular coupling), to altered autonomic neural activity (Cassaglia et al., 2009; Cassaglia et al., 2008a), and in response to systemic metabolism via changes in blood gases (cerebrovascular reactivity). Regulation of CBF is thus highly controlled and involves a wide spectrum of regulatory mechanisms that together work to provide adequate oxygen and nutrient supply (Ainslie & Duffin, 2009; Ogoh & Ainslie, 2009a; Edvinsson & Krause, 2002; Panerai et al.,
1999; Querido & Sheel, 2007; Ainslie & Tzeng, 2010b; Lucas et al., 2010). Indeed, the cerebral vasculature is highly sensitive to changes in arterial blood gases, in particular the partial pressure of arterial carbon dioxide (PaCO$_2$) (Ainslie & Duffin, 2009; Sato et al., 2012; Willie et al., 2012). It is thought that CA acts to change cerebral vascular resistance via vasomotor effectors, principally at the level of the cerebral arterioles and pial vessels (Edvinsson & Krause, 2002). Additionally, neuronal metabolism elicits an effect on CBF as necessitated by changes to regional oxygen consumption, with the sympathetic nervous system likely playing a protective role in preventing over-perfusion in the cerebral vasculature (Ainslie & Tzeng, 2010b; Tzeng et al., 2010c; Tzeng et al., 2010d; Wilson et al., 2010; Cassaglia et al., 2009; Cassaglia et al., 2008a). This thesis focuses on the role of arterial blood gases in the regulation of CBF.

There is a pronounced effect of PaCO$_2$ on the cerebrovasculature, yielding an approximate 3–6% increase and/or 1–3% decrease in flow mmHg change in CO$_2$ above and below eupnoeic PaCO$_2$, respectively. Alveolar ventilation, by virtue of its direct effect on PaCO$_2$, is consequently tightly coupled to CBF. The response of CBF to PaCO$_2$ is of vital homeostatic importance as it directly influences central CO$_2$/pH, which is the central chemoreceptor stimulus (Chapman et al., 1979). For example, ventilation and CBF both increase in response to rising PaCO$_2$. Tissue PCO$_2$ is reduced by the augmented CBF, curbing further ventilatory stimulation and stabilizing the negative feedback loop between PaCO$_2$ and ventilation (Ainslie & Duffin, 2009; also see Fan et al., 2010a; Fan et al., 2010b). Indeed, previous studies have found a correlative link between blunted cerebrovascular CO$_2$ reactivity and the occurrence of central sleep apnea in patients with congestive heart failure (Xie et al., 2005), and also in the pathophysiology of obstructive sleep apnea (Burgess et al., 2010; Reichmuth et al., 2009).

Hypoxia is a cerebral vasodilator as reflected by a proportional increase in CBF with decreasing PaO$_2$ in conditions of isocapnia, but only when PaO$_2$ is reduced below ~50 mmHg (Shapiro et al., 1970). This threshold sensitivity is clinically relevant given the arterial hypoxemia encountered during exercise in elite athletes (Ogoh & Ainslie, 2009a; Ogoh & Ainslie, 2009b) and at high altitude (Lucas et al., 2011; Willie et al., 2014a; Ainslie & Ogoh, 2010). Related changes may also occur in severe stages of certain pathologies such as
chronic lung disease and heart failure (reviewed in Ainslie & Duffin, 2009; Ainslie & Ogoh, 2010; see also Galvin et al., 2010). Because the ventilatory response to hypoxia causes an attendant decrease in PaCO$_2$, exposure to hypoxia of sufficient severity produces opposing effects on the cerebrovasculature. There is moreover an interactive effect between blood gases where hypercapnia increases and hypocapnia decreases cerebrovascular reactivity to hypoxia (Mardimae et al., 2012).

1.2 Objective

The objective of the present thesis was to systematically examine mechanisms of cerebrovascular regulation during changes in arterial blood gases. This was carried out in four distinct studies utilizing both laboratory-based methods of independently manipulating PaO$_2$ and PaCO$_2$, and natural paradigms of hypoxia that elicit concomitant hypocapnia (high altitude) and hypercapnia (breath hold diving).

1.3 Specific Aims and hypotheses

1.3.1 Study 1 (Chapter 4)

On the basis of a review of the literature, limited data suggest the CBF responses to alterations in arterial blood gases differ between the brainstem and cortex.

**Aims:** To explicate the independent effects of PaO$_2$ and PaCO$_2$ on regional CBF.

**Hypothesis:** That the vertebral artery (VA) and internal carotid artery (ICA) would yield disparate flow-reactivity to changes in both PaCO$_2$ and PaO$_2$.

1.3.2 Study 2 (Chapter 5)

Ascent and acclimatization to high altitude produces progressive changes in arterial blood gases and acid base balance as a result of ventilatory and metabolic compensation for hypoxia. Regional CBF was therefore examined in the context of a field-based experiment to high altitude (5050 m).

**Aims:** To assess if the variability in CBF during ascent and acclimatization was attributable to ventilatory acclimatization (i.e., oxyhemoglobin saturation) and/or regional differences in CBF.
Hypothesis: Based on the finding in Chapter 4 that VA reactivity to both hypoxia and hypocapnia was greater than of the ICA, we hypothesized that brain stem blood flow would be more closely related to ventilatory acclimatization as indexed by SpO₂.

1.3.3 Study 3 (Chapter 6)
Acclimatization to high altitude involves metabolic compensation for respiratory alkalosis and a resultant leftward shift of the CO₂-pH relationship, increased ventilatory reactivity to CO₂, and greater prevailing sympathetic nervous system activity.

Aims: To clarify the effect of altered acid-base balance and chronic hypocapnia on CBF reactivity to changes in blood gases following partial acclimatization to 5050 m.

Hypothesis: We hypothesized that metabolic compensation for respiratory alkalosis with ascent to high altitude would yield steeper cerebrovascular reactivity with changes in PaCO₂.

1.3.4 Study 4 (Chapter 7)
Breath-hold divers are capable of volitionally attaining levels of hypoxia and hypercapnia beyond the tolerable limits of most humans. In addition to these dramatic changes in arterial blood gases, prolonged apnea also yields dramatic increases in blood pressure and sympathetic nervous activity.

Aims: To identify the regional CBF response to severe hypercapnic hypoxaemia during prolonged apnea in elite breath hold divers, and its relationship to duration of maximal apnea. We further aimed to isolate the effects of arterial blood gases per se from other cardiovascular consequences of prolonged apnea such as increased blood pressure and sympathetic nervous activity by replicating an identical temporal pattern of PaO₂ and PaCO₂ while the subject was breathing.

Hypothesis: That the magnitude of CBF increase would be greater during apnea than during breathing due to larger increases in mean arterial pressure (MAP), and that the duration of breath-hold would be related to the balance of hypoxemia to acidosis.

1.4 Presentation
This dissertation contains seven further chapters. Following this overview chapter, Chapter 2 reviews mechanisms regulating CBF, with specific focus on humans. The relationship between regulatory mechanisms is emphasized, but the following three broad categories of
control are explicated: (1) the effect of blood gases and neuronal metabolism on CBF; (2) buffering of CBF with changes in blood pressure, termed cerebral autoregulation; and (3) the role of the autonomic nervous system in CBF regulation. The state of our knowledge with respect to these areas is summarized, important gaps in the literature are outlined and avenues for future research suggested. Chapter 3 reviews CBF methodology. Key metrics of CBF quantification are first discussed, followed by their application in various metrics for assessing cerebrovascular function in health and disease. Chapter 4 describes a laboratory study in which the independent effects of altered PaO₂ and PaCO₂ were assessed. Anterior and posterior CBF and reactivity were quantified through the widest range of steady state PaO₂ and PaCO₂ tolerated in humans. Chapter 5 assessed regional CBF to the hypocapnic hypoxia experienced during ascent to 5050 m and every 12 hours during the first 3 days of acclimatization. Chapter 6 evaluates the effect of acclimatization on CBF regulation to changes in arterial blood gases. The CBF response to steady state independent changes in PaO₂ and PaCO₂ were assessed at sea level and again following 8-10 days at 5050 m. Chapter 7 examined the roles of hypercapnic hypoxaemia and increases in MAP in regulation of CBF during prolonged apnea in elite breath hold divers. Finally, Chapter 8 summarizes these studies, interprets their collective conclusions, and suggests important avenues for future research.
Chapter 2: Literature review

Due to limited capacity for substrate storage (Brown & Ransom, 2007), and the high metabolic rate of brain tissue, the precise regulation of cerebral blood flow (CBF) is critical for maintenance of constant nutrient and oxygen supply to the brain. Substantial reductions in CBF quickly lead to unconsciousness (Van Lieshout et al., 2003), and if maintained, brain damage and death ensues (Smith et al., 2011). Fastidious control of CBF involves a wide spectrum of overlapping regulatory mechanisms that together work to ensure optimum oxygen and nutrient delivery (Figure 2.1). That these mechanisms are present and largely unique to the cerebrovasculature does not obviate the importance of maintained systemic cardiovascular control. Yet, as will be explicated in this review, CBF regulation is often assumed to be so efficacious that it is treated separately, instead of as an integral component of the cardiovascular system.

The partial pressure of arterial carbon dioxide (PaCO₂), mean arterial pressure (MAP), and cerebral metabolism are the principal regulators of CBF, with the autonomic nervous system contributing a somewhat lesser role. The regulation of CBF should not, therefore, be viewed as being limited to mechanisms within the cranium. Rather, the regulation of CBF should be noted as an integrative process that involves the marked influence of pulmonary gas exchange and cardiovascular function in addition to intracranial mediators of cerebral vessel resistance (and therefore flow).

This literature review aims to concentrate on human cerebrovascular control from an integrative standpoint in the hope of stimulating resurgent interest in a field of study that lags behind those of other systems of the body. We endeavor to synthesize an expansive scope of information into a brief review rather than detail specific areas or mechanisms of cerebrovascular control; there are specific reviews on many of the topics we cover herein to which the reader will be referred throughout.
Figure 2.1 Schematic illustrating the anatomy and key mechanisms of regulation in the cerebrovasculature.
Figure 2.1 (continued)

Central figure: The cerebrovasculature is comprised of two pairs of large arteries that branch from the sublavian arteries: the internal carotid arteries (ICA) that carry ~70% of total cerebral blood flow (CBF) and the vertebral arteries (VA) that distribute ~30% of total CBF to the brainstem, cerebellum and occipital cortex. Both the ICA and VA anastomose to form the circle of Willis before branching out into the main intracerebral arteries that ramify extensively en route to the brain surface. At the surface the vessels form a dense network of highly vasoactive arterioles within the pia mater before they penetrate into the cortex (Inlay II). The driving pressure in this system is the cerebral perfusion pressure (CPP) that is determined by the difference between MAP and intracranial pressure (ICP), under conditions where central venous pressure (CVP) is lower than ICP. Under these conditions MAP approximates CPP. Thus, it is important to note that the figure shows a schematic of the cerebrovascular state in a resting supine human, and does not consider the myriad complex adjustments that take place with orthostatic stress (Gisolf et al., 2004; Hicks & Munis, 2005). Because of the enclosed nature of the skull, ICP acts as a Starling resistor for cerebral venous outflow, a mechanism likely of greater importance with marked elevations in ICP or central venous pressure, or both. The cerebral arteries (including the internal carotid and vertebral arteries) are sensitive to changes in blood gases (Heistad et al., 1978a; Faraci et al., 1987a; Willie et al., 2012) and to changes in perfusion pressure, thus serving as a ‘first-line’ defense in maintaining brain perfusion (Faraci et al., 1987a; Faraci et al., 1987b) (Inlay I). These arteries are also densely innervated with branches of the cranial nerves, carotid sinus nerve and branches from the superior cervical ganglion. The role of these nerves is contentious but evidence favors cerebral constriction in response to increased sympathetic outflow and/or increased MAP, particularly at the tortuous segments where the ICA and VA vessels enter the skull. Turbulent blood flow through these segments increases resistance for a given luminal diameter according to Poiseuille’s law; constriction of the vessel in these sections in the face of increased MAP attenuates pressure increases distal to the tortuous segment (Inlay I).

Inlay II shows a neurovascular unit. The pial vessels respond to changes in PaCO₂, PaO₂, and perfusion pressure (Kontos et al., 1978; Wolff & Lennox, 1930) (Inlay III). The pial arteriole penetrates the pia mater through the Virchow-Robin space where it becomes encapsulated by glial processes termed end-feet and pericytes that release vasoactive substances and mechanical constrict/dilate, respectively, in response to changes in metabolic demand of the surrounding neural matrix. Gap junctions between the endothelial and vascular smooth muscle cells allow for retrograde intramural vascular signal conductance such that vasodilatory or constrictive signals pass to the pial arterioles. Thus the neurovascular unit titrates blood flow to the metabolism of discrete cortical areas.

Inlay III shows a qualitative schematic of pial cross sections against a hypothetical metabolic milieu spectrum. Note the vessels are not only exposed to arterial conditions but also to that of the cerebrospinal fluid that completely surrounds the pial vessel, tethered on all sides by thin processes to the pia mater. The vessels dilate with decreases in perfusion pressure, PaO₂, and/or CaO₂ and increases in PCO₂ and/or [H⁺].
Four broad sections, each focusing on an important facet of cerebrovascular control, are delineated below. Section 2.1 discusses the metabolic control of CBF, from systemic metabolism and the consequent coupling of arterial blood gases and CBF, to the effect of local neuronal metabolism on local CBF. Section 2.2 provides an overview of how the cerebrovasculature buffers changes in blood pressure, termed cerebral autoregulation (CA); and how systemic and local metabolism might in turn affect CA. Section 2.3 covers the role of the autonomic nervous system in CBF regulation. Finally, section 2.4 summarizes the state of our knowledge on cerebrovascular regulation, reiterating key gaps in our understanding and suggesting avenues for future research.

### 2.1 Metabolic regulation of cerebral blood flow

#### 2.1.1 Regulation by arterial blood gases

Brain perfusion is highly sensitive to changes in changes in PaCO$_2$. Studies using transcranial Doppler ultrasound of the middle (Ide et al., 2003b; Battisti-Charbonney et al., 2011) and posterior cerebral arteries, and basilar artery (Skow et al., 2013), and Duplex ultrasound of the internal carotid and vertebral arteries (Sato et al., 2012; Willie et al., 2012) all show an approximate 3-6% increase, and/or 1-3% decrease in flow per mmHg change in CO$_2$ above and below eupnic PaCO$_2$, respectively (see Figure 2.2, left). Imaging studies show broadly comparable results (Kemna et al., 2001; Mandell et al., 2008a; Piechnik et al., 2008). It is important to recognize that methodological differences make comparison between studies difficult. These differences include: Steady state versus rebreathing methods of CO$_2$ manipulation; linear versus non-linear analyses of the cerebrovascular response to hypocapnia, hypercapnia, or the entire manipulated range of CO$_2$; whether PaO$_2$ is maintained during CO$_2$ manipulation; and the method of CBF measurement all influence the values of CBF reactivity to changes in PCO$_2$ (extensively reviewed in Ainslie & Duffin, 2009; Fierstra et al., 2013). Regardless, this high vascular sensitivity to CO$_2$ is unique to the cerebrovasculature (Ainslie et al., 2005) and is manifest throughout, from the large arteries of the neck (Willie et al., 2012) through the large intracerebral arteries (Giller et al., 1993; Wilson et al., 2011; Willie et al., 2014a) to the smallest pial arterioles (Wolff & Lennox, 1930) and parenchymal vessels (Binks et al., 2008; Mandell et al., 2008b; Nöth et al., 2008; Piechnik et al., 2008). This sensitivity appears to be similar between brain regions in the
hypercapnic range, but dissimilar with hypocapnia (Sato et al., 2012; Willie et al., 2012), as assessed by flow through the arteries of the neck. Based on MRI data, CO₂ reactivity of the microvasculature in grey matter is greater than that of white matter, likely because of relatively less vascularization (Mandell et al., 2008b; Nöth et al., 2008).

Figure 2.2. Blood flow through the internal carotid and vertebral arteries during steady-state changes in arterial CO₂ and oxygen. Cerebrovascular reactivity to changes in CO₂ and to hypoxia (%∆CBF/mmHg CO₂; %∆CBF/%SaO₂) was found to be similar between vessels in the hypercapnic range, ~10% greater for VA than ICA in the hypocapnic range, and 50% greater for the VA with extreme hypoxia. ICA, internal carotid artery; VA, vertebral artery.

The cerebrovasculature is sensitive to hypoxia, but only below a PaO₂ of ~50 mmHg (see Figure 2.2, right). The response is dependent on the prevailing PaCO₂ – hypercapnia increases and hypocapnia decreases cerebrovascular sensitivity to hypoxia (Mardimae et al., 2012). Studies of hypoxic cerebrovascular reactivity are thus confounded by the ventilatory response to hypoxia, which produces hypocapnia and results in cerebrovascular constriction (i.e., poikilocapnia). Studies incorporating a range of techniques that have assessed the CBF response to isocapnic hypoxia have reported CBF reactivities ranging from 0.5-2.5% increase in CBF per percent reduction in in SaO₂ (Cohen et al., 1967; Jensen et al., 1996; Reichmuth et al., 2009; Shapiro et al., 1970; Querido et al., 2013; Querido et al., 2008; Willie et al., 2012). Variability in methods of blood gas manipulation, consequent changes in BP, and degree of – or lack of – PaCO₂ clamping necessitated by the ventilatory response to hypoxia
(Kolb et al., 2004; Shapiro et al., 1970; Willie et al., 2012) lead to variation in population norms, and values in the literature vary considerably. For a given severity of isocapnic hypoxia blood flow to the brain stem increases more than that to the middle and anterior regions, as assessed by flow through the vertebral and internal carotid arteries, respectfully (Ogoh et al., 2013; Willie et al., 2012). Congruous PET scan data collected during isocapnic hypoxia reveal that cortical blood flow is less responsive to hypoxia than phylogenetically older areas of the brain (Binks et al., 2008). Unlike for PaCO$_2$, the CBF response to oxygen appears to be determined by oxygen content rather than PaO$_2$ per se, as reduced oxygen content with carbon monoxide exposure, hemodilution, and acute or chronic anemia produces increased CBF (Brown et al., 1985; Gottesman et al., 2012; Hare, 2004; Metry et al., 1999; Paulson et al., 1973; Todd et al., 1994; Tomiyama et al., 1999).

2.1.2 Locations of CO$_2$ and O$_2$ sensitivity

Although the entire cerebrovasculature – from the large arteries of the neck to the penetrating cortical arterioles – is sensitive to changes in blood gases, the pial arterioles are generally considered to be the site of resistance modulation. The pial vessel response (dilation) to asphyxia was observed ~150 years ago in rabbits (Donders, 1851). Pial arteries dilate up to 40% in response to both increased PaCO$_2$ and increased cerebrospinal fluid (CSF) PCO$_2$ (Kontos et al., 1977b; Wolff & Lennox, 1930). Increased PaCO$_2$ produces smooth muscle relaxation, vessel dilation and increased flow, whereas hypocapnia increases cerebrovascular resistance and decreases CBF (Kety & Schmidt, 1948a; Wasserman & Patterson, 1961). Their anatomical position in the sub-arachnoid space, tethered to the abutting mater and surrounded by CSF (Figure 2.1, Inlays II, III) makes them readily exposed to local metabolic conditions. Thus the tone of the pial vessels is a function of arterial blood gases, arterial pH, and local CSF. As described below (see Neurovascular coupling), pial vessel resistance is also coupled to downstream metabolic activity via retrograde intramural propagation of vascular signals (Kawamura et al., 2003; Lagaud et al., 2002; Segal, 2000; Attwell et al., 2010; Itoh & Suzuki, 2012).

The cerebral arteries (including the internal carotid and vertebral arteries) are also sensitive to changes in blood gases (Heistad et al., 1978a; Faraci et al., 1987a; Willie et al., 2012) and perfusion pressure; whereas the pial arteriolar bed serves to modulate regional blood flow,
the large vessels serve as a ‘first-line’ defense in maintaining brain perfusion (Faraci et al., 1987a; Faraci et al., 1987b) (Figure 2.1; Inlay I). It has been recently demonstrated that the ICA and VA of humans are reactive to changes in arterial blood gases with the ICA showing a ~20% change in luminal diameter through a PaCO$_2$ range of 15-65 mmHg (Willie et al., 2012). That the entire cerebrovascular arterial tree is vasoactive is a key feature of this system. Unified vasomotion allows microvascular pressure to remain constant. For example, large artery constriction only yields decreased pial artery pressure; whereas, constriction along the cerebrovascular tree reduces flow with no changes in pial pressure (Baumbach & Heistad, 1983). Hypocapnia, for example, therefore produces no changes in small arteriole pressure despite attenuated flow because small and large arteries both constrict to reduced PCO$_2$ (Faraci et al., 1987a).

2.1.2.1 Mechanisms of cerebrovascular sensitivity to arterial blood gases

The cellular mechanisms responsible for the cerebrovascular response to changes in PaCO$_2$ and PaO$_2$ have been subject of countless studies. Yet, the resulting diversity of conclusions serves largely to demonstrate a mechanistic redundancy inherent to the precise cerebrovascular regulation by arterial blood gases. Next we will focus on human and select in vivo animal studies. Studies employing various in vitro vessel preparations are difficult to compare and apply to in vivo physiology; for detailed reviews of the in vivo literature see Yoon et al., 2012; Faraci & Heistad, 1998.

Arterial PCO$_2$: Altered alveolar gas exchange (either by changes in alveolar ventilation and/or metabolic CO$_2$ production) elicits concomitant changes in both PaCO$_2$ and pH. The latter is thus influenced by both respiratory alkalosis or acidosis in the short term and over longer periods by the degree of renal compensation. However, a long-standing question has been which of these (PaCO$_2$ or pH), or both, are responsible for the resultant change in CBF. Direct manipulation of arterial pH does not alter CBF under conditions of maintained PaCO$_2$ (Harper & Bell, 1963; Lambertsen et al., 1961). In contrast, manipulation of extravascular pH induces changes in arteriolar diameter (Kontos et al., 1977a; Kontos et al., 1977b; Wahl et al., 1970). These observations suggest the CO$_2$ mechanism is independent of arterial pH, and therefore likely to be dependent on the diffusion of non-polar CO$_2$ molecules across the cerebrovascular blood-brain barrier that can induce a change in pH in the extracellular space.
of the vessel (Lambertsen et al., 1961; Lassen, 1968). This paradigm is supported by animal work utilizing in vivo cranial windows and superficial application of solutions with varying PCO₂ and pH (Kontos et al., 1977b; Wolff & Lennox, 1930). For example, hypercapnic and hypocapnic solutions respectively cause pial arteriole dilation and constriction, providing the superfusate pH is correspondingly acidic or alkaline. In contrast, application of neutral pH solutions elicits no vasomotion regardless of superfusate PCO₂ (Kontos et al., 1977a; Kontos et al., 1977b; Wahl et al., 1970). Moreover, the vasodilatory effect of arterial hypercapnia is nullified with external (i.e., around the pial vessel) application of alkaline superfusate in rat (Liu et al., 2012), dog (Koehler & Traystman, 1982), and cat (Kontos et al., 1977b). Such evidence for the importance of extracellular pH has been more recently corroborated by in vivo vascular preparations as well (e.g., Dabertrand et al., 2012; Peng et al., 1998). It also bears consideration that hypercapnia induces increases in CBF that increase vascular wall shear stress likely to elicit release of vasodilatory compounds such as nitric oxide (Peebles et al., 2008; Toda & Okamura, 1998), and that the net CBF response will thus be a balance between the effect of CO₂ per se and endothelial signals in response to changes in flow.

Arterial PO₂: Unlike the cerebrovascular response to changes in PaCO₂, hypoxemia causes little change in CBF until a threshold at the steep portion of the oxyhemoglobin dissociation curve (~80% SaO₂). Studies in baboons (James et al., 1969) and dogs (Kogure et al., 1970; McDowall, 1966) showed CBF to begin increasing at a PaO₂ of approximately 50 mmHg. Similar data have been published for humans (Ainslie & Poulin, 2004; Willie et al., 2012). The processes involved in the hypoxemia-induced increase in CBF are multifaceted, probably comprising 1) a retrograde stimulus arising at the neurons/glia of the neurovascular unit – i.e., neurovascular coupling – in response to local decreases in tissue oxygen (Gordon et al., 2008; Iadecola & Nedergaard, 2007; Leithner & Royl, 2013; Pelligrino et al., 1995; Thompson et al., 2003); 2) neuronal extracellular acidosis concomitant to vascular dilation (Kogure et al., 1970; Nolan et al., 1982) and direct vascular mechanisms (see below), but the relative contribution and importance of these processes remain unknown. Particularly with respect to the direct neuronal/glial contribution to hypoxic cerebrovascular dilation there are few data (Gordon et al., 2008), but a direct CNS effect of hypoxia in ventilatory regulation has been described (reviewed in Joseph & Pequignot, 2009; Powell et al., 2000). In humans,
however, there have been surprisingly few studies that have attempted to examine potential mechanisms by which hypoxia leads to cerebral vasodilation. Those that have been done in humans have focused principally on the role of adenosine and NO and are considered in detail next.

Adenosine is popularly held to mediate hypoxic cerebral vasodilation. This is based on the evidence that: 1) adenosine is released with hypoxemia (Winn et al., 1981; Meno et al., 1993); 2) that in most animal studies adenosine receptor antagonists attenuate the increase in CBF during hypoxemia (Laudignon et al., 1990; Pinard et al., 1989; Haller & Kuschinsky, 1987; Coney & Marshall, 1998; Morii et al., 1987; Miekisiak et al., 2008); and in vitro data indicate adenosine blocks vasoconstrictive signals within the parenchyma (Gordon et al., 2008). The few studies assessing the role of adenosine on the human cerebrovasculature have reported a 20-30% decrease in CBF and cerebral oxygen delivery in normoxia (Wechsler et al., 1950; Magnussen & Hoedt-Rasmussen, 1977; Gottstein & Paulson, 1972), during competitive adenosine receptor antagonism with aminophylline. Nevertheless, following adenosine receptor blockade, the cerebral hypoxic-vasodilatory response in fact remains intact – CBF decreases with aminophylline administration in normoxia but increases with hypoxic exposure, albeit to values approximately equal to normoxic control conditions (Bowton et al., 1988; Nishimura et al., 1993; Nishimura et al., 1992). Parenthetically, there is an opposite effect of adenosine antagonism on the peripheral vasculature, with brachial artery blood flow increasing by ~150% in normoxia following aminophylline infusion; the effect of adenosine receptor blockade following hypoxia at rest is difficult to interpret because of this massive increase in baseline blood flow that renders such data difficult to interpret (Casey et al., 2009). So, while the release of adenosine during hypoxia certainly implicates it in the normal hypoxic cerebral vasodilatory response, it cannot be the sole mediator of hypoxemia induced CBF increase in humans. It seems that adenosine serves different functions in the cerebral and peripheral vasculatures.

There is similar confusion concerning the role of nitric oxide in mediating hypoxic cerebrovascular dilation. Studies in animals have reported substantial effects of NOS inhibition on the CBF response to moderate hypoxia (Hudetz et al., 1998; Bauser-Heaton & Bohlen, 2007; Santizo et al., 2000), whereas others have reported little effect (Pelligrino et
al., 1993; Pelligrino et al., 1995). Only two studies in humans have assessed NOS inhibition (both via N\textsuperscript{G}-monomethyl-L-arginine [L-LMMA] infusion) on the cerebrovascular response to 20 minutes hypoxemia. Using phase-contrast MRI to measure CBF in healthy young men, Van Mil et al. (2002) reported CBF returned to near-normoxic values following 1-LMMA administration in hypoxia (SpO\textsubscript{2} = 80%). Conversely, Ide et al. (2007) found 1-LMMA to have no effect on the increased CBF with slightly less severe hypoxia (PET\textsubscript{O\textsubscript{2}} = 50 mmHg; SpO\textsubscript{2} not reported) as assessed with transcranial Doppler ultrasound of the MCA. Aside from a larger 1-LMMA dose used by Ide et al., and the slightly more severe hypoxia in van Mil et al., there was no apparent difference in either the methodology or the reported physiological response of the subjects in these studies (except for CBF), including end-tidal PCO\textsubscript{2}. A speculative explanation for these discrepant findings is a change in MCA diameter following hypoxia and/or 1-LMMA infusion that would confound the estimations of CBF by Ide et al. (2007); indeed, MCA dilation in hypoxia is now well documented (Willie et al., 2012; Wilson et al., 2011; Willie et al., 2014a) and after glycerol trinitrate administration (Hansen et al., 2007).

Local CBF is tightly coupled to neural metabolism, a function of the exquisite physical association between neurons, glia, and the microvasculature – together termed the neurovascular unit (see Neurovascular Coupling, below). Excitation of astrocytes by hypoxia in vitro stimulates the direct and indirect production arachidonic acid metabolites associated with local vasodilation (Liu & Alkayed, 2005; Yamaura et al., 2006). It is difficult to identify mechanisms of neurovascular coupling in humans involved in the hypoxic vasodilatory response. Given that transient local hypoxia following increased local metabolism is thought to partially mediate the BOLD MRI response during cognitive activation (Offenhauser et al., 2005; Thompson et al., 2003; Vanzetta & Grinvald, 1999), it is likely the neurovascular unit is minimally one mechanism involved in cerebral vasodilation in hypoxia. This finding suggests both mechanistic redundancy of mediators of hypoxic vasodilation and synergism between the neurovascular unit and the larger arteries and arterioles of the cerebrovasculature due to their similar responses to hypoxia.
2.1.3 Neurovascular Coupling

That local cerebral metabolism is tightly coupled to local brain perfusion has been known – though not understood – for over a century (Donders, 1851; Mosso, 1880; Roy & Sherrington, 1890). This coupling is a product of the anatomical and metabolic relationship between neurons, glial cells, and cortical penetrating arterioles (Figure 2.1, inlay II) that together comprise the neurovascular unit. Excitatory and inhibitory neurons synapse on both astrocytes and GABA interneurons, these interneurons being in close association with astrocytes with processes that terminate in end-feet enveloping cortical penetrating arterioles. By way of gap-junctions between adjacent vascular smooth muscle cells intramural propagation of vascular signals produce remote vasodilation of upstream pial arterioles (Figure 2.1, Inlay II) (Kawamura et al., 2003; Lagaud et al., 2002; Iadecola, 2004). The result is a robust coupling of neuronal activation to regional CBF that can be easily observed with TCD, for example, by activation of the occipital cortex by visual stimulation that elicits an immediate ~20-30% increase in the blood velocity through the posterior cerebral artery feeding the posterior lobe (Boms et al., 2010; Rosengarten et al., 2001; Rosengarten et al., 2003; Willie et al., 2011c). This signaling between local neuronal metabolic state and the vasculature that feeds it is involved in the response to systemic stimuli, such as hypoxemia (Liu & Alkayed, 2005; Yamaura et al., 2006). There is a rich body of literature based on in vitro experimental data devoted to the mechanisms of communication within the neurovascular unit. Numerous excellent reviews have been written on the topic which, being beyond the purview of this review, will not be detailed here (Girouard & Iadecola, 2006; Hamel, 2006; Iadecola, 2004; Iadecola & Nedergaard, 2007; Jakovcevic & Harder, 2007). Despite this extensive knowledge of the molecular mechanisms based from numerous in vivo studies, it should be noted that to the best of our knowledge, no studies to date have attempted to delineate the mechanisms of neurovascular coupling in humans.

2.2 Cerebral autoregulation

In 1895 Bayliss, Hill and Gulland concluded in this Journal that “In all physiological conditions a rise in arterial pressure accelerates the flow of blood through the brain, and a fall slackens it” (Bayliss et al., 1895). This concept prevailed until, in a review paper published in 1959, Lassen constructed a plot of average blood pressure and total brain blood
flow from seven studies involving 11 different patient groups having a range of drug and/or pathology-induced blood pressure levels (Lassen, 1959); see Figure 2.3 left). The plot revealed a plateau region wherein cerebral blood flow appears to be completely stable across a relatively wide range of blood pressures (~60 to 150 mmHg). Such a physiological relationship requires reflex adjustments in cerebrovascular resistance concomitant with changes in blood pressure, and was termed static cerebral autoregulation. Lassen’s curve continues to be cited and illustrated in numerous high impact publications and textbooks (Dagal & Lam, 2009; Barret et al., 2010); the potential consequences of such a parochial view in fields such as anesthesiology are obvious as stated previously (Drummond, 1997).

Figure 2.3. Stylized representation of the classical and contemporary paradigms of cerebral autoregulation.

Left. Stylized representation of the classical view of the relationships between mean arterial pressure and cerebral blood flow, i.e., autoregulation, put forward by Lassen et al., 1959 based on the between-subjects analysis of patients during various pharmacological interventions or pathologies. Right, Schematic based on contemporary data indicating a small plateau region (Tan et al., 2012) and CA hysteresis. Based on the within-subject reanalysis of 41 studies that reported concomitantly measured CBF and MAP during increases (circles) or decreases (squares) in blood pressure the slope of the $\%\Delta$CBF/$\%\Delta$MAP relationship was determined at 0.81 ± 0.77 in the hypotensive range, and 0.21 ± 0.47 in the hypertensive range. This indicates a far more pressure-passive CBF than is conventionally believed, and more efficacious buffering capacity against increases than decreases in perfusion pressure (unpublished observations). See text for details.
Unfortunately, very few attempts have been made to characterize the normal within-subject relation between pressure and flow across the brain. The major challenge is that normal baroreflex function limits the effective range of blood pressures, necessitating the use of vasoactive drugs or physical manipulation of central blood volume, both of which confound interpretation of cerebrovascular reflexes per se. Although recent studies have shown a more pressure passive relationship between MAP and CBF in healthy individuals (reviewed in Numan et al., 2013), this is not a universal finding (e.g., Liu et al., 2013). However, limitations of the majority of these studies are three-fold. First, CBF velocity quantified by transcranial Doppler (TCD) represents CBF only if the diameter of the insonated vessel remains constant. This assumption has been evidenced to be violated under very high PaCO₂ (Willie et al., 2012), hypoxia (Willie et al., 2013; Wilson et al., 2011), and may be violated when MAP is changed dramatically (based on animal data that the large intracranial vessels react to changes in perfusion pressure (Faraci & Heistad, 1990; Mchedlishvili, 1964; Mchedlishvili et al., 1973). Second, many studies have pharmacologically manipulated CBF with anesthetics (Ogawa et al., 2008; Ogawa et al., 2010; McCulloch et al., 2005), angiotensin (Krejcy et al., 1997), and alpha adrenergic receptor agonists and/or nitric oxide donors (Liu et al., 2013; Lucas et al., 2010; Willie et al., 2013; Zhang et al., 2009) the influence of which on cerebrovascular tone is not well understood and subject of controversy [e.g., (Drummond, 2012; Stewart et al., 2013)]. Finally, although PaCO₂ is markedly altered during pharmacological manipulation of BP (Liu et al., 2013), only a few studies have attempted to control for this confounding effect [e.g., Chan et al., 2011; Gelinhas et al., 2012; Lucas et al., 2010]. Static autoregulation is thus likely a function of the experimental conditions under which is it assessed, and its definitive efficacy in healthy humans remains uncertain. Despite this caveat, however, the available within-subject human data indicates the CBF – MAP relationship is not flat through a broad range of MAP (Figure 2.3). We recently reanalyzed 41 studies in healthy humans reporting concurrently steady-steady state changes in MAP and CBF for the slope of the %ΔCBF/% ΔMAP relationship above and below resting MAP, which was found to be 0.81 ± 0.77 in the hypotensive range, and 0.21 ± 0.47 in the hypertensive range (Numan et al., 2013). Of the 41 studies, 33 estimated CBF by transcranial Doppler ultrasound; however, separate analysis of the studies using other modalities (e.g., MRI, PET, ¹³³Xenon technique) yielded similar CA values. These data
indicate that the cerebrovasculature does have autoregulatory capacity, but that its efficacy is not perfect, and is dependent on the severity and direction of change in perfusion pressure, as outlined next.

The study of the human cerebrovascular response to rapid changes in MAP has evolved in the last two decades following the introduction of BP and CBF quantification techniques that afford a high temporal resolution. Static versus dynamic CA is an experimental not physiological distinction where static CA refers to the steady state relationship between MAP and CBF and dynamic CA to the cerebral pressure flow relationship during transient changes in MAP, such as during changes in posture, for example. There are no data, however, that explicitly indicate that short and long term regulation of CBF – dynamic and static CA, respectively – are separate mechanistic entities (Tan & Taylor, 2013). Early methods of CBF quantification lacking sufficient temporal resolution to assess the CBF response to rapid changes in MAP were subsequently referred to as static CA, with the inception of the dynamic CA concept arising out of the ability to concomitantly measure beat-to-beat CBF and MAP. Indeed frequency domain analysis of CBF was born out of a single technique – transcranial Doppler ultrasound – that allows beat-to-beat analysis of blood velocity in the intracerebral vessels, typically the MCA. Two seminal studies in particular have provided for a foundation for our understanding of dynamic CA: Aaslid et al. (1989) was the first to study the temporal relationships between middle cerebral artery blood velocity and MAP during the release of inflated thigh occlusion cuffs that induces rapid transient hypotension. That cerebral blood velocity tracked the drop in MAP but recovered more quickly, clearly demonstrated the conceptual inadequacies of the classical view that autoregulation was nearly perfect by showing that CA yielded relative, not absolute, flow buffering. Further characterization of CA as a relative flow buffer followed when Birch et al. (1995) showed that the transfer function characteristics between blood pressure and cerebral blood velocity fluctuations resemble a high pass filter wherein higher frequency blood pressure fluctuations are more linearly transferred to the cerebral circulation than lower frequency fluctuations.

The above inferences on the nature of dynamic CA are based on the implicit assumption that active and linear changes in cerebrovascular resistance are the primary determinants of dynamic pressure-flow relationships. However, recent studies employing the Windkessel
model suggest that the compliant nature of intracranial blood vessels may play an important role in mechanically buffering against dynamic blood pressure fluctuations, and that this ability of vessels to transiently “store” blood through a cardiac cycle depends on the speed of MAP change (Chan et al., 2011; Tzeng et al., 2011; Zhang et al., 2009). Transfer function analysis also assumes the cerebrovascular response to changing MAP is identical whether MAP is rising or falling, but there is clear evidence of hysteresis in CA – i.e., the brain defends more effectively against acute hypertension than hypotension (Aaslid et al., 2007; Schmidt et al., 2009; Tzeng et al., 2011; Schmidt et al., 2012). At a practical level, the above suggests that metrics of CA derived from pressure-flow recordings need to consider not only absolute blood pressure as an input to the cerebral circulation, but also whether blood pressure is accelerating or decelerating, and rising or falling (Chan et al., 2011; Tzeng et al., 2011). Such a comprehensive and universal metric does not yet exist but the need to consider important non-stationary components is needed (Panerai, 2013). Recently Tan (2012) utilized projection pursuit regression purported to circumvent some of these linear limitations and reported a small autoregulatory plateau of only ~10 mmHg when blood pressure oscillations were induced at 0.03Hz. If the autoregulatory range is in fact so limited, characterization of static CA through incremental changes in MAP may miss the autoregulatory region altogether (e.g. Liu et al., 2013; Lucas et al., 2010; Zhang et al., 2009).

It is important to recognize that there remain numerous methods of CA quantification, each with inherent assumptions and caveats, each specific to some experimental paradigm, and no one method is considered a “gold-standard” measure. Indeed, the available metrics of CA have been evidenced to yield largely divergent results for the same data (Tzeng et al., 2012) and should be thus be scrutinized carefully. There is, however, enough data to support consensus that CA does not maintain constant perfusion through a MAP range of 60-150 mmHg as is so often cited in the literature.

2.2.1 Mechanisms of Cerebral Autoregulation

Changes in cerebrovascular resistance must necessarily underlie CA, whether static or dynamic, yet there remains little consensus, especially in humans, on the mechanisms and location of this cerebral resistance modulation. Most of the attention paid to CA in the last two decades has aimed to characterize CA, to deduce a role of neural regulation of CA,
to study the effects of stimuli ranging from exercise to pathology on CA. These studies almost universally ignore the question of *where* exactly this regulation takes place (e.g. Jordan & Powers, 2012; Claassen & Zhang, 2011). The pial arterioles are dogmatically accepted to serve in this capacity (Fog, 1938; Lassen, 1959). But there are data to the contrary that indicate only the largest of these vessels respond to physiological ranges of MAP, and that the large intracranial arteries as well as the even larger vessels of the neck contribute to a substantial portion of cerebrovascular resistance (Faraci *et al.*, 1987a; Heistad *et al.*, 1978a; Heistad *et al.*, 1978a; Kontos *et al.*, 1978). Notwithstanding the exact site(s) where CA is modulated, there seems to be inbuilt mechanistic redundancy. For example, in addition to the apparent roles for the autonomic nervous system (see Section III, Autonomic regulation of cerebral blood flow, below) there is a myogenic role in the regulation of CA. Only two studies in humans have addressed CA following myogenic “block” using Ca\(^{2+}\)-channel inhibitors: Tzeng *et al.* (2011) used the cerebrovascular-specific Ca\(^{2+}\) antagonist (nimodipine) and Tan *et al.* (Tan *et al.*, 2013) used the systemically acting vascular smooth muscle cell Ca\(^{2+}\) channel blocker (nicardipine). Both studies demonstrated altered CA, but only during low-frequency (~20-30s) oscillations of MAP as driven by oscillating lower body negative pressure. Given that buffering of CBF against changes in perfusion pressure must necessarily entail changes in vascular resistance somewhere within the cerebrovasculature, it is perhaps not surprising that compromising the vascular smooth muscle cells alters CA. Equally, however, alterations of CA by Ca\(^{2+}\) channel blockers must not be of elemental importance, as widespread orthostatic intolerance is not necessarily observed in patients prescribed these drugs (Grunig *et al.*, 2013). The relative importance and relations between myogenic, autonomic, and local neuronal mechanisms in CA remains to be holistically understood, and will certainly require more sophisticated methods of CBF assessment that consider cerebral vasomotion (such as Duplex ultrasound or MRI, rather than TCD alone).

### 2.2.1.1 Large conduit arteries and cerebral autoregulation

There is a convincing body of evidence demonstrating the large arteries of the brain play a much larger and important role in the regulation of CBF than generally ascribed (Mchedlishvili, 1964; Mchedlishvili *et al.*, 1973; Faraci & Heistad, 1990). Indeed, using a
canine in situ ICA where the inlet pressure could be controlled, ICA constriction maintained pressure at the circle of Willis nearly constant in the face of increasing perfusion pressure (Mchedlishvili et al., 1973); the feline VA, in contrast, does not buffer increases in MAP (Faraci et al., 1987a). Another group using a different in vivo technique that allowed calculation of the lumped resistance for all the large arteries in dogs and cats (Faraci et al., 1987a; Heistad et al., 1978a) found similar results, concluding that the large arteries were responsible for a quarter to half of the total cerebrovascular resistance during resting conditions. Perhaps because these larger arteries of the neck are generally considered to be “conduit” arteries, the idea they can actively participate in CBF regulation has not been embraced. However, in rabbits and dogs the internal geometry of the ICA and VA was found to change considerably where the vessels bend – at the cavernous sinus for the ICA (the carotid siphon), and at the V3 segment of the VA at the entrance of the foramen magnum (Mchedlishvili, 1964). The turbulent flow resulting from such luminal diameter changes within tortuous segments must dramatically increase resistance compared to non-tortuous segments. That is to say that a smaller decrease in lumen diameter would be required to produce a given increase in resistance within the carotid siphon and V3 segment of the VA (Figure 2.1, Inlay I). Recent human MRI data showing complex non-Newtonian flow and attenuated pulsatility along the carotid siphon support this theory (Schubert et al., 2011; Takeuchi & Karino, 2010). In humans these vessels change diameter in response to changes in PaCO$_2$ and PaO$_2$ (Willie et al., 2012; Wilson et al., 2011); future studies using such advanced imaging techniques should assess if they are also involved in the cerebrovascular response to acute changes in MAP.

### 2.2.1.2 Interaction between arterial blood gases and cerebral autoregulation

Traditional tests to assess cerebrovascular CO$_2$ reactivity or CA treat these concepts as separate entities. Clearly they are not: there are persuasive data that both CA and CO$_2$ responses may utilize the same vascular reserve. In a landmark study, Harper & Glass (1965) examined the brain vascular reactivity to changes in PaCO$_2$ in dogs at various blood pressures (see Figure 2.4). Severely hypotensive animals (~60% MAP) showed no change in cerebral vessel diameter in response to increases or decreases in PaCO$_2$ (i.e., CBF reactivity was abolished), due to the vessels being already maximally dilated (Harper &
Glass, 1965). The reciprocal instance was also demonstrated where, in hypocapnia, CBF was well maintained in the face controlled-hemorrhage induced hypotension (Iwabuchi et al., 1973). In contrast, with hypercapnia, CBF fell linearly with MAP (Iwabuchi et al., 1973). Broadly comparable findings in both healthy humans (Ainslie et al., 2012; Przybylowski et al., 2003) and those with pathology [e.g., carotid stenosis (Nishimura et al., 1999)] indicate that CBF reductions with transient hypotension lead to a blunting of the CBF response to hypercapnia – hypotension selectively attenuated cerebrovascular CO₂ reactivity to hypercapnia but not hypocapnia. Thus, the compromised capacity of the cerebral vessels to dilate under hypotensive conditions when PaCO₂ is elevated indicates that the maintenance of cerebral perfusion takes precedence over the maintenance of a normal tissue PCO₂; or, that there is limited vasodilatory reserve regardless of the combined stimulus. Equally, the latter process may take place regionally. For instance, during times of decreased perfusion or global cerebral vasodilatory stimulus, vascular beds with reduced vasodilatory reserve may have limited ability to dilate sufficiently to successfully compete for limited blood flow to the brain (Mandell et al., 2008a). As a result, despite global vasodilation, regional CBF in these vessels may even decrease resulting in a cerebrovascular “steal” phenomenon (Faraci & Heistad, 1990; Fierstra et al., 2010; Mandell et al., 2008a).
Figure 2.4. Schematic of the effect on prevailing mean arterial pressure on cerebrovascular reactivity to changes in \( \text{PaCO}_2 \).

Note the abolishment of cerebrovascular reactivity with progressive hypotension. Values are calculated from the data of Harper and Glass, 1965.

Moreover, because the brain does not autoregulate perfectly (Figure 2.4) and since hypoxia and elevations in \( \text{PaCO}_2 \) also ‘impair’ the brain’s capability to defend against BP changes (Tzeng et al., 2012), BP is clearly a critical component of CBF. An example of these integrated changes in \( \text{PaCO}_2 \) and BP occur in a myriad of everyday activities: changing posture, laughing, exercise, straining, sexual activity, coughing, to name but a few. Although there is extensive evidence in support of this proposed interaction between pressure and chemical regulation of the cerebrovasculature (Ainslie & Tzeng, 2010a; Ainslie & Smith, 2011; Tzeng et al., 2012; Willie et al., 2012; Jordan et al., 2000; Przybylowski et al., 2003;
Kety & Schmidt, 1948a; Kety & Schmidt, 1946) integrated consideration of these players is seldom incorporated into contemporary research design. The use of cerebrovascular conductance index is sometimes used for this reason, as it may provide for more precise estimation of CO$_2$ reactivity that considers the effect of arterial BP on CBF; however, this does not take into account factors that may be manifest during alterations in ventilation such as large changes in intrathoracic, intracranial, and central venous pressures that in turn effect cerebral perfusion pressure. It also assumes that the relationship of PaCO$_2$ and MAP on CBF is linear when this is likely not the case (Battisti-Charbonney et al., 2011). As such, cerebrovascular conductance may oversimplify the issue. In our view, expressing the effects of CO$_2$ and MAP individually (e.g., Willie et al., 2012) gives a better impression of the relative effects of each on CBF.

2.3 Autonomic regulation of cerebral blood flow

The entire cerebrovasculature is extensively innervated by adrenergic and cholinergic fibers of diverse extrinsic (e.g., cervical, sphenopalatine, and trigeminal ganglia) and intrinsic (e.g., locus coeruleus, fastigial nucleus, dorsal raphe nucleus) origins. Cerebral arteries show three layers of nerve plexi with relative distribution varying with the vessel location: 1) the most superficial layer is a paravascular layer of nerve bundles longitudinally arranged superficially to the adventitia; 2) a dense perivascular plexus within the adventia; and, 3) a deep perivascular plexus following a transverse course at the adventitial-medial border (Bleys et al., 1996). The extracranial arteries feature a higher proportion of longitudinally arranged paravascular nerve bundles, whereas intracranially there is a greater total density of perivascular nerve fibers, with more following a spiral or annular course, and far more neuron terminals – particularly at bifurcations, communicating arteries and the intracranial curvatures of the ICA and VA (Bleys et al., 1996; Borodulya & Pletchkova, 1973; Borodulya & Pletchkova, 1976; Mchedlishvili, 1986). The ICA territory appears to possess denser sympathetic innervation than the vertebrobasilar system (Edvinsson & Hamel, 2002). The necessary anatomy is therefore extant for a neural role in the regulation of CBF.

2.3.1 Sympathetic nervous system and CBF

Human studies assessing this role are broadly of two types – studies of patients with various diseases treated by ganglionectomy - or studies in healthy humans employing local
subcutaneous blockade of a ganglion(s), or oral pharmacological blockade of sympathetic ganglia or systemic receptor group. Four studies have assessed CBF following ganglion excision and each showed increased CBF (Jeng et al., 1999; Shenkin, 1969; Shenkin et al., 1951; Suzuki et al., 1975). Following local cervical ganglion block five studies reported increased CBF (Ide et al., 2000; Linden, 1955; Treggiari, 2003; Umeyama et al., 1995; Yokoyama et al., 2004), whereas three reported no change in CBF (Harmel et al., 1949; Ohta et al., 1990; Scheinberg, 1950); and in one study a decrease in CBF was found (Kang et al., 2010). Local anesthesia can result in a partial block of the targeted ganglion, which could explain the lack of effect on CBF in three studies. Although the pathological conditions should be acknowledged, that ganglionectomy universally increased CBF is convincing evidence for a role of sympathetic nerves in CBF regulation, though these data shed little light on the precise nature of such a role.

Invasive animal experiments suggest the SNS is most important during changes in blood pressure. Most well controlled animal studies have observed some decrease in CBF at baseline, but especially during hypertension with stimulation of the superior cervical ganglion. This role of the SNS seems especially important in buffering surges in perfusion pressure (Ainslie, 2009; Cassaglia et al., 2009; Cassaglia et al., 2008a; Cassaglia et al., 2008b; Tzeng et al., 2010a; Mayhan et al., 1987). Unilateral resection of the superior cervical ganglion during induced hypertension produced ipsilateral disruption of cortical vessel integrity (Heistad et al., 1978b; Ponte & Purves, 1974). This apparent protective function of the SNS to increases in perfusion pressure seems to be principally a function of the larger arteries. For example, only the largest pial arterioles respond to sympathetic activation (Wei et al., 1975) and do so to a smaller degree than the large cerebral arteries (Baumbach & Heistad, 1983). The ability of these larger vessels to maintain flow during changes in perfusion pressure is dependent on their innervation as the vessels become pressure-passive once denervated; autoregulation in the large vessels is perhaps modulated, at least in part, neurogenically (Mchedlishvili et al., 1973; Tamaki & Heistad, 1986). Interpretation of studies utilizing animal models to assess the SNS role in cerebrovascular function should be done with caution as profound differences have been reported between species (Busija et al.,
1980; Heistad et al., 1978b), and nothing is known of the corresponding relation to human physiology.

2.3.2 Sympathetic nervous system and CA

Studies employing pharmacological blockade benefit from being completed in healthy humans, but are potentially confounded by the systemic effects of the drug. Nonetheless, a number of elegantly studies have reported similar findings, indicating impairment of CA following SNS blockade. Both sympathetic ganglion blockade with trimethaphan (Zhang et al., 2004) and α-adrenoreceptor block with phentolamine (Kimmerly et al., 2003) resulted in a greater rise in CBF for a given increase in MAP produced during a Valsalva maneuver or norepinephrine infusion, respectively. Likewise, both these pharmacological interventions increase transfer function gain and decrease phase lead at low frequencies (0.03Hz), indicating impaired CA (Hamner et al., 2010; Zhang et al., 2002), and α₁-adrenoreceptor block with Prazosin impairs CBF recovery to transient hypotension induced by thigh-cuff release (Ogoh et al., 2008). It is nonetheless difficult to dissociate the systemic effects of SNS blockade (ganglionic or receptor group) on MAP and/or peripheral vascular reflexes from direct effects on CA per se.

2.3.3 Sympathetic nervous system and arterial blood gases

The relationship between SNS and the cerebrovascular response to CO₂ and hypoxia also remains vague because it is difficult to dissociate the peripheral (e.g., MAP) from direct cerebrovascular effects of SNS activity. A convincing study in baboons reported that hypoxemic stimulation of the carotid bodies increased ipsilateral CBF, and that this response was abolished following resection of cranial nerve VII (Ponte & Purves, 1974). Human data are inconsistent. CO₂ reactivity has been reported to be unchanged following augmentation of SNS by handgrip (Ainslie et al., 2005) and lower body negative pressure (LeMarbre et al., 2003); but conversely, attenuated CO₂ reactivity was reported during lower body negative pressure (Zhang et al., 2010) and following ganglionic blockade with trimethaphan (Jordan et al., 2000). Systemic pharmacological manipulation of SNS activity is likely confounded, however, by the consequent blunted MAP response to increased PaCO₂ or PaO₂. For example, since MAP may influence CBF (Section II), lowering of reactivity is likely confounded by an attenuated hypercapnia-induced pressure response (Ainslie et al., 2012;
Przybylowski et al., 2003). Moreover, to our knowledge no study has examined whether sympathetic blockade attenuates the hypoxia-induced CBF increase, or conversely of SNS activity might serve to restrain large increases in CBF observed in severe hypercapnia or hypoxia (Willie et al., 2012; Wilson et al., 2011).

2.3.4 Parasympathetic nervous system and CA

There are limited data available assessing the cholinergic control of CBF in humans, but again anatomical studies of cerebral vessels indicate rich distribution of cholinergic nerve terminals throughout the intracranial vessels proximal to the Virchow-Robin space (Florence & Bevan, 1979; Hamel, 2004; Sato et al., 2001; Heistad et al., 1980). As with the SNS, cholinergic control seems to be species specific, as petrosal nerve resection or stimulation does not affect baseline CBF in cats but does in dogs (D'Alecy & Rose, 1977) and rats (Pinard et al., 1979). Only one study has assessed cerebrovascular control at rest in healthy humans. Hamner et al. (2012) reported increased transfer function coherence between MAP and CBF (as estimated by TCD) following systemic cholinergic blockade with glycopyrrolate, suggesting impaired CA. As explained in Section II, the precise physiological meaning of transfer function metrics remains ambiguous and while these data do indicate a cholinergic role in cerebrovasculature function, more work needs certainly be directed to this end.

2.4 Synopsis

Our aim in the present review is to call attention to dated concepts in accepted understanding of cerebrovascular regulation. Despite ample data and past reviews in high-impact journals that have highlighted these erroneous views, they continue to be taught to medical professionals, neuroscientists and physiologists across the globe. Below we outline four principal points that should be incorporated into contemporary cerebrovascular physiology knowledge, as well as future directions of research pertaining to each:

1. Cerebral autoregulation does not maintain constant perfusion through a MAP range of 60-150 mmHg. The cerebral circulation does have more effective autoregulatory ability in the range above baseline MAP, and to a lesser extent below baseline MAP; however, CBF is nonetheless directly affected by changes in perfusion pressure. A definitive, within-individual assessment of global and regional CBF
across a range of non-pharmacologically and pharmacologically perturbed blood pressures with maintained PaCO$_2$ has yet to be completed. How this relationship may alter in pathological conditions is therefore also unknown.

2. **There is important stimulatory synergism and regulatory interdependence of arterial blood gases and blood pressure on CBF regulation.** The study of cerebrovascular regulation needs to consider this relationship – if BP and PaCO$_2$ or PaO$_2$ change simultaneously, interpretation of the relative impact of each becomes difficult. The relationship between MAP and cerebrovascular reactivity to PaCO$_2$ remains unknown in the hypertensive range. Moreover, advanced imaging techniques should facilitate study of the effects of vascular reactivity and resistance heterogeneity in the cerebral vascular bed on the distribution of CBF in response to vasoactive stimuli.

3. **Cerebral autoregulation and cerebrovascular sensitivity to changes in arterial blood gases are not modulated solely at the pial arterioles.** The large arteries of the neck and cerebrum are critically involved, serving the first-line defense such that the pial and cortical vessels see minimal changes in pressure and can respond principally to prevailing systemic and local neural metabolism. High resolution MRI/MRA should be utilized to assess the response of the large vessels to non-pharmacologically driven changes in blood pressure and controlled dynamic and steady state changes in arterial blood gases.

4. **Neurogenic control of the cerebral vasculature is an important player in autoregulatory function, particularly in the large vessels, and acts to buffer surges in perfusion pressure.** Although the precise role of cerebral vasomotor nerves are poorly understood after more than 80 years of study it remains premature to dismiss their role. There are numerous studies yet to be completed in humans to determine precisely the capacity for sympathetic and cholinergic nervous input on the cerebrovasculature. Future studies in healthy humans should not rely solely on TCD in assessing these questions. Newer imaging modalities, and direct modification of cerebral SNS outflow, rather than global perturbation of SNS receptors, will help to elucidate the role of the autonomic nervous system in cerebrovascular control. Particularly during transient bouts of hypertension – such as that commonly
experienced during activity, strain (defecation, lifting, etc.), pathology (autonomic
dysreflexia, subarachnoid hemorrhage, etc.) and during rapid-eye movement sleep –
sympathetically mediated buffering of global CBF, and constriction of large cerebral
arteries should be assessed. One methodological possibility to this end would be to
use a centrally acting \( \alpha_2 \)-adrenoreceptor agonist (e.g., clonidine) to diminish SNS
outflow whilst quantifying regional (cerebral) noradrenaline spillover (Mitchell et al.,
2009) during non-pharmacologically induced changes in MAP. Another approach
would be assessing CBF and large artery vasomotion (by ultrasound or MRI) during
transient hypotension and hypertension before and following cervical ganglion block.

Answering these questions in healthy humans will provide new insight into the fundamental
mechanisms that regulate CBF. Only by first understanding these mechanisms can we
subsequently decipher the role of CBF regulatory impairment in myriad cerebrovascular
diseases, such as Alzheimer’s disease and dementia (Qiu et al., 2003b; Faraci et al., 1987b;
Hebert et al., 2004), and even neurogenic hypertension (Waki et al., 2011).
Chapter 3: Techniques for the measurement of cerebral blood flow, cerebral blood velocity, and cerebrovascular function

3.1 Metrics of cerebral blood flow

3.1.1 The Kety-Schmidt Technique

Kety and Schmidt (1945) were the first to quantify CBF using an inert tracer (e.g., nitrous oxide, N\textsubscript{2}O). The reference method for the measurement of global CBF, the Kety-Schmidt method is based on the Fick principle, whereby the arterio-venous difference of an inert tracer is proportional to the volume of blood flow through the brain (Kety & Schmidt, 1948b). The tracer is infused until tension equilibrium is attained (the saturation phase) and then terminated, after which the concentration falls toward zero (the desaturation phase). Simultaneous arterial and jugular venous samples are withdrawn during either phase and CBF calculated by the Kety-Schmidt equation:

$$ CBF = 100 \times \lambda \times \frac{C_{JV} \text{ (equilibrium)}}{\int_{t=0}^{t=\infty} (C_{JV}(t) \times dt) - \int_{t=0}^{t=\infty} (Ca(t) \times dt)$$

[1]

where $C_{JV}(t)$ and Ca(t) are the jugular-venous and arterial concentration, respectively, of the tracer at time t (in minutes), and $\lambda$ is the brain-blood partition coefficient (in mL g\textsuperscript{-1}) . The global cerebral metabolic rate (CMR) of substance $x$ is given by the Fick principle as:

$$ CMR = CBF_x \cdot D(x_a - x) = CBF_x \cdot D(C(x)_a - C(x)_v) $$

[2]

where $D(x_a-x_v)$ is the arterial to jugular-venous concentration of x. This provides a valid CMR due to the identical sampling sites (regions of interest) for the CBF measurement and the $D(x_a-x_v)$ (see Figure 3.1)
Figure 3.1. The Kety-Schmidt method of measure CBF using N2O.
N2O concentration is given on the y-axis as the percent of equilibrium concentration (mean ± SD) versus time, by discontinuous blood sampling in the desaturation phase during normocapnia.

Theoretically, this principle can be exploited using any freely diffusible tracer; indeed N2O, 133Xe, hydrogen, and iodoantipyrine have all been utilized (Edvinsson & Krause, 2002). While this method did stimulate seminal research in cerebrovascular physiology (Kety, 1999), there are several important limitations: measurements are taken over the course of minutes, making it impossible to assess dynamic changes in CBF; only a global, but not regional, measure of CBF, cerebral metabolic rate, or blood-brain substrate exchange is possible; internal jugular and peripheral arterial lines are necessary making it quite invasive; and, finally, the value of cerebral oxygen consumption must be assumed (Kety & Schmidt, 1948). Furthermore, venous outflow from the brain may not be symmetrical, with 50% of individuals exhibiting cortical drainage of venous blood mainly through the right internal jugular vein, and subcortical largely through the left. Two decades later, the measurement of cerebral oxygen consumption was improved using radioactive inert gases 85Kr (Lassen et al., 1963) and 133Xe (Harper & Glass, 1965) that allow extracranial imaging of gamma emission from the cerebral cortex. However, the aforementioned temporal resolution combined with potential problems of extra-cranial tissue sampling and inadequate desaturation are limitations that remain with these approaches.
3.1.2 Transcranial Doppler ultrasound

The use of Doppler ultrasound was first described as early as 1959 for assessing blood velocity in the extracranial vessels (Miyazaki & Kato, 1965). The thickness of the skull bones greatly attenuates the penetration of ultrasonic waves making noninvasive use of the technique difficult. Ultrasound was therefore limited to surgical procedures, or to use in children with open fontanels. However, Aaslid et al. (1982) demonstrated that the attenuation of sound by bone within the frequency range of 1-2MHz was far less than conventional frequencies of 3-12 MHz. Indeed, insonation is possible through thinner regions of the skull, termed “acoustic” windows, making it feasible to measure static and dynamic blood velocities within the major cerebral arteries. For the first time, a non-invasive measure of beat-to-beat changes in blood velocity in the vessels of the brain with superior temporal resolution than indicator-dilution techniques was available. However, it is imperative to note that TCD cannot measure CBF per se. Rather, TCD measures the velocity of red blood cells within the insonated vessel. Moreover, only the larger basal arteries provide an adequate signal for measurement of cerebral blood velocity with TCD. Because these arteries tend to deliver oxygenated blood to large regional areas of the brain, TCD gives an index of global, rather than local, stimulus-response. This is an important distinction given that local changes in CBF likely differ (Hendrikse et al., 2004; Nöth et al., 2008; Piechnik et al., 2008). Certainly, there is a notable dissimilarity in vasoreactivity across the cerebrovasculature. At least in hypercapnia, small vessels and capillaries possess much higher reactivity to CO$_2$ (Mandell et al., 2008a), and vasculature residing within gray matter show higher reactivity than vasculature within white matter (Nöth et al., 2008; Piechnik et al., 2008). This lack of spatial resolution in brain hemodynamics is the principal limitation of TCD. There are a variety of modern imaging techniques that allow sufficient spatial resolution to discern localized brain perfusion. Detailed description of these techniques is beyond the scope of this thesis; however, see Wintermark et al. (2005) for a detailed review.

3.1.2.1 Principles of Doppler ultrasound

The principles of TCD are the same as extracranial Doppler ultrasound: the Doppler probe emits sound waves that are reflected off moving red blood cells, which are subsequently detected by the transducer. The resultant Doppler-shift is proportional to the velocity of the
blood (DeWitt & Wechsler, 1988; Aaslid, 1986a). Duplex ultrasound (simultaneous two dimensional B-mode and pulse-wave velocity) typically used in vascular ultrasound to measure both vessel luminal diameter and blood velocity (and therefore volumetric flow; see Section VI) is not possible with current TCD systems due to lack of resolution. Because the diameter of the insonated vessel is unknown, TCD only measures cerebral blood velocity (CBV) not absolute volumetric flow. The velocity of blood through a vessel is proportional to the fourth power of vessel radius; the measurement of CBV by TCD assumes constant diameter of the insonated vessel – this assumption has been found to be valid in various studies (Serrador et al., 2000; Bishop et al., 1986; Nuttall et al., 1996; Peebles et al., 2008; ter Minassian et al., 1998; Valdueza et al., 1997). Despite these validations, however, it remains possible that the cerebral conduit vessels do, in fact, change diameter, and as such, any TCD data should be openly interpreted with this possibility in mind. In addition, other problems remain for meaningful velocity quantification. For example, the velocity of blood through a vessel – in the presence of laminar flow – is approximately parabolic in shape, with the fastest velocity in the center of the vessel. The Doppler signal consequently represents not a single value but rather a distribution of velocities, therefore requiring mathematical manipulation to extract meaningful velocity values. Typically, a power spectrum distribution is produced from segments of ~5 seconds using a Fast Fourier transform, and maximum or mean velocity is calculated from the maximum or intensity weighted mean, respectively (Lohmann et al., 2006; Aaslid, 1986b).

A frequency of 2 MHz is typically used for TCD because higher frequencies do not sufficiently penetrate the bones of the skull (DeWitt & Wechsler, 1988; Aaslid et al., 1982). Despite the increased penetration of TCD, an acoustic window in the skull is necessary for adequate insonation of intracerebral arteries. The choice of window, however, can dramatically affect the type of recording possible. For example, insonation through the transtemporal or foramen magnum windows allow use of a headpiece for securing the Doppler probe, whereas this is not possible when insonating through the optic canal.

Imaging modalities such as colour-coded Doppler and power Doppler, significantly increase the reliability of TCD as direct visualization of the target vessel facilitates better insonation angle correction (Martin et al., 1995). Studies have demonstrated that the use of colour-coded
and/or power TCD facilitates: (1) an improved signal-to-noise ratio with transcranial insonation (Postert et al., 1997); (2) insonation in the presence of poor acoustic windows, particularly when combined with the use of contrast (Nabavi et al., 1999; Gerriets et al., 2000); (3) the measurement of arterial diameter threshold for collateral flow in the circle of Willis (Hoksbergen et al., 2000); and, (4) an increase in the diagnostic sensitivity for cerebral vasospasm (Sloan et al., 2004); and direct measurement of MCA diameter (Willie et al., 2014a; Wilson et al., 2011).

3.1.3 Vascular ultrasound of extracranial neck arteries
Measurement of CBF by quantification of inflowing blood through the neck is not a new technique, but has not seen the prolific utilization of TCD or MRI, for example. Nonetheless, the technique has recently seen a resurgent popularity because it facilitates estimation of both flow proper (as opposed to blood velocity), and regional distribution of CBF during a variety of conditions (exercise, standing, environmental stress, etc.) not possible with MRI. The technique is nonetheless especially prone to measurement error, both for technical reasons and because of the apparent ease with which an untrained individual can pick up a vascular ultrasound probe, image a carotid artery, and believe the resulting measurement is accurate.

The aim of this section is to outline the virtues and caveats of the measurement of CBF via quantification of neck artery blood flow using high-resolution vascular ultrasound. First, we will discuss the vascular anatomy providing blood to the head. Then, we will address principals of linear vascular ultrasound, methods of analysis, sources of measurement error and our perspective on the appropriate use of the technique. Finally, the utility of the technique will be discussed in conjunction with an overview of the current literature, with normative values provided in tabular form.

3.1.3.1 The arteries of the neck
Early Greek physicians termed the principal arteries of the neck Karatides, after the adjective for stupefying, because their compression yielded unconsciousness. Such were these vessels’ importance recognized early, even before the physiological function of blood was
understood. Indeed, the carotid arteries are the principle conduit for blood transport to the brain, carrying approximately 70% of global CBF.

The carotid system originates with the common carotid arteries (CCA), that branch from the aortic arch and brachiocephalic trunk on the left and right sides, respectively. The CCA bifurcates into the external (ECA) and internal (ICA) carotid arteries (see Figure 2.1). The position and morphology of the carotid bifurcation exhibits some variation, lying somewhere between the level of the thyroid cartilage and hyoid bone in the majority of individuals. The ECA is most often positioned either anteromedial or medial to the ICA (Al-Rafiah et al., 2011), giving off the superior thyroid artery and ascending pharangeal artery within the first two centimeters distal from the bifurcation. There is, however, variation in the loci of these arteries origins, occasionally branching from the CCA or ICA, and more often from the bifurcation itself. The ICA normally does not give off any branches until after entering the base of the skull through the foramen lacerum. The ophthalmic artery and a number of smaller arteries branch prior to the circle of Willis, where the ICA terminates to form the middle, anterior, and posterior communicating arteries. Because in the majority of individuals there are no branches off of the ICA prior to entering the skull, ICA blood flow is an accurate metric of CBF.

The vertebral arteries (VA) arise as the most proximal branches off the subclavian arteries, then courses through the foremen of the transverse processes of C6-C2, into the spinal canal and skull to join bilaterally forming the basilar artery. The vessel can be imaged proximal to entering C6, and between each of the vertebra until entering the skull. There are numerous anastomoses with the VA both extra- and intracranially. The VA communicates with branches of the deep cervical artery, and inferior thyroid artery extracranially, and upon entering the skull gives off branches to the cerebellum before joining to form the basilar artery (BA). A number of arteries project from the BA to supply the cerebellum and pons before the BA bifurcates to form the poster circle of Willis as the posterior communicating arteries. Figure 3.2 below provides typical ultrasound example of the different waveforms commonly observed in the ICA, ECA, and VA.
Figure 3.2. Typical waveforms from the extracranial neck arteries.
From left to right, ultrasound examples of the ECA (left), ICA (middle) and VA (right). Note: 1) The ECA waveform should have a sharp upstroke and a low end-diastolic velocity, as it supplies a high resistance vascular bed. ECA may have a flow reversal component (flow below the baseline) in late systole or early diastole; 2) ICA waveform should have a more gradual upstroke (slower acceleration) in systole, and an elevated end-diastolic velocity. This is because it supplies a low resistance vascular bed (i.e., the brain). Thus flow should be above the baseline for the entire cardiac cycle; and 3) The VA waveform should have a gradual upstroke and relatively high end-diastolic velocity as it also supplies a low resistance vascular bed.

3.1.3.2 Technical aspects of vascular ultrasound
Neck artery ultrasound accurately quantifies global and regional CBF non-invasively and with high spatial and temporal resolution; indeed, it is the only known method of CBF measurement possessing these attributes. The principal limitation is consequently that of the operator, not the technique per se. But in fact the technique is so easily confounded by user error that this limitation obviates flippant dismissal. For example, a one degree error in the angle of insonation; a 0.1 mm error in diameter measurement; and, one cm/s error in mean blood velocity each yield a 3, 4, and 4% error in the measurement of flow. It is obvious that very minor operator errors during insonation and during subsequent analysis quickly compound and can easily produce significant inaccuracies (Schoning et al., 1994). For every 1-degree error in the insonation angle an approximate 3% error in velocity, and therefore in flow, results. But, whereas the velocity and flow error are linearly related, inaccuracies in the measurement of diameter have an exponentially larger effect (because the diameter is squared to calculate luminal area). The appropriate measurement of diameter is a topic of extensive debate within other fields reliant on ultrasonic measure of blood flow (Black et al., 2008; Green et al., 2011). It is unfortunate, then, that the accurate measurement of luminal diameter presents a number of problems that have largely been unaddressed since the invention of the technique. The majority of investigators utilize calipers typically part of the ultrasound software to manually measure from one luminal surface to the other. Some authors have accounted for the pulsatile nature of most arteries by measuring both systolic
and diastolic diameters, and calculate a mean diameter based on a third to two-thirds systolic-diastolic ratio (Sato et al., 2011; Sato & Sadamoto, 2010). Other authors have discordantly reported the ICA, ECA, and VA to be without pulsatile changes throughout the cardiac cycle (Scheel et al., 2000a; Scheel et al., 2000b; Schoning & Hartig, 1996; Schoning et al., 2005; Schoning et al., 1994). Proprietary and commercially available software that automatically tracks the vessel internal walls on the B-mode image with high temporal resolution facilitates calculation of other metrics such as pulsatility and shear stress, and moreover dramatically increases the precision of the diameter measurement. Manual measurement of vessel lumen is also likely to prevent observation of any change in diameter, as arterial response to changes in shear or blood gases involves a temporal latency with stimulus related and inter – individual variability impossible to observe when only a few measures are taken.

3.2 Assessment of cerebrovascular function

3.2.1 Assessment of cerebral autoregulation
In this section we provide a practical overview of methods used to assess cerebral autoregulation (CA) including the use of suprasystolic thigh cuff, postural alterations, lower body negative or oscillatory pressure, the Valsalva maneuver, the Oxford technique and transfer function analysis.

3.2.1.1 Suprasystolic thigh cuffs
The rapid release of thigh cuffs inflated to suprasystolic pressures for ≥2 minutes elicits a transient hypotension (Aaslid et al., 1989; Mahony et al., 2000; Tiecks et al., 1995a). The rate of regulation (RoR), quantifies the rate at which cerebrovascular resistance (CVR), or conductance changes in response to a perturbation in MAP and can be given by Equation 1:

\[
RoR = \frac{\Delta CVR/\Delta t}{\Delta MAP}
\]

where \( \Delta CVR \) is given by MCAv-mean/MAP, and \( \Delta MAP \) by control MAP-MAP-mean. \( \Delta t \) is taken as the 2.5 second period one second following thigh cuff release (Figure 3.3).
Figure 3.3. Cerebrovascular and cardiovascular changes following suprasystolic thigh-cuff deflation.

Typical changes in arterial blood pressure (ABP), cerebral blood flow-velocity (CBFV) and cerebrovascular reactivity (CVR) in response to thigh cuff deflation, for determining dynamic cerebral autoregulation (CA). All tracings are shown in normalized units relative to control pre-release values from -4 to 0 seconds. Straight line (bold line) through CVR (bottom figure) curve is determined by regression analysis of data obtained in the interval from 1 to 3.5 seconds after thigh cuff release and is used for calculating rate of regulation (RoR).

This time interval was originally put forth by Aaslid et al. (Aaslid et al., 1989) based on two factors: (1) the change in CVR is relatively linear during this period, allowing a slope of the response to be taken; and, (2) it was thought that the latency of the baroreflex response was such that within the first 3.5 seconds of the cerebrovascular response to a hypotensive challenge, only cerebrovascular mechanisms (i.e., dCA) would be involved in regulation of CBF. However, the drop in arterial pressure following thigh cuff deflation engages the arterial baroreflex within 0.44 seconds of baroreceptor unloading by neck pressure (Eckberg,
1980) causing transient tachycardia. Although unilateral thigh cuff deflation was reported to not alter central venous pressure (Fadel et al., 2001), unpublished observations from our laboratory indicate this may not be the case for bilateral thigh-cuff release. It is unclear, consequently, how cardiac output is affected following bilateral thigh-cuff release. Some authors (Ogoh et al., 2003; Ogoh et al., 2007), but not all (Deegan et al., 2010), have reported that increases in cardiac output can augment CBF; thus, it is plausible that baroreflex function may exert a modulating influence on dynamic CBF regulation such that RoR reflects the integrated response of both the baroreflex and dCA (Ogoh et al., 2009b).

The thigh cuff technique is also somewhat painful, with inflation often associated with an increase in MAP that is maintained until cuff release. The influence of sympathetic nervous system activity in response to discomfort is not known, but a minimum 8-minute recovery period has been recommended following cuff deflation (Mahony et al., 2000).

Another prevalent method to quantify the dCA response to thigh cuff release, termed the autoregulatory index, was proffered by Tiecks et al. (1995a). This approach uses a second order differential equation to relate changes in MAP and three predefined model parameters (T: time constant; D: damping factor; and k: autoregulatory gain) to generate ten templates of CBV-response to a non-pharmacologically induced transient hypotension (Figure 3.4).

Typically, rapid thigh-cuff deflation is used to induce a transient hypotension. According to the Tiecks model, the autoregulatory index assigns an integer value to each of ten template curves (0-9). These coefficients are generated using a second-order linear differential equation:

\[
\begin{align*}
dP_n &= \frac{\text{MAP} - \text{MAP}_{\text{base}}}{\text{MAP}_{\text{base}} - \text{CCP}} \\
x2_n &= x2_{n-1} + \left(\frac{x1_n - 2D\cdot x2_{n-1}}{f\cdot T}\right) \\
x1_n &= x1_{n-1} + \left(\frac{dP_n - x2_{n-1}}{f\cdot T}\right) \\
mV_n &= \text{MCAv}_{\text{base}} \cdot \left(1 + dP_n - k\cdot x2_n\right)
\end{align*}
\]

where \(dP_n\) is the normalized change in mean arterial blood pressure (MAP) relative to baseline MAP (MAP_{base}) and adjusted for estimated critical closing pressure (CCP); \(x2_n\) and
$x_{1n}$ are state variables (equal to 0 at baseline); $mV_n$ is modeled mean velocity; $MCA_{v_{base}}$ is baseline $MCA_{v_{mean}}$; $f$ is the sampling frequency, and $n$ is the sample number. The $mV_n$ generated from ten predefined combinations of parameters $T$ (time constant), $D$ (dampening factor) and $k$ (autoregulatory gain) that best fit the actual $MCA_{v_{mean}}$ recording is taken as an index of dynamic CA. A value of 0 represents no autoregulation where CBV passively follows perfusion pressure, and a value of 9 represents perfect CA where changes in perfusion pressure produce no alteration to CBV. The autoregulatory index has also been derived from spontaneously occurring blood pressure and cerebral blood vessel velocity fluctuations using transfer function analysis (Panerai et al., 1999; Panerai et al., 2001). However, the validity of comparison between a linear model and that from a transient hypotensive stimulus may be questionable (see Transfer function analysis below).

**Figure 3.4. Modeling parameters of the autoregulatory index.** (A) Responses of CA model (ARI) to step changes in blood pressure. The CBFV response curve model with 10 different degrees of dynamic CA is calculated by this method. 9 is the highest degree of dynamic CA. (B) Tabular comparison of ARI, and associated constants, with percentage RoR; T indicates time constant; D, damping factor; K, autoregulatory dynamic gain; ARI, autoregulation index; and dROR, dynamic rate of regulation.

### 3.2.1.2 Postural alterations

Because of the confounders inherent to both pharmacological and non-physiological methods of BP alteration (e.g., thigh-cuff release) postural maneuvers to alter blood pressure have been utilized for CA assessment. The simple act of standing from a sitting, supine, or squat
position is enough to elicit a transient drop in BP of ~35 mmHg and associated drop in CBV (Thomas et al., 2009; Murrell et al., 2009; Murrell et al., 2007). RoR (Sorond et al., 2005), ARI, and transfer function analysis (Claassen et al., 2009) have all been used to quantify the dCA response to postural changes in BP.

3.2.1.3 Valsalva maneuver

Forced expiration against a closed glottis regularly occurs during normal daily activities such as during defecation and lifting. The Valsalva maneuver has been well described in the literature (Smith et al., 1996; Tiecks et al., 1996) and consists of four phases: (1) increased MAP due to increased intrathoracic pressure; (2a) impaired atrial filling and resultant drop in MAP, followed by; (2b) a baroreceptor mediated tachycardia and increase in MAP; (3) release of strain and drop in intrathoracic pressure which decreases MAP; and (4) baroreceptor mediated sympathetic activity that drives MAP above baseline in the face of transient hypotension. Due to impaired atrial filling, combined with raised intracranial pressure induced by increased intrathoracic pressure during strain, there is a marked decrease in cerebral perfusion pressure during the onset of phase 2a. This drop in perfusion pressure provides an adequate stimulus for measurement of dCA (Tiecks et al., 1996; Tiecks et al., 1995b; Zhang et al., 2002). The Valsalva maneuver may, however, be confounded by its inherent physiological complexity. Changes in PaCO$_2$ are likely to occur over the course of the breath-hold, and although it has been suggested that there is sufficient time delay between changes in end-tidal PCO$_2$ (PetCO$_2$) and subsequent changes in CBF to preclude the breath-hold from effecting measured values of CA, this is not known for certain (Hetzel et al., 2003). Furthermore, changes in intrathoracic pressure likely vary between individuals, and throughout the maneuver there are changes in intracranial pressure, venous outflow pressure and resistance. And though there is a period of relatively stable intracranial pressure, the possibility of these changes confounding measures of CA certainly exist. Nonetheless, Tiecks et al. (1996) described the Valsalva ARI as the ratio between the relative changes in cerebral blood flow velocities and blood pressure, calculated as:

$$ARI_{Valsalva} = \frac{(CBFV(IV)/CBFV(I))/(BP(IV)/BP(I))}$$
where I and IV signify phases one and four of the Valsalva response, respectively. CBF is modified proportionally more than BP, implying that autoregulation is preserved, if the ratio is found to be >1. Because minor variations in expiratory pressure results in changes in the various stages of the maneuver, it is imperative to standardize the technique between and within subjects.

3.2.1.4 Transfer function analysis

Transfer function analysis (TFA) for the assessment of dynamic CA is based on analysis of the coherence, frequency and phase components of spontaneous changes in MAP, and the resultant degree to which these changes are reflected in CBV (Zhang et al., 1998). It is thought that CA acts as a high pass-filter, effectively dampening low-frequency oscillations (<0.07Hz) (Panerai et al., 1998; Zhang et al., 1998). An advantage to this method is the ability to complete the measurement in a subject at baseline without the need for any pharmacological or physiological manipulation of BP. However, the corollary is that TFA cannot be used to analyze the CA response to a transient and directional change in BP (i.e., it cannot distinguish between “upward” and “downward” fluctuations in BP). Furthermore, TFA assumes that the dynamic autoregulatory responses to spontaneous fluctuations in BP are linear. This is to say that TFA assumes CA is equally effective in attenuating changes in cerebral perfusion in response to both hyper and hypotensive changes in MAP; however, this may not be the case (Aaslid et al., 2007; Tzeng et al., 2010c). If hysteresis is a natural characteristic of CA (i.e., differential CA depending on directionality of the blood pressure change), than the assumptions of linear techniques such as TFA may not be valid. An extension of TFA is impulse response analysis, whereby spontaneous changes in MAP are inversely transformed back to the time domain (Panerai, 2008). In other words, the impulse response function is an inverse algorithm of the Fast Fourier analysis of frequency shifts of blood velocity, as a time domain function. This allows time-domain models such as the ARI to be applied to spontaneous data (Czosnyka et al., 2009). Again, if the CA response is not linear, comparison of ARI’s generated from linear models, to those generated from a transient hypotensive or hypertensive stimulus, may also not be valid. A limitation of CA assessment using spontaneous data is the small magnitude and inconsistency of spontaneous pressure oscillations (Taylor et al., 1998). See below.
3.2.1.5 The Oxford Technique

The Oxford technique is the method of using vasoactive drug injections (most often phenylephrine hydrochloride and sodium nitroprusside; the modified Oxford technique) to provoke baroreflex responses, and has been widely used since (Smyth et al., 1969) utilized bolus angiotensin injected intravenously during wake and sleep; the R-R interval response to changes in arterial BP provides an index of cardiac baroreflex sensitivity. Despite its prevalence in baroreflex research (Willie et al., 2011a), until recently, the technique had not been utilized for assessment of CA, despite providing some distinct advantages in CA quantification. Blood pressure can be raised or lowered, allowing both positive and negative CA gains to be assessed independently – an important consideration given evidence that CA may be more effective at dealing with increases in blood pressure than decreases in blood pressure (Aaslid et al., 2007; Tzeng et al., 2010c). The Oxford technique is also largely painless, reducing the influence of pain induced sympathetic activity. dCA is quantified by taking the slope of the linear regression between CBV and MAP – the slope is inversely proportional to the efficacy of cerebral autoregulation in maintaining CBV (Willie et al., 2013). This is to say, a slope of zero would imply perfect autoregulation where CBF remains constant across the entire range of MAP, while a gain equal to 1 would reflect the total absence of autoregulation (Tzeng et al., 2010c). The limitations inherent to any pharmacological approach remain manifest with this technique, and although there is supportive evidence (Greenfield & Tindall, 1968) the assumption that there is no direct drug-effect on the cerebral vasculature has been questioned (Brassard et al., 2010). However, direct effects of PE and SNP on cerebral vasculature are considered unlikely given that the blood-brain barrier normally prevents endogenous circulating catecholamine from binding to \( \alpha_1 \)-adrenoreceptors in small cerebral vessels (Ainslie & Tzeng, 2010b; MacKenzie et al., 1976; McCalden et al., 1977).

3.2.1.6 Oscillating blood pressure

A criticism of TFA is that it analyses relatively small natural swings in blood pressure. Coherence between pressure and CBF is often low (<0.5) making it difficult to ascertain the statistical and TFA model reliability, as well as the causal relationship between these variables. These difficulties have been partially overcome by inducing large-amplitude blood
pressure oscillations through either repeated squat-standing or oscillatory lower body negative pressure at frequencies associated with CA (Claassen et al., 2009; Hamner et al., 2004; Smirl et al., 2014).

3.2.2 Cerebrovascular Reactivity

Cerebrovascular reactivity gives an index of reactivity of the intracranial vessels in response to a stimulus – typically either pharmaceutical (e.g., acetazolamide) or through ventilatory alterations of PaCO$_2$. There is differential reactivity to CO$_2$ across the cerebral vasculature. Cerebrovascular CO$_2$ reactivity assessed by TCD gives a global measure of reactivity compared to more sophisticated techniques such as pulsed arterial spin labeling MRI and positron emission tomography that both allow a specific brain area to be assessed. Typically, a hypercapnic stimulus is utilized to assess reactivity, and using TCD to assess CBV reactivity can be given by:

\[
\text{Cerebrovascular reactivity} = \frac{\Delta \text{CBV}}{\Delta \text{PCO}_2}
\]

Similarly, volitional hyperventilation can be utilized decrease PCO$_2$, such that reactivity to hypo and hypercapnia can be assessed. From a clinical perspective, impairment of cerebrovascular reactivity to CO$_2$ – as assessed by TCD – has been linked to such pathologies as obstructive and central sleep apnea (Burgess et al., 2010; Reichmuth et al., 2009), carotid artery stenosis (Widder et al., 1994), hypertension (Serrador et al., 2005), congestive heart failure (Xie et al., 2005), and cerebral ischemic events (Wijnhoud et al., 2006). It is also an established independent predictor of ischemic stroke (Markus & Cullinane, 2001; Silvestrini et al., 2000; Vernieri et al., 2001).

Acetazolamide can also be used to assess cerebrovascular reactivity. Acetazolamide inhibits carbonic anhydrase, the enzyme responsible for reversible catalyzation of H$_2$CO$_3$ formation from CO$_2$ + H$_2$O. Consequently it increases tissue PCO$_2$, leads to metabolic acidosis, and increased CBF. Although the exact mechanisms of acetazolamide-induced increases in CBF are not fully understood they likely involve both metabolic factors and direct as well as indirect vascular effects (Pickkers et al., 2001); and reviewed by Settakis et al., 2003). The use of acetazolamide for cerebrovascular reactivity assessment necessitates intravenous
administration. However, in this form the drug can be costly, and depending on the country, difficult to procure. Regardless, confounds associated with cerebrovascular reactivity quantification using acetazolamide are not well understood, but it may directly affect the cerebral vasculature and can indirectly drive increased ventilation, which when combined with the need for intravenous administration and high-cost, makes acetazolamide less utilized than alteration of inspired CO₂. Regardless of the stimulus used, when assessing cerebrovascular reactivity, an absolute measurement of CBV is not as important as resolution of beat-to-beat changes in CBF from a pre-stimulus baseline.

3.2.3 Neurovascular Coupling
Functional hyperemia describes the increased CBF to active areas of the brain where the demand for both nutrient delivery, and clearance of metabolic by-products is increased. The functional anatomy of the brain allows this neurovascular coupling to be easily and reliably examined by measurement of the sensorimotor or cognitive stimulatory effects on CBV – a method termed functional TCD (fTCD; Figure 3.5). This technique was first utilized by Aaslid et al. (1987) who showed that blood velocity in the PCA changed with visual stimulation (see Hubel & Wiesel, 2005 for a comprehensive report of visual system physiology), but there are numerous studies in the neuro-cognitive literature that demonstrate consistent CBF changes in response to cognitive, verbal, and motor tasks (Rosengarten et al., 2003; Aaslid, 1987; Deppe et al., 2004; Klingelhöfer et al., 1997; Silvestrini et al., 1993; Stroobant & Vingerhoets, 2000).
Figure 3.5. Cerebrovascular response to visual stimulation.
Mean time course of peak systolic PCAv during visual stimulation (reading) while at upright-seated rest in 10 healthy young volunteers. Smooth line generated by locally weighted polynomial regression.

Despite the poor spatial resolution inherent to TCD, many studies have examined the relationship between cognitive activation and CBF. For example, chronic hypotension depresses cognitive activity (Jegede et al., 2009; Duschek et al., 2008; Duschek & Schandry, 2007). Conversely, cognitive activity can be improved with pharmacological treatment of hypotension (Duschek et al., 2007). These studies demonstrate that cognitive activity is positively related to neural tissue oxygen delivery, but the scope of fTCD is very broad. Studies have examined the effect of pharmacological agents (Rosengarten et al., 2002a), Type I diabetes (Rosengarten et al., 2002b), Alzheimer’s disease (Rosengarten et al., 2007), voluntary movements (Orlandi & Murri, 1996; Sitzer et al., 1994), hemispheric language lateralization (Knecht et al., 1998b; Dorst et al., 2008; Markus & Boland, 1992; Knecht et al., 1998a; Knecht et al., 1998b), emotional processing (Troisi et al., 1999), and attentional processes (Schnittger et al., 1996; Schnittger et al., 1997; Helton et al., 2007; Knecht et al.,
1997) on neurovascular coupling. It has also been well characterized in clinical populations (Silvestrini et al., 1993; Silvestrini et al., 1995; Silvestrini et al., 1998; Silvestrini et al., 2000; Thie et al., 1992; Njemanze, 1991; Bruneau et al., 1992), and may be a useful paradigm for the evaluation of cerebrovascular function in certain disease states (Boms et al., 2010).

3.2.4 Estimation of intracranial and critical closing pressure using TCD
The critical closing pressure is the theoretical pressure at which blood flow within the cerebral vessels drops to zero, due to failure of the transmural pressure across a vessel to counteract the tension created by the vessel’s smooth muscle. Measures of cerebrovascular resistance or compliance assume proportional linearity between blood flow and pressure, and that flow through a vessel ceases when the pressure is zero. Aaslid et al. (Aaslid et al., 2003) demonstrated in humans that flow stops due to vessel collapse when perfusion pressure remains positive, making CCP a potentially better measure of cerebrovascular tone. CCP can be estimated by extrapolation of the CBV – blood pressure relationship to the pressure at which zero flow would theoretically occur. However, regardless of whether the entire pressure and velocity waveforms are used (Aaslid et al., 2003), or the systolic and diastolic values only (Ogoh et al., 2010), this technique typically yields an underestimate of CCP. Indeed, in some individuals the estimated CCP may even be negative, which is difficult to interpret physiologically. Furthermore, most studies have used peripheral blood pressure recordings that do not take into account pulse wave amplification in the periphery, which further contaminate CCP estimation. The reader is referred to (Panerai, 2003) for a detailed review of the concept.

3.2.5 Clinical Applicability of TCD
The low cost, excellent temporal resolution, and bedside availability of TCD make it an ideal tool for clinical diagnosis of acute and chronic cerebrovascular diseases. The principle area of clinical application of TCD is the assessment of pathologies that alter blood velocity within the intracranial arteries or veins. We particularly focus on vasospasm, stenosis, intracranial occlusions, thrombosis, critical closing pressure, brain death, and patent foramen ovale.
3.2.5.1 Vasospasm

Vasospasm is observed as a complication of subarachnoid hemorrhage with an incidence ranging between 30% and 70% depending if the vasospasm is symptomatic or angiographic, respectively. Because blood velocity within a vessel is inversely proportional to its cross-sectional area, the primary pathological condition that affects flow-velocity is vasospasm, which is therefore detectable with TCD (Aaslid et al., 1982). Vasospasm can remain asymptomatic, but the factors leading to symptom presentation are largely unknown. Although diagnosis of vasospasm requires the presence of hyperaemia in addition to increased blood flow velocities (see the Lindegaard index, below), at least within the MCA, threshold values of MCAv are fairly well accepted. Velocities between 120 and 200 cm/s are indicative of a reduction in lumen diameter between 25% and 50%, and serious vasospasm and lumen diameter reduction greater than 50% is indicated with velocities above 200 cm/s (Tsivgoulis et al., 2009). Hyperaemia must also be present to diagnose vasospasm; the Lindegaard Index is a ratio between the mean flow velocity in the MCA and that in the ICA, where values greater than 6 indicate severe vasospasm, between 3 and 6 indicate moderate vasospasm, and less than 3, hyperaemia (Rasulo et al., 2008). The disadvantage of using the Lindegaard ratio is that it assumes a dichotomous condition – where there is either vasospasm or not – which may be misleading in certain patients. A promising diagnostic criterion is the use of a daily increase in the systolic pressure of more than 50 cm/sec; this avoids dichotomous classification of vasospasm, informs about the physiopathological trend towards vasospasm, thereby allowing the early identification of patients at risk. To further increase the accuracy of transcranial Doppler in the identification of cerebral vasospasm, thresholds in mean velocities of more than 160 cm/sec have accurately diagnosed cerebral vasospasm (Mascia et al., 2003).

3.2.5.2 Stenosis

Typically TCD does not provide sufficient data for accurate identification of stenosis of a cerebral vessel, particularly in the posterior vessels that are more tortuous and have greater anatomic variability. Diagnosis of stenosis using TCD requires: (1) acceleration of flow velocity through the stenotic segment, 2) decrease in velocity below the stenotic segment, (3) bilateral asymmetry in flow, and (4) disturbances in flow (i.e., turbulence and murmurs)
(Rasulo et al., 2008). Diagnosis of stenosis using TCD has greater sensitivity and specificity in the anterior than in the posterior circulation due to the lower anatomic variability and relative ease of insonation of the anterior vessels.

### 3.2.5.3 Intracranial occlusion

TCD has excellent utility in diagnosis of occlusion within the cerebral vessels with sensitivity and specificity over 90% – particularly in patients where cerebral ischemia is present (Camerlingo et al., 1993). Diagnosis is through absence or a profound reduction of flow at the normal position and depth, and/or consequent lack of signal for the vessels in the immediate vicinity of the occluded region. Furthermore, due to its non-invasiveness, TCD can easily be used to track the progression of an occlusion both before and after treatment (Rasulo et al., 2008). Furthermore, recent data suggest an independent effect of the ultrasound in augmenting the thrombolysis of the occlusion in patients with acute MCA thrombosis (Eggers et al., 2003; Eggers et al., 2009). The Clotbust trial (Alexandrov et al., 2004) demonstrated that the presence of residual flow signal, dampened waveform, and microembolic signals prior to thrombolysis was associated with increased likelihood of complete recanalisation after thrombolysis. Furthermore, in patients with acute ischemic stroke, continuous TCD significantly increased tissue plasminogen activator-induced arterial recanalization (Alexandrov, 2009). See (Alexandrov, 2006) for a review of the use of TCD in thrombolytic treatment of stroke.

### 3.2.5.4 Sickle cell disease and risk of arterial thrombosis

Sickle cell disease is associated with an increased risk of stroke in children (Adams et al., 1998). Level I evidence has been established for the use of TCD in the diagnostic screening of patients with sickle cell anemia. A MCAv threshold of 170 cm/sec was identified as indicative for the need of blood transfusion, such that a 30% reduction in circulating hemoglobin-s was achieved (Adams et al., 1997). A randomized trial subsequently demonstrated that application of the above Doppler criteria yielded a 92% absolute reduction in the risk of stroke in children (Adams et al., 1998). Additionally, reference CBV values were recently outlined for the purpose of screening for intracranial vessel narrowing in children with sickle cell disease (Krejza et al., 2000).
3.2.5.5 Brain death

Electroencephalography or angiography can be utilized for clinical diagnosis of brain death. Angiography is typically preferred because EEG gives little information regarding brainstem function and signals can be difficult to attain within the intensive care unit. However, angiography requires injectable contrast media, and cannot be completed bedside at all. Typically, increased intracranial pressure concomitant with brain death reduces diastolic blood flow velocity in the intracerebral vessels. Further increases in intracranial pressure produce reversed flow during diastole in the circle of Willis, and finally, spiked and reverberating flow is considered indicative of brain death (de Freitas & André, 2006; Ropper et al., 1987; Tsivgoulis et al., 2009).

3.2.5.6 Shunt and emboli detection

In the presence of right-to-left cardiac shunt microbubbles injected into the venous circulation – that are largely filtered out in the lungs – will appear in the cerebral circulation within 5-15 seconds. There are reports of up to 100% sensitivity in right-to-left shunt detection (Droste et al., 2002); however, it seems unlikely that the TCD technique can differentiate between various forms of shunt. For example, that microbubbles appear in the systemic circulation could be indicative of a patent foramen ovale, pulmonary arteriovenous malformation, or an atrial septal defect. But given that up to 60% of the normal population may present with right-to-left shunt of either intracardiac or pulmonary arteriovenous malformations (Woods et al., 2010), an inexpensive means of screening such as TCD, as part of a diagnostic battery, may be very useful.

Both gaseous and solid microemboli can be detected using TCD through recognition of irregularities within the Doppler signal (Padayachee et al., 1987; Deverall et al., 1988; Ringelstein et al., 1998). Although these microemboli are often clinically silent, their detection may be of prognostic value in assessing risk of stroke, and of use during cardiac or vascular surgeries where gaseous emboli may originate from the oxygenator. The detection of emboli using TCD is complicated and relies on 10 technical parameters and Ringelstein et al. provide a detailed description of the technique (Ringelstein et al., 1998). There is some difficulty in distinguishing between gaseous and solid emboli. This is of clinical importance as each has distinct clinical relevance, particularly in cases where both types of emboli may
be present (e.g., mechanical heart valve patients, patients with carotid stenosis (reviewed in: (Rodriguez et al., 2009; Markus & Punter, 2005).

3.3 Synopsis

Many methods are available for the assessment of CBF, but the high temporal resolution, non-invasiveness, and relative low-cost of ultrasound make it functional in both clinical and research settings. The ability to assess cerebral reactivity, CA, and neurovascular coupling, makes TCD extremely useful for the assessment of integrative cerebrovascular function. That the diameter of the insonated intracranial vessel is likely not static in cases of extreme changes in arterial blood gases needs to be careful weighed in the context of the experimental question; the measurement of ICA and VA flow circumvent this caveat of TCD. Four principle components of cerebrovascular regulation should be assessed if possible, as collectively these provide insight into a complex physiology. Measurement of (1) velocities within the major cerebral vessels, (2) measurement of ICA and VA blood flow, (3) assessments of autoregulation, (4) cerebrovascular reactivity and (5) neurovascular coupling together facilitate holistic appraisal of cerebrovascular function.
Chapter 4: Regional brain blood flow in man during acute changes in arterial blood gases

4.1 Overview
Despite the importance of blood flow on brainstem control of respiratory and autonomic function, little is known about regional cerebral blood flow (CBF) during changes in arterial blood gases. We quantified: 1) anterior and posterior CBF and reactivity through a wide range of steady-state changes in the partial pressures of CO$_2$ (PaCO$_2$) and O$_2$ (PaO$_2$) in arterial blood; and 2) determined if the internal carotid (ICA) and vertebral arteries (VA) change diameter through the same range. We used near-concurrent vascular ultrasound measures of flow through the ICA and VA, and blood velocity in their downstream arteries [the middle (MCA) and posterior (PCA) cerebral arteries]. Part A (n=16) examined iso-oxic changes in PaCO$_2$, consisting of three hypocapnic stages (PaCO$_2$ = ~15, ~20, ~30 mmHg) and four hypercapnic stages (PaCO$_2$ = ~50, ~55, ~60, ~65 mmHg). In Part B (n=10), during isocapnia, PaO$_2$ was decreased to ~60, ~44, and ~35 mmHg and increased to ~320 mmHg and ~430 mmHg. Stages lasted ~15 minutes. Intra-arterial pressure was measured continuously; arterial blood gases were sampled at the end of each stage. There were three principal findings: 1) Regional reactivity: the VA reactivity to hypocapnia was larger than the ICA, MCA and PCA; hypercapnic reactivity was similar. With profound hypoxia (35 mmHg) the relative increase in VA flow was 50% greater than the other vessels. 2) Neck vessel diameters: changes in diameter (~25%) of the ICA was positively related to changes in PaCO$_2$ ($R^2$, 0.63 ± 0.26; $P < 0.05$); VA diameter was unaltered in response to changed PaCO$_2$ but yielded a diameter increase of +9% with severe hypoxia. 3) Intra- vs. extra-cerebral measures: MCA and PCA blood velocities yielded smaller reactivities and estimates of flow than VA and ICA flow. Findings respectfully indicate: 1) disparate blood flow regulation to the brainstem and cortex; 2) cerebrovascular resistance is not solely modulated at the level of the arteriolar pial vessels; and, 3) transcranial Doppler ultrasound may underestimate measurements of CBF during extreme hypoxia and/or hypercapnia.

4.2 Background
Brain perfusion is highly sensitive to changes in the partial pressure of carbon dioxide in arterial blood (PaCO$_2$) and, to a lesser degree, the partial pressure of oxygen in arterial blood.
(PaO₂) (Kety & Schmidt, 1948a). These high sensitivities, especially to PaCO₂, are unique to the cerebrovasculature when compared with the peripheral vasculature (Ainslie et al., 2005; Lennox & Gibbs, 1932). Elevations in PaCO₂ (hypercapnia) cause a decrease in cerebrovascular resistance and consequent increases in cerebral blood flow (CBF), whereas hypocapnia causes increased cerebrovascular resistance and related decreased in CBF (Kety & Schmidt, 1948a; Wasserman & Patterson, 1961). This response of the brain’s circulation to PaCO₂ partly serves to stabilize respiratory and cardiovascular autonomic control, which are regulated by specific regions of the brainstem sensitive to the pH of surrounding tissue (Ainslie & Duffin, 2009; Xie et al., 2009). Thus, the increase in CBF with hypercapnia mitigates rising tissue CO₂, and the decreased CBF with hypocapnia attenuates falling tissue CO₂. Under normal physiological circumstances, hypoxia-induced increases in ventilation results in hypocapnia; changes in PaO₂ and PaCO₂ therefore influence both ventilation and CBF (Ainslie & Ogoh, 2010; Lucas et al., 2011). Despite the importance of this reciprocal regulation at the level of the brainstem to central respiratory and autonomic control, most studies to date have measured the velocity response of the middle cerebral artery (MCAv), assuming blood gas reactivity is similar for different brain regions. But there has been limited study of regional differences in brain blood flow regulation with which to verify this assumption.

Jansen et al. (2002) reported greater increases in middle cerebral artery blood velocity (MCAv) than basilar artery blood velocities (BAv) during 7% O₂ gas exposure; however, these data were collected under dynamic poikilocapnic conditions, precluding analysis of cerebrovascular CO₂ and O₂ sensitivities independently of each other. Conversely, with changes in PaCO₂, previous studies have found a comparable cerebrovascular sensitivity between the MCAv and BAv (Hida et al., 1996; Ogawa et al., 1988). Hauge et al. (1980) also reported similar CO₂ reactivity differences in velocity between the vertebral and internal carotid arteries (VA and ICA). However, all previous studies are limited by their measurement of blood velocity (rather than flow) and may consequently have underestimated changes and/or differences in CO₂ sensitivities, and no study has assessed reactivity in the posterior cerebral artery (PCA). Recent data indicate that within 3 hours of hypoxic exposure (12% O₂), the MCA may dilate (Wilson et al., 2011). Moreover, MCA diameter may change
by 4 - 18% during a ~15 mmHg range in PaCO₂ (Giller et al., 1993). It is unknown if these diameter changes are more pronounced through a broader PaCO₂ range, but if so may lead to a marked error in estimations of flow. Sato et al. (2010) reported different regional blood flow changes through the ICA and VA during progressive exercise (and concomitant changes in PaCO₂); interpretation with respect to sensitivities to blood gases per se is confounded by the myriad other changes manifest during exercise. Moreover, if neck arteries are directly sensitive to changes in arterial blood gases, estimation of cerebrovascular reactivity, and hence physiological interpretations, could be broadly inaccurate as well.

The current model of cerebrovascular function is a single-resistor model, with changes in cerebrovascular resistance occurring at the precapillary arterioles of the pia mater – the pial vessels (Forbes & Wolff, 1928; Wolff & Lennox, 1930; Harper & Glass, 1965; Kontos et al., 1978). There are several animal studies, however, demonstrating that the large cerebral arteries, and distal arteries of the neck (ICA and VA) contribute significantly to total changes in cerebrovascular resistance during alterations of blood pressure and arterial gases (Faraci & Heistad, 1990; Heistad et al., 1978a), but to our knowledge, the relative sensitivities of ICA and VA to altered blood gases is not known, and moreover, this concept has never been intentionally explored in humans.

We investigated the regional CBF distribution through a wide range of steady state progressive changes in PaCO₂ and PaO₂ near the limits of human tolerance in conscious humans. Second, we aimed to determine if the large extra-cerebral arteries (ICA and VA) change diameter through the same range of arterial blood gases, indicating their potential role in cerebrovascular regulation. Based on the different ICA and VA flow responses reported by Sato et al. (2010) during exercise we hypothesized that VA and ICA flow would exhibit disparate reactivity to changes in arterial blood gases. Based on highly-controlled animal studies (Faraci & Heistad, 1990; Heistad et al., 1978a), we further speculated that the ICA and VA would dilate in hypoxia, and hypercapnia, and constrict with hypocapnia.
4.3 Methods

4.3.1 Participants
Twenty-six adults volunteered for the study and gave written informed consent. Part A (carbon dioxide investigations), included sixteen adults (5 female) with a mean age of $22 \pm 3.2$ (mean ± SD), and body mass index of $23.8 \pm 1.8$ kg/m$^2$. Eleven subjects were studied between 0700-1200, four subjects between 1200-1700; there were no statistical differences found for time of day, data were therefore pooled. Part B (oxygen investigations), included ten adults (5 female) with a mean age of $28 \pm 4.5$, and body mass index of $24 \pm 2.2$ kg/m$^2$. Parts A and B were completed at different centres on a different group of participants. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any medications other than contraception; females were studied during the follicular phase. The study was approved by the Clinical Research Ethics Board of the University of British Columbia (Study A) and the Institutional Review Board of Duke University Medical Center (Study B) and conformed to the standards set by the Declaration of Helsinki.

4.3.2 Experimental design
After familiarization with the experimental procedures outlined below, each participant was studied on one occasion. Before each experimental session, participants were required to abstain from exercise and alcohol for 24 h, caffeine for 12 h and a heavy meal for 4 h prior. After local anesthesia (1% lidocaine), a 20-gauge catheter (Arrow, Markham Ontario) was placed into the radial artery and attached to a pressure transducer (AD Instruments, Colorado Springs, USA) positioned at the level of the right atrium in the midaxillary line for the measurement of beat-to-beat arterial blood pressure and arterial blood gases. Following cannulation, subjects rested quietly in the supine position, breathing room air, for $\geq 30$ minutes to allow the setup of monitoring equipment, which included calibration of this pressure transducer. An automated gas blender adjusted the composition and flow to a sequential gas delivery mask and breathing circuit according to the method described by Slessarev et al. (2007) (RespirAct; Thornhill Research, Toronto, Canada). This apparatus enables prospective control of the individual’s end-tidal CO$_2$ and O$_2$ (PETCO$_2$ and PETO$_2$), and minute ventilation independently of one and another. At least during modest changes in
PaCO$_2$ (35 mmHg – 55 mmHg), the breathing circuit has been shown to operate such that the PETCO$_2$ is equal to PaCO$_2$ (Slessarev et al., 2007). Two protocols were conducted, described below.

**Part A (carbon dioxide investigations):** Following a 15-minute baseline at 40 mmHg PaCO$_2$, either progressive hypocapnia or hypercapnia was randomly begun. In either case, sequential iso-oxic ~15-minute steps approximating 15, 20 mmHg (hypocapnia), and 50, 55, 60, and 65 mmHg (hypercapnia) were conducted. Due to technical limitations, the maximal hyperventilation stage (PaCO$_2$ ~15 mmHg) was carried out without oxygen clamping. Each step was separated by a recovery period long enough to allow PaCO$_2$ to return to baseline values – typically ~15-30 minutes. Arterial blood gases were sampled at the end of each stage, the exact duration of which depended on subject tolerance, the time needed to stabilize at the desired PaCO$_2$ level, and the time needed to make neck blood flow measurements; in most cases stages lasted 12-15 minutes.

**Part B (oxygen investigations):** Following a 15-min breathing room air baseline measurements were made before PaO$_2$ was sequentially decreased to 60, 44, and 35 mmHg PaO$_2$ for exactly 15 minutes each. Subjects then breathed room air again for 15-min, before PaO$_2$ was increased to ~320 mmHg and ~430 mmHg. PaCO$_2$ was clamped at room air levels throughout all tests.

**4.3.3 Measurements**

*Cerebral blood velocity:* The left middle cerebral artery blood velocity (MCAv) and right posterior cerebral artery blood velocity (PCAv) were measured using a 2-MHz pulsed transcranial Doppler ultrasound (TCD) system (Spencer Technologies, Seattle, WA, USA). The left MCA was measured through the temporal window, at a depth 1cm distal to the MCA-anterior cerebral artery bifurcation. The right P1 segment of the PCA was insonated from the right anterior temporal window. The probes were fixed and held in place using a specialized headband fixation device (M600 Bilateral Head frame, Spencer Technologies, USA). Standardized search techniques were used to optimize signal quality as detailed previously (Willie et al., 2011b). Our test-retest reliability for baseline measures of MCAv and PCAv are approximately 3% and 2% respectfully.
Extracranial ultrasound of blood flow in conduit vessels: Continuous diameter and blood flow recordings in the left internal carotid artery and right vertebral artery were obtained using a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason 3000™, Teratech, Burlington, MA). Imaging of the extracranial arteries commenced when end-tidal clamping was stable for at least 1 minute. One sonographer on the left side of the participant conducted all left ICA measures, while another sonographer on the right side performed imaging of the right VA. The left ICA was measured at least 2cm from the carotid bifurcation, whilst ensuring there was no evidence of turbulent or retrograde flow. The right VA was measured within 1cm either proximal or distal (but at the same location within each subject) to the transverse process of C3. Average diameter and blood flow recordings were made from a minimum of 10 cardiac cycles (see below), and care was taken to ensure probe position was stable so that the angle of insonation did not vary from 60°. The sample volume was positioned in the centre of the vessel and adjusted to cover the width of the vessel diameter. Measurement settings for each extracranial artery within an individual were standardized for all gas stages. Test-retest reliability for baseline measures of ICA and VA flow are approximately 5% and 11% respectfully.

All extracranial vascular images were directly stored as a DICOM file for offline analysis. As described in depth elsewhere, analysis involved continuous measurements of arterial diameter synchronous with measurements of blood velocity at 30 Hz performed using an off-line custom-designed edge-detection and wall-tracking software (Black et al., 2008). Mean blood flow was determined as half the time averaged maximum velocity (Evans, 1985) multiplied by the cross-sectional lumen area. This method is used instead of the intensity weighted mean because the latter is more susceptible to noise and other distorting influences; the maximum follower is only affected by noise above the maximum frequency, noise that is visually obvious. Reproducibility of diameter measurements using this software is significantly better than manual methods as it reduces observer error significantly, and possesses an intra-observer coefficient of variation of 6.7% (Black et al., 2008).

Blood pressure and heart rate: Electrocardiogram, non-invasive beat-to-beat blood pressure by finger photopleysymography (Finometer, Amsterdam, the Netherlands), intra-radial artery pressure, and PETCO₂ were sampled continuously at 1 kHz via an analogue to digital
data acquisition system (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA).

*Arterial blood gases:* Arterial blood gas samples from the radial artery were drawn into a preheparinised syringe, and analysed immediately. Following standardised calibration, all blood samples were analysed using an arterial blood-gas analysing system (ABL-80 Co-Ox, Radiometer, Copenhagen, Denmark) for pH, PaO$_2$, PaCO$_2$ and SaO$_2$.

4.3.4 **Calculations**

Values for mean arterial pressure (MAP), MCAv, PCAv, heart rate (HR), and end-tidal gases were averaged over the last 3 minutes of each stage. Cerebrovascular conductance indices (CVC) were calculated by dividing MAP by the mean cerebral blood velocities (CBV; for MCAv and PCAv), or flow (for ICA and VA). Two forms of the hypoxic cerebrovascular reactivity were calculated for each individual as the slopes of the linear regressions between: 1) absolute CBF (CBV or flow) vs. SaO$_2$; and 2) the relative (percentage) change from baseline CBF vs. SaO$_2$. SaO$_2$ is conventionally used in place of PaO$_2$ to calculate hypoxic cerebrovascular reactivity because: 1) the former gives an approximately linear (rather than exponential) relationship with CBF, and 2) SaO$_2$ is clinically meaningful and less invasively obtained than PaO$_2$. Cerebrovascular CO$_2$ reactivity was similarly calculated both in absolute and relative terms as the slopes of the linear regression between CBF vs. PaCO$_2$ and %ΔCBF vs. PaCO$_2$, for the entire PaCO$_2$ range. All $R^2$ values were greater than 0.65. Cerebrovascular CO$_2$ reactivity was also calculated separately for hypo- and hypercapnia. Arterial blood gas sampling was not possible in two subjects (one due to lack of catheter, one to technical issues with the gas analyser). In these individuals PaO$_2$ and PaCO$_2$ were estimated from their measured end-tidal gases using the group relationship between arterial and end-tidal gases that yielded the regression equations: PaCO$_2$ = 0.882 (PetCO$_2$) + 2.47 ($R^2 = 0.98$); PaO$_2$ = 0.806 (PetO$_2$) + 8.3 ($R^2 = 0.724$). The relationship between arterial and end-tidal CO$_2$ is shown in Figure 4.1.
Arterial oxygen delivery was calculated as the product of the arterial content of oxygen \((\text{CaO}_2)\) and flow through the ICA and VA. \(\text{CaO}_2\) was calculated as:

\[
\text{CaO}_2 \text{ (mL \, dL}^{-1}) = [\text{hemoglobin}] (g \cdot \text{dL}^{-1}) \times 1.36 (\text{mL} \cdot \text{O}_2 \cdot \text{g hemoglobin}^{-1}) \times \text{SaO}_2 \text{ (%) ÷ 100} + 0.003 (\text{mL} \cdot \text{dL}^{-1}) \times \text{PaO}_2 \text{ (mmHg)}
\]

![Figure 4.1. Relationship between arterial (PaCO\(_2\)) and end-tidal (PetCO\(_2\)) partial pressures of carbon dioxide during targeted steady state changes in PetCO\(_2\). A. Regression equation: \(\text{PaCO}_2 = 0.882 \times \text{(PETCO}_2\) + 2.47; \((R^2 = 0.98)\). B, Bland-Altman plot of differences between PaCO\(_2\) and PetCO\(_2\), and the mean value of both. Dashed lines represents the 95\% confidence intervals and the mean bias.]

4.3.5 Statistical Analysis

Normal distribution of variables was confirmed with Shapiro-Wilk normality test. One-way ANOVA was used to compare differences across blood gas conditions, and where appropriate, post hoc comparisons to baseline blood gases were made using Dunnet’s post-test. One-way repeated measures ANOVA was used to compare ICA diameter with PaCO\(_2\) stage in the 8 subjects who completed all stages between -20 mmHg and 55 mmHg PaCO\(_2\). To make comparisons between ICA and VA, and between MCA and PCA, for a given blood gas stage, student’s \(t\)-test were performed with appropriate Bonferroni correction for multiple comparison. Relationships between selected variables were determined using Pearsons
correlations. There were no statistical differences in any variable between males and females, these data were therefore pooled. Values are presented as mean ± SD.

4.4 Results

4.4.1 Subjects

CO₂ investigations: There were marked variation in tolerances to the extremes of both hypo- and hypercapnia. For example, with hypercapnia, the 50, 55, 60, 65 mmHg steps were completed by 16, 15, 9 and 5 individuals, respectively. With hypocapnia, the 30, 20 and maximal hypocapnic (15 mmHg) steps were completed by 16, 15, and 11 individuals, respectively. Inadequate quality in ICA images were obtained in 4 subjects. Three of these subjects’ images lacked clearly demarcated vessel walls throughout the cardiac cycle, and in one subject large changes in the position of the vessel with respiration limited imaging of the vessel over more than a few concurrent cardiac cycles. Adequate images of the VA were not obtained in 4 subjects due in all cases to inadequate vessel wall sharpness; two of these subjects were amongst those excluded from ICA analysis. Those subjects with inadequate ICA and/or VA images were excluded only from ICA and/or VA analysis. Thus, group means for MAP, HR, PetCO₂, MCAv, and PCAv were all based on n = 16, whereas VA and ICA metrics were calculated on their respective n=12. Data from the individuals who completed all stages showed the same blood flow and reactivity trends as that of the group means. Final sample sizes for each of the vessel measures at each stage are given in Table 4.1. In one subject a radial arterial catheter was not possible; blood pressure values from finger photoplethysmography were substituted in this subject only. Automated blood pressure measures were used to confirm these finger photoplethysmography values.

O₂ investigations: 10 subjects began the protocol. One subject could not tolerate the mask at the 70% SaO₂ stage; her data were excluded from analysis. In another subject post-analysis revealed technical problems with the blood pressure recording; therefore these data were also excluded from analysis. Of the n=8 (age 28.6 ± 4.5) remaining, in one subject poor ICA images were attained; ICA analysis was therefore based on n = 7.
4.4.2 Cardiorespiratory data

$CO_2$ investigations: There were no differences between the pre-hypocapnia and pre-hypercapnia baselines; thus full recovery was obtained between the progressive hypocapnic and hypercapnic steps. As expected, $PaCO_2$ was altered at each stage ($P<0.05$; Table 4.1). Although MAP did not differ from baseline during hypocapnia, it was significantly increased during each stage of hypercapnia (Table 4.1). Heart rate was not different at 10 mmHg $PaCO_2$ above or below baseline, but was elevated at every other stage.

$O_2$ investigations (Tables 4.2 and 4.3): There were no differences in any variable between pre-hypoxia and pre-hyperoxia trials. Full recovery was therefore achieved between the hypoxia and hyperoxia stages. Heart rate was elevated from baseline at each stage of hypoxia but was unaltered during hyperoxia. Mean arterial pressure did not change during any stage.
Table 4.1. Cardiorespiratory and cerebrovascular variables during iso-oxic PaCO\(_2\) alterations.

<table>
<thead>
<tr>
<th>Variable</th>
<th>15 mmHg</th>
<th>20 mmHg</th>
<th>30 mmHg</th>
<th>40 mmHg (Baseline)</th>
<th>50 mmHg</th>
<th>55 mmHg</th>
<th>60 mmHg</th>
<th>65 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO(_2)†</td>
<td>16.6 ± 1.7*</td>
<td>20.0 ± 3.6*</td>
<td>29.2 ± 4.2*</td>
<td>39.2 ± 3.7</td>
<td>49.2 ± 3.7*</td>
<td>53.7 ± 3.5*</td>
<td>58.2 ± 4.7*</td>
<td>62.2 ± 3.8*</td>
</tr>
<tr>
<td>PaO(_2)</td>
<td>109 ± 31</td>
<td>100 ± 25</td>
<td>92.6 ± 9</td>
<td>91.3 ± 17</td>
<td>95.9 ± 14</td>
<td>94.6 ± 14</td>
<td>93.2 ± 17</td>
<td>93.5 ± 21</td>
</tr>
<tr>
<td>HR (beats / min)</td>
<td>89 ± 14*</td>
<td>79 ± 11*</td>
<td>68 ± 9</td>
<td>65 ± 1</td>
<td>72 ± 8.3</td>
<td>76 ± 11*</td>
<td>79 ± 5.8*</td>
<td>85 ± 6*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81.1 ± 3.6</td>
<td>82.1 ± 5.2</td>
<td>82.5 ± 4.5</td>
<td>82.4 ± 3.6</td>
<td>86.5 ± 5.3</td>
<td>91.7 ± 7.4*</td>
<td>93.9 ± 8.3*</td>
<td>100 ± 5.2*</td>
</tr>
<tr>
<td>Q(_{ICA}), mL min(^{-1})</td>
<td>122 ± 30.0*</td>
<td>121 ± 24.3*</td>
<td>151 ± 25.0*</td>
<td>222 ± 47.6</td>
<td>341 ± 75.2*</td>
<td>418 ± 92.1*</td>
<td>482 ± 109*</td>
<td>489 ± 84.9*</td>
</tr>
<tr>
<td>DO(_2) (mL O(_2) / min)</td>
<td>26.3 ± 6.3*</td>
<td>26.4 ± 5.4*</td>
<td>32.4 ± 5.6</td>
<td>47.1 ± 11.0</td>
<td>72.5 ± 16.4*</td>
<td>87.4 ± 23.1*</td>
<td>102.7 ± 25.3*</td>
<td>108.3 ± 19.9*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.47 ± 0.05</td>
<td>0.48 ± 0.05</td>
<td>0.50 ± 0.07</td>
<td>0.52 ± 0.06</td>
<td>0.55 ± 0.07</td>
<td>0.57 ± 0.08</td>
<td>0.58 ± 0.06</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>∆% Diameter</td>
<td>-6.6 ± 2.9</td>
<td>-6.0 ± 5.0</td>
<td>-3.2 ± 5.3</td>
<td>2.6 ± 4.3</td>
<td>5.8 ± 6.1</td>
<td>10.0 ± 6.0</td>
<td>11.5 ± 4.8</td>
<td>11.5 ± 4.8</td>
</tr>
<tr>
<td>(CVC, mL/mmHg)</td>
<td>(1.5 ± 0.4) *</td>
<td>(1.5 ± 0.4) *</td>
<td>(1.8 ± 0.4) *</td>
<td>(2.6 ± 0.7)</td>
<td>(4.0 ± 1.2) *</td>
<td>(4.4 ± 1.0) *</td>
<td>(4.8 ± 1.3) *</td>
<td>(4.5 ± 1.3) *</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=7</td>
<td>n=11</td>
<td>n=12</td>
<td>n=12</td>
<td>n=10</td>
<td>n=7</td>
<td>n=10</td>
<td>n=3</td>
</tr>
<tr>
<td>Q(_{VA}), mL min(^{-1})</td>
<td>39.1 ± 25.4</td>
<td>39.9 ± 21.5</td>
<td>47.2 ± 22.7</td>
<td>71.4 ± 27.0</td>
<td>99.5 ± 35.3</td>
<td>118 ± 41.3*</td>
<td>158 ± 58.9*</td>
<td>187 ± 30.4*</td>
</tr>
<tr>
<td>DO(_2) (mL O(_2) / min)</td>
<td>8.4 ± 5.4</td>
<td>8.4 ± 4.1</td>
<td>8.7 ± 4.4</td>
<td>14.0 ± 5.4</td>
<td>19.6 ± 6.6</td>
<td>24.0 ± 8.3*</td>
<td>32.0 ± 10.7*</td>
<td>39.2 ± 3.2*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.40 ± 0.07</td>
<td>0.39 ± 0.07</td>
<td>0.40 ± 0.07</td>
<td>0.41 ± 0.07</td>
<td>0.41 ± 0.05</td>
<td>0.42 ± 0.04</td>
<td>0.43 ± 0.04</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>∆% Diameter</td>
<td>-2.9 ± 9.7</td>
<td>-6.1 ± 8.8</td>
<td>0.3 ± 8.9</td>
<td>2.4 ± 6.3</td>
<td>3.1 ± 7.4</td>
<td>-0.5 ± 9.5</td>
<td>2.8 ± 6.7</td>
<td>2.8 ± 6.7</td>
</tr>
<tr>
<td>(CVC, mL/mmHg)</td>
<td>(0.47 ± 0.3)</td>
<td>(0.49 ± 0.2)</td>
<td>(0.56 ± 0.27)</td>
<td>(0.87 ± 0.33)</td>
<td>(1.2 ± 0.45)</td>
<td>(1.3 ± 0.45)*</td>
<td>(1.8 ± 0.81)*</td>
<td>(2.0 ± 0.30)*</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=4</td>
<td>n=6</td>
<td>n=11</td>
<td>n=12</td>
<td>n=9</td>
<td>n=3</td>
<td>n=10</td>
<td>n=2</td>
</tr>
<tr>
<td>MCA(_v), cm/s</td>
<td>37.9 ± 6.9*</td>
<td>40.5 ± 6.8*</td>
<td>46.6 ± 6.4*</td>
<td>62.4 ± 10.1</td>
<td>88.0 ± 14.5*</td>
<td>101 ± 14.3*</td>
<td>111 ± 18.7*</td>
<td>111 ± 19.6*</td>
</tr>
<tr>
<td>(CVC, cm/s/mmHg)</td>
<td>(0.47 ± 0.08)*</td>
<td>(0.50 ± 0.09)*</td>
<td>(0.56 ± 0.07)*</td>
<td>(0.76 ± 0.13)</td>
<td>(1.0 ± 0.18)*</td>
<td>(1.1 ± 0.18)*</td>
<td>(1.2 ± 0.20)*</td>
<td>(1.1 ± 0.16)*</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=11</td>
<td>n=15</td>
<td>n=16</td>
<td>n=16</td>
<td>n=15</td>
<td>n=15</td>
<td>n=15</td>
<td>n=5</td>
</tr>
<tr>
<td>PCA(_v), cm/s</td>
<td>28.8 ± 6.4*</td>
<td>31.0 ± 7.1*</td>
<td>34.9 ± 7.1*</td>
<td>46.7 ± 11.5</td>
<td>67.4 ± 15.6*</td>
<td>76.8 ± 12.4*</td>
<td>84.3 ± 14.5*</td>
<td>86.3 ± 16.8*</td>
</tr>
<tr>
<td>(CVC, cm/s/mmHg)</td>
<td>(0.36 ± 0.09)*</td>
<td>(0.38 ± 0.10)*</td>
<td>(0.42 ± 0.09)*</td>
<td>(0.57 ± 0.15)</td>
<td>(0.78 ± 0.19)*</td>
<td>(0.84 ± 0.14)*</td>
<td>(0.90 ± 0.17)*</td>
<td>(0.86 ± 0.14)*</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=10</td>
<td>n=14</td>
<td>n=16</td>
<td>n=16</td>
<td>n=15</td>
<td>n=15</td>
<td>n=15</td>
<td>n=5</td>
</tr>
</tbody>
</table>

† Values are arterial PaCO\(_2\) and PaO\(_2\), except for six blood gas values distributed amongst the subjects, and in two subjects in whom arterial blood sampling was not possible. PaCO\(_2\) and PaO\(_2\) values were estimated in these cases from the regression equations between PaCO\(_2\), PaO\(_2\) and PetCO\(_2\) for all subjects; see methods section. Sample sizes vary between measured vessels due to inadequate image quality for VA and ICA measures, and loss of PCA.
signal in one subject during hypocapnia. PaCO$_2$ and PaCO$_2$, partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; Q$_{ICA}$, internal carotid artery blood flow; DO$_2$, Oxygen delivery through vessel; Q$_{VA}$, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity / MAP); * $P < 0.05$ vs. 40 mmHg (Baseline).
Table 4.2. Cardiorespiratory and cerebrovascular variables during isocapnic hypoxemia.

<table>
<thead>
<tr>
<th></th>
<th>100% SaO₂</th>
<th>90% SaO₂</th>
<th>80% SaO₂</th>
<th>70% SaO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PaO₂ (mmHg)</strong></td>
<td>106 ± 8.0</td>
<td>59 ± 4.0*</td>
<td>43 ± 2.8*</td>
<td>36 ± 4.3*</td>
</tr>
<tr>
<td><strong>SaO₂ (%)</strong></td>
<td>98.7 ± 0.39</td>
<td>92.4 ± 2.0*</td>
<td>80.3 ± 2.0*</td>
<td>70.1 ± 3.0*</td>
</tr>
<tr>
<td><strong>PaCO₂ (mmHg)</strong></td>
<td>40 ± 1.6</td>
<td>40 ± 2.4</td>
<td>41 ± 0.7</td>
<td>41 ± 1.7</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>65 ± 9</td>
<td>72 ± 11*</td>
<td>82 ± 10*</td>
<td>88 ± 10*</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>89 ± 5</td>
<td>91 ± 6</td>
<td>93 ± 10</td>
<td>95 ± 12</td>
</tr>
<tr>
<td><strong>Q ICA, mL min⁻¹</strong></td>
<td>230 ± 67.5</td>
<td>222 ± 66.9</td>
<td>284 ± 62.8</td>
<td>322 ± 95.9*</td>
</tr>
<tr>
<td>DO₂, mL O₂ min⁻¹</td>
<td>48.7 ± 16.2</td>
<td>42.4 ± 12.5</td>
<td>50.8 ± 17.4</td>
<td>46.2 ± 14.4</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.54 ± 0.09</td>
<td>0.54 ± 0.09</td>
<td>0.55 ± 0.07</td>
<td>0.49 ± 0.21</td>
</tr>
<tr>
<td>Δ% Diameter</td>
<td>-1.0 ± 8.1</td>
<td>1.4 ± 8.6</td>
<td>6.3 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>CVC, mL min⁻¹</td>
<td>2.7 ± 0.90</td>
<td>2.6 ± 0.86</td>
<td>3.3 ± 1.0</td>
<td>3.0 ± 1.8*</td>
</tr>
<tr>
<td>mmHg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Q VA, mL min⁻¹</strong></td>
<td>48.7 ± 16.5</td>
<td>52.6 ± 22.4</td>
<td>66.9 ± 30.8</td>
<td>92.4 ± 21.3*</td>
</tr>
<tr>
<td>DO₂, mL O₂ min⁻¹</td>
<td>9.4 ± 2.8</td>
<td>9.5 ± 4.2</td>
<td>10.4 ± 4.0</td>
<td>13.0 ± 2.3*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.33 ± 0.05</td>
<td>0.33 ± 0.06</td>
<td>0.35 ± 0.06</td>
<td>0.36 ± 0.06*</td>
</tr>
<tr>
<td>Δ% Diameter</td>
<td>0.0 ± 4.8</td>
<td>5.3 ± 6.9</td>
<td>8.3 ± 9.2</td>
<td></td>
</tr>
<tr>
<td>CVC, mL min⁻¹</td>
<td>0.55 ± 0.19</td>
<td>0.58 ± 0.24</td>
<td>0.75 ± 0.39</td>
<td>1.0 ± 0.31*</td>
</tr>
<tr>
<td>mmHg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCAv, cm s⁻¹</strong></td>
<td>59.9 ± 10.8</td>
<td>66.3 ± 14.1</td>
<td>76.2 ± 16.6*</td>
<td>83.6 ± 18.4*</td>
</tr>
<tr>
<td>CVC, cm s⁻¹</td>
<td>0.67 ± 0.12</td>
<td>0.73 ± 0.16</td>
<td>0.82 ± 0.17*</td>
<td>0.89 ± 0.18*</td>
</tr>
<tr>
<td>mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCAv, cm s⁻¹</strong></td>
<td>38.0 ± 8.8</td>
<td>40.6 ± 10.8</td>
<td>46.2 ± 10.7*</td>
<td>50.7 ± 12.0*</td>
</tr>
<tr>
<td>(CVC, cm s⁻¹)</td>
<td>0.43 ± 0.12</td>
<td>0.45 ± 0.14</td>
<td>0.51 ± 0.16*</td>
<td>0.55 ± 0.18*</td>
</tr>
<tr>
<td>mmHg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PaCO₂ and PaCO₂, partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; Q ICA, internal carotid artery blood flow; DO₂, Oxygen delivery through vessel; Q VA, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity / MAP). * P < 0.05 vs. 100% SaO₂ (Baseline).
Table 4.3. Cardiorespiratory and cerebrovascular variables during isocapnic hyperoxia.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>320 mmHg PaO$_2$</th>
<th>430 mmHg PaO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO$_2$ (mmHg)</td>
<td>104 ± 10.3</td>
<td>321 ± 7.8*</td>
<td>434 ± 33.2*</td>
</tr>
<tr>
<td>SaO$_2$ (%)</td>
<td>98 ± 0.74</td>
<td>99.6 ± 0.25*</td>
<td>99.6 ± 0.23*</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
<td>40 ± 1.7</td>
<td>40 ± 2.6</td>
<td>41 ± 2.3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>68 ± 10</td>
<td>66 ± 11</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 ± 8</td>
<td>92 ± 8</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>QICA, mL/ min</td>
<td>242 ± 86.4</td>
<td>245 ± 110</td>
<td>243 ± 86.4</td>
</tr>
<tr>
<td>DO$_2$ (mL O$_2$/min)</td>
<td>51.9 ± 15.6</td>
<td>54.5 ± 21.9</td>
<td>51.0 ± 20.0</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.56 ± 0.08</td>
<td>0.53 ± 0.08</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>CVC, mL/ min/mmHg</td>
<td>2.9 ± 0.86</td>
<td>2.9 ± 1.2</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>QVA, mL/ min</td>
<td>53.6 ± 15.4</td>
<td>52.1 ± 24.7</td>
<td>42.8 ± 12.3</td>
</tr>
<tr>
<td>DO$_2$ (mL O$_2$/min)</td>
<td>10.8 ± 2.7</td>
<td>10.5 ± 5.1</td>
<td>8.9 ± 2.7</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.36 ± 0.06</td>
<td>0.33 ± 0.08</td>
<td>0.32 ± 0.07</td>
</tr>
<tr>
<td>CVC, mL/ min/mmHg</td>
<td>0.61 ± 0.18</td>
<td>0.56 ± 0.26</td>
<td>0.47 ± 0.15</td>
</tr>
<tr>
<td>MCAv, cm/s</td>
<td>64.0 ± 12.8</td>
<td>60.4 ± 11.8*</td>
<td>57.5 ± 10.7*</td>
</tr>
<tr>
<td>CVC, cm/s/ mmHg</td>
<td>0.70 ± 0.12</td>
<td>0.66 ± 0.11*</td>
<td>0.62 ± 0.11*</td>
</tr>
<tr>
<td>PCAv, cm/s</td>
<td>41.4 ± 11.1</td>
<td>39.6 ± 10.3</td>
<td>38.8 ± 9.8*</td>
</tr>
<tr>
<td>(CVC, cm/s/mmHg)</td>
<td>0.44 ± 0.14</td>
<td>0.42 ± 0.13</td>
<td>0.41 ± 0.12*</td>
</tr>
</tbody>
</table>

PaCO$_2$ and PaCO$_2$, partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; QICA, internal carotid artery blood flow; QVA, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity / MAP). * Denotes differences from baseline (P<0.05).

4.4.3 Cerebrovascular data: CO$_2$ investigations

Changes in flow and velocity with PaCO$_2$ (Figures 4.2 and 4.3; Table 4.1): QICA, MCAv and PCAv, and their respective CVC values, were significantly increased during all stages of hypercapnia, and decreased during all stages of hypocapnia. QVA and VA CVC were increased during hypercapnia; however, the decrease during hypocapnia did not reach statistical significance (Table 4.1). With the extreme level of hypercapnia (65 mmHg) the
relative increases in CBF from baseline were: \( Q_{\text{ICA}}: 146 \pm 23.7\%; \) \( Q_{\text{VA}}: 158 \pm 35\%; \) MCAv: 99.1 \( \pm \) 16.8\%; and PCAv: 108 \( \pm \) 22.7\. Conversely, at the extreme level hypocapnia CBF decreases were: \( Q_{\text{ICA}}: -41.0 \pm 9.8\%; \) \( Q_{\text{VA}}: -49.8 \pm 8.2\%; \) MCAv: -39.1 \( \pm \) 6.9\%; PCAv: -39.5 \( \pm \) 7.3\. Oxygen delivery through the ICA was decreased at 15 and 20 mmHg \( \text{PaCO}_2 \), and increased at 50 mmHg \( \text{PaCO}_2 \) and above. Posterior cerebral (VA) oxygen delivery was increased above 50 mmHg \( \text{PaCO}_2 \); there was a trend for reduced VA oxygen delivery during hypocapnia (Table 4.1).

**Figure 4.2.** Absolute cerebral blood flow during changes in arterial blood gases. Blood flow (top panels; ICA and VA) and blood velocity (bottom panels; MCA and PCA) during steady state changes in arterial \( \text{CO}_2 \) (left panels) and oxygen (right panels). * Difference from baseline (40 mmHg \( \text{PaCO}_2 \) or 100 \( \text{PaO}_2 \)), \( P < 0.05 \). † Indicates differences between vessel flow (ICA vs. VA) or velocity (MCA vs. PCA) at a given stage; \( \text{PaCO}_2 \): \( P < 0.006 \); \( \text{PaO}_2 \): \( P < 0.012 \). All values are mean \( \pm \) SD. ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. Note: the number of subjects comprising each mean value is different between stages and vessels. Please refer to Tables 4.1 and 4.2 for these values.
Figure 4.3. Relative changes in cerebral blood flow during changes in arterial blood gases.
Percentage change from baseline in blood flow (Q; ICA and VA) and blood velocity (CBV; MCA and PCA) during steady state changes in arterial CO₂ (top panels) and oxygen (bottom panels). All values are mean ± SD. ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. The relative flow change in the neck arteries were ~50% greater than velocity change in the intracranial vessels during hypercapnia, suggesting either anatomical flow redistribution or dilation of the MCA and PCA – or both. Note the much greater increase in VA flow during hypoxia compared to the other vessels; see Figure 4.4 for comparative CO₂ and O₂ reactivities. Note: the number of subjects comprising each mean value is different between stages and vessels. Please refer to Tables 4.1 and 4.2 for these values.
**Comparisons between vessels** (Figure 4.3): Absolute flow/velocity values for each vessel for a given stage were different from each other ($P < 0.006$). Overall cerebrovascular reactivity to CO$_2$ based on percent changes in CBF from baseline was ~25% greater ($P<0.05$) for the neck arteries (ICA: $4.0 \pm 0.38 \%$/mmHg; VA $4.4 \pm 2.1 \%$/mmHg versus that of the cerebral arteries (MCA: $2.9 \pm 0.47 \%$/mmHg; PCA: $3.0 \pm 0.62 \%$/mmHg). However, overall reactivity did not differ between ICA and VA, or between MCA and PCA. Reactivity in the hypercapnic range did not differ between vessels, whereas in the hypocapnic range VA reactivity was greater than the other vessels (Figure 4.4). For comparison, Figure 4.4 (bottom panel) also depicts CO$_2$ reactivity when calculated on absolute CBF values.

**Relationships with PaCO$_2$** (Figures 4.5 and 4.6): Changes in PaCO$_2$ were reflected in significant changes in the diameter of the ICA (individual regression mean $R^2$, $0.63 \pm 0.26$; Figure 4.5). In the eight subjects who completed all PaCO$_2$ stages from 20 mmHg to 55 mmHg, repeated measured ANOVA, revealed that a >10 mmHg change in PaCO$_2$ produced a significant change in ICA diameter, in both the hyper- and hypocapnic ranges. There was no significant change in VA diameter. The hypercapnic-induced elevations in MAP were positively ($P<0.05$) related to elevations in CBF or CBV in all arteries ($R^2$ 0.8-0.91; Figure 4.6).

### 4.4.4 Cerebrovascular data: O$_2$ investigations

**Changes in flow and velocity with SaO$_2$** (Figures 4.2 and 4.3): $Q_{\text{ICA}}$ and $Q_{\text{VA}}$ (and related CVC) were increased at 36 mmHg PaO$_2$ (SaO$_2$ ~70%) only. In contrast, $MCA_{v}$ and $PCA_{v}$ (and related CVC) were increased at both 44 mmHg (SaO$_2$ ~80%) and 36 mmHg PaO$_2$ (SaO$_2$ ~70%) stages. Both stages of hyperoxia decreased velocities and CVC of the MCA and PCA relative to baseline, but had no significant effect on the arteries of the neck (Table 4.3). Anterior cerebral (ICA) oxygen delivery was unchanged in hypoxia, whereas VA oxygen delivery was increased at 70% SaO$_2$ (Table 4.2). Hyperoxia elicited no change in oxygen delivery in either vessel.
Figure 4.4. Mean cerebral blood flow reactivity to CO\(_2\) and hypoxemia.
The top row depicts the slope of the normalized CBF change (\(\Delta\%\), relative to baseline) and PaCO\(_2\) or SaO\(_2\); the bottom row depicts absolute flow (Q; ICA and VA) or blood velocity (CBV; MCA and PCA) and PaCO\(_2\) or SaO\(_2\). Horizontal bars indicate significant relationships, \(P < 0.05\).
Figure 4.5. Response of the internal carotid artery to changes in PaCO₂.
Mean (squares; ± SD) and individual (circles) percent change in luminal diameters of the internal carotid artery during steady state change in PaCO₂. Linear regression of individual data $y = 3.6 \times 10^{-1}(\text{PaCO}_2) - 13.3; r^2 = 0.63$. Through a large PaCO₂ range, internal carotid diameter changes by ~20%, indicating that cerebrovascular resistance is not solely modulated at cerebral arterioles with changes in the partial pressure of arterial blood gases.
Figure 4.6. Relative changes in mean arterial pressure and cerebral blood flow during changes in PaCO₂.
Percent change from baseline in MAP (A) and relationship between ∆MAP and ∆CBF or ∆CBV (B) during hypercapia and hypoxia. A, percentage change in (∆MAP) relative to baseline for PaCO₂ (top row) and hypoxaemia (bottom row) trials. Right hand eight plots (B) depict ∆MAP in the hypercapnic or hypoxic ranges (shown boxed in A). ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. The hypercapnia related hypertension was positively related (P < 0.05) to elevations in CBF/CBV in all vessels; in contrast, hypoxia induced increases in MAP were not related to CBF/CBV.
Comparisons between vessels (Figures 4.2-4.4): Absolute values for each vessel at each PaO$_2$ stage were different from each other ($P < 0.006$). Cerebrovascular reactivity, expressed as the relative percent change in CBF with SaO$_2$, was ~50% greater in the VA (-3.3 ± 1.4) relative to the other arteries. Reactivity in these arteries were not different from one another (ICA: -1.71 ± 1.3; MCA -1.39 ± 0.5; PCA -1.19 ± 0.3). However, when expressed as the absolute value of flow (ICA and VA), or velocity (MCA and PCA), reactivity in the ICA was the highest; absolute reactivity was not different between the other three vessels.

Relationships with $O_2$: The diameter of the VA increased at ~36 mmHg PaO$_2$ (SaO$_2$ ~70%) whereas ICA diameter was unaffected by altered PaO$_2$. In contrast to the PaCO$_2$ relationships, there was no relationship between the (non-significant) increase in MAP and CBF or CBV in any vessel (Figure 4.6).

4.4.5 Proximal vs. distal measures of CBF
Figure 4.7 shows Bland-Altman plots of the difference in percent change in CBF between VA and PCA, and ICA and MCA, plotted against their respective means. TCD provides an accurate estimate of CBF at lower CBF velocities but likely underestimates flow at the high CBF values during hypoxia or hypercapnia.

4.5 Discussion
This is the first study to examine regional cerebrovascular response differences over the tolerable extremes of acute arterial blood gas changes in humans. The novel findings of this study were: 1) VA reactivity to isocapnic hypoxia, and hypocapnia was greater than the other arteries (i.e., ICA, MCA and PCA); 2) overall cerebrovascular reactivity to PaCO$_2$ was greater in the neck arteries than the cerebral arteries (i.e., ICA and VA vs. MCA and PCA); 3) VA diameter increased during extreme hypoxemia, whereas ICA diameter was sensitive to changes in PaCO$_2$, indicating proximal resistance to the cerebral circulation is not constant during changes in blood gases; and, 4) at high blood flows due to both isocapnic hypoxia and iso-oxic hypercapnia, TCD underestimates changes in global CBF. Collectively these findings indicate disparate blood gas sensitivity to the brainstem and cortex, challenge the dogma that cerebrovascular resistance is solely modulated at the level of the arteriolar pial
vessels, and highlights limitations in the use of TCD for the measurement of CBF during extreme hypoxia and/or hypercapnia.

Figure 4.7. Bland–Altman plots of differences between neck artery blood flows and the respective downstream intracranial vessel, and the mean value of both. Values are percentage change from baseline. Dashed line represents the 95% confidence intervals, dotted line the mean bias. All plots show systematic error proportional to the increase in CBF; A and B suggest systematic and random error. Data show that at lower CBF values TCD estimates of intracranial blood velocity accurately reflect CBF for both the vertebral–PCA systems and ICA–MCA systems. With high CBF with hypoxia or hypercapnia MCAv and PCAv are likely to underestimate CBF.
4.5.1 Cerebrovascular reactivity to PaCO₂ and PaO₂

The cerebrovasculature is exquisitely sensitive to PaCO₂ and, to a lesser extent, PaO₂. This sensitivity serves to buffer brain tissue pH, thereby stabilizing chemosensory and autonomic control. We examined the slope of the CBF-PaCO₂ / PaO₂ relationships, as well as the separate CBF reactivities to hypocapnia and hypercapnia. The neck arteries displayed greater overall CO₂ reactivity than the intracranial vessels, and the VA displayed higher reactivity to hypoxia than both the ICA and intracranial vessels. The explanation for differences in reactivity between the neck vessels and their downstream arteries likely has either an anatomical or technical basis – or both.

Anatomical: In addition to the MCA, the ICA also supplies the anterior cerebral artery. Thus the assumption of unity between ICA and MCA flow/velocity is contingent on a constant distributive relationship between the middle and anterior cerebral arteries. Similarly, the VA feed a number of other vessels in addition to the PCA, which in regards to the vertebrobasilar system, has a higher degree of complexity. There are numerous anastomoses with the VA both extra- and intracranially. The VA communicates with branches of the deep cervical artery, and inferior thyroid artery extracranially, and upon entering the skull gives off multiple branches to the cerebellum, medulla and pons before joining to form the basilar artery (Edvinsson & Krause, 2002). A number of arteries project from the basilar artery to supply the cerebellum and pons before it ramifies to form the posterior circle of Willis and posterior cerebral arteries (Willie et al., 2011b). Thus, particularly in the case of the posterior vessels, the different reactivities in the neck vs. intracranial vessels could be a result of disparate changes in downstream resistance of regions not supplied by the MCA or PCA. In other words, for a given change in PaCO₂ or PaO₂ blood may be diverted away from MCA or PCA through intracranial vessels not directly measured in the current study. There are no data, however, to suggest that this is the case, and given the similar differences in ICA/MCA and VA/PCA reactivities, we feel the following technical argument is more likely.

Technical: Diameter changes of the MCA and PCA themselves provide the second explanation for the difference in reactivity between neck and intracranial vessels. That the overall PaO₂ or PaCO₂ reactivity of the neck vessels was greater than the intracranial vessels could be evidence of vasodilation at the site of insonation. Hypercapnic (or hypoxic)-
induced dilation would reduce blood velocity resulting in an underestimation of flow (see: Implications; Flow vs. Velocity with TCD).

We observed that in the hypercapnic range all vessels exhibit similar cerebrovascular reactivity, yet in the hypocapnic range the VA had greater CO\textsubscript{2} reactivity than the ICA, MCA, and PCA, which were not different from one another. No previous study has reported reactivities for the ICA, VA or PCA but our MCA values are consistent with that previously reported (Ainslie & Duffin, 2009). Our values of PaCO\textsubscript{2} reactivity for MCA and PCA were similar to that reported for the basilar artery (Hida et al., 1996). As highlighted in the current study, it is known that steady-state cerebrovascular reactivity is more sensitive to increases in PaCO\textsubscript{2} (i.e., hypercapnic reactivity) than decreases (i.e., hypocapnic reactivity) (Ide et al., 2003a; Peebles et al., 2007; Xie et al., 2005). The mechanisms underlying this greater reactivity to hypercapnia compared with hypocapnia may be related to a greater influence of vasodilator mediators on intracranial vascular tone compared with vasoconstrictive mediators (Toda & Okamura, 1998). It is also likely that increased MAP in the hypercapnic range influences reactivity, though there was no obvious relationship between MAP and CBF with hypoxia.

4.5.2 Brain stem blood flow regulation
Relative to all other vessels, the VA exhibited greater reactivity to hypoxia and to CO\textsubscript{2} in the hypocapnic range. Because of the complexity and distribution of the vertebrobasilar system, these results are difficult to interpret. There are also no values, to our knowledge, in the literature of VA reactivity to either CO\textsubscript{2} or hypoxia to compare our findings. Nevertheless, the greater reactivity of the VA, particularly to severe hypoxemia and hypocapnia, is interesting in light of it being the principal blood conduit to the brainstem, and highlights the importance of regional blood flow distribution in the study of chemoreflex and autonomic control.

4.5.3 A Proximal Resistor for the Human Cerebral Circulation?
We observed a 27% change in ICA diameter through a wide PaCO\textsubscript{2} range at the limits of our subjects’ tolerance, and a 9% change in VA diameter with extreme hypoxemia; to our knowledge this has not been previously reported in the literature. These data indicate that the
large changes in CBF manifest through broad PaCO\textsubscript{2} and PaO\textsubscript{2} ranges may be partially affected by diameter modulation of the neck arteries themselves. That the regulation of brain blood flow is mediated by the pial arterioles has remained dogma for more than seventy years (Fog, 1938; Fog) despite evidence from animals suggesting that, unlike other vascular beds, the large arteries of the brain contribute significantly to total (cerebral) resistance. Indeed, this was proposed by Mchedlishvili sixty years ago, but his ideas did not reach western scientific literature until much later (Mchedlishvili, 1980; Mchedlishvili \textit{et al.}, 1973). There are a number of studies demonstrating that the large cerebral vessels of cats and dogs contribute up to 30-45\% of total cerebrovascular resistance (Faraci & Heistad, 1990; Gross \textit{et al.}, 1980; Heistad \textit{et al.}, 1978a). The present data indicate that both the proximal cerebral arteries and distal cerebral arterioles dilate with hypercapnia and constrict with hypocapnia. Consistent with the results of the above animal studies, it is therefore likely that the entire cerebral arterial tree is sensitive to altered blood gases. Because the relative change in proximal and distal cerebral artery resistance ultimately determines cerebral microvasculature pressure, calculating the resistance changes based on the observed changes in diameter is not a meaningful quantification. But with union between small and large vessel vasomotion, microvascular pressure would be kept relatively constant despite large changes in flow. The ability of large cerebral arteries to act as ‘protective’ resistors (as opposed to static tubes) seems teleologically important. We acknowledge that it is possible that the increase in ICA diameter may not be due to the direct influence of PaCO\textsubscript{2} or hypoxia, but is rather a secondary endothelial-mediated response to changes in shear stress concomitant to flow changes, or passively during hypercapnia with increased MAP. At least at the level of brain arterioles in rats, however, vasodilation with hypercapnia appears to be mediated through non-endothelial mechanisms (Wang \textit{et al.}, 1994), and we observed a significant decrease in ICA diameter during hypocapnia, when MAP did not change.

Our study was not designed to clarify the mechanism(s) or the specific site of PaCO\textsubscript{2} reactivity. Such a study will be difficult to design \textit{in vivo}, however, given that subjecting only the arteries of the neck to changes in CO\textsubscript{2} but not their downstream associations is of obvious difficulty in humans. Regardless of the mechanism, however, our data clearly indicate that with severe changes in arterial blood gases the large vessels of the neck are not
mere conduit vessels, and rather act as active resistance vessels. We speculate that these findings are not simply evidence that the proximal vessels are vasoactive, but rather that the entire arterial structure of the cerebrovasculature is responsive to changes in arterial blood gases. This finding requires validation with higher resolution imaging modalities through a normal range of physiological changes, but if true, represents a new paradigm in our understanding of human CBF regulation.

4.5.4 Methodological considerations

Assessment of reactivity: absolute versus relative comparisons: Our findings exemplify the marked differences in PaCO₂ and PaO₂ reactivity between vessels if absolute flow/velocity values are compared directly (Figures 4.2 and 4.3). Because different vessels have inherently different baseline flow through them, the use of absolute values for reactivity calculation yields values that are largely dependent on the baseline flow. Because there is no analytical interpretational standard for comparison of vessel reactivities, we recommend both absolute and relative reactivities be shown.

4.5.5 Pressure-flow relationships across the cerebrovasculature

The brain is able to modulate its flow to some degree in the face of changes in arterial pressure (Ainslie & Tzeng, 2010a), an ability conventionally termed cerebral autoregulation. However, it is likely that hypercapnia and hypoxia both impair cerebral autoregulation (Subudhi et al., 2009; Aaslid et al., 1989; Zhang et al., 1998). Figure 4.6 highlights this phenomenon, where CBF increases concomitantly with MAP during hypercapnia. Such findings are broadly consistent to a well-controlled experiment by Przybyowski and co-workers (2003) who reported that apnea-induced elevations in MCAv were attenuated by ganglionic blockade that blunted increases in MAP. However, in this study, we cannot distinguish between the effects of PaCO₂ or elevations in MAP per se because they occur concurrently, hypertension being caused by the chemoreflex response to hypercapnia. Although the MAP increase during hypoxia is much lower than with hypercapnia, it is interesting to note the lack of obvious MAP-CBF relationship during hypoxia.
4.5.6 Flow verses velocity with TCD

During both hypercapnia and hypoxia, our findings clearly show a greater increase in flow in the ICA and VA flow compared with velocity changes in the MCA and PCA, respectively. Bland-Altman plots (Figure 4.7) reveal a systematic underestimation of flow via TCD proportional to the higher ranges of CBF during hypercapnia and hypoxia. As we observed CO$_2$ reactivity in the ICA, and others have shown high CO$_2$ reactivity in the M2 segment of the MCA (Giller et al., 1993), we feel it is likely the diameter of the MCA changes with PaCO$_2$. There are two salient points: there is now ample support in the literature to preclude the assumption of constant cerebral vessel diameter at extremes of blood gas changes (Giller et al., 1993; Wilson et al., 2011), and these cerebral vessel characteristics need to be directly characterized with the most sophisticated imaging modalities available.

4.5.7 End-tidal vs. Arterial PO$_2$ and PCO$_2$

A strength of this study is that target PetCO$_2$ and PetO$_2$ values were confirmed PaCO$_2$ and PaO$_2$ by arterial blood samples. Breathing via a sequential gas delivery circuit (Slessarev et al., 2007) reduces the CO$_2$ and O$_2$ alveolar-arterial difference in the lung (Ito et al., 2008), but this has not been assessed at the extreme values presented in this study. This validation is important, especially during curve fitting, since PetCO$_2$ is known to underestimate PaCO$_2$ at rest (Robbins et al., 1990) and overestimate PaCO$_2$ during breathing of hypercapnic air in both animals (Tojima et al., 1988) and humans (Peebles et al., 2007). Our data indicate that PetCO$_2$ overestimates PaCO$_2$ through the entire hypercapnic range (Figure 4.1). Thus, arterial blood gas collection is necessary for the true representation and physiological interpretation of cerebrovascular reactivity to O$_2$ and CO$_2$, at the extremes of physiological tolerance to hypoxia, alkalosis and acidosis presented herein.

4.5.8 Assessment of blood flow

Although a number of previous studies have measured blood flow in the ICA and VA at rest and during exercise (Sato et al., 2011; Sato & Sadamoto, 2010), this is the first report to integrate novel edge detection and wall tracking software (Black et al., 2008). This software allows operator-independent simultaneous assessment of conduit artery diameter changes and velocity, and thus quantitative assessment of blood flow through the arteries of interest at high resolution (30 Hz). We were however limited to near-concurrent insonation of the right
ICA and left VA, and cannot therefore account for side-to-side differences in flow between the arteries. Indeed, Schoning (1994) showed no difference between the right and left ICA, whereas the right VA (the vessel measured in the present study) was found to have a ~20% lower flow than the left VA. Regardless of potential differences in flows, we are unaware if any data suggesting there are bilateral differences in reactivity between the internal carotid arteries or vertebral arteries. Thus, we feel that our technological limitation of only being able to measure one ICA and one VA is unlikely to detract from our main study conclusions.

4.5.9 Curve fitting
As used extensively elsewhere for steady-state changes in PaCO$_2$ (Ainslie & Duffin, 2009) and SaO$_2$ (Jensen et al., 1996), we used linear regression to determine the relationship with CBF/CBV ($R^2$ mean: 0.86 ± 0.07). In the case of PaCO$_2$, this approach allows both the determination of the full reactivity to PaCO$_2$ and for hypercapnia and hypocapnia separately. Because the cellular mechanisms likely differ between the hypercapnia vs. hypocapnia ranges (reviewed in Ainslie & Duffin (2009)), consideration of them separately is important, especially when changes in both ranges are not comparable in many aspects of pathophysiology (Xie et al., 2005), physiology (e.g., with exercise (Ogoh et al., 2009a), and high altitude (Lucas et al., 2011). However, our capacity for more complex curve fitting was consequently limited. Although technically challenging because of the prolonged vessel imaging necessitated, future studies are now needed to compare volumetric flow measures in the ICA and VA during Duffin-type rebreathing (Battisti-Charbonney et al., 2011).

4.6 Conclusion
In summary, we have characterized regional cerebrovascular responses through the widest range of PaCO$_2$ and PaO$_2$ reported in the literature to date. This is the first study in humans to implicate the large cerebral vessels of the neck in blood gas related modulation of CBF, and moreover identify regional differences in neck artery blood-gas reactivity. That the VA demonstrated greater sensitivity to hypoxia, and CO$_2$ in the hypocapnic range, has significant implications for our understanding of brain stem control of respiratory and autonomic function. These data need to be considered in future studies of cerebrovascular function, as the long-held paradigm that blood flow regulation is modulated solely at the pial arterioles is likely an inadequately simplistic regulatory model.
Chapter 5: Regional cerebral blood flow in humans at high altitude: gradual ascent and two weeks at 5050 m.

5.1 Summary
The inter-individual variation in ventilatory acclimatization to high altitude (HA) is likely reflected in variability in the cerebrovascular responses to HA, particularly between brain regions displaying disparate hypoxic sensitivity. We assessed regional differences in CBF measured with Duplex ultrasound of the left internal carotid and vertebral arteries. End-tidal PCO₂, SpO₂, blood pressure and heart rate were measured during a trekking ascent to, and during the first two weeks at 5050 m. Transcranial color-coded Duplex-ultrasound (TCCD) was employed to measure flow and diameter of the middle cerebral artery (MCA). Measures were collected at 344 m (TCCD-baseline), 1338 m (CBF-baseline), 3440 m, and 4371 m. Following arrival to 5050 m regional CBF was measured every 12 hours during the first three days, once at 5-9 days, and once at 12-16 days. Total CBF was calculated as twice the sum of internal carotid and vertebral flow and increased steadily with ascent, reaching a maximum of 842 ± 110 mL/min (+53 ± 7.6% vs. 1338 m; mean ± SE) at ~60 hours after arrival at 5050 m. These changes returned to +15 ± 12% after 12-16 days at 5050 m and were related to changes in SpO₂ (R²=0.36; P<0.0001). TCCD-measured MCA-flow paralleled the temporal changes in total CBF. Dilation of the MCA was sustained on days two (+12.6 ± 4.6%) and eight (+12.9 ± 2.9%) after arrival at 5050 m. We observed no significant differences in regional CBF at any time point. In conclusion, the variability in CBF during ascent and acclimatization is related to ventilatory acclimatization as reflected in changes in SpO₂.

5.2 Introduction
Ascent to high altitude (HA) is a potent physiological stressor that manifests in acute and chronic ventilatory and acid-base adaptations that serve to elevate the partial pressure of arterial oxygen (PaO₂) and maintain pH (respectfully). Cerebral blood flow (CBF) is intrinsically linked to ventilatory acclimatization by virtue of its sensitivity to PaO₂ and arterial carbon dioxide (PaCO₂). Changes in CBF vary proportionally to changes in PaCO₂ and inversely with reduced oxyhemoglobin saturation (SpO₂). Moreover, the change in CBF in response to altered arterial blood gases may influence the ventilatory response to the related blood gas perturbations (Xie et al., 2006; Xie et al., 2009). Laboratory studies at sea
level have shown a ~3-4% decrease in CBF per mmHg reduction in PaCO₂ from eupnic PaCO₂ to ~20 mmHg and a ~4-5% per mmHg increase in CBF above eupneic PaCO₂ (Ainslie & Duffin, 2009; Sato et al., 2012; Willie et al., 2012). Isocapnic hypoxemia increases CBF ~3-4% per unit decrease in SaO₂ in order to maintain adequate oxygen delivery to the brain (Ainslie & Ogoh, 2010; Willie et al., 2012). Yet each of these sensitivities is altered during the course of acclimatization (Lucas et al., 2011; Robbins, 2007; Sato et al., 1994), and differ between the internal carotid (ICA) and vertebral arteries (VA) at sea level (Ogoh et al., 2013; Sato et al., 2012; Willie et al., 2012). Flow through the ICA and VA are estimates of anterior and posterior (e.g., brain stem) perfusion, respectively.

Severinghaus et al. (1966) first reported that total CBF increased by ~24% within the first 6-12 hours of arrival at 3810 m before decreasing to 13% above sea-level values by 3-5 days; these data have been largely confirmed (Huang et al., 1987; Lucas et al., 2011; Baumgartner et al., 1994; Jensen et al., 1990). The time course of these changes varies both within individuals and between studies and is likely reflective of the known inter-subject variation in the ventilatory and cerebrovascular responses to hypoxia (Hirshman et al., 1975; Willie et al., 2012), differing altitudes, ascent profiles and measurement techniques utilized (see Figure 5.1 for details).

In sum, variability in ventilatory acclimatization likely reflects a spectrum of proportional contributions from central and peripheral chemoreceptor drives (Hirshman et al., 1975), variable rates of metabolic compensation for respiratory alkalosis, and variation in regional cerebrovascular reactivity to these collective changes. Whether this variation remains when CBF is considered regionally remains unknown. To provide insight into this question, we quantified regional CBF at three distinct sites – the middle cerebral artery (using transcranial color-coded Duplex ultrasound [TCCD]) and the ICA and VA [using linear array vascular Duplex ultrasound] – during an eight-day ascent to 5050 m. We repeated ICA and VA measures every ~12 hours during the first three days, and following 5-9 days, and 12-16 days at 5050 m; TCCD measures were repeated after 2, 8, and 14 days at 5050 m. Our goal was to assess if the variability in CBF during ascent and acclimatization was attributable to ventilatory adjustments (i.e., SpO₂) and/or regional differences in CBF. Based on greater posterior CBF sensitivity to hypoxia reported at sea-level (Ogoh et al., 2013; Willie et al.,
2012), we hypothesized that changes in brainstem blood flow would be more closely related to ventilatory acclimatization as indexed by SpO$_2$.

5.3 Methods

5.3.1 Subjects and Protocols
Eight subjects were tested as part of Protocol 1, and 13 tested for Protocol 2; six subjects partook in both studies making the total sample size 15 sea-level residents (4 female; aged 28 ± 6 [mean ± SD], range: 20-38; body mass index 23.9 ± 3.3). The study was approved by the clinical ethical review board of the University of British Columbia. All volunteers provided written informed consent. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any cardiovascular medications. Prior to inclusion into the study, participants were screened by means of a 12-lead ECG stress test, transthoracic echocardiogram, pulmonary function testing, and full polysomnography; no subject exhibited any sleep disorder breathing at sea level. All participants were born and lived close to sea level (<1000 m) and none had been to HA for >2 years. This study was part of a larger research expedition conducted in April-June in 2012. As such, participants took part in a number of studies conducted during the three weeks at the Ev-K2-CNR Pyramid Laboratory. The experimental question addressed in this paper was \emph{a priori} driven and the data included herein will not be duplicated in future reports. The recovery time between the various testing sessions was managed to prevent any potentially confounding results (e.g., >48 hours between all drug and/or exercise intervention studies).

5.3.2 Ascent to high altitude
All participants spent one week in Kathmandu (1338 m) prior to flying to Lukla (2860 m) to begin the trek to 5050 m over 6-8 days. One acclimatization day was taken at 3440 m, 3860 m and 1-3 days at 4371 m. Additionally, during the first 6-7 days of the trek to 5050 m, participants were given low-dose acetazolamide (125 mg, oral) twice a day as an acute mountain sickness prophylactic (Basnyat \textit{et al.}, 2006; Ritchie \textit{et al.}, 2012). Treatment of acetazolamide was discontinued on day 8 of the trek (i.e., 4371 m) to allow sufficient time (e.g., > 24 hours) for the drug to clear participants’ system prior to the first data collection.
session at 5050 m, as the half-life of acetazolamide is reported to be ~10 hours (Ritschel et al., 1998) and this low-dose is typically 90-100% passed through the system within 24 hours of administration (Richalet et al., 2005). This approach was utilized to ensure the safety of the experimental volunteers at 5050 m.

5.3.3 Protocol 1
In eight subjects baseline TCCD measurements (see below), MAP, HR, and SpO₂ and were collected in Kelowna (344m), and repeated after two, eight, and fourteen days following arrival at 5050 m (the Ev-K2-CNR Pyramid Laboratory; barometric pressure 413 ± 1 mmHg).

5.3.4 Protocol 2
In 13 participants baseline measures were collected in Kathmandu (1338 m), Nepal, two days prior to flying to Lukla (2860 m). Measurements were repeated following at least three hours rest the evening of arrival and during the first morning at 3440 m (n=13); three hours following arrival to 4371 m (n=7); and again during the first three evenings and mornings at 5050 m (n=13). Measures were repeated again between days 5-9 (n=6) and between days 12-16 at 5050 m (n=6).

5.3.5 Measurements
Subjects abstained from caffeine and alcohol for the duration of the study, but were permitted acetaminophen ad libitum. In supine position, subjects rested in a pre-warmed sleeping bag for a minimum of 10 minutes before each measurement session. Efforts were made to ensure subjects were warm and calm before measures were taken. Evening measurements were collected between 1700-2200h, at least one hour after eating. Morning data were collected between 0400-0800h, before eating. End-tidal partial pressure of CO₂ (PETCO₂; EMMA capnometer, Masimo, Sweden), SpO₂, heart rate (HR; Nonin Onyx oximeter, Plymouth Minnesota, USA), and mean arterial pressure (MAP; manual auscultation) were measured in triplicate at each point described above.

5.3.6 Metrics of Cerebral Blood Flow
In Protocol 1, diameter and blood flow velocity in the MCA were measured using TCCD (GE Vivid-E-Ultrasound; 2.5 MHz probe; GE Healthcare, United States). The MCA was
identified using color Doppler in the same plane as the mesencephalon, with flow towards the probe. Diameter (by manual caliper placement) and velocity (EchoPac, GE Healthcare, United States) was measured 1 cm distal to the ICA-MCA-anterior cerebral artery trifurcation and were averaged over 10 cardiac cycles. Blood flow through the MCA (QMCA) was calculated as the product of mean MCA cross sectional area and mean blood velocity. One experienced sonographer (AB) collected all TCCD measures. Regional CBF was measured in Protocol 2 and analyzed as previously described (Willie et al., 2012). In brief, left internal carotid and vertebral artery flows (QICA and QVA) were determined using high-resolution duplex ultrasound (10 MHz multifrequency linear array probe; Terson t3000 ultrasound machine, Teratech, Burlington, MA, USA). Continuous diameter and velocity recordings were obtained at least 2cm distal to the carotid bifurcation for QICA, and between C6-C4 for QVA. Screen capture videos were recorded for subsequent offline analysis at 30Hz using custom edge-tracking software as detailed elsewhere (Black et al., 2008). Two experienced sonographers (KJS and CKW) scanned the same subjects at each time point and care was taken to obtain the same angle of insonation and vessel location within-subjects. Reproducibility of diameter measurements using this software is better than manual methods as it reduces observer error significantly, and possesses an intra-observer coefficient of variation of 6.7% (Black et al., 2008). Global CBF was calculated as twice the sum of QICA and QVA.

5.3.7 Statistics

Normal distribution of variables were confirmed by Shapiro-Wilk test (P <0.05). One-way ANOVA assessed differences with ascent/time, and Dunnett’s post-hoc test was used where appropriate to compare means to Kelowna (344m; Protocol 1) or Kathmandu (1338 m; Protocol 2). Repeated measures t-tests were used to compare the relative change in ICA to VA flow at each point during the first 3 days at 5050 m. Pearson’s correlation determined relationships between CBF and SpO2. Inter-individual coefficient of variation was calculated for the percent change in CBF values as √SD/mean for all individuals at a given time point (see discussion). No statistical differences were noted between sexes and data were therefore pooled. Values are shown as mean ± SE.
5.4 Results

5.4.1 Subjects

All subjects were included in analysis. For protocol 2, it was not possible to collect data on four subjects in Kathmandu (1338 m) due to time constraints and illness. Since these subjects were part of the UBC research team, data obtained in Kelowna (344m) one-month prior as part of another study was available in these subjects and was substituted in these individuals only. Data that were available for both 344m and 1338 m for nine of the 13 subjects showed that no variable significantly differed between the two altitudes. Likewise, the substituted values (n=4) did not differ significantly from that of the other nine subjects at 1338 m.

5.4.2 Changes with altitude:

5.4.2.1 Protocol 1

Figure 5.1 and Table 5.1 show the change in QMCA that increased to a maximum of +42 ± 14% after two days at 5050 m, 35 ± 8% after 8 days, and 19 ± 12% after two weeks at 5050 m. At 5050 m the diameter of the MCA significantly increased ($P < 0.05$) by $+12.5 \pm 4.6\%$ and $+12.9 \pm 2.9\%$ on days two and eight, respectfully, and trended to remain increased on day 14 ($+9.8 \pm 3.6\%$).

<table>
<thead>
<tr>
<th></th>
<th>Kelowna (344m)</th>
<th>Ev-K2-CRN Research Pyramid (5050 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 8</td>
</tr>
<tr>
<td>HR (b/min)</td>
<td>58 ± 3</td>
<td>76 ± 4*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>90 ± 2</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>99 ± 0.3</td>
<td>80 ± 0.9*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>4.3 ± 0.2</td>
<td>4.8 ± 0.4*</td>
</tr>
<tr>
<td>Velocity (cm/s)</td>
<td>72 ± 2</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>Flow (mL/min)</td>
<td>650 ± 68</td>
<td>892 ± 160*</td>
</tr>
</tbody>
</table>

HR, heart rate; MAP, mean arterial pressure; SpO₂, percent saturation of hemoglobin. Values are Mean ± SE based on n=8 at each time point. * $P < 0.05$ vs. Kelowna (344m).
Figure 5.1. Percent increases in cerebral blood flow with time at high-altitude from six studies at various altitudes and durations.

Severinghaus et al. (1966) studied CBF using the Kety-Schmidt technique in five subjects brought rapidly by car to 3810 m. Using the Xe$^{133}$ method Jensen et al. (1990) measured CBF in 12 subjects at 3475 m. Huang et al. (1987) measured ICA and VA blood velocities as a metric of CBF on Pikes Peak (4300 m). Baumgartner et al. (1994) studied 24 subjects who rapidly ascended to 3200 m by cable car, slept one night at 3600 m, and ascended by foot to 4559 m the next day. Cerebral blood flow was estimated by transcranial Doppler ultrasound. About 2/3 of the subjects developed symptoms of AMS, data above is the mean of all subjects. Lucas et al. (2011) employed an identical ascent profile as the present study but estimated changes in CBF by transcranial Doppler ultrasound of the middle cerebral artery. TCCD; transcranial colour-coded duplex ultrasound. Global CBF was calculated as twice the sum of flow through the internal carotid and vertebral arteries. ∆%CBF, percent change in cerebral blood flow with time.

5.4.2.2 Protocol 2

There was a significant effect with ascent to altitude for all variables (Tables 5.1-5.3). Increases with ascent were noted for HR and CBF, whereas PetCO$_2$ and SpO$_2$ decreased with ascent to 5050 m (Tables 5.1 and 5.3; Figure 5.2). End-tidal partial pressure of CO$_2$ increased ~2-3 mmHg from its nadir after the first night at 5050 m. Results for post-hoc multiple comparisons are shown in Table 5.1. Total CBF increased steadily with ascent.
reaching a maximum of 842 ± 110 mL/min (53 ± 7.6%; mean ± SE) at ~60 hours after arrival at 5050 m; these changes returned to 15 ± 12% above baseline values after 12-16 days at 5050 m (Table 5.3, Figure 5.1). Figure 5.2 shows the changes in global CBF, SpO₂, and PETCO₂ with ascent and time at 5050 m; the change in SpO₂ with ascent was related to the elevation in global CBF ($P < 0.0001$; $R^2 = 0.36$; Figure 5.3). There were no statistical differences between ICA and VA percent increase (from Kathmandu, 1338 m) at any time point; global CBF only is therefore depicted in Figures 5.1 and 5.2. The coefficient of variation of the percent change in global CBF from baseline is shown in Tables 5.2 and 5.3.
Table 5.2. Cardiorespiratory and regional cerebral blood flow variables upon ascent to 4371 m.

<table>
<thead>
<tr>
<th></th>
<th>Kathmandu (1338 m)</th>
<th>Namche (3440 m)</th>
<th>Pheriche (4371 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PM</td>
<td>AM</td>
<td>PM</td>
</tr>
<tr>
<td>HR (b/min)</td>
<td>58 ± 2</td>
<td>62 ± 2</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>88 ± 2</td>
<td>86 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>96 ± 0.4</td>
<td>91 ± 0.9*</td>
<td>90 ± 0.9*</td>
</tr>
<tr>
<td>PETCO₂ (mmHg)</td>
<td>40 ± 1</td>
<td>31 ± 0.8*</td>
<td>27 ± 0.9*</td>
</tr>
<tr>
<td>QICA (mL/min)</td>
<td>214 ± 13</td>
<td>246 ± 22</td>
<td>239 ± 19</td>
</tr>
<tr>
<td></td>
<td>Δ% vs. 1338 m</td>
<td>+16.8 ± 6.8</td>
<td>+4.2 ± 4.5</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=13</td>
<td>n=10</td>
<td>n=7</td>
</tr>
<tr>
<td>QVA (mL/min)</td>
<td>89.0 ± 7.7</td>
<td>99.8 ± 6.7</td>
<td>96.5 ± 12</td>
</tr>
<tr>
<td></td>
<td>Δ% vs. 1338 m</td>
<td>+15.4 ± 9.8</td>
<td>+8.8 ± 7.0</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=13</td>
<td>n=10</td>
<td>n=7</td>
</tr>
<tr>
<td>Q_total (mL/min)</td>
<td>622.5 ± 40</td>
<td>684 ± 45</td>
<td>665 ± 60</td>
</tr>
<tr>
<td></td>
<td>Δ% from 1338 m</td>
<td>+16.2 ± 7.0</td>
<td>+4.2 ± 3.7</td>
</tr>
<tr>
<td>Sample size</td>
<td>n=13</td>
<td>n=9</td>
<td>n=6</td>
</tr>
<tr>
<td>CoV Δ% from 1338 m</td>
<td>640%</td>
<td>131%</td>
<td>218%</td>
</tr>
</tbody>
</table>

HR, heart rate; MAP, mean arterial pressure; SpO₂, percent saturation of hemoglobin; PETCO₂, partial pressure of end-tidal carbon dioxide; QICA, internal carotid artery blood flow; QVA, vertebral artery blood flow; Q_total, estimated total cerebral blood flow; CoV, coefficient of variation. Values are Mean ± SE. * P < 0.05 vs. Kathmandu (1338 m).
Table 5.3. Cardiorespiratory and regional cerebral blood flow variables during acclimatization to 5050 m.

<table>
<thead>
<tr>
<th></th>
<th>PM1</th>
<th>AM1</th>
<th>PM2</th>
<th>AM2</th>
<th>PM3</th>
<th>AM3</th>
<th>Day 5-9</th>
<th>Day 12-16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (b/min)</strong></td>
<td>77 ± 4*</td>
<td>68 ± 4</td>
<td>71 ± 4*</td>
<td>71 ± 4*</td>
<td>73 ± 3*</td>
<td>70 ± 3</td>
<td>69 ± 4</td>
<td>68 ± 3</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>90 ± 2</td>
<td>91 ± 3</td>
<td>94 ± 2</td>
<td>92 ± 2</td>
<td>89 ± 2</td>
<td>90 ± 2</td>
<td>98 ± 2</td>
<td>94 ± 2</td>
</tr>
<tr>
<td><strong>SpO₂ (%)</strong></td>
<td>81 ± 0.9*</td>
<td>82 ± 0.8*</td>
<td>81 ± 1*</td>
<td>82 ± 1*</td>
<td>80 ± 0.9*</td>
<td>81 ± 1*</td>
<td>79 ± 0.9*</td>
<td>82 ± 0.8*</td>
</tr>
<tr>
<td><strong>PETCO₂ (mmHg)</strong></td>
<td>27 ± 0.8*</td>
<td>29 ± 0.7*</td>
<td>29 ± 0.5*</td>
<td>30 ± 0.6*</td>
<td>30 ± 0.5*</td>
<td>28 ± 0.7*</td>
<td>30 ± 0.8*</td>
<td>28 ± 0.7*</td>
</tr>
<tr>
<td><strong>QICA (mL/min)</strong></td>
<td>278 ± 25</td>
<td>284 ± 18</td>
<td>292 ± 21*</td>
<td>312 ± 27*</td>
<td>330 ± 20*</td>
<td>325 ± 22</td>
<td>322 ± 17*</td>
<td>303 ± 51</td>
</tr>
<tr>
<td><strong>QVA (mL/min)</strong></td>
<td>118 ± 10</td>
<td>97.5 ± 5.5</td>
<td>117 ± 10</td>
<td>118 ± 11</td>
<td>129 ± 12*</td>
<td>133 ± 14*</td>
<td>85.3 ± 7.2</td>
<td>118 ± 11</td>
</tr>
<tr>
<td><strong>Qtotal (mL/min)</strong></td>
<td>814 ± 60*</td>
<td>769 ± 45</td>
<td>818 ± 54*</td>
<td>859 ± 62*</td>
<td>910 ± 51*</td>
<td>871 ± 69*</td>
<td>815 ± 50</td>
<td>842 ± 110</td>
</tr>
<tr>
<td><strong>CoV</strong></td>
<td>79%</td>
<td>68%</td>
<td>67%</td>
<td>57%</td>
<td>48%</td>
<td>61%</td>
<td>117%</td>
<td>184%</td>
</tr>
</tbody>
</table>

HR, heart rate; MAP, mean arterial pressure; SpO₂, percent saturation of hemoglobin; PETO₂, partial pressure of end-tidal carbon dioxide; QICA, internal carotid artery blood flow; QVA, vertebral artery blood flow; Qtotal, estimated total cerebral blood flow; CoV; coefficient of variation. Values are Mean ± SE. * P < 0.05 vs. Kathmandu (1338 m).
Figure 5.2. Cerebral blood flow and end-tidal PCO2 during ascent and over time at high altitude.
Mean (± SE) values at each altitude and time at a given altitude. Upper plot (A) depicts end-tidal partial pressure of CO2 (P\text{ETCO}_2; left axis) and percent oxyhemoglobin saturation (SpO\textsubscript{2}; right axis). Bottom plot (B) shows total cerebral blood flow. Note that sample sizes are different at each time point (see Table 5.1), which is responsible for the apparent discrepancy in relative change from 1338 m at days 5-9 and 12-16 if using the mean data (this figure) versus relative change for each individual (Figure 1). The five subjects included at these points gave a mean total CBF of 764 ± 98 mL/min at 1338 m.
Figure 5.3. Relationship between total cerebral blood flow and percent oxyhemoglobin saturation.
Values of total cerebral blood flow ($Q_{CBF_{TOTAL}}$) and oxyhemoglobin saturation ($SpO_2$) represent all altitudes and durations at altitudes. Regression equation: $Q_{CBF_{TOTAL}} = 2121 - 15.85 \cdot (SpO_2)$; $R^2 = 0.36$.

5.5 Discussion
This study is the first to: 1) volumetrically quantify global CBF during an ascent and over time at HA inclusive of the normal physical activity, speed of ascent, and pharmacological intervention typical of most sea-level sojourners to HA; 2) assess regional differences in CBF during ascent. Contrary to our hypothesis, we observed no differences between VA and ICA percent changes in flow with ascent at any time point.

5.5.1 Inter-individual variability in cerebral blood flow at high altitude
We aimed to characterize CBF changes during a sojourn to HA inclusive of exercise, standard prophylactic acute mountain sickness treatment (see below), and gradual ascent profile. To our knowledge, this study is the sixth in the literature to assess the time-course of CBF changes with exposure to HA, but the first to make multiple measurements per day during the first 3 days after arrival and during partial pre-acclimatization to 5050 m. Figure 5.1 demonstrates that following ~60 hours at altitude CBF begins to fall from its zenith, returning to near-sea level values by two weeks. Importantly our data show the largest CBF increase to date, despite our gradual ascent profile (versus the other studies which employed a rapid ascent by car or tram to altitude), and that this maximum occurred at ~60 hours at 5050 m.
m. This is in contrast to the other studies that showed a less pronounced peak in CBF that more promptly began to decrease. This is likely a result of the higher temporal resolution employed here (every 12 hours for the first 3 days) and greater altitude and degree of hypoxemia.

Whether cerebral metabolism changes with acclimatization remains unknown. The studies of Severinghaus (1966) and Jensen (1990) used the Kety-Schmidt and Xe\textsuperscript{133} methods (respectively) of CBF estimation, which assume constant brain metabolic rate; thus, if brain metabolism increases during acclimatization these methods could underestimate CBF. Of interest is the more modest CBF (as indexed by MCA blood velocity) increase we observed previously following an identical ascent profile (Lucas et al., 2011). Wilson et al. (Wilson et al., 2011) recently reported a ~24% dilation of the middle cerebral artery at 6400 m, we suggested the same with extreme hypoxemia (\(\text{PaO}_2 = 43 \text{ mmHg}\)) (Willie et al., 2012); both studies are consistent with our findings of a sustained ~9-12% dilation of the MCA at 5050 m. Thus, relative to the present data, the attenuated CBF increase previously reported (as estimated by transcranial Doppler ultrasound that assumes constant diameter of the insonated vessel) (Lucas et al., 2011) is likely explained by dilation of the middle cerebral artery.

As highlighted by previous reports (Ogoh et al., 2013; Willie et al., 2012), the variability in CBF with hypoxia was substantial. Indeed, the inter-subject coefficient of variation for the percent increase in CBF during the first night at 3440 m was 640%. This coefficient of variation in delta-CBF decreased to 48% during the third night at 5050 m when absolute global CBF, and the relative change from 1338 m in global CBF, were greatest. The increase in CBF with hypoxia presumably serves to maintain cerebral oxygen delivery (West et al., 2004; Wolff et al., 2002), as reflected in a strong correlation between \(\text{SpO}_2\) with CBF, that accounts for ~40% of the statistical variability \((R^2 = 0.36)\). The remaining variance in individual CBF with altitude/hypoxia exposure is perhaps not surprising given the known variability in the hypoxic ventilatory response (Hirshman et al., 1975), speed of acclimatization (Bailey et al., 2009b; Roach & Hackett, 2001), and cerebrovascular response to both \(\text{CO}_2\) and hypoxia (Willie et al., 2012; Lucas et al., 2011). However, two aspects of the present data indicate oxygen delivery to the brain is critical for successful acclimatization to high altitude: 1) the strong relationship between CBF and \(\text{SpO}_2\); and, 2) that the increase in
CBF during ascent (i.e., at 3440 m and 4371 m) showed marked variability, whereas at 5050 m the increase in CBF was homogeneous between subjects.

5.5.2 Regional CBF changes at HA
Recent reports indicate greater reactivity to hypoxia in the brainstem than cortex (Sato et al., 2012; Willie et al., 2012; Ogoh et al., 2013), and greater grey matter relative to white matter hypoxic blood flow sensitivity (Binks et al., 2008). In contrast, we found no significant difference between QVA and QICA at any point. These findings are consistent with those of Huang et al. (Huang et al., 1987) who measured VA and ICA velocities (as an estimate of flow) at 4300 m. While it is possible that the inter-subject variability in our data reduced the precision needed to observe the relatively small differences between brain regions, there were no statistical differences at any point at 5050 m when the between-subjects coefficient of variation was least and the mean difference greatest. Indeed, at these time points, in order to achieve a power of 0.8 and \( \alpha=0.05 \) a sample size >70 would be required (Atkinson & Nevill, 1998), which is clearly not practical in a field study of this nature. Moreover, based on previously reported data from our laboratory (Willie et al., 2012) collected by the same two sonographers and equipment, we were able to demonstrate regional CBF differences during severe step-changes in arterial PCO\(_2\) and PO\(_2\). While advanced imaging modalities (not yet logistically possible in a high altitude field study) would likely yield lower variability, the high inter-subject variability observed in these data is likely representative of normal variation in the human response to a multifaceted stress such as altitude.

5.5.3 Acetazolamide, cerebral blood flow, and ventilatory acclimatization
Acetazolamide is a carbonic anhydrase inhibitor that increases ventilation and SaO\(_2\) through enhanced renal excretion of bicarbonate, resulting in metabolic acidosis (West et al., 2004). Cerebral blood flow is reciprocally affected by acidosis-induced cerebrovascular dilation on the one hand (Fencl et al., 1969; Kontos et al., 1977b), but hyperventilation-induced decreases in PaCO\(_2\) that cause cerebral vessel constriction on the other (Kontos et al., 1977b; Willie et al., 2012). Thus, the use of acetazolamide in the present study somewhat obfuscates the interpretation of these data. To our knowledge, this is the first study to document the altered ventilatory acclimatization pattern at 5050 m following cessation of acetazolamide. Figure 5.2 shows that at 5050 m, PETCO\(_2\) increased from its nadir at PM1 (evening 1).
is not consistent with the steady increase in ventilation and PaO₂ and consequent reduction in PETCO₂ typically seen with acclimatization (Rahn & Otis, 1949). Termination of acetazolamide may have resulted in a transient hypoventilation as acetazolamide-induced metabolic acidosis was withdrawn. Oral acetazolamide per se does not produce altered CBF at sea-level (Huang et al., 1988), but because we did not measure or manipulate base-excess it is difficult to determine the potential influence of acid-base balance on prevailing CBF at HA.

5.6 Conclusion
Following gradual ascent to 5050 m over 5-9 days, CBF continued to increase over the first ~60 hours at 5050 m to +53 ± 7.6% of low altitude values; it then began to decrease to approximately +20% of low-altitude values after two weeks at 5050 m. The sustained MCA dilation observed for the duration of exposure to 5050 m indicates intra-cerebral vessel dilation facilitates increased brain oxygen delivery and should be taken into consideration in future studies utilizing conventional TCD at high altitude. We have previously reported VA dilation with 70% SpO₂ (arterial PO₂ ~36mmHg). That no dilation of the neck arteries was observed at 5050 m indicates this vessel may only dilate under conditions of extreme isocapnic hypoxia with minimum acid-base compensation, and suggests greater relative hypoxic sensitivity of the intracerebral arteries. Finally, there is tremendous inter-individual variability in both the CBF increase with ascent, and CBF decrease with acclimatization; SpO₂ appears to explain approximately 40% of the statistical variation in CBF.
Chapter 6: Cerebral Blood Flow and Fuel Utilization in Man at High Altitude

6.1 Summary

We hypothesized: 1) that cerebrovascular reactivity (CVR) and ventilatory sensitivity would be increased at high altitude (HA; 5050 m) due to a steeper PCO$_2$-H$^+$ relationship; 2) the pressor response to hypercapnia would be greater at HA; and, 3) that manipulation of CO$_2$ would affect cerebral metabolism more than hypoxia. The partial pressures of arterial O$_2$ (PaO$_2$) and CO$_2$ (PaCO$_2$) were manipulated at sea-level (SL) to acutely simulate HA exposure; at HA, arterial blood gases at SL were simulated, and CVR and ventilatory sensitivity via steady-state euoxic PaCO$_2$ alterations were assessed. At each stage, arterial-jugular venous differences were measured to directly calculate cerebral metabolic rates. Cerebral blood flow (CBF) was measured by the Fick principle and estimated by transcranial Doppler ultrasound (TCD). At SL, the isocapnic reduction in PaO$_2$ to 40 ± 1 mmHg (79 ± 1 % SaO$_2$) elicited a 45 ± 16% increase in CBF, whereas hypocapnic hypoxia caused a 21 ± 16 % reduction in CBF. At 5050 m (PaO$_2$ = 41 ± 2 mmHg) normalization of PaO$_2$ (99.0 ± 3.5 mmHg) decreased CBF by 17 ± 6.7%. Despite a steeper PCO$_2$-H$^+$ relationship, both arterial and jugular venous CVR to hypercapnia were unaltered with ascent to HA; TCD underestimated CVR in hypercapnia at both altitudes implying dilation of the middle cerebral artery. Ventilatory sensitivity increased at HA (SL 2.3 ± 1.3; HA 5.9 ± 1.6 l.min$^{-1}$/mmHg). Although the ΔMAP–PaCO$_2$ relationship doubled at HA (SL 1.2 ± 0.74 %/mmHg versus HA 2.8 ± 1.0 %/mmHg), the slope of the ΔCBF vs. MAP regression was reduced – indicative of enhanced cerebral autoregulation. Independent of altitude, acute hypercapnia reduced the cerebral metabolic rate of glucose. In conclusion, despite an increased PCO$_2$-H$^+$ slope and elevated MAP-reactivity at HA, the brain adapts effectively to chronic hypoxia and hypocapnia: It retains similar fuel utilization and sensitivity to changes in PaCO$_2$, and displays more effective autoregulation than at SL.
6.2 Introduction

Acclimatization to high altitude (HA) is a multifaceted process involving cardiorespiratory and renal compensatory changes that are most dynamic during the first few weeks of exposure (Rahn & Otis, 1949; Haldane et al., 1919; Barcroft et al., 1923). Hypoxic stimulation of the peripheral chemoreceptors leads to hyperventilation, resulting in hypocapnia and respiratory alkalosis that is slowly compensated for by renal bicarbonate excretion. Thus, for example, despite the partial pressure of arterial carbon dioxide (PaCO$_2$) normally being reduced to ~25 mmHg at altitudes of ~5000 m, blood pH returns to near sea level (SL) values within 2-3 weeks. The balance between neuronal substrate demand, blood pressure, and arterial blood gases and pH determine the volume of blood flow to the brain (Lassen, 1959; Willie et al., 2014b). Ascent to HA thus represents a complex stimulus to the cerebrovasculature, the nature of which is not well understood, particularly in light of sustained alterations and compensations in arterial blood gases and pH.

Metabolic compensation for respiratory alkalosis results in marked decreases in plasma bicarbonate ion concentration that while helping to return pH to normal levels also reduces buffering capacity. Cerebrovascular reactivity to changes in PaCO$_2$ following ascent to HA have been reported to rise (Fan et al., 2010a; Fan et al., 2013), fall (Rupp et al., 2014; Lucas et al., 2011; Villien et al., 2013), or remain unchanged (Ainslie et al., 2007; Ainslie & Burgess, 2008) with ascent to high altitude. Between-study differences in altitude, degree of acclimatization, and method to assess reactivity likely underscore many of these reported differences. Moreover, most of these data come from transcranial Doppler ultrasound estimations of CBF that may be confounded by constriction or dilation of the insonated vessel in response to both changes in blood gases and ascent to HA (Subudhi et al., 2014; Willie et al., 2012; Willie et al., 2014a; Wilson et al., 2011). In addition, ventilatory acclimatization elicits slightly different ramifications to arterial than cerebral extracellular fluid (ECF) (Dempsey et al., 1978; Fencl et al., 1969); internal jugular venous blood provides a better estimation of brain ECF parameters than does arterial blood (Bradley & Semple, 1962).
Hypoxic cerebral vasodilation affects the initial increase in CBF with ascent to HA, which prevails in the face of hypocapnia. A number of studies have measured CBF over the course of acclimatization to HA and have found CBF to return to SL values, or remain slightly elevated, after two weeks (Baumgartner et al., 1994; Huang et al., 1987; Jensen et al., 1990; Lucas et al., 2011; Severinghaus et al., 1966; Subudhi et al., 2014; Willie et al., 2014a). Additionally, Severinghaus et al. (1966) reported in four subjects that acute correction of hypoxia (via administration of 30% O₂) following 5 days at 3810 m returned CBF to SL values when PaCO₂ was maintained at HA values (~31 mmHg). Similarly at 4300 m in Andean natives, CBF was reduced by 18% following 60 minutes of oxygen breathing (Milledge & Sorensen, 1972). Interestingly, oxygen delivery appears to be maintained during ascent and acclimatization to HA, with the initial rise in CBF compensating for reductions in arterial oxygen content (CaO₂) until oxygen carrying capacity of blood improves after a few days at altitude (Ainslie, 2014; In Press).

The majority of studies have reported unchanging CMRO₂ (cerebral metabolic rate of oxygen) with severe (PaO₂ ~ 35 mmHg; Cohen et al., 1967; Kety & Schmidt, 1948a; Overgaard et al., 2012; Ainslie et al., 2013) or moderate (PaO₂ ~ 45 mmHg; Bailey et al., 2009a) acute hypoxia at sea level; and following three weeks acclimatization to HA (5260 m; PaO₂ ~ 51 mmHg) in lowlanders (Moller et al., 2002) or in high-altitude residents exposed to acute hypoxia at 3800 m (PaO₂ ~ 41 mmHg; Sorensen et al., 1974). Only one study has reported a 5% increase in CMRO₂ (using MRI) during acute hypoxia roughly equivalent to 4000 m HA (SaO₂ was not reported but was likely ~85%; Xu et al., 2012) – the discrepancy likely reflect different methodologies and their respective assumptions. Consistent with a stable CMRO₂, cerebral carbohydrate utilization has been reported to be relatively consistent in acute (Bailey et al., 2009a; Ainslie et al., 2013) and chronic hypoxia (Sorensen et al., 1974); however, this is not a universal finding as other studies have reported increased cerebral carbohydrate metabolism during acute hypoxia (Cohen et al., 1967; Overgaard et al., 2012). Conversely, older studies employing positron emission tomography indicate that cerebral glucose metabolism might decrease at altitude in populations evolutionarily less adapted to high altitude (Hochachka et al., 1994) than the Sherpa in whom it is similar to sea level dwellers at sea level (Hochachka et al., 1996). It thus remains unclear if the prevailing
CBF following partial acclimatization is regulated by oxygen delivery, sustained hypocapnia, altered acid-base buffering, or the balance between all three. Studies in animals indicate decreased cerebral carbohydrate metabolism (Folbergrová et al., 1974; Folbergrová et al., 1975), and in humans small decreases (~13%) in CMRO₂ with hypercapnia (Xu et al., 2011), although this is not a universal finding (Chen & Pike, 2010; Jain et al., 2011), and has not been assessed using cerebral arteriovenous differences in humans at sea level or at high altitude.

We aimed to clarify the importance of blood gases and cerebral metabolism on CBF at HA by measuring arterial – jugular venous differences across the brain during acute changes in blood gases at SL and following partial acclimatization to HA. To achieve this, at SL we induced changes to PaO₂ and PaCO₂ to approximate those that would be experienced at HA; and following 6-10 days at 5050 m blood gases were altered at HA to acutely simulate those at SL. We were thus able to assess the effect of partial acclimatization and altered acid-base balance on brain blood flow and metabolism. We further induced steady state iso-oxic changes in PaCO₂ at both SL and 5050 m to address how cerebrovascular reactivity to changes in arterial and internal jugular PCO₂ and [H⁺] is altered at HA. We hypothesized that metabolic compensation for respiratory alkalosis with ascent to HA – and the resultant leftward shift in the CO₂-pH relation – would yield steeper cerebrovascular reactivity, and ventilatory sensitivity to changes in PaCO₂, but not when expressed as [H⁺]. Because of this greater acidic response and hence chemoreflex drive, we also reasoned there might be a great pressor response (Willie et al., 2012) leading to an additional elevation in CBF (Willie et al., 2014b). Finally, we hypothesized that while acute correction of hypoxia at HA would not affect cerebral metabolism, the markedly enhanced CBF with relative hypercapnia would lead to an enhanced delivery of oxidative and non-oxidative fuels in excess of the metabolic requirements.

6.3 Methods

6.3.1 Participants

Eleven healthy young subjects (1 female; age 30.4 ± 7; BMI 24.9 ± 3.7) gave written informed consent before participating in baseline data collection. The University of British
Columbia Clinical Review Ethical Board granted ethical approval, and the study conformed to standards set by the Declaration of Helsinki. None of the volunteers were smokers, had no history of cardiovascular diseases, were taking any medications, or had been to HA in the six months prior to testing. Before commencement of testing subjects were screened via a 12-lead electrocardiogram, pulmonary function testing, full polysomnography (to rule out any sleep-disordered breathing) and transthoracic echocardiogram. This study was one component of a large expedition to 5050 m – sea level data was collected during April 2012 in Kelowna, British Columbia (altitude 344m) and HA experiments completed over three weeks at the Ev-K2-CRN Pyramid laboratory, Khumbu region, Nepal during May 2012. Some studies from this expedition have been reported elsewhere (Foster et al., 2014; Smirl et al., 2014; Willie et al., 2014a; Lewis et al., 2014a). Expedition members participating as subjects had a minimum of 48 hours between studies involving pharmaceutical interventions or exercise to mitigate contamination.

6.3.2 Experimental Design

Subjects were first familiarized with the experimental protocol, as detailed below. Testing was completed on two days, once at SL and once at 5050 m, separated by ~2 months. On both days subjects abstained from any caffeine containing beverages or alcohol at least 24h prior to testing. The HA protocol was completed between 6-10 days following arrival to 5050 m during which time no subjects displayed any signs or symptoms of acute mountain sickness. At both altitudes the subject’s preparedness and physical status was reassessed, after which two catheters were placed under local anesthesia (1% lidocain) and by ultrasound guidance by an experienced anesthesiologist. A 20G arterial catheter (Arrow, Markham, Ontario, Canada) was inserted into the left radial artery, and a jugular bulb catheter (Edwards PediaSat Oximetry catheter) was placed by the Seldinger technique into the right internal jugular vein (IJV) and advanced to the jugular bulb. Both arterial and IJV catheters were attached to pressure transducers and isolated sampling reservoirs for sampling of arterial and jugular venous blood (Edwards Lifesciences VAMP system, Mississauga, Canada). The radial arterial transducer was calibrated at the level of the right atrium for the measurement of beat-to-beat blood pressure. Following cannulations subjects rested supine for at least 30 minutes breathing ambient air while they were equipped with measurement apparatuses.
End-tidal partial pressures of CO₂ (P\text{ET CO}_2) and O₂ (P\text{ET O}_2) were controlled in order to target specific PaCO₂ and PaO₂ values using a portable end-tidal forcing system (AirForce, GE Foster, Kelowna, BC, Canada). The system prospectively targets inspirate gases by a feedback control and error reduction algorithm to achieve desired arterial values (Bain et al., 2013; Querido et al., 2013). At both sea level and 5050 m four permutations of P\text{ET O}_2 and P\text{ET CO}_2 were targeted: 100 mmHg P\text{ET O}_2 / 40 mmHg P\text{ET CO}_2; 100 mmHg P\text{ET O}_2/ 25 mmHg P\text{ET CO}_2; 40 mmHg P\text{ET O}_2/ 40 mmHg P\text{ET CO}_2; and, 40 mmHg P\text{ET O}_2 / 25 mmHg P\text{ET CO}_2 (see Figure 6.1). These are described in detail next.

### 6.3.2.1 End-tidal forcing at SL

Steady state arterial blood gases (ABG) were maintained for 3-5 minutes with each stage separated by ten minutes breathing ambient air. **Clamp 1**: First, subjects hyperventilated while P\text{ET O}_2 was clamped at 100 mmHg, reducing P\text{ET CO}_2 which was clamped to 25 mmHg (equivalent to normal baseline PaCO₂ at 5050 m); P\text{ET CO}_2 was then increased in 5 mmHg increments for 3-5 minutes at each level until a P\text{ET CO}_2 15 mmHg above eupnic P\text{ET CO}_2 was reached. **Clamp 2**: P\text{ET O}_2 was lowered to 45 mmHg and P\text{ET CO}_2 to 25 mmHg with subjects instructed to hyperventilate further if the hypoxic ventilatory response was insufficient to reduce PaCO₂ to the desired level (in order to bring both PaO₂ and PaCO₂ to normal baseline values at 5050 m as measured during our earlier studies: (Fan et al., 2010a; Lucas et al., 2011)). Finally, in **Clamp 3**, P\text{ET O}_2 was reduced to 40 mmHg (normal PaO₂ at 5050 m) while P\text{ET CO}_2 was maintained at sea level baseline values of 40 mmHg (Figure 6.1).

### 6.3.2.2 End-tidal forcing at 5050 m

Per SL protocol clamping was maintained for 3-5 minutes at each end-tidal target. The study was originally designed to target resting SL PaCO₂ (~40 mmHg), but individuals were generally unable to cope with such relative hypercapnia. Consequently, hypercapnic stages at HA reached P\text{ET CO}_2’s of ~35 mmHg. Subjects first hyperventilated with maintenance of euoxia (40 mmHg PaO₂) to drop P\text{ET CO}_2 to 10 mmHg below eupnic levels. P\text{ET CO}_2 was then increased in a stepwise fashion in 5 mmHg increments to 35 mmHg to mimic sea level normocapnic hypoxia (**Clamp 1**), followed by 10 minutes rest breathing ambient air. Next, P\text{ET O}_2 was increased to 100 mmHg (**Clamp 2**) with maintenance of isocapnia (at 25 mmHg) for three minutes, after which P\text{ET CO}_2 was increased directly to 35 mmHg (**Clamp 3**) for > 3
minutes, to replicate sea level hypocapnia and baseline blood gases, respectively. Clamp 2 and 3 were completed back-to-back due to a limited supply of compressed gases (Figure 6.1).

Figure 6.1. Schematic of protocol at sea level and 5050 m. Top, sea level. Bottom, 5050 m. Solid lines indicate clamping of end-tidal gases; dashed lines indicate breathing of ambient air. Data were recorded for 3-5 minutes once a steady state had been achieved. Each clamped period was separated by 10 minutes breathing ambient air, except between clamp 2 and 3 at 5050 m where PETCO₂ was increased to enter clamp 3 directly from clamp 2. Please see methods for further details. In brief, the partial pressures of arterial O₂ (PaO₂) and CO₂ (PaCO₂) were manipulated at sea-level to acutely simulate high altitude exposure at 5050 m; at high altitude, arterial blood gases at sea level were simulated.
6.3.3 Measurements

6.3.3.1 Data acquisition

All data except internal carotid and vertebral artery flows and arterial blood gases were collected continuously at 1 kHz via an analogue to digital data acquisition system (Powerlab/16SP ML795; AD instruments). Beat-to-beat blood pressure was measured from the radial artery and the mean arterial pressure (MAP) calibrated offline by manual sphygmomanometry. Heart rate (HR) was measured from the three-lead electrocardiogram. The left middle cerebral artery blood velocity (MCAv) and right posterior cerebral artery blood velocity (PCAv) were measured by transcranial Doppler ultrasound (Spencer technologies, Seattle, WA, USA) using a 2mHz pulsed probe. Standard search techniques that optimize signal quality and reproducibility were utilized as detailed by us previously (Willie et al., 2012; Willie et al., 2011b).

6.3.3.2 Blood gases

Arterial and IJV blood samples drawn into pre-heparinised syringes were analyzed either immediately, or were kept on ice if there was a delay greater than 5 minutes. All samples were analyzed within 30 minutes of collection for pH; PO$_2$; PCO$_2$; percent saturation of hemoglobin (SO$_2$); total hemoglobin (tHb); and, plasma glucose, lactate and electrolyte concentrations (ABL-90, Radiometer, Copenhagen, Denmark).

6.3.4 Quantification of cerebral blood flow

6.3.4.1 Extracranial assessment of CBF

Duplex vascular ultrasound (10 MHz multifrequency linear array probe, Terason 3000, Teratech, Burlington, MA, USA) was used to measure continuous diameters and velocities in the internal carotid (ICA) and vertebral (VA) arteries as detailed previously (Willie et al., 2012; Willie et al., 2014a). Briefly, the left ICA was measured at least 2cm from the bifurcation ensuring there was no turbulent or retrograde flow at the site of velocity measurement. The right VA was measured between C6 and C4. Continuous screen capture was saved from subsequent offline analysis using proprietary edge-tracking software. Use of this software reduced observer error and bias over manual methods of analysis, possessing an intra-observer coefficient of variation of 6.7% (Black et al., 2008; Willie et al., 2012; Willie
et al., 2014a). Accurate quantification of flow through a vessel requires extremely good image quality, and the repeated measured design of the present study necessitated that the exact same point of a vessel is insonated with identical insonation angles during each measure. We were unable to capture images in every subject at each blood gas target that met these criteria, and in two subjects no usable ICA or VA images could be collected at either altitude. We consequently calculated the cerebral metabolic rate of oxygen (CMRO$_2$) from ICA and VA flows, and used these resultant mean CMRO$_2$ values and the cerebral arteriovenous difference for oxygen to calculate CBF by the Fick equation, as detailed below.

6.3.5 Calculations

6.3.5.1 Cerebral blood flow quantification by the Fick equation

Volumetric global cerebral blood flow was estimated by:

$$\text{CBF (mL min}^{-1}) = (Q_{\text{ICA}} \cdot 2) + (Q_{\text{VA}} \cdot 2).$$

(1)

$Q_{\text{ICA}}$ and $Q_{\text{VA}}$ is the blood flow through the left ICA and right VA, where, assuming bilateral symmetry of flow through these vessels, the total CBF is equal to twice their sum. Thus both ICA and VA for a given target blood gas stage must be known to estimate total CBF.

CMRO$_2$ was calculated according to the equation:

$$\text{CMRO}_2 (\text{mL O}_2 \text{ min}^{-1}) = \text{CBF} \cdot (\text{CaO}_2 - \text{CvO}_2),$$

(2)

where CBF is that calculated in equation (1) and CaO$_2$ – CvO$_2$ is the cerebral arteriovenous oxygen content difference in mL dL$^{-1}$ (Equations 4 and 5). CMRO$_2$ was calculated in this manner for each target blood gas stage at both SL and 5050 m when both $Q_{\text{ICA}}$ and $Q_{\text{VA}}$ were known, but therefore with differing sample sizes (see below). These mean CMRO$_2$ values were used to quantify total CBF using the Fick principle by reorganizing equation (2):

$$\text{Total CBF (mL min}^{-1}) = \frac{\text{CMRO}_2}{\text{CaO}_2 - \text{CvO}_2},$$

(3)
where CMRO\textsubscript{2} is that calculated in equation (2) and \(\text{CaO}_2 - \text{CvO}_2\) is the cerebral arteriovenous oxygen content difference in mL dL\textsuperscript{-1} (Equations 4 and 5).

Cerebral substrate delivery is often reported as units per 100g of brain tissue. In order to facilitate comparison of values presented herein, and assuming an average brain mass of 1.4 kg, CBF was also calculated as:

\[
\text{Total CBF (mL 100 g min}^{-1}) = \frac{\text{CBF (mL min}^{-1})}{14}. \tag{3b}
\]

Arterial content of oxygen (\(\text{CaO}_2\)) was calculated by:

\[
\text{CaO}_2 (\text{mL dL}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SaO}_2(\%)}{100} + 0.003 \cdot \text{PaO}_2, \tag{4}
\]

where [Hb] is the arterial blood hemoglobin concentration, 1.36 is the affinity for \(\text{O}_2\) to hemoglobin, and 0.003 is the solubility of \(\text{O}_2\) dissolved in blood.

Venous content of oxygen (\(\text{CvO}_2\)) was calculated by:

\[
\text{CvO}_2 (\text{mL dL}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SvO}_2(\%)}{100} + 0.003 \cdot \text{PvO}_2, \tag{5}
\]

where [Hb] is the venous blood hemoglobin concentration, 1.36 is the affinity for \(\text{O}_2\) to hemoglobin, and 0.003 is the solubility of \(\text{O}_2\) in blood.

Cerebrovascular conductance (CVC) was calculated from:

\[
\text{CVC(mmHg mmol 100 g min}^{-1}) = \frac{\text{CBF}}{\text{MAP}}. \tag{6}
\]

where MAP is the mean arterial pressure (which approximates cerebral perfusion pressure) and CBF is calculated from equation 3.

### 6.3.5.2 Calculation of cerebral metabolism metrics:

Oxygen extraction fraction (\(\text{O}_2\)EF) was calculated by:

\[
\text{O}_2\text{EF}(%) = \frac{\text{CaO}_2 - \text{CvO}_2}{\text{CaO}_2} \cdot 100\%,
\]
where $\text{CaO}_2$ and $\text{CvO}_2$ are calculated from equations 4 and 5, respectively.

Glucose extraction fraction (GluEF) was calculated by:

$$\text{GluEF} \% = \frac{\text{Glu}_a - \text{Glu}_v}{\text{Glu}_a} \cdot 100\%.$$  

(8)

Lactate extraction fraction (LacEF) was calculated by:

$$\text{LacEF} \% = \frac{\text{Lac}_a - \text{Lac}_v}{\text{Lac}_a} \cdot 100\%.$$  

(9)

where $\text{Glu}_a$ and $\text{Lac}_a$ equal the arterial concentration of glucose and lactate, and $\text{Glu}_v$ and $\text{Lac}_v$ equal jugular venous concentration of glucose and lactate, respectively.

Cerebral delivery of oxygen (CaDO$_2$) was calculated from:

$$\text{CaDO}_2 (\text{mL min}^{-1}) = \text{CBF} \cdot \text{CaO}_2$$  

(10)

where CBF is calculated from equation 3, and CaO$_2$ from equation 4.

Cerebral delivery of Glucose was calculated by:

$$\text{CD}_{\text{Glu}} (\text{mM 100 g min}^{-1}) = \text{CBF} \cdot \text{Glu}_a.$$  

(11)

Cerebral delivery of Lactate was calculated by:

$$\text{CD}_{\text{Lac}} (\text{mM 100 g min}^{-1}) = \text{CBF} \cdot \text{Lac}_a,$$  

(12)

where CBF from equation 3b gives the blood flow per unit of tissue and $\text{Glu}_a$ and $\text{Lac}_a$ are the arterial concentrations of glucose and lactate, respectively.

Cerebral metabolic rate for glucose (CMR$_\text{glu}$) was calculated by:

$$\text{CMR}_{\text{glu}} (\text{mM 100 g min}^{-1}) = \text{gCBF} \cdot (\text{Glu}_a - \text{Glu}_v).$$  

(13)

Cerebral metabolic rate for lactate (CMR$_\text{lac}$) was calculated by:
\[ CMR_{\text{lac}}(\text{mM}.100 \text{ g min}^{-1}) = \text{gCBF} \cdot (\text{Lac}_a - \text{Lac}_v), \]  

(14)

where the CBF is the blood flow per unit of brain tissue (equation 3b), and Glu_a-Glu_v and Lac_a-Lac_v are the arteriovenous differences of glucose and lactate, respectively.

Oxygen Glucose Index (OGI) was calculated by:

\[ \text{OGI} = \frac{(\text{CaO}_2 - \text{CvO}_2)}{\text{Glu}_a - \text{Glu}_v}. \]  

(15)

The OGI indicates the molar cerebral uptake ratio of oxygen to glucose. Perfect cellular respiration requires six oxygen molecules per molecule of glucose, thus purely aerobic cerebral metabolism would theoretically yield an OGI equal to six, OGI < six indicates not all glucose was aerobically metabolized, and an OGI > 6 indicates oxidation of other substrates.

The brain metabolizes lactate in addition to glucose (Van Hall et al., 2009). The Oxygen Carbohydrate Index (OCI) indicates the ratio of oxygen to glucose plus lactate, calculated as:

\[ \text{OCI} = \frac{(\text{CaO}_2 - \text{CvO}_2)}{(\text{Glu}_a - \text{Glu}_v) + \frac{1}{2}(\text{Lac}_a - \text{Lac}_v)}. \]  

(16)

The carboxylation of lactate to pyruvate into the citric acid cycle results in only one molecule of pyruvate, compared to the two derived from the breakdown of glucose to pyruvate during glycolysis. Half the molar arteriovenous lactate difference is used in the calculation of OCI because there are half the number carbon atoms involved in the oxidation of lactate than of glucose.

### 6.3.5.3 Calculation of reactivity

Cerebrovascular reactivity to CO\(_2\) was assessed separately for hypo- and hypercapnia as the slope of the linear regression between %ΔCBF and PaCO\(_2\), arterial [H\(^+\)], P_{IJV}CO\(_2\), and IJV [H\(^+\)]. Although at SL a +15 mmHg PCO\(_2\) step was conducted, this was not used in the calculation of SL hypercapnic CVR in order that hypercapnic CVR considered the same ∆PCO\(_2\) at both altitudes (subjects were unable to tolerate +15 mmHg PaCO\(_2\) above eupnia at
5050 m). The $R^2$ of every individual regression line for the calculation of reactivity was greater than 0.6.

**6.3.6 Statistical analysis**

That data were normally distributed was confirmed by the Shapiro-Wilk test. Repeated-measures ANOVA was used to compare differences across blood gas targets, and selected bonferroni corrected post-hoc comparisons of *a priori* interest were made between blood gas targets at both SL and 5050 m. Comparisons were made between SL and 5050 m for a given blood gas target, and between targets within elevation. Cerebrovascular reactivity between hypo and hypercapnia, or between altitudes were compared with *t*-tests. Alpha was 0.05, and values are shown as mean ± SD.

**6.4 Results**

**6.4.1 Subjects**

All eleven subjects completed all ABG tests at sea level. Two subjects were airlifted from 5050 m prior to data collection due to one developing acute appendicitis; both of these subjects were removed from the data set yielding an $n = 9$, all male, aged $31.9 ± 6.7$ years, with a BMI of $25.5 ± 3.8$ kg/m$^2$.

**6.4.2 Cardiovascular effects of HA and blood gas clamping (Tables 6.1 and 6.2)**

Mean arterial pressure at SL was not significantly altered relative to baseline with any of the PaO$_2$ manipulations. Conversely, hypoxia at SL elevated HR irrespective of PaCO$_2$, whereas at HA clamping elicited no significant changes in HR. At rest, MAP was elevated following 6-10 days at 5050 m and was further increased under conditions of euoxic and hyperoxic hypercapnia (+28.4 ± 11% MAP and +13.7 ± 10, respectively). Conversely, hypocapnia at HA elicited a 10.0 ± 6.0 % decrease in MAP whereas it was unaltered at SL during this condition (Figure 6.2). The mean slope of the percent increase in MAP versus PaCO$_2$ in the hypercapnic range was significantly greater at HA (SL: $1.2 ± 0.7$ Δ%/mmHg versus HA $2.8 ± 1.0$ Δ%/mmHg; Figure 6.2). If the difference between SL and HA was accounted for solely by the change in PCO$_2$-H$^+$ relationship at HA, than MAP reactivity as a function of [H$^+$] should be similar between altitudes; however, MAP reactivity as a function of [H$^+$] was actually greater than that at SL. Hyperoxic hypercapnic at HA produced a similar increase in
MAP as euoxic hypercapnia at SL (HA ΔMAP 13.7 ± 10%; SL ΔMAP 12.3 ± 9.0%), suggesting background hypoxia caused the greater increase in MAP with hypercapnia at HA.
Table 6.1. Cardiorespiratory and cerebrovascular variables at sea-level and 5050m during various arterial blood gas manipulations.

<table>
<thead>
<tr>
<th></th>
<th>Altitude</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target Blood gases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO$_2$ (mmHg)</td>
<td></td>
<td>86.8 ± 5.8</td>
<td>99.9 ± 2.7</td>
<td>100 ± 4.7</td>
<td>99.0 ± 3.5</td>
<td>40.0 ± 1.8</td>
<td>41.4 ± 2.0</td>
<td>39.9 ± 1.1</td>
<td>41.8 ± 1.9</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
<td></td>
<td>39.7 ± 2.0</td>
<td>36.8 ± 2.5</td>
<td>30.9 ± 1.9</td>
<td>25.4 ± 2.4</td>
<td>26.0 ± 1.5</td>
<td>24.7 ± 2.3</td>
<td>41.4 ± 2.5</td>
<td>34.1 ± 2.5</td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td></td>
<td>621 ± 52</td>
<td>1036 ± 145*</td>
<td>420 ± 50</td>
<td>540 ± 80</td>
<td>493 ± 132</td>
<td>653 ± 81</td>
<td>795 ± 92</td>
<td>1179 ± 232*</td>
</tr>
<tr>
<td>CVC (ml/min / mmHg)</td>
<td></td>
<td>6.2 ± 0.9</td>
<td>8.5 ± 1.4*</td>
<td>4.9 ± 0.8</td>
<td>6.7 ± 0.8</td>
<td>4.9 ± 1.5</td>
<td>7.1 ± 0.7</td>
<td>8.2 ± 1.5</td>
<td>7.2 ± 1.2*</td>
</tr>
<tr>
<td>MCAv (cm/s)</td>
<td></td>
<td>68.6 ± 10</td>
<td>112 ± 17.3*</td>
<td>50.3 ± 9.3</td>
<td>63 ± 9.4*</td>
<td>54.3 ± 9.0</td>
<td>76.3 ± 12*</td>
<td>87.1 ± 12</td>
<td>113 ± 17.8*</td>
</tr>
<tr>
<td>PCAv (cm/s)</td>
<td></td>
<td>48.4 ± 7.6</td>
<td>81.5 ± 12.4*</td>
<td>37.0 ± 7.5</td>
<td>43 ± 9.2*</td>
<td>36.4 ± 6.2</td>
<td>51.2 ± 9.8*</td>
<td>62.9 ± 12</td>
<td>82.4 ± 12.8*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td>97 ± 10</td>
<td>115 ± 14*</td>
<td>89 ± 12</td>
<td>95 ± 9</td>
<td>94 ± 10</td>
<td>101 ± 9</td>
<td>107 ± 10</td>
<td>129 ± 6.6*</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td></td>
<td>64 ± 8</td>
<td>79 ± 18*</td>
<td>73 ± 12</td>
<td>68 ± 15</td>
<td>82 ± 12</td>
<td>76 ± 15</td>
<td>79.4 ± 11</td>
<td>81.6 ± 21.1</td>
</tr>
</tbody>
</table>

* $P < 0.05$ versus Sea-level for same target blood gases.
Table 6.2. Arterial and internal jugular venous variables at sea-level and 5050m during various arterial blood gas manipulations.

<table>
<thead>
<tr>
<th>Target Blood gases</th>
<th>Altitude</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
<th>344m</th>
<th>5050m</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>86.8 ± 5.8</td>
<td>99.9 ± 2.7</td>
<td>100 ± 4.7</td>
<td>99.0 ± 3.5</td>
<td>40.0 ± 1.8</td>
<td>41.4 ± 2.0</td>
<td>39.9 ± 1.1</td>
<td>41.8 ± 1.9</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td></td>
<td>39.7 ± 2.0</td>
<td>36.8 ± 2.5</td>
<td>30.9 ± 1.9</td>
<td>25.4 ± 2.4</td>
<td>26.0 ± 1.5</td>
<td>24.7 ± 2.3</td>
<td>41.4 ± 2.5</td>
<td>34.1 ± 2.5</td>
</tr>
<tr>
<td>Arterial</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td></td>
<td>86.8 ± 5.8</td>
<td>99.9 ± 2.7*</td>
<td>100 ± 4.7</td>
<td>99.0 ± 3.5</td>
<td>40.0 ± 1.8</td>
<td>41.4 ± 2.0</td>
<td>39.9 ± 1.1</td>
<td>41.8 ± 1.9*</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td></td>
<td>39.7 ± 2.0</td>
<td>36.8 ± 2.5*</td>
<td>30.9 ± 1.9</td>
<td>25.4 ± 2.4*</td>
<td>26.0 ± 1.5</td>
<td>24.7 ± 2.3</td>
<td>41.4 ± 2.5</td>
<td>34.1 ± 2.5*</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td></td>
<td>97.3 ± 0.5</td>
<td>97.3 ± 0.2</td>
<td>98.6 ± 0.2</td>
<td>97.9 ± 0.6*</td>
<td>85.9 ± 2.0</td>
<td>80.7 ± 2.8*</td>
<td>78.8 ± 1.4</td>
<td>76.6 ± 2.2*</td>
</tr>
<tr>
<td>CaO₂ (ml O₂/dL)</td>
<td></td>
<td>19.5 ±0.6</td>
<td>21.1 ± 1.3*</td>
<td>19.8 ± 1.1</td>
<td>21.1 ± 1.6*</td>
<td>17.5 ± 1.0</td>
<td>17.4 ± 1.2</td>
<td>16.1 ± 0.8</td>
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<tr>
<td>tHb (g/dL)</td>
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<td>14.6 ± 0.5</td>
<td>15.7 ± 1.0*</td>
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<td>15.6 ± 1.2*</td>
<td>14.9 ± 0.6</td>
<td>15.8 ± 1.0*</td>
<td>14.9 ± 0.6</td>
<td>15.9 ± 1.0*</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.40 ± 0.01</td>
<td>7.35 ± 0.04*</td>
<td>7.48 ± 0.02</td>
<td>7.45 ± 0.03*</td>
<td>7.55 ± 0.01</td>
<td>7.47 ± 0.04*</td>
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<td>7.39 ± 0.04</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td></td>
<td>24.8 ± 1.0</td>
<td>20.5 ± 1.0*</td>
<td>23.0 ± 1.0</td>
<td>17.7 ± 1.4*</td>
<td>22.6 ± 1.1</td>
<td>18.0 ± 1.3*</td>
<td>25.7 ± 1.0</td>
<td>20.5 ± 1.3*</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td></td>
<td>-0.03 ± 0.9</td>
<td>-4.58 ± 1.7*</td>
<td>0.64 ± 0.9</td>
<td>-4.04 ± 1.6*</td>
<td>1.84 ± 0.7</td>
<td>-3.85 ± 1.6*</td>
<td>0.11 ± 0.7</td>
<td>-4.30 ± 1.6*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td></td>
<td>5.4 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>5.4 ± 0.4</td>
<td>5.2 ± 0.3</td>
<td>5.2 ± 0.3</td>
<td>5.4 ± 0.4</td>
<td>5.2 ± 0.3</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td></td>
<td>0.7 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>K⁺ (mM)</td>
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<td>3.7 ± 0.2</td>
<td>3.4 ± 0.3*</td>
<td>3.7 ± 0.3</td>
<td>3.3 ± 0.4*</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.4*</td>
<td>3.7 ± 0.3</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Na⁺ (mM)</td>
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<td>142 ± 0.8</td>
<td>142 ± 1.5</td>
<td>141 ± 1.1</td>
<td>141 ± 1.6</td>
<td>141 ± 0.9</td>
<td>142 ± 1.5</td>
<td>141 ± 1.0</td>
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<tr>
<td>Ca²⁺ (mM)</td>
<td></td>
<td>1.17 ± 0.03</td>
<td>1.17 ± 0.03</td>
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<td>1.13 ± 0.04</td>
<td>1.14 ± 0.05</td>
<td>1.18 ± 0.05</td>
<td>1.18 ± 0.03</td>
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<td>Cl⁻ (mM)</td>
<td></td>
<td>110.1 ± 1.5</td>
<td>116 ± 2.4*</td>
<td>111 ± 1.5</td>
<td>118 ± 2.5*</td>
<td>111 ± 1.5</td>
<td>118 ± 4.5*</td>
<td>109 ± 1.8</td>
<td>118 ± 6.2*</td>
</tr>
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<td>Jugular Venous</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PvO₂ (mmHg)</td>
<td></td>
<td>33.5 ± 1.2</td>
<td>52.4 ± 2.7*</td>
<td>25.2 ± 1.5</td>
<td>34.0 ± 2.9*</td>
<td>20.6 ± 2.5</td>
<td>26.6 ± 0.7*</td>
<td>27.1 ± 0.7</td>
<td>33.3 ± 1.2*</td>
</tr>
<tr>
<td>PvCO₂ (mmHg)</td>
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<td>50.0 ± 4.3</td>
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<td>47.4 ± 3.4</td>
<td>32.9 ± 2.6*</td>
<td>38.1 ± 3.6</td>
<td>30.4 ± 2.9*</td>
<td>49.0 ± 3.7</td>
<td>36.4 ± 2.7*</td>
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<td>SvO₂ (%)</td>
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<td>83.4 ± 1.4*</td>
<td>51.6 ± 4.2</td>
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<td>45.0 ± 8.2</td>
<td>52.7 ± 1.7*</td>
<td>54.6 ± 2.8</td>
<td>62.0 ± 2.0*</td>
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<tr>
<td>CvO₂ (ml O₂/100ml)</td>
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<td>13.3 ± 0.9</td>
<td>17.2 ± 1.1*</td>
<td>10.4 ± 0.9</td>
<td>13.5 ± 1.2*</td>
<td>9.4 ± 2.3</td>
<td>11.2 ± 0.9*</td>
<td>11.2 ± 0.8</td>
<td>13.2 ± 0.9*</td>
</tr>
<tr>
<td>tHb (g/dL)</td>
<td></td>
<td>14.6 ± 1.0</td>
<td>150 ± 0.9</td>
<td>148 ± 0.7</td>
<td>153 ± 0.7</td>
<td>150 ± 0.8</td>
<td>155 ± 0.8</td>
<td>149 ± 0.5</td>
<td>155 ± 0.8</td>
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<tr>
<td>pH</td>
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<td>7.39 ± 0.01</td>
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<td>7.46 ± 0.04</td>
<td>7.46 ± 0.04</td>
<td>7.37 ± 0.01</td>
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<td>HCO₃⁻ (mEq/L)</td>
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<td>27.9 ± 1.9</td>
<td>21.0 ± 1.4*</td>
<td>28.7 ± 1.6</td>
<td>20.5 ± 1.8*</td>
<td>27.1 ± 1.8</td>
<td>20.2 ± 1.7*</td>
<td>28.1 ± 1.8</td>
<td>21.3 ± 1.8*</td>
</tr>
<tr>
<td>BE (mEq/L)</td>
<td></td>
<td>0.2 ± 1.2</td>
<td>-5.2 ± 1.9*</td>
<td>1.3 ± 1.1</td>
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<td>1.8 ± 0.8</td>
<td>-4.1 ± 1.9*</td>
<td>0.3 ± 1.3</td>
<td>-4.6 ± 2.0*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
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<td>4.9 ± 0.34</td>
<td>4.9 ± 0.4</td>
<td>4.8 ± 0.5</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>4.8 ± 0.5</td>
<td>5.0 ± 0.4</td>
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<tr>
<td>Target Blood gases</td>
<td>Altitude</td>
<td>344m</td>
<td>5050m</td>
<td>344m</td>
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<tr>
<td>PaO₂ (mmHg)</td>
<td>86.8 ± 5.8</td>
<td>99.9 ± 2.7</td>
<td>100 ± 4.7</td>
<td>99.0 ± 3.5</td>
<td>40.0 ± 1.8</td>
<td>41.4 ± 2.0</td>
<td>39.9 ± 1.1</td>
<td>41.8 ± 1.9</td>
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<tr>
<td>PaCO₂ (mmHg)</td>
<td>39.7 ± 2.0</td>
<td>36.8 ± 2.5</td>
<td>30.9 ± 1.9</td>
<td>25.4 ± 2.4</td>
<td>26.0 ± 1.5</td>
<td>24.7 ± 2.3</td>
<td>41.4 ± 2.5</td>
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</tr>
<tr>
<td>Lactate (mM)</td>
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</tr>
<tr>
<td>K⁺ (mM)</td>
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<td>3.2 ± 0.3*</td>
<td>3.8 ± 0.2</td>
<td>3.1 ± 0.4*</td>
<td>3.9 ± 0.2</td>
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<tr>
<td>Na⁺(mM)</td>
<td>143 ± 1.1</td>
<td>143 ± 1.1</td>
<td>143 ± 1.3</td>
<td>143 ± 1.2</td>
<td>142 ± 1.2</td>
<td>143 ± 1.2</td>
<td>142 ± 0.9</td>
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</tr>
<tr>
<td>Ca²⁺(mM)</td>
<td>1.2 ± 0.07</td>
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<td>1.1 ± 0.04</td>
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<tr>
<td>Cl⁻(mM)</td>
<td>108 ± 2.9</td>
<td>117 ± 3.3*</td>
<td>107 ± 2.6</td>
<td>117 ± 2.5*</td>
<td>107 ± 2.4</td>
<td>118 ± 6.0*</td>
<td>107 ± 2.6</td>
<td>117 ± 4.2*</td>
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</table>

* P < 0.05 versus Sea-level for same target blood gases
Figure 6.2. Mean arterial pressure during changes in PaCO$_2$ at sea level and high altitude. Percent difference in MAP from baseline (A) during euoxic changes in PaCO$_2$ at SL (solid squares) and HA (hollow squares), and the individual slopes of this relationship in the hypercapnic range (B). Mean slopes for this relationship were: SL hypercapnic range 1.2 ± 0.7 %/mmHg; HA, hypocapnic range 1.1 ± 0.7 %/mmHg (not-shown); HA, hypercapnic range 2.8 ± 1.0 %/mmHg.

6.4.3 Acid base balance at 5050 m (Table 6.2)
Exposure to 5050 m resulted in a partial metabolic compensation (i.e., decrease in arterial BE and HCO$_3^-$) for the respiratory alkalosis (arterial pH at 5050 m was 7.47 ± 0.04 versus 7.40 ± 0.01 at sea level). The plasma concentration of potassium ion was decreased, and chloride increased at 5050 m, but neither were influenced by ABG clamping. Acute hypo- and hypercapnia changed arterial and jugular venous pH at both altitudes. Arterial and internal jugular venous HCO$_3^-$ was decreased at HA relative to SL. Arterial HCO$_3^-$ was increased during hypercapnia, and decreased during hypocapnia at both altitudes. Conversely, jugular HCO$_3^-$ was not significantly affected by any acute change to ABG at SL, and at HA was altered only at +10 mmHg PaCO$_2$. Figure 6.3 shows that a given iso-oxic change in PCO$_2$ elicits a greater change in proton concentration at HA and that this difference is augmented in internal jugular venous blood.
Figure 6.3. Relationship between proton concentration and PCO2 in arterial and internal jugular venous blood at sea level and high altitude.
Proton concentration in arterial (A) and internal jugular venous blood (B) at SL and HA during acute euoxic changes in PETCO2. Slopes of the regression were significantly less at SL than at HA for both arterial and internal jugular venous blood ($P < 0.05$).

6.4.4 Effects of O2 (Tables 6.1, 6.2, and 6.3)
At SL, the isocapnic reduction in PaO2 to 39.9 ± 1.1 mmHg (78.8± 1.4 % SaO2) elicited a 45 ± 16% increase in CBF, reduced cerebral a-vPaO2 from 53 ± 5.2 mmHg to 12.9 ± 0.9 mmHg and maintained O2 EF. At 5050 m normalization of PaO2 to SL values decreased CBF in all subjects (Figure 6.4D). As expected, normalization of O2 resulted in a corresponding increase in a-vPaO2 from 14.9 ± 2.2 to 65.0 ± 4.9 mmHg. Compared to SL hypocapnic normoxia, O2 normalization at HA yielded greater CDO2 and correspondingly lower O2EF.
### Table 6.3. Cerebral metabolism at sea-level and 5050m during various arterial blood gas manipulations.

<table>
<thead>
<tr>
<th>Altitude</th>
<th>344m</th>
<th>5050m</th>
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<tr>
<td><strong>Target Blood gases</strong></td>
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<tr>
<td>PaO$_2$ (mmHg)</td>
<td>86.8 ± 5.8</td>
<td>99.9 ± 2.7</td>
<td>100 ± 4.7</td>
<td>99.0 ± 3.5</td>
<td>40.0 ± 1.8</td>
<td>41.4 ± 2.0</td>
<td>39.9 ± 1.1</td>
<td>41.8 ± 1.9</td>
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<tr>
<td>PaCO$_2$ (mmHg)</td>
<td>39.7 ± 2.0</td>
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<td>30.9 ± 1.9</td>
<td>25.4 ± 2.4</td>
<td>26.0 ± 1.5</td>
<td>24.7 ± 2.3</td>
<td>41.4 ± 2.5</td>
<td>34.1 ± 2.5</td>
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<tr>
<td><strong>Cerebral substrate delivery</strong></td>
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<tr>
<td>CDO$_2$ (ml/min)</td>
<td>124 ± 12</td>
<td>222 ± 25*</td>
<td>84.5 ± 7.5</td>
<td>116 ± 12*</td>
<td>89.0 ± 28</td>
<td>116 ± 10*</td>
<td>131 ± 15</td>
<td>200 ± 34*</td>
</tr>
<tr>
<td>CD$_{\text{glucose}}$ (µmol/100g/min)</td>
<td>240 ± 23</td>
<td>398 ± 74*</td>
<td>164 ± 26</td>
<td>202 ± 34</td>
<td>183 ± 46</td>
<td>274 ± 35*</td>
<td>292 ± 27</td>
<td>399 ± 47*</td>
</tr>
<tr>
<td>CD$_{\text{lactate}}$ (µmol/100g/min)</td>
<td>30.0 ± 13</td>
<td>70.1 ± 18*</td>
<td>21.5 ± 8.6</td>
<td>37.0 ± 8.0*</td>
<td>274 ± 35*</td>
<td>34.6 ± 0.9*</td>
<td>275 ± 35</td>
<td>77.6 ± 15*</td>
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<tr>
<td><strong>Arterial-venous differences</strong></td>
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<tr>
<td>PO$_2$ (mmHg)</td>
<td>53 ± 5.2</td>
<td>47.4 ± 2.0*</td>
<td>75.0 ± 5.0</td>
<td>65.0 ± 4.9*</td>
<td>19.1 ± 3.5</td>
<td>14.9 ± 2.2</td>
<td>12.9 ± 0.9</td>
<td>8.5 ± 1.5</td>
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<td>PCO$_2$ (mmHg)</td>
<td>-10 ± 4</td>
<td>-2.6 ± 1.4*</td>
<td>-16 ± 2.8</td>
<td>-7.4 ± 1.8*</td>
<td>-12 ± 3.4</td>
<td>-5.7 ± 1.5*</td>
<td>-7.6 ± 2.8</td>
<td>-2.3 ± 1.7*</td>
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<tr>
<td>SO$_2$ (%)</td>
<td>31 ± 2.2</td>
<td>14 ± 1.4*</td>
<td>47 ± 4.2</td>
<td>34 ± 4.2*</td>
<td>40 ± 9.5</td>
<td>28 ± 3.1*</td>
<td>24 ± 1.8</td>
<td>15 ± 1.3*</td>
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<tr>
<td>O$_2$ content (ml/dL)</td>
<td>6.4 ± 0.53</td>
<td>4.0 ± 0.6*</td>
<td>9.5 ± 1.1</td>
<td>7.7 ± 1.2*</td>
<td>8.2 ± 2.0</td>
<td>6.4 ± 0.8*</td>
<td>5.0 ± 0.6</td>
<td>3.6 ± 0.7</td>
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<tr>
<td>Glucose (mM)</td>
<td>0.62 ± 0.34</td>
<td>0.43 ± 0.2</td>
<td>0.63 ± 0.3</td>
<td>0.67 ± 0.3</td>
<td>0.67 ± 0.3</td>
<td>0.61 ± 0.3</td>
<td>0.36 ± 0.3</td>
<td>0.30 ± 0.2</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>0.01 ± 0.06</td>
<td>0.01 ± 0.2</td>
<td>-0.01 ± 0.1</td>
<td>-0.01 ± 0.1</td>
<td>-0.01 ± 0.4</td>
<td>-0.03 ± 0.1</td>
<td>0.00 ± 0.07</td>
<td>0.00 ± 0.2</td>
</tr>
<tr>
<td>Glucose + ½ lactate (mM)</td>
<td>0.63 ± 0.4</td>
<td>0.40 ± 0.2</td>
<td>0.63 ± 0.3</td>
<td>0.65 ± 0.3</td>
<td>0.73 ± 0.4</td>
<td>0.58 ± 0.3</td>
<td>0.37 ± 0.4</td>
<td>0.20 ± 0.3</td>
</tr>
<tr>
<td><strong>Cerebral metabolism</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O$_2$EF (%)</td>
<td>32 ± 3.1</td>
<td>19 ± 2.4*</td>
<td>47 ± 4.2</td>
<td>36 ± 4.2*</td>
<td>46 ± 11</td>
<td>36 ± 3.3*</td>
<td>31 ± 3.4</td>
<td>21 ± 2.9*</td>
</tr>
<tr>
<td>Glucose EF (%)</td>
<td>11 ± 6.1</td>
<td>8.0 ± 3.5</td>
<td>12 ± 5.4</td>
<td>12 ± 5.7</td>
<td>13 ± 5.2</td>
<td>11 ± 5.0</td>
<td>6.9 ± 5.5</td>
<td>5.7 ± 3.0</td>
</tr>
<tr>
<td>Lactate EF (%)</td>
<td>1.7 ± 10</td>
<td>1.7 ± 17</td>
<td>-3.7 ± 16</td>
<td>-0.42 ± 14</td>
<td>-12 ± 32</td>
<td>-3.8 ± 11</td>
<td>-2.2 ± 13</td>
<td>-0.55 ± 20</td>
</tr>
<tr>
<td>CMR glucose (µmol/100g/min)</td>
<td>27.1 ± 15</td>
<td>32.2 ± 16</td>
<td>18.7 ± 6.8</td>
<td>24.9 ± 10</td>
<td>21.9 ± 5.8</td>
<td>27.6 ± 12</td>
<td>18.8 ± 15</td>
<td>22.0 ± 10</td>
</tr>
<tr>
<td>CMR lactate (µmol/100g/min)</td>
<td>0.45 ± 2.6</td>
<td>0.91 ± 9.5</td>
<td>-0.38 ± 3.0</td>
<td>-0.70 ± 4.7</td>
<td>10.5 ± 46</td>
<td>-1.4 ± 4.7</td>
<td>-0.37 ± 4.0</td>
<td>-1.5 ± 18</td>
</tr>
<tr>
<td>OGI (%)</td>
<td>7.5 ± 5.8</td>
<td>5.9 ± 4.3</td>
<td>7.7 ± 3.3</td>
<td>8.0 ± 8.7</td>
<td>6.4 ± 1.7</td>
<td>5.9 ± 2.8</td>
<td>9.4 ± 6.6</td>
<td>8.0 ± 5.2</td>
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<tr>
<td>OCI (%)</td>
<td>8.7 ± 9.1</td>
<td>6.0 ± 4.5</td>
<td>8.5 ± 5.2</td>
<td>8.1 ± 8.7</td>
<td>7.2 ± 3.8</td>
<td>6.1 ± 2.7</td>
<td>9.2 ± 6.6</td>
<td>5.3 ± 6.6</td>
</tr>
<tr>
<td>CMRO$_2$ (µmol/100g/min) [n]</td>
<td>1.2 ± 0.2 [7]</td>
<td>1.2 ± 0.4 [4]</td>
<td>1.2 ± 0.2 [5]</td>
<td>1.5 ± 0.6 [6]</td>
<td>1.2 ± 0.3 [7]</td>
<td>1.4 ± 0.6 [6]</td>
<td>1.4 ± 0.3 [6]</td>
<td>1.0 ± 0.3 [4]</td>
</tr>
</tbody>
</table>

* P < 0.05 versus Sea-level for same target blood gases. ‡ Values calculated from Duplex ultrasound-quantified CBF; see Methods for details.
Figure 6.4. Individual cerebral blood flow values during isocapnic changes in PaO\textsubscript{2} at SL and HA.

The top plots (A, B) show CBF\textsubscript{total} data from SL; the bottom plots (C, D) show data from 5050 m. The left side of each plot depicts CBF while PaO\textsubscript{2} was ~100 mmHg (except at SL – plot A – where ambient PO\textsubscript{2} yielded a smaller PaO\textsubscript{2}), whereas hypoxia is shown on the right side of each plot. The left hand plots (A, C) show isocapnia clamped at ~40 mmHg at SL and ~35 mmHg at HA (the lower PaCO\textsubscript{2} values at HA were due to inability of subjects to tolerate higher values; see results and discussion). Right side plots (B, D) show isocapnic clamp at ~25 mmHg. Please see methods for further details on gas permutations.

6.4.5 Effects of CO\textsubscript{2} (Tables 6.1, 6.2, and 6.3)

Iso-oxic hypercapnia and hypocapnia resulted in significant increases and decreases, respectively, in CBF at both SL and HA (Figure 6.5). Hypercapnic reactivity was greater
than hypocapnic reactivity at both elevations. In the hypocapnic range the decrease in CBF was similar between SL and HA, decreasing 25.1 ± 7.0 % (-5 mmHg PaCO$_2$) and 40.0 ± 18 % (-10 mmHg PaCO$_2$) at SL, and 19.7 ± 9.0 (-5 mmHg PaCO$_2$) and 36.0 ± 6.8 (-5 mmHg PaCO$_2$) at 5050 m. The hypocapnic CVR was therefore not different between SL and HA (4.0 ± 1.0 %/mmHg at both SL and HA). In the hypercapnic range at SL CBF increased 23.9 ± 15 % (+5 mmHg PaCO$_2$) and 67.2 ± 29 % (+10 mmHg PaCO$_2$), whereas at HA CBF increased 38.2 ± 24 % (+5 mmHg PaCO$_2$) and 77.0 ± 27 (+10 mmHg PaCO$_2$) giving a hypercapnic reactivity at SL of 6.8 ± 2.9 %/mmHg versus 9.0 ± 3.6 %/mmHg at HA (P = 0.27; Figure 6.6A). Consideration of the altered acid-base buffering capacity at HA by determination of the hypercapnic CVR as ∆CBF versus [H$^+$] did not change these relationships (Figure 6.6C).

**Figure 6.5.** Total cerebral blood flow during steady state euvolic changes in PCO$_2$ at SL and HA. Hollow squares represent CBF plotted against arterial PCO$_2$ and solid squares against internal jugular vein PCO$_2$. CBF was significantly altered from baseline at all levels of PCO$_2$ at both SL and 5050 m ($P < 0.05$).
Figure 6.6. Cerebrovascular reactivity to CO$_2$ at sea level and high altitude.
Cerebrovascular reactivity to CO$_2$ at sea level and high altitude. Individual (circles, grey) and mean (squares, black) cerebrovascular reactivity (CVR) to euoxic changes in arterial (left) and jugular (right) PCO$_2$ (Top) and [H$^+$] (bottom). CVR in the hypocapnic range was lower than in the hypercapnic at both elevations when determined as a function of PaCO$_2$ or arterial [H$^+$] (A and C; $P < 0.05$). $P_{IJV}$CO$_2$ CVR did not differ between hypo- and hypercapnia nor between altitudes (B), whereas [H$^+$]$_{IJV}$ was greater in the hypercapnic range at HA. The very high arterial CVR in one individual at HA (11.2 %/mmHg) was more than two standard deviations above the mean (6.8 ± 5.4 %/mmHg); however, this individual’s very high CBF with hypercapnia at HA is supported by his very low a-v Glucose difference as can be seen as the outlier in Figure 10, bottom.
Figure 6.7 depicts the substantial underestimation of CO₂ reactivity when based on MCAv. In the hypercapnic range MCAv-CVR was approximately half of actual CVR at both SL and HA. In the hypocapnic range MCAv underestimated reactivity at sea level but not at 5050 m.

![Figure 6.7. Comparison of cerebral blood flow (CBF) versus middle cerebral artery velocity (MCAv) reactivities to change in PaCO₂ at SL and HA.](image)

MCAv CVR was lower than CBF CVR in the hypercapnic range at both altitudes, likely due to dilation of the MCA during increases in CO₂, slowing of MCA blood velocity and consequent underestimation of reactivity to increases in PaCO₂.

The effect of returning PaCO₂ to SL values at HA (relative hypercapnia) was anecdotally more unpleasant and difficult at HA for the majority of the volunteers, likely because at HA hypercapnia elicited very high ventilation in most subjects (e.g., +10 mmHg hypercapnia at HA yielded Ve = 83.8 ± 23 L/min; whereas at SL hypercapnia elicited Ve = 40.6 ± 17.4 L/min). Ventilatory sensitivity to CO₂ increased from SL values of 2.3 ± 1.3 mL.min⁻¹ / mmHg (3.7 ± 2.8 mL.min⁻¹ / [H⁺]) to 5.9 ± 1.6 mL.min⁻¹ / mmHg (7.7 ± 2.2 mL.min⁻¹ / [H⁺]) at HA. Ventilatory sensitivity as a function of jugular venous blood was similar to arterial values, except for IJV PCO₂ sensitivity that was greater than PaCO₂ at HA (Figure 6.8). The O₂ EF varied inversely with CBF (and PaCO₂) at both SL and HA, ranging at SL from 47 ± 4.2 % to 12.9 ± 3.1 %, and at HA from 51.5 ± 4.8 % to 21.0 ± 2.9 (-10 mmHg PETCO₂ to +10 mmHg PETCO₂, respectively).
Ventilatory sensitivity to increases in PCO$_2$ (left) and [H$^+$] (right) in arterial and jugular venous blood. Ventilatory sensitivity was increased from SL to HA regardless of the metric (i.e., arterial or jugular) and was greater at HA as indexed by jugular PCO$_2$ than PaCO$_2$ ($P < 0.05$).

Despite the greater MAP-PaCO$_2$ reactivity at HA, the CBF for a given change in MAP was smaller at HA than at SL in every individual but one (Figure 6.9c). There was a significant relationship between the change in hypercapnic MAP-CO$_2$ reactivity from SL to HA and the change from SL to HA in hypercapnic CBF-CO$_2$ reactivity ($R^2 = 0.47$).
Figure 6.9. Relationships between CBF and ∆MAP at SL and HA.
Plots A and B depict CBF versus the percent change in MAP from baseline at SL (A) and HA (B). Each individual’s change in CBF per percent ∆MAP within the hypercapnic range is given in plot C. This CBF-MAP reactivity was observed to decrease in every subject but one, despite a greater MAP-PCO2 reactivity at HA that elicited greater hypertension than at SL.

6.4.6 Combined effects of O2 and CO2 (Tables 6.1, 6.2, and 6.3)
At SL the CBF increase observed during normocapnic hypoxia was neutralized with concomitant hypocapnia (i.e., CBF was not significantly different between normoxic or hypoxic hypocapnia; Figure 6.4b). Whereas at HA, increasing PaO2 to SL values during concomitant hypercapnia caused CBF to decrease in five of nine individuals (Figure 6.4C); changes in PaCO2 elicited significant changes in CBF and MAP regardless of prevailing PaO2. Thus, although hyperoxia decreased CBF at HA by 17 ± 6.7%, CBF decreased by 18 ± 5.1% with hypocapnia despite this eliciting a 31.5 ± 6.0 decrease in CDO2. These findings indicate that oxygen is not as potent a stimulant to cerebral vasomotion as CO2 and that CBF is reset to a lower prevailing PaCO2 at HA.

6.4.7 Cerebral metabolism (Table 6.3)
No metric of cerebral carbohydrate metabolism was significantly altered during any change in PaO2 at SL or at HA (i.e., CMR lactate, CMR glucose, OGO, OCI, or arteriovenous differences for glucose or lactate). At SL, but not at HA, CMR glucose and CMR lactate were reduced with hypercapnia (+10 mmHg). The arteriovenous difference for glucose decreased with hypocapnia and increased with hypercapnia at both SL and HA (Figure 6.10, top) and the
slope of this relationship was steeper at HA than at SL (Figure 6.10 bottom). Lactate arteriovenous differences increased significantly only with hypercapnia at SL.

![Graph showing individual cerebral arteriovenous glucose differences at sea level and high altitude versus PaCO₂](image)

Figure 6.10. Individual cerebral arteriovenous glucose differences at sea level and high altitude versus PaCO₂.
At both SL (left) and HA (right) the a-v Glucose difference is inversely related to PaCO₂ (and to hence CBF, data not shown); however, at HA this relationship is steeper i.e., more negative slope (bottom; \( P < 0.05 \)).

### 6.5 Discussion

The principal findings of this study were: 1) partial acclimatization to HA yields a steeper CO₂-H⁺ relation in both arterial and jugular venous blood; yet, 2) whereas ventilatory sensitivity to euoxic ΔPCO₂ increased at HA, contrary to our hypothesis, cerebrovascular reactivity did not change, despite 3) MAP-CO₂ reactivity being augmented at HA, indicating enhanced cerebral autoregulation. 4) Acute hypoxia at SL increased CBF, and acute restoration of oxygen at HA reduced CBF, but neither had any effect on cerebral metabolism;
in contrast, 5) hypercapnia decreased the cerebral metabolic rate of glucose irrespective of background oxygen.

6.5.1 Metabolic compensation and CBF
Partial metabolic compensation following 6-10 days at 5050 m (bicarbonate excretion) to respiratory alkalosis steepened the relationship between PCO$_2$ and pH (proton concentration) – i.e., a given change in PCO$_2$ elicited a greater change in arterial and internal jugular venous [H$^+$] than at SL. We initially hypothesized that changes in buffering status would be responsible for any changes to reactivity (cerebrovascular, MAP, ventilatory), yet the increase in MAP and ventilatory reactivity at HA was similar when reactivity was assessed as a function of proton concentration. Reduced buffering capacity is therefore not the principal mechanism that mediates elevations in MAP and ventilatory reactivity. We did not administer the entire PCO$_2$ range under conditions of hypoxia at SL or hyperoxia at HA. However, that the increase in MAP and ventilation during hyperoxic hypercapnia at HA increased similarly as during normoxic hypercapnia at SL suggests that background hypoxia is responsible for the observed augmentation in MAP and ventilatory reactivity at HA likely via activation of the peripheral chemoreflex and/or increased sensitivity of the carotid body (Howard & Robbins, 1995; Wilson et al., 2005).

6.5.2 Arterial, tissue, and venous PCO$_2$
That the mammalian cerebral vasculature is highly sensitive to changes in PaCO$_2$ has been readily accepted for 150 years (Bayliss et al., 1895; Donders, 1851; Mosso, 1880; Roy & Sherrington, 1890; Wolff & Lennox, 1930). At sea-level there is a ~4-5% increase in CBF per mmHg increase in PaCO$_2$ above eupnic PaCO$_2$, and 3-4% decrease in CBF below eupnic PaCO$_2$, affected principally by dilation and constriction (respectively) of the pial arterioles on the brain’s surface (Ainslie & Duffin, 2009; Sato et al., 2012; Willie et al., 2012; Wolff & Lennox, 1930). How CO$_2$ precisely exerts its effects on the cerebrovasculature is not definitively known. Altered arterial pH without changes in PaCO$_2$ does not effect CBF (Harper & Bell, 1963; Lamberts et al., 1961), but extravascular application of acidic or basic solutions alters vessel tone (Kontos et al., 1977a; Kontos et al., 1977b; Wahl et al., 1970). The sensitivity to CO$_2$ thus appears to rely on diffusion of molecular CO$_2$ into the vascular wall where the resultant shift in extracellular pH drives changes in smooth muscle
tone (Lassen, 1968). That CBF is a function of PaCO$_2$ rather than arterial pH is further supported by the present data where CBF at HA was equivalent to SL despite chronic alkalosis, indicating CBF CO$_2$ sensitivity is reset over time at HA.

Conversely, ventilatory sensitivity and MAP sensitivity both increased at HA. Figure 6.3 shows the reduction in buffering capacity at HA was particularly manifest in jugular venous blood, perhaps reflecting differential buffering on the tissue/venous side of the cerebral circulation. The pH, PCO$_2$, and PO$_2$ of jugular venous blood is equivalent to that of cerebral spinal fluid and is a much closer approximation of brain tissue (Bradley & Semple, 1962), and the ventilatory response to CO$_2$ is principally a function of the central chemoreceptor environment (Ainslie & Duffin, 2009). The significant increase in the slope of the CO$_2$-H$^+$ relationship in jugular venous blood therefore provides a likely explanation for the augmented ventilatory sensitivity at HA, in addition to augmented carotid body sensitivity, although the nature of the interaction between peripheral and central chemoreceptors are poorly understood (Duffin & Mateika, 2013; Wilson & Day, 2013).

6.5.3 Cerebral blood flow and arterial blood gases

Hypoxia below a PaO$_2$ ~50 mmHg is a potent vasodilator of the cerebral circulation; however, the present study clearly demonstrates the comparatively greater importance of CO$_2$ in regulating CBF. For example, at SL hypocapnia reduced CBF equally in normoxia and hypoxia. In contrast, during normocapnia at HA returning PaO$_2$ to SL values decreased CBF and under hypercapnic conditions normalization of PaO$_2$ caused no uniform change in CBF as CBF was already elevated by ~80%. Indeed, oxygen delivery is well maintained during even severe hypoxia (PaO$_2$ 36 ± 4.3 mmHg; reviewed Ainslie, 2014, In Press), whereas it becomes significantly attenuated during hypocapnia at both SL and HA (present data, and Willie et al., 2012). Yet despite the decreased delivery, EF increases proportionally to maintain cerebral O$_2$ delivery. To our knowledge, this is the first direct evidence that informs previous observations of “graying” of vision, carpal-pedal spasm, and profound sleepiness during severe euoxic hypocapnia (PaCO2 16.6 ± 1.7 mmHg Willie et al., 2012), suggesting these symptoms are due to local vasodilation in brain regions associated with these symptoms but due to global reductions in O$_2$ delivery.
6.5.4 Relationship between enhanced MAP reactivity and CBF

We observed a greater increase in MAP for a given increase in PaCO$_2$ or [H+] at HA, likely reflecting the additive chemoreceptor stress of hypercapnia in background hypoxia. This is evidenced by the fact that removal of the hypoxic stimulus during hypercapnia at HA significantly attenuated the increase in MAP (euxoxic hypercapnia: +28.4 ± 11%, versus hyperoxic hypercapnia: +13.7 ± 10%). In fact, the hyperoxic hypercapnia at HA elevated MAP to a similar extent as did SL euoxic hypercapnia (HA ΔMAP 13.7 ± 10%; SL ΔMAP 12.3 ± 9.0%).

Despite the greater MAP reactivity at HA, CBF was altered less for a given change in MAP at HA relative to SL. This suggests that the intrinsic cerebrovascular mechanisms serving to buffer changes in perfusion pressure – conventionally termed cerebral autoregulation – were not impaired but were in fact enhanced. Many studies have concluded that autoregulation is impaired at HA (Subudhi et al., 2009; Subudhi et al., 2013) (Ainslie et al., 2007; Jansen et al., 2007; Van Osta et al., 2005), although this is not a universal finding (Smirl et al., 2014). A number of studies have found an important function of the sympathetic nervous system in attenuating surges in CBF (Cassaglia et al., 2009; Cassaglia et al., 2008a; Cassaglia et al., 2008b; Mayhan et al., 1987; Tzeng et al., 2010a). Muscle sympathetic activity is increased at HA (Hansen & Sander, 2003; Reeves et al., 1992) and, if extrapolated to the cerebral circulation, we speculate that this is responsible for the augmented cerebral autoregulation to increased MAP we observed during rising PaCO$_2$ at HA. There did, however, appear to be a within-individual relationship between MAP and CBF reactivity increase at HA because ΔMAP reactivity (from SL to HA) was significantly related to the ΔCBF reactivity ($R^2 = 0.47$), likely reflecting the influence of perfusion pressure on CBF.

6.5.5 Velocity vs. flow CVR

Transcranial Doppler ultrasound remains a popular tool for the estimation of CBF. Its use assumes a constant diameter of the insonated vessel, an assumption we have previously found appropriate within a relatively narrow range of arterial blood gases (Willie et al., 2012). During extreme changes in arterial blood gases (Giller et al., 1993), exposure to high altitude (Wilson et al., 2011; Willie et al., 2014a) or following glycerol trinitrate administration (Hansen et al., 2007) the middle cerebral artery dilates. Herein we show that
hypercapnic CVR estimated by transcranial Doppler ultrasound of the middle cerebral artery is approximately half of that based on total CBF, consistent with dilation of the vessel, and indicating that previous measurements of hypercapnic CVR at HA have been grossly underestimated (Fan et al., 2010a; Fan et al., 2013; Lucas et al., 2011; Jensen et al., 1996).

6.5.6 Comparison to other studies
To our knowledge this is the first study to collect arterial blood gases (and IJV blood gases) during a series of steady-state blood gas permutations at both SL and HA. Collection of arterial blood allows for precise quantification of reactivity to changes in blood gases compared to commonly used estimation by end-tidal PCO₂ and PO₂. We observed an increased PaCO₂ – P_end-tidal CO₂ gradient that increased from -2.1 ± 1.5 at SL baseline to -4.0 ± 2.2 at HA baseline, ranging at HA from -2.7 ± 1.3 during -10 mmHg hypocapnia to -5.1 ± 2.1 during hypercapnia (data not shown; to be published in a future manuscript). This finding has important implications for interpretation of previous studies where arterial blood gases were unknown during the CO₂ manipulation (Fan et al., 2010a; Fan et al., 2013; Lucas et al., 2011; Rupp et al., 2014). Contrary to the present data, some studies have reported a lower hyper- than hypocapnic CVR at HA (Lucas et al., 2011; Jansen et al., 1999). Overestimation of PaCO₂ in the hypercapnic range may provide an explanation for this finding, as it would produce an artificially low hypercapnic CVR. Figure 6.7 shows how this error is further exacerbated by MCAv-based underestimations of CBF and likely explains why MCAv hypercapnic CVR was not different from hypocapnic CVR at HA.

6.5.7 Cerebral metabolism during changes in blood gases
Our finding of reduced CMR for glucose and lactate during hypercapnia at SL is the first reported in conscious humans. Cohen et al. (1964) similarly found a reduced rate of glucose metabolism with hypercapnia during halothane-induced anesthesia in healthy men. Studies in animals later suggested this is due to inhibition of phosphofructokinase by CO₂ per se and consequent inhibition of glycolysis (Borgstrom et al., 1976; Miller et al., 1975). In the present study during hypercapnia at SL some individuals showed a small efflux of glucose from the brain. To our knowledge this has not been previously demonstrated. Although our methodology cannot elucidate the mechanisms responsible, hypercapnia increases the capillary permeability to glucose and amino acid transport in humans (Hertz & Paulson,
Glucose transport is bidirectional with similar transport velocities (Pardridge & Oldendorf, 1975). Impaired glycolysis during steady state hypercapnia increases the intracellular concentration of glucose in rats (Folbergrová et al., 1975) and in conjunction with increased transport of alternative metabolic substrates (e.g., amino acids) it is theoretically possible the gradient for cerebral glucose metabolism was inverted. Given we observed no negative glucose arteriovenous differences at HA we speculate a higher degree of absolute hypercapnia is necessitated to elicit such profound changes to cerebral metabolism. But, studies on human cerebral metabolism during hypercapnia have focused exclusively on CMRO$_2$ (Chen & Pike, 2010; Jain et al., 2011; Xu et al., 2011). The study of Hertz and Paulson (1982) is the only other study, to our knowledge, assessing cerebral substrate metabolism during acute hypercapnia in conscious humans (who required carotid angiography). They reported a decrease in mean a-vGlucose but there was no mention of a negative a-vGlucose difference in any subjects despite a similar magnitude of hypercapnia. Moreover, acute and chronic hypercapnia actually increased CMR$_{\text{glucose}}$ in sheep (Yang & Krasney, 1995) and had no effect on CMR in pigs (van Hulst et al., 2004). These results are difficult to reconcile but likely reflect known between-species anatomical and physiology variability, as well as differing methodologies. Regardless, a definitive study in humans utilizing advanced imaging and tracer substrate modalities during suitably elevated PaCO$_2$ has not been conducted.

### Technical limitations

Our intention was to measure regional CBF in the internal carotid and vertebral arteries using Duplex ultrasound, a technique we have previously found to reliably quantify CBF during experimentally altered arterial blood gases (Ainslie et al., 2013; Willie et al., 2012) and during ascent to 5050 m on this same expedition (Willie et al., 2014a). We impose strict requirements for image quality to ensure all factors are kept constant for repeated measures within subjects (e.g., section of vessel, steering angle etc.) and carefully discard images that do not meet these standards. Such standards are difficult – if not impossible - to attain when very high ventilation recruits accessory neck muscles such as during severe hypo- or hypercapnia (especially at HA). With the numerous blood gas permutations, the repeated measures design in the field at 5050 m, and the loss of one sonographer and two subjects
early in the study at 5050 m, we were unable to produce enough measures that passed our quality control to report repeatedly measured regional CBF changes within individuals for all blood gas permutations at both altitudes. We consequently chose to use those accurate measures of total CBF we did collect, to estimate CMRO₂ in order to quantify CBF by the Fick principle in all subjects for every blood gas permutation.

Previous studies have often used a fixed CMRO₂ (typically 3.2 ml/100g/min Kety & Schmidt, 1948a) in order to estimate CBF from arterial and cerebral venous samples (e.g., Peebles et al., 2008) based on evidence that CMRO₂ remains constant during profound changes in arterial blood gases (Kety & Schmidt, 1948a; Wasserman & Patterson, 1961)). In the subjects in whom we successfully measured total CBF by Duplex ultrasound we observed a trend for CMRO₂ to increase \((P = 0.35)\) at 5050 m, and in 4 subjects CMRO₂ varied inversely with changes in PaCO₂ at both elevations. We pooled CMRO₂ measures at SL and HA \((N = 7\) with different subjects contributing a different number of values to the mean) and calculated CBF from these SL and HA mean CMRO₂ values. If CMRO₂ does, in fact, vary inversely with PaCO₂ than it is possible we under and over estimated CBF values during hypocapnia and hypercapnia, respectively; indeed, there is some evidence for small (<10%) increase and decrease in CMRO₂ with moderate hypo- and hypercapnia respectively (Chen & Pike, 2010; Jain et al., 2011). However, our CVR values at SL are highly consistent with previous reports (Willie et al., 2012) (Ide et al., 2003a; Sato et al., 2012) and we do not think that subtle under and over estimated CBF values during hypocapnia and hypercapnia detract from our main conclusions.

6.6 Conclusion

Despite some loss of buffering capacity and left shift of the pH-H⁺ relationship that produced a significantly greater change in pH for a given change in CO₂ at HA, we observed no alteration to cerebrovascular responses to CO₂ at HA. This was in contrast to ventilatory and MAP reactivities to CO₂, both of which increased at HA, but seemingly not due to the change in acid-base buffering, but rather to sensitization by background hypoxia. Importantly, we report evidence that cerebral autoregulation to hypercapnia-induced increases in MAP is effective at HA, a finding that challenges a number of previous data suggesting the opposite but confounded by the use of TCD and largely spontaneous analysis of dynamic
autoregulation between MAP and MCAv. Indeed, MCAv likely underestimated cerebrovascular responses at HA, consistent with a number of recent reports that the vessel diameter is not, in fact, static as is so often assumed. Finally, our data indicate that cerebral metabolism is altered with changes in blood gases – particularly that glucose metabolism is altered with changes in PaCO$_2$. That brain glucose metabolism may be altered by respiratory acidosis and alkalosis is a fascinating phenomenon that due to its practical application to various disease states most certainly warrants further investigation.
Chapter 7: Brain blood flow in elite breath-hold divers during changes in arterial blood gases

7.1 Summary
Cerebral blood flow (CBF) is principally regulated by the partial pressures of arterial oxygen (PaO$_2$) and carbon dioxide (PaCO$_2$), perfusion pressure, and autonomic nervous activity. The study of CBF during extreme changes in arterial blood gases is limited and would inform our understanding of brain regulatory mechanisms during pathophysiological events involving life-threatening hypoxia. Breath-hold divers are capable of volitionally attaining levels of hypoxia and hypercapnia beyond the tolerable limits of most humans, but the mechanisms by which this is possible are unknown. Arterial blood gases, regional CBF (vascular ultrasound) and cardiorespiratory metrics were measured during maximum dry apneas in seventeen elite breath-hold divers. End-tidal forcing (“clamp”) was then used to replicate an identical temporal pattern of PaO$_2$ and PaCO$_2$ while the subject was breathing. Maximum apnea times ranged from 212 - 435 seconds (mean 316 ± 60 sec) and yielded end-apnea PaO$_2$ ranging from 23 mmHg to 37mmHg (mean 30 ± 7 mmHg). Clamp replicated the arterial blood gas profile during the apnea with near-identical levels of, PaO$_2$ and PaCO$_2$ that were matched within 3 mmHg throughout the protocol. The increase in mean arterial pressure was greater during apnea than during clamp reaching +54 ± 24% and 34 ± 26%, respectively. Although CBF increased by ~90 ± 30% at end-apnea, it increased similarly during the clamp. Despite 40-50% reductions in arterial oxygen content, the elevations in CBF maintained cerebral oxygen delivery. Cerebral oxygen delivery is maintained during prolonged apnea despite profound hypoxaemia in elite breath hold divers by commensurately increased CBF. Apnea produced larger increases in mean arterial pressure than clamp, suggesting apnea per se augments sympathetic nerve activity. CBF was buffered in the face of escalating perfusion pressure during apnea indicating maintained cerebral autoregulation during maximum apnea.

7.2 Introduction
The regulation of cerebral blood flow (CBF) with hypoxia is complicated due to the ventilatory response to hypoxia and resultant hypocapnia. Hypoxia per se causes cerebral vasodilation (and increases CBF), whereas hypocapnia induces vasoconstriction and a
reduction in CBF (Kety & Schmidt, 1948a; Wolff & Lennox, 1930). Thus, the CBF response to hypoxia is a balance between the degree of hypoxia, which is influenced by an individual’s ventilatory sensitivity to hypoxia, and the cerebrovascular reactivity to the hypocapnia (Lucas et al., 2011; Willie et al., 2014b). Indeed, CBF was reported to increase by ~200% at 7950 m (SaO₂ ~ 65%) despite profound hypocapnia (Wilson et al., 2011). Data on CBF in humans is generally in the context of either hypobaric hypoxia (Severinghaus et al., 1966; Pagani et al., 2000; Subudhi et al., 2014; Willie et al., 2014a; Wilson et al., 2011) or by various means of altering inspired gases to induce acute poikilocapnic or isocapnic hypoxia (Ogoh et al., 2013; Willie et al., 2012; Lewis et al., 2014b). Recently, during various hypoxic conditions, regional differences have been reported reflecting a preferential increase in flow to the brainstem (as indexed via ultrasound measures of the vertebral artery) (Ogoh et al., 2013; Willie et al., 2012).

Elite breath-hold divers experience a similar or greater magnitude of transient hypoxemia as do individuals exposed to very high altitudes, but with concomitant hypercapnia – two factors that together can generate an extremely potent cerebral vasodilatory stimulus (Cross et al., 2013; Palada et al., 2007). Prolonged apneas of >4 minutes may attain values of arterial partial pressures of oxygen (PaO₂) and carbon dioxide (PaCO₂) of ~19-25 mmHg and ~30-50 mmHg, respectively (Lindholm & Lundgren, 2006; Overgaard et al., 2006). The current world record for static apnea is an outstanding 11 minutes 35 seconds; such apnea durations likely result in even greater changes to arterial blood gases; extremely low peripheral oxyhemoglobin saturations (SaO₂%) are regularly reported anecdotally (personal correspondence, Croatian National Free-diving Coach). Elite breath-hold divers are therefore an inimitable model for assessing the cerebrovascular response to extreme changes in arterial blood gases because of their unique ability to generate and tolerate profound hypoxemia.

Breath hold diving (BHD) includes a number of related disciplines characterized by prolonged apneas while either floating face down in water (static), swimming a maximum distance under water on one breath (dynamic), descending to a maximum depth and returning to the surface on one breath (in which there are a range of disciplines), and for the collection of food (e.g., spear fishing) (Dujic et al., 2013). Elite BHD athletes therefore regularly endure hypoxia and acidosis at the limits of human physiological tolerance. Moreover, in
disciplines such as spear fishing, intermittent apneas over the course of a 5-6 hour competition may yield 60-90 minutes total apnea time (Dujic & Breskovic, 2012).

A significant physiological characteristic of maximum apnea is the occurrence of involuntary breathing movements (IBM) – increasingly powerful contractions of the respiratory muscles that result in elevations in venous return and cardiac output. The profound changes in arterial blood gases with prolonged apnea cause CBF (as indexed by velocity in the middle cerebral artery) to increase by > 100%, but IBM in conjunction with progressive increases in sympathetic nervous activity (Heusser et al., 2009) and mean arterial pressure (MAP) (Palada et al., 2008) may additionally facilitate cerebral perfusion (Cross et al., 2013). Conversely, cerebral sympathetic nerve activity in other paradigms that induced transient surges in MAP (e.g., rapid eye movement sleep) attenuates surges in CBF (Cassaglia et al., 2009; Cassaglia et al., 2008a), and chemoreflex augmentation of muscle sympathetic nerve activity is nearly double during apnea than with breathing for the same values of PaO₂ and PaCO₂ (Steinback et al., 2010). The specific effects of the apnea-induced changes in blood gases versus the combined blood gas and circulatory effects of IBM on CBF per se remain ambiguous.

To explicate the relative impact of arterial blood gases and IBM on CBF we measured arterial blood gases and regional CBF throughout maximum dry static apneas in elite BHD athletes. On an individual basis, we then replicated each individual breath-hold time course of arterial blood gas “profiles” (i.e., declining PaO₂, increasing PaCO₂) during breathing by end-tidal clamping. We hypothesized that the magnitude of CBF increase would be greater during apnea than during clamped breathing due to larger increases in MAP, and that the duration of BH would be related to the balance of hypoxemia to acidosis.

7.3 Methods

7.3.1 Participants
Seventeen competitive and elite breath-hold divers (4 female; age 29.5 ± 6.5 years; BMI 24 ± 2.5 kg/m²) were selected from the Croatian cities of Split and Zagreb, most of whom were being coached by the Croatian national apnea coach at the time of testing and had been practicing competitive breath hold diving for 2-8 years (mean 4.7 ± 2.5 years). The majority
of subjects were competitive in pool disciplines of free diving, with half additionally involved in various depth disciplines (and consequent regular exposure to high pressure). Seven of the subjects were world-class free diving competitors, having placed top-ten within the last three years in international competition in at least one event. Three subjects had recently set new official world records. Subject characteristics are presented in Table 7.1. The experimental procedures were approved by the ethical committee of the University of Split School and Medicine, and by the Clinical Research Ethics Board of the University of British Columbia, and conformed to the standards set by the Declaration of Helsinki.

Table 7.1. Anthropometric and performance characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.9 ± 6.8</td>
<td>19-48</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.2 ± 16</td>
<td>53.2-98.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180 ± 12</td>
<td>156.8-194</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 2.5</td>
<td>19.1-27.1</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>6.8 ± 1.4</td>
<td>3.9-8.5</td>
</tr>
<tr>
<td>FVC₁ (L)</td>
<td>5.4 ± 1.0</td>
<td>3.9-8.5</td>
</tr>
<tr>
<td>Body fat index (%: body fat/kg)</td>
<td>10.6 ± 2.5</td>
<td>7.3-17.3</td>
</tr>
<tr>
<td>VO₂ max (L/min)</td>
<td>4.4 ± 1.1</td>
<td>2.5-5.8</td>
</tr>
<tr>
<td>Personal best static apnea (sec)</td>
<td>377 ± 75</td>
<td>305-558</td>
</tr>
<tr>
<td>Personal best dynamic apnea (m)</td>
<td>187 ± 38</td>
<td>125-281</td>
</tr>
<tr>
<td>Years breath-hold diving</td>
<td>4.7 ± 2.5</td>
<td>2-8</td>
</tr>
<tr>
<td>Breath hold duration (sec)</td>
<td>316 ± 60</td>
<td>212-435</td>
</tr>
<tr>
<td>End apnea End-tidal PO₂ (mmHg)</td>
<td>37 ± 14</td>
<td>24-77</td>
</tr>
<tr>
<td>End apnea End-tidal PCO₂ (mmHg)</td>
<td>45 ± 7</td>
<td>34-60</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index; FVC, forced vital capacity, FVC₁, forced vital capacity in one second; body fat index, based on 7-site skin folds; VO₂ max, maximal oxygen uptake (running); static apnea performed floating prone in a pool; dynamic apnea performed in a pool.
7.3.2 Experimental Design

Subjects visited the laboratory on one occasion. The procedures were explained in the subjects’ first language and signed informed consent was obtained. Standard pulmonary function measures were first collected (Table 7.1) before the subject lay supine for the placement of a 20-gauge radial arterial catheter (Arrow, Markham, Ontario, Canada) by ultrasound guidance under local anesthesia (1% lidocaine). The catheter was attached to an in-line waste-less sampling system and a pressure transducer at the level of the right atrium (Edwards Lifesciences VAMP, and TruWave transducer, Irvine, CA, USA). After cannulation, subjects rested for ~30-minutes during the set up of the monitoring equipment.

Breath-hold divers typically complete a number of preparatory apneas before attempting a maximum apnea. All subjects therefore completed two such preparatory apneas with two minutes of resting breathing between. The first apnea was held long enough to elicit seven IBM, the second apnea was held through ten IBM. Then, following seven minutes of rest measurements were obtained while the subject completed a maximum apnea with verbal encouragement from their coach. Arterial samples were drawn every 30-seconds starting 30-60 seconds before apnea onset, to 2-minutes following completion of apnea. Following a full recovery of at least 30-minutes rest, the end-tidal clamping procedure began. Based on the PaO₂ and PaCO₂ profile determined during the apnea, the exact time profile was followed for each individual (see end-tidal forcing below; see Figure 7.1), and verified with arterial samples every 30-seconds.
7.3.3 Measurements

7.3.3.1 Cerebrovascular Measures

Blood velocities in the right middle cerebral artery (MCAv; measured 1-cm from anterior cerebral artery-MCA bifurcation) and left posterior cerebral (PCAv; P1 segment) artery were
measured by 2MHz pulsed transcranial Doppler ultrasound (Spencer Technologies, Seattle, WA, USA). Signal quality was optimized using standardized search techniques as previously described that produce test-retest reliability of ~3% and 2% for MCAv and PCAv, respectfully (Willie et al., 2011b).

Extracranial blood flow in the right internal carotid (ICA) and left vertebral (VA) arteries were measured with Duplex vascular ultrasound. Right ICA was measured ~2 cm from the carotid bifurcation with a 10 MHz linear array probe (Terason 3000, Teratech, Burlington, MA), and the left VA at the C5-C6 or C5-C4 intravertebral space with a variable frequency high resolution harmonic probe (Vivid Q, GE, Fairfield, CT, USA). Great care was taken to ensure the same settings and anatomical position of measurement within subjects. With the steering angle set to 60-degrees, the sample volume was placed in the center of the vessel adjusted to cover the vascular lumen. The entire breath hold or clamp protocol was collected as an AVI file with analyses of simultaneous luminal diameter and velocity over ≥ 10 cardiac cycles every 30-seconds subsequently completed offline at 30 Hz using custom designed software (Black et al., 2008). This semi-automated method of analysis results in better reproducibility of baseline measurements, giving test-retest reliability for ICA and VA flows of ~5% and ~11%, respectively (Willie et al., 2012).

7.3.3.2 Hemodynamics

Manual blood pressure measures were collected before the start of each protocol. Three-lead electrocardiogram (ECG) and beat-to-beat blood pressures were collected at 1 kHz by both finger photoplethysmography (Finometer, Finipress medical systems, Amsterdam, Netherlands) and direct intra-radial arterial pressure. However, due to the frequency of arterial blood sampling, radial artery pressure could only be measured for ~5-10 seconds every 30-seconds. Heart rate (HR) was calculated online from the R-R interval measured by ECG.

7.3.3.3 Ventilation and end-tidal clamping

Subjects breathed through a mouthpiece for collection of end-tidal gases before and at the break point of apnea, and during the entire end-tidal clamping procedure (see below). Gases were sampled from the mouthpiece and analyzed by a calibrated gas analyzer (ML206
ADinstruments, Colorado Springs, CO, USA) and respiratory flows by pneumotachograph (HR 800L, Hans Rudolph, Shawnee, KS, USA). Custom software (written in Labview, Austen, TX, USA) determined the breath-by-breath tidal volumes and end-tidal partial pressures of oxygen and carbon dioxide (PETO₂ and PETO₂). A portable end-tidal forcing system prospectively delivered inspired gases to clamp PETO₂ and PETO₂ at desired levels. Independently controlled solenoid valves delivered the desired volumes of O₂, CO₂, and N₂ as determined by an error reduction algorithm incorporating PETO₂, PETO₂, and inspiratory and expiratory tidal volume from the last breath (AirForce, GE Foster, University of British Columbia – Okanagan, Kelowna, Canada).

7.3.3.4 Calculations
Mean values for MAP, HR, MCAv, PCAv were averaged over 30 seconds at the time of each arterial blood draw, from which values of pH, PaO₂, PaCO₂, and SaO₂ were obtained. Cerebrovascular conductance was calculated individually for the ICA and VA as MAP/flow through the respective vessel. Because SaO₂ and PaCO₂ change concomitantly during apnea, cerebrovascular reactivity was calculated within individuals as the slope of the linear regression between flow and the ratio SaO₂/PaCO₂. SaO₂, instead of PaO₂, was used because CBF is linearly related to SaO₂ (whereas the CBF-PaO₂ relationship is exponential) and because SaO₂ provides a clinically meaningful but more easily obtained metric of arterial oxygen content than does PaO₂.

Arterial content of oxygen (CaO₂) was calculated by:

\[
CaO₂ (\text{mL} \cdot \text{dL}^{-1}) = [\text{Hb}] \cdot 1.36 \cdot \frac{\text{SaO}_2(\%)}{100} + 0.003 \cdot \text{PaO}_2
\]

[1]

7.3.3.5 Estimations of volumetric cerebral blood flows
All but one of the subjects demonstrated IBM that elicited severe contractions of the accessory breathing muscles in the neck and chest. Toward the end of apnea (when IBM became most severe) it was consequently impossible in most individuals to collect accurate ultrasound images and Doppler waveforms of ICA and VA vessel diameter and blood velocity. Likewise at the extremely high rates of ventilation near the end of clamp accurate
neck ultrasound was not possible. For each subject a regression equation was thus calculated between QICA and MCAv, and between QVA and PCAv during each condition (apnea/clamp) and used to estimate the missing flow values. Of the total 768 cerebral blood flow bins collected across both conditions, 558 were quantified by neck vascular ultrasound, with the remaining 27% imputed from these regression equations. R² values were 0.78 ± 0.17 and were not significantly different between arteries or conditions.

7.3.3.6 Analysis
That data were normally distributed was confirmed with a Shapiro-Wilk test. Baseline cardiovascular variables did not differ between male and female subjects and data were therefore pooled. To assess the relationship between changes in blood gases and CBF, the slopes of the regressions between the ratio SaO₂/PaCO₂ and total CBF were calculated for each individual duration apnea and clamp. These values were compared by student’s paired t-test. Huynh-Feldt corrected two-way repeated measured ANOVA was used to assess interactions between conditions (apnea versus clamp) and time-point of apnea/clamp. A priori defined comparisons relative to baseline, and between apnea and clamp, were tested by one-way repeated measures ANOVA (Dunnet’s post-hoc tests) and paired t-tests, respectively (two-tailed, α = 0.05). Analyses were completed with SPSS 16.02 (SPSS Inc.) and Prism 5.0 (GraphPad Software Inc., La Jolla, CA). All values in text and tables are mean ± SD; figures show mean ± SEM.

7.4 Results
Every subject completed the apnea and clamp protocols, however, in two subjects neither ICA nor VA blood flow was successfully measured due to poor quality images. These two subjects were therefore excluded from all further analyses. All data presented are based on n = 15 (4 female; age 30 ± 6.8 years; BMI 23.6 ± 2.5 kg/m²). Although females had significantly smaller anthropometric variables and apnea durations, the level of hypoxaemia at end-apnea was not different between sexes, and consequently subjects were pooled.

7.4.1 Efficacy of clamping
The arterial blood gas profiles between apnea and clamping were generally very well matched. Due to the nature of end-tidal forcing (and unknown end-tidal to arterial
differences), precisely matching the very low PaO\textsubscript{2} values attained during apneas was difficult with the extremely large ventilations and necessarily severely low fraction of inspired oxygen. Nonetheless, PaO\textsubscript{2} was lower by 2.7 mmHg in clamp only at 80%; SaO\textsubscript{2} was lower in clamp at 80% and 100%; PaCO\textsubscript{2} was slightly higher (+2.6, +3.2 mmHg, respectively) during clamp at 80 and 100%; and, pH was slightly lower in apnea at 40% and 60% (Figure 7.1, Table 7.2). These differences were therefore small relative to the magnitude of hypoxic stimulus generated with both apnea and clamping (see Table 7.2).

### 7.4.2 Cardiovascular and respiratory effects of apnea and clamping

Table 7.2 displays the cardiovascular variables during apnea and clamp. All subjects hyperventilated to some extent prior to apnea; PaCO\textsubscript{2} before apnea was 34.2 ± 7.1 mmHg versus 37.1 ± 7.4 mmHg before clamp. All other baseline variables shown in Table 7.2 were statistically similar between apnea and clamp. Maximum apnea times ranged from 212 - 435 seconds (mean 316 ± 60 sec) and yielded end-apnea PaO\textsubscript{2} ranging from 23 mmHg to 36.9 mmHg (mean 29.5 ± 6.5 mmHg). Single-breath PET\textsubscript{O\textsubscript{2}} and PET\textsubscript{CO\textsubscript{2}} were 37 ± 14 mmHg and 45 ± 7 mmHg, respectively (Table 7.1), over estimating PaO\textsubscript{2} and under estimating PaCO\textsubscript{2} – the mean ET-aPO\textsubscript{2} and ET-aPCO\textsubscript{2} differences were 7.5 ± 12.9 mmHg and -5.1 ± 6.1 mmHg, respectively. All but one subject had involuntary breathing movements that began at 50 ± 11% of the total apnea time.

With onset of apnea all subjects displayed a transient decrease in MAP (-11.9 ± 12%), which remained below baseline at 10% apnea duration in all but three subjects. In all subjects MAP increased throughout the apnea to a maximum of 54 ± 24% above baseline values, a significantly greater increase than during clamp where MAP increased 34 ± 26%; in fact, the increase in MAP was greater in apnea at all times from 20% through 120% of apnea/clamp (Figure 7.2). At 80% and 100% apnea HR decreased to below baseline values at which points it was significantly less than HR during clamp (Table 7.2).
<table>
<thead>
<tr>
<th>Condition</th>
<th>N=15</th>
<th>Baseline</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>80%</th>
<th>100%</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>Apnea</td>
<td>7.48 ±0.06†</td>
<td>7.47 ± 0.05</td>
<td>7.42 ± 0.04*†</td>
<td>7.39 ± 0.03*†</td>
<td>7.37 ± 0.03*</td>
<td>7.35 ± 0.03*</td>
<td>7.45 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>7.45 ± 0.06</td>
<td>7.48 ± 0.06</td>
<td>7.45 ± 0.05</td>
<td>7.41 ± 0.03*</td>
<td>7.36 ± 0.04*</td>
<td>7.35 ± 0.04*</td>
<td>7.44 ± 0.05</td>
</tr>
<tr>
<td><strong>PaCO₂</strong> (mmHg)</td>
<td>Apnea</td>
<td>34.2 ± 7.1†</td>
<td>32.8 ± 6.0</td>
<td>38.2 ± 5.3*</td>
<td>44.6 ± 4.9*†</td>
<td>49.0 ± 5.7*†</td>
<td>51.0 ± 6.7*†</td>
<td>35.1 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>37.1 ± 7.4</td>
<td>34.3 ± 6.7</td>
<td>37.8 ± 6.2</td>
<td>43.0 ± 4.8*</td>
<td>51.8 ± 6.9*</td>
<td>54.3 ± 7.0*</td>
<td>36.6 ± 7.5</td>
</tr>
<tr>
<td><strong>PaO₂</strong> (mmHg)</td>
<td>Apnea</td>
<td>104 ± 19.2</td>
<td>109 ± 11.4</td>
<td>81.9 ± 11.0*</td>
<td>55.7 ± 7.6*</td>
<td>40.0 ± 6.6*†</td>
<td>29.5 ± 6.5*</td>
<td>103 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>103 ± 14.7</td>
<td>111 ± 20.9</td>
<td>77.4 ± 16.9*</td>
<td>54.9 ± 9.0*</td>
<td>37.0 ± 6.6*</td>
<td>26.9 ± 8.9*</td>
<td>102 ± 22.2</td>
</tr>
<tr>
<td><strong>SaO₂ (%)</strong></td>
<td>Apnea</td>
<td>98.3 ± 2.0</td>
<td>98.9 ± 0.5</td>
<td>96.7 ± 1.7</td>
<td>89.3 ± 4.6*</td>
<td>75.4 ± 7.2*†</td>
<td>56.7 ± 11.3*†</td>
<td>98.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>98.4 ± 1.1</td>
<td>98.8 ± 1.1</td>
<td>95.9 ± 3.2</td>
<td>89.5 ± 4.8*</td>
<td>70.7 ± 8.5*</td>
<td>48.3 ± 16.8*</td>
<td>97.7 ± 3.3</td>
</tr>
<tr>
<td><strong>HR</strong> (b/min)</td>
<td>Apnea</td>
<td>77 ± 14</td>
<td>83 ± 22</td>
<td>78 ± 19</td>
<td>69 ± 16</td>
<td>61 ± 16*†</td>
<td>67 ± 13†</td>
<td>76 ± 18</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>74 ± 12</td>
<td>76 ± 14</td>
<td>73 ± 14</td>
<td>75 ± 14</td>
<td>84 ± 11*</td>
<td>90 ± 11*</td>
<td>77 ± 16</td>
</tr>
<tr>
<td><strong>MAP</strong> (mmHg)</td>
<td>Apnea</td>
<td>89.3 ± 9.1</td>
<td>96.8 ± 10†</td>
<td>104 ± 13*†</td>
<td>115 ± 14*†</td>
<td>130 ± 14*†</td>
<td>135 ± 19*†</td>
<td>96.8 ± 13†</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>83.7 ± 13</td>
<td>82.9 ± 13</td>
<td>85.4 ± 3.0</td>
<td>92.3 ± 6.3*</td>
<td>108 ± 8.0*</td>
<td>111 ± 8.5*</td>
<td>81.8 ± 0.7</td>
</tr>
</tbody>
</table>

PaCO₂ and PaCO₂₂, partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure. * P < 0.05 vs. baseline. † P < 0.05 vs. Clamp.
Table 7.2 Cardiovascular variables (continued).

<table>
<thead>
<tr>
<th></th>
<th>Condition</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.507</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>0.011</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>0.169</td>
<td>&lt;0.001</td>
<td>0.892</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>0.015</td>
<td>&lt;0.001</td>
<td>0.037</td>
</tr>
<tr>
<td>HR (b/min)</td>
<td>0.045</td>
<td>0.098</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PaCO₂ and PaCO₂, partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure. * $P < 0.05$ vs. baseline. † $P < 0.05$ vs. Clamp.
7.4.3 Cerebrovascular effects of apnea and clamping

Global CBF (gCBF) began to increase immediately following the drop in MAP with apnea onset. During clamp gCBF did not increase significantly until 60% of clamp duration. Conversely, during apnea, gCBF was significantly increased from baseline by 40% apnea duration (Figures 7.2 and 7.3). Until 60% duration, apnea caused greater increases in gCBF than did clamp, at which point the CBF change became similar between interventions, despite the consistently greater MAP in apnea than clamp. Flow in the ICA and VA, as well as MCAv and PCAv showed similar trends for both apnea and clamp (Table 7.3). Cerebral oxygen delivery was never attenuated during either apnea or clamp i.e., the progressively increasing CBF compensated for the decrease in CaO$_2$; indeed, cerebral O$_2$ delivery significantly increased from baseline at 60% and 80% duration of apnea, and at 80% duration of clamp. The slopes of SaO$_2$/PaCO$_2$ versus total $\Delta$CBF (%) were not different between apnea and clamp (apnea 79.9 ± 21 mmHg$^{-1}$; clamp 73.3 ± 24 mmHg$^{-1}$). SaO$_2$, PaCO$_2$, and $\Delta$MAP during both apnea ($R^2 = 0.54, 0.62, 0.57$) and clamp ($R^2 = 0.57, 0.37, 0.61$) were positively related to the increase in CBF ($P < 0.05$; Figure 7.4).
Table 7.3. Cerebrovascular variables.

<table>
<thead>
<tr>
<th>Condition (sample size)</th>
<th>Baseline</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>+QICA, mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (12)</td>
<td>189 ± 60†</td>
<td>181 ± 60†</td>
<td>265 ± 153*</td>
<td>329 ± 172*</td>
</tr>
<tr>
<td>Clamp (11)</td>
<td>244 ± 91.6</td>
<td>218 ± 75.1</td>
<td>246 ± 88.0</td>
<td>328 ± 120*</td>
</tr>
<tr>
<td>DO₂ (mL O₂/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (12)</td>
<td>38.0 ± 11.0†</td>
<td>35.3 ± 12.4</td>
<td>50.1 ± 27.0</td>
<td>59.6 ± 28.9*</td>
</tr>
<tr>
<td>Clamp (11)</td>
<td>48.0 ± 16.4</td>
<td>43.0 ± 13.63</td>
<td>47.6 ± 13.7</td>
<td>58.1 ± 19.5</td>
</tr>
<tr>
<td>CVC, mL/min/mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (12)</td>
<td>2.1 ± 0.6†</td>
<td>1.8 ± 0.5†</td>
<td>2.5 ± 1.1</td>
<td>2.8 ± 1.2*†</td>
</tr>
<tr>
<td>Clamp (11)</td>
<td>2.9 ± 0.8</td>
<td>2.7 ± 0.8</td>
<td>2.9 ± 0.9</td>
<td>3.6 ± 0.9*</td>
</tr>
<tr>
<td>+QVA, mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (13)</td>
<td>81.1 ± 32†</td>
<td>80.1 ± 29*</td>
<td>98.3 ± 32*</td>
<td>124 ± 44*</td>
</tr>
<tr>
<td>Clamp (15)</td>
<td>92.2 ± 25</td>
<td>84.7 ± 21</td>
<td>95.6 ± 27</td>
<td>122 ± 47*</td>
</tr>
<tr>
<td>DO₂ (mL O₂/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (13)</td>
<td>16.3 ± 6.4</td>
<td>15.6 ± 5.9</td>
<td>19.0 ± 6.0</td>
<td>22.3 ± 6.9*</td>
</tr>
<tr>
<td>Clamp (15)</td>
<td>18.2 ± 5.3</td>
<td>16.7 ± 4.6</td>
<td>18.2 ± 5.3</td>
<td>21.7 ± 9.3</td>
</tr>
<tr>
<td>CVC, mL/min/mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (13)</td>
<td>0.9 ± 0.3†</td>
<td>0.8 ± 0.2†</td>
<td>0.9 ± 0.2†</td>
<td>1.1 ± 0.3*†</td>
</tr>
<tr>
<td>Clamp (15)</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>MCAv, cm/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (15)</td>
<td>50.9 ± 8.6†</td>
<td>56.1 ± 12.3*</td>
<td>70.4 ± 15*†</td>
<td>85.4 ± 13.2*†</td>
</tr>
<tr>
<td>Clamp (15)</td>
<td>58.0 ± 12</td>
<td>52.5 ± 9.8</td>
<td>60.0 ± 12</td>
<td>78.4 ± 15.4*</td>
</tr>
<tr>
<td>PCAv, cm/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnea (15)</td>
<td>33.3 ± 9.3†</td>
<td>35.0 ± 9.6*</td>
<td>44.4 ± 14.2*†</td>
<td>54.6 ± 15.6*</td>
</tr>
<tr>
<td>Clamp (15)</td>
<td>38.3 ± 9.7</td>
<td>34.8 ± 6.5</td>
<td>38.9 ± 8.0</td>
<td>50.4 ± 13*</td>
</tr>
</tbody>
</table>

Values are quantified flow through the respective vessel except in most subjects at 80% and 100% where flows were estimated from the regression equation derived between QICA – MCAv and QVA – PCAv; see methods. Sample sizes vary between measured vessels due to inadequate image quality for VA and ICA measures. QICA, internal carotid artery blood flow; DO₂, Oxygen delivery through vessel; QVA, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity / MAP); * P < 0.05 vs. baseline. † P < 0.05 vs. Clamp.
Table 7.3 Cerebrovascular variables (continued).

<table>
<thead>
<tr>
<th></th>
<th>80%</th>
<th>100%</th>
<th>Recovery</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Condition</td>
<td>Time</td>
<td>Interaction</td>
<td></td>
</tr>
<tr>
<td>+QICA, mL/min</td>
<td>380 ± 167*</td>
<td>396 ± 176*</td>
<td>245 ± 128</td>
<td>0.224</td>
</tr>
<tr>
<td></td>
<td>444 ± 125*</td>
<td>446 ± 153*</td>
<td>273 ± 126</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DO₂ (mL O₂/min)</td>
<td>57.4 ± 22.8*</td>
<td>43.6 ± 17.8*</td>
<td>50.3 ± 25.8</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>61.8 ± 15.2*</td>
<td>44.3 ± 16.0*</td>
<td>53.7 ± 25.8</td>
<td>0.256</td>
</tr>
<tr>
<td>CVC, mL/min/mmHg</td>
<td>2.9 ± 1.1†</td>
<td>2.9 ± 1.3†</td>
<td>2.6 ± 1.5†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4.1 ±0.8*</td>
<td>4.0 ±1.1*</td>
<td>3.4 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+QVA, mL/min</td>
<td>145 ± 43*</td>
<td>149 ± 40*</td>
<td>103 ± 28</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>149 ± 53*</td>
<td>154 ± 52*</td>
<td>116 ± 45</td>
<td>0.421</td>
</tr>
<tr>
<td>DO₂ (mL O₂/min)</td>
<td>22.2 ± 6.2*</td>
<td>16.9 ± 5.5</td>
<td>21.2 ± 7.1*</td>
<td>0.797</td>
</tr>
<tr>
<td>CVC, mL/min/mmHg</td>
<td>1.1 ± 0.3†</td>
<td>1.1 ± 0.3†</td>
<td>1.1 ± 0.4*†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.5*</td>
<td>1.4 ± 0.4*</td>
<td>1.4 ± 0.4*</td>
<td>0.323</td>
</tr>
<tr>
<td>MCAv, cm/s</td>
<td>99.2 ± 13*</td>
<td>101 ± 12*</td>
<td>60.2 ± 14*</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>98.6 ± 12*</td>
<td>98.7 ±13*</td>
<td>59.8 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCAv, cm/s</td>
<td>64.2 ± 12.7*</td>
<td>66.9 ± 10.8*</td>
<td>41.7 ± 12.6*</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
<td>65.3 ± 13*</td>
<td>68.3 ± 12*</td>
<td>41.2 ± 12</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are quantified flow through the respective vessel except in most subjects at 80% and 100% where flows were estimated from the regression equation derived between QICA – MCAv and QVA – PCAv; see methods. Sample sizes vary between measured vessels due to inadequate image quality for VA and ICA measures. QICA, internal carotid artery blood flow; DO₂, Oxygen delivery through vessel; QVA, vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity / MAP); * P < 0.05 vs. baseline. † P < 0.05 vs. Clamp.
Figure 7.2. Percent changes in cerebral blood flow and MAP.
The top plot depicts changes in total CBF (top) during apnea (solid line) and clamp (dashed lines) against the percent duration of apnea. The bottom plot shows changes in MAP. Despite MAP being greater during apnea than during clamp at every time point, CBF is only greater 30-50% into the apnea, suggesting a cerebral autoregulatory attenuation of maximum CBF.
Figure 7.3. Cerebral blood flows over time during maximal apnea and end-tidal forcing. Apnea (left) times ranged from 212-435 seconds. During clamp (right) the identical duration and changes in arterial blood gases were replicated while the subjects breathed.

Figure 7.4. Relationship between $\Delta$CBF and $\Delta$MAP during apnea and clamp. CBF percentage change from baseline was positively related to the percentage change from baseline of MAP ($P < 0.05$) both for apnea (left) and clamp (right).

**7.5 Discussion**

This is the first study to combine measures of regional cerebral blood flow and arterial blood sampling during maximal dry apneas in elite breath hold divers, and also to precisely simulate a maximal apnea using dynamic end-tidal forcing. Our principal findings were that 1) cerebral oxygen delivery was never attenuated by maximal dry apnea as it was maintained by CBF increases of up to 100% of baseline values; 2) changes in CBF were relatively homogenous between regions perfused by the ICA and VA; and, 3) despite significantly
greater increases in MAP during apnea relative to clamped (breathing) conditions, the increase in CBF was similar suggesting that CBF regulatory mechanisms remain effective during maximal dry apnea.

7.5.1 Arterial blood gases
A number of studies have previously estimated changes in arterial blood gases based on sampling of end-tidal gases on the first breath following apnea. We observed a 7.5 ± 12.9 mmHg overestimation of PaO$_2$ by end-tidal sampling. Our P$_{ET}$O$_2$ values (37 ± 14 mmHg) were much higher than those reported in similar studies with similar breath hold durations (e.g., 26.9 ± 7.5 mmHg, 284 ± 25 seconds Lindholm & Lundgren, 2006; 26 ± 4.5 mmHg, 309 ± 38 seconds Overgaard et al., 2006) suggesting that either the subjects in these studies experienced a greater degree of hypoxaemia than our subjects, or a methodological problem with our single breath end-tidal tension method. With respect to the latter, effort was made to ensure each subject expired fully but some individuals experienced an impaired level of consciousness at end-apnea making it difficult to ensure this was the case. Given that neither of the studies above reported oxygen saturation it is difficult to further assess their degree of hypoxaemia relative to that reported here; however, certainly it can be seen in the present data that some individuals desaturated more quickly than others, so it is possible differences between cohorts account for these disparate P$_{ET}$O$_2$ findings. Regardless, arterial analysis of PO$_2$ is a far more robust measure of hypoxaemia than P$_{ET}$O$_2$ as it reflects any unquantifiable degree of shunt or ventilation-perfusion mis-match that may be manifest during apnea.

7.5.2 Regulation of CBF during apnea
Despite a number of studies assessing the relative sensitivity of the ICA and VA to changes in arterial blood gases, their heterogeneous methodologies belie definitive elucidation of regional cerebrovascular responses to hypoxia and PCO$_2$ flux. At high altitude three studies have found no difference between ICA and VA flow increase with ascent to altitudes ranging from 4300 m to 5260 m (Huang et al., 1987; Subudhi et al., 2014; Willie et al., 2014a). Conversely, sea level sensitivity to isocapnic or poikilocapnic hypoxia is greater in the VA (Willie et al., 2012; Lewis et al., 2014b). Euoxic hypocapnia also elicits greater decreases in VA flow through a broad range of PaCO$_2$ (~15-40 mmHg) (Willie et al., 2012), however this
is not a universal finding through smaller changes in PCO$_2$ (Sato et al., 2012). That ICA and VA showed similar profiles during clamp confirms their similar reactivities to simultaneous changes in PaCO$_2$ and PaO$_2$; that both vessels also had similar profiles during apnea demonstrates their additive response to altered perfusion pressure. The IBM of prolonged apnea are associated with oscillations in intrathoracic pressure that augment left ventricular stroke volume (Costalat et al., 2013; Cross et al., 2013; Palada et al., 2008), suggesting that CBF (as indexed by MCAv) is augmented by IBM during the latter half of a prolonged apnea. The present study does not support this conclusion. We were able to dissociate the effects of blood gases and IBM during apnea: CBF increased more with apnea only during the first 50% of apnea/clamp duration (preceding onset of IBM) due to the greater apnea-induced increase in MAP. Initiation of apnea decreases right heart filling (Costalat et al., 2013; Palada et al., 2008) producing a transient MAP decrease that in turn facilitates baroreflex mediated increases in sympathetic activity (Breskovic et al., 2011). Inspiration inhibits sympathetic outflow, but in the absence of ventilatory restraint, it increases unchecked until the end of apnea (Breskovic et al., 2011; Steinback et al., 2010) thus driving peripheral vasoconstriction and sustained increases in MAP. The augmentation of CBF by MAP could be interpreted to serve maintenance of cerebral oxygenation, but the present data show this has no effect on cerebral oxygen delivery at apnea termination when presumably CBF is most critical. Thus it seems IBM are not necessary for prolonged apnea – indeed, the one individual who never experienced an IBM maintained apnea for 366 seconds reaching PaO$_2$ = 27.7 mmHg. Involuntary breathing movements may therefore instead be a byproduct of chemoreceptor stimulation and consequent efferent respiratory motor output (Breskovic et al., 2012). Given that the delivery of oxygen never fell below baseline values in either vessel, the present data indicate that even during radical hypoxaemia commensurate increases in CBF sate the entire brain’s requirement for oxygen and, by extension, that oxygen delivery cannot be the mechanism stimulating apnea break point.

### 7.5.3 Apnea break point

As early as 1908, Hill and Flack (Hill & Flack, 1908) observed that – in healthy volunteers – the break point of volitional apnea was not solely a function of chemoreflex stress as identical PETO$_2$ and PETCO$_2$ values could be sustained for longer and with greater ease
during rebreathing. Indeed, our volunteers consistently noted they felt they could continue breathing at the end of clamp – despite their blood gas levels being at the same level (or, in a number of trials, even more hypoxaemic) as their apnea break point. What factors are responsible for the break point remain to be clearly explicated, but clearly involve to a large extent the athlete’s will to sustain asphyxia apnea (Dujic et al., 2013). Moreover, we could not identify a threshold PaO$_2$, PaCO$_2$, or ratio of SaO$_2$/PaCO$_2$ common amongst individuals at end apnea, though most of the subjects reached PaO$_2$ values in the mid-twenties. The factors that allow some individuals to maintain apnea longer than others is their greater oxygen stores (i.e., lung volume, blood, and tissue) and lower oxygen consumption (Lindholm & Lundgren, 2006). Therefore, while a threshold PaO$_2$ per se may not stimulate apnea break point, oxygen metabolism clearly plays a crucial role in apnea duration in elite breath hold divers.

7.5.4 Cerebral autoregulation during apnea

Despite a greater surge in MAP, and the very similar changes in PaO$_2$ and PaCO$_2$, the CBF increase during apnea was similar to that during clamp. A mechanism buffering further increases in CBF was therefore necessarily present. Cerebral autoregulation is the term given to the buffering of changes in CBF from changes in perfusion pressure, and is principally facilitated by myogenic (Tan et al., 2013; Tzeng et al., 2011) and autonomic mechanisms (reviewed in Willie et al., 2014b). Sympathetic activity restrains augmented perfusion during surges in MAP (Cassaglia et al., 2009; Cassaglia et al., 2008a; Cassaglia et al., 2008b; Mayhan et al., 1987; Tzeng et al., 2010a), and is increased by ~20% more during apnea compared to breathing at similar arterial blood gas tensions as indexed by muscle sympathetic nerve activity (Steinback et al., 2010). It therefore seems likely that the observed similarly in CBF increase between apnea and clamp, despite greater MAP during the former, is a function of increased SNS activity during apnea. Previous CBF estimations by TCD may be interpreted to conflict with the present findings. For example, Kjeld et al. (2009) showed enhanced CBF during maximum BH with concomitant facial immersion in cold water based on TCD estimation of CBF. This apparent CBF increase during facial immersion manifest in spite of lower cardiac output, stroke volume heart rate and MAP during facial immersion (an effect further augmented during full body immersion (Costalat et
As shown in the present study, and numerous others (e.g., (Lucas et al., 2010; Tan, 2012; Willie et al., 2012)), CBF increases approximately 0.4-0.6 % per mmHg increase in MAP. The finding by Kjeld et al. (2009) that CBF increases more when these variables are lower is therefore somewhat surprising. One possibility is that increased sympathetic activity during immersion constricted the MCA, yielding an artifactually augmented estimate of CBF. Although that the diameter of the MCA is not static has been recently supported (Hansen et al., 2007; Willie et al., 2012; Willie et al., 2014a; Wilson et al., 2011), it remains to be explicitly demonstrated that it constricts with increased SNS activity. Cross et al. (2014) interpreted increased phase synchronization during apnea to indicate impaired cerebral autoregulation, but various metrics that ostensibly quantify the efficacy of cerebral autoregulation have been shown to yield disparate results (Tzeng et al., 2012). However, that CBF did not increase further than during clamp despite a much greater increase in MAP is evidence of cerebral autoregulatory efficacy without the confounding influence of more quantitatively complex metrics of autoregulation. Nevertheless, a worthwhile future study is to determine whether volumetric CBF is further augmented during maximum apneas with facial immersion, as the similar CBF increase during apnea and clamp in the present study suggest a threshold for CBF increase.

7.6 Conclusion
The present data indicate that the profound hypoxaemia of extreme breath holding does not produce marked brain hypoxia. On the contrary, cerebral oxygen delivery is never attenuated through the duration of breath hold, suggesting an alternative mechanism triggering apnea breakpoint. Despite an approximate 50% increase in MAP at end-apnea, CBF is buffered from increasing more than ~100% of baseline values indicating cerebral autoregulation is not impaired during prolonged apnea in elite breath hold divers.
Chapter 8: Conclusion

8.1 Introduction
The regulation of CBF is multifactorial. Arterial blood gases, mean arterial pressure (MAP), and cerebral metabolism are the principal regulators of CBF, with the autonomic nervous system contributing a somewhat lesser role (Willie et al., 2014b). Given the high metabolic rate of brain tissue that demands constant oxygen and nutrient supply, these regulatory mechanisms are crucial to normal cerebral function. The present work demonstrates the efficacy of cerebrovascular regulation – in the face of dramatic acute or chronic alterations to arterial blood gases and acid-base balance, and large increases in MAP, the delivery of oxygen to the brain is almost universally defended. The genesis of the research presented herein was developed in view of the limited data describing regional differences in CBF during changes in arterial blood gases in humans; the lack of consideration and related interaction of MAP; and that the majority of evidence is largely based on methods of CBF quantification with insufficient temporal resolution or using assumptions related to velocity reflecting flow.

The independent effects of PaO₂ and PaCO₂ on regional CBF were first quantified in Chapter 4. Duplex vascular ultrasound combined with automatic wall-tracking software of the vertebral artery (VA) and internal carotid artery (ICA) provide accurate means of regional CBF measurement not susceptible to error caused by intra-cranial vessel vasomotion, and indeed capable of measuring extra-cranial vessel dilation or constriction. Combined with direct sampling of arterial blood gases [as opposed to estimation by end-tidal sampling which may overestimate true arterial values; (Peebles et al., 2007)] the effect of extreme changes in PaO₂ and PaCO₂ on regional CBF was reliably determined for the first time. The finding that the VA exhibited greater reactivity to hypoxia and hypocapnia than the ICA suggested important implications to our understanding of the control of breathing and autonomic function. For example, because the sensitivity of the respiratory centers is partially affected by CBF (Xie et al., 2006; Xie et al., 2009), differential changes in flow through the VA (which supplies blood to the brain stem) may influence ventilatory control. This study also provided the first data in humans showing the major cerebral vessels alter diameter with
changes in PaCO₂ and/or flow, demonstrated the importance of MAP in the cerebrovascular response to hypoxia and hypercapnia, and provided evidence cerebral oxygen delivery is prioritized and maintained in all conditions but the most severe levels of hypocapnia.

Independent manipulation of arterial blood gases permits the carefully controlled setting required to understand the specific influence of PaO₂ and PaCO₂, but precludes identifying their integrated role during natural paradigms of altered blood gas levels. For example, ascent to high altitude and exposure to hypoxia results in progressive hyperventilation and subsequent reductions in PaCO₂ (Haldane et al., 1919). During ascent and acclimatization to high altitude the cerebrovasculature is thus exposed to the opposing effects of hypoxia-induced vasodilation and hypocapnic vasoconstriction. Chapter 5 aimed to measure regional volumetric CBF during ascent (at 3050m and 4050m) and every twelve hours during the first three days, and again after two weeks at 5050m. This study is the sixth in the literature to assess the time course of CBF changes with exposure to HA, but the first to make multiple measurements of flow per day during the first 3 days after arrival and during partial preacclimatization to 5,050 m. Following ~60 h at altitude, CBF begins to fall from its zenith as the slowly increasing oxygen carrying capacity of the blood increases, returning to near sea level values by 2 weeks. Importantly, these data show the largest CBF increase to date, despite the gradual ascent profile (vs. the other studies, which employed a rapid ascent by car or tram to altitude [e.g., (Baumgartner et al., 1994; Huang et al., 1987; Jensen et al., 1990; Severinghaus et al., 1966; Subudhi et al., 2014]), and that this maximum occurred at ~60 h at 5,050 m. Contrary to our hypothesis, we observed no differences between the changes in flow in the VA and ICA with ascent at any time point. There was a strong correlation between SpO₂ with CBF, that accounted for ~40% of the statistical variability. The increase in CBF was facilitated by dilation of the middle cerebral artery (and presumably the downstream pial arterioles also), which compensated for hypoxemia such that cerebral oxygen delivery was maintained.

Due to the partial metabolic compensation for the respiratory alkalosis, CBF returns to near sea level values within 10-14 days at 5050m despite significant hypoxemia. It is well established that ventilatory sensitivity to CO₂ is augmented at high altitude (Mathew et al., 1983; Schoene et al., 1990), but the precise reasons for this have remained ambiguous.
Probable mechanisms include the left shift in the pH-CO$_2$ due to chronic hypocapnia and associated reduction in buffering capacity, the additive effect of background hypoxia on the chemoreflex (Hornbein et al., 1961), and increases in the intrinsic sensitivity of the carotid body (Nielsen et al., 1988; Vizek et al., 1987). Whether these mechanisms likewise affect cerebrovascular reactivity to CO$_2$ are unknown, as reactivity has been shown to increase (Fan et al., 2010a; Fan et al., 2013), decrease (Rupp et al., 2014; Lucas et al., 2011; Villien et al., 2013), or not change (Ainslie et al., 2007; Ainslie & Burgess, 2008; Somogyi et al., 2005) at altitude. The disparate results reflect methodological differences (i.e., altitude, duration of exposure, method of assessment of cerebrovascular reactivity to CO$_2$), but are also likely a function of the application of Transcranial Doppler ultrasound (TCD) to vasoactive arteries and the consequent error in CBF measurement. Chapter 6 presents a field study where the entire protocol was conducted in a laboratory at sea level and then replicated following partial acclimatization to 5050m. Here, we measured the ventilatory and cerebrovascular responses to acute changes in PaCO$_2$ and PaO$_2$ before and following partial acclimatization to 5050m in the same subjects. Arterial and internal jugular venous blood gases and substrates (e.g., glucose, lactate) were directly measured and CBF calculated using the Fick principle. We observed that the increase in the ventilatory response to CO$_2$ at altitude was not due to the left shift in the pH-CO$_2$ relationship, and that augmented MAP-CO$_2$ reactivity at altitude was ameliorated with restoration of oxygen to sea level values suggesting background hypoxia accounts for augmented ventilatory and MAP CO$_2$ reactivity at altitude. We also observed that CBF reactivity is not augmented at altitude, despite the greater increase in MAP for a given change in PCO$_2$, indicating maintenance of cerebral autoregulation at HA. Cerebral oxygen delivery was also well maintained with acute or chronic hypoxia, being only mildly decreased during acute hypocapnia. Data also indicate, again, that TCD dramatically underestimates hypercapnic CBF reactivity, particularly at altitude, and presumably due to cerebral artery dilation.

Altitude provided an excellent model to examine the interactions of acid-base balance and hypoxia on the cerebral circulation; however, the CBF response to concomitant hypercapnic hypoxia had not been studied using methods that account for dilation of cerebral vessels (i.e., all studies to date have used TCD). Elite breath-hold divers are capable of volitionally
attaining levels of hypoxemia and hypercapnia beyond the tolerable limits of most humans, and are therefore an inimitable model for assessing the cerebrovascular response to extreme changes in arterial blood gases (Dujic et al., 2013). Likewise the massive sympathetic-induced MAP augmentation during apnea (Steinback et al., 2010) provided the opportunity to partially isolate the effects of MAP from arterial blood gases on CBF and moreover probe the relationship between apnea duration and cerebral oxygen delivery. For the first time, this was achieved by replicating each individual’s breath-hold time course of arterial blood gas “profiles” (i.e., declining PaO₂, increasing PaCO₂) during breathing by end-tidal clamping. Despite a 20% greater increase in MAP during apnea than during a temporally identical arterial blood gas profile, the maximum increase in CBF was identical. The efficacy of cerebral autoregulation during apnea was therefore maintained, and was possibly facilitated by augmented sympathetic nervous activity. Cerebral oxygen delivery was never attenuated during either apnea or clamp with the progressively increasing CBF compensating for the decrease in CaO₂.

8.2 Overall summary and interpretation
The studies comprising this thesis each assess the role of arterial blood gases on CBF regulation in a variety of conditions. Several fundamental physiological conclusions warrant discussion.

8.2.1 Regional CBF
Greater VA reactivity was observed to isocapnic hypoxemia and to euoxic hypocapnia in Chapter 4. No regional differences in CBF were observed during gradual ascent and acclimatization to high altitude during conditions of both hypoxemia and partial respiratory alkalosis (Chapter 5). This finding of a lack of regional CBF differences was also consistent with those during acute hypercapnic hypoxia and hypertension of prolonged breath holding (Chapter 6). We were unfortunately unable to collect regional volumetric ICA and VA flows in all subjects at both sea level and HA, so it is not possible to say if VA flow reactivity is greater than ICA flow reactivity during independently altered arterial blood gases at HA. However, that regional CBF changes were similar during early acclimatization and during breath hold indicate that in these contexts anterior and posterior CBF was
uniformly affected by changes in blood gases induced through normal ventilatory challenges where \( \text{PaO}_2 \) and \( \text{PaCO}_2 \) are uncontrolled.

Recent studies help clarify these findings. An expedition to Mt Chacaltaya (5200m) observed slight (9%) but disparate changes in cerebral regional brain oxygen delivery immediately (2-4 hours) following a rapid ascent (whilst breathing \( \text{O}_2 \)) by automobile without progressive acclimatization (Subudhi et al., 2014). The authors reported a significant dilation of the VA but not ICA. Moreover, despite a lesser increase in VA flow than ICA flow, oxygen delivery through the VA was significantly enhanced (~20%) whereas ICA oxygen delivery was maintained. The authors did not address how it is possible that the relative VA flow increase could be lower yet still yield a greater increase in oxygen delivery. Nonetheless, their observed dilation of the VA is in accordance with the data presented in Chapter 4, and is further supported by a recent study assessing the effects of poikilocapnic hypoxia on regional CBF. Lewis et al (2014b) observed greater dilation of the VA than ICA following 60 minutes of exposure to a \( \text{FiO}_2 \) of 11%. Following 5.5 hours VA flow remained significantly elevated whereas ICA flow decreased toward baseline. Conversely, Ogoh et al (2013) found that 15 min of acute normobaric poikilocapnic hypoxia produced a selective increase in VA flow (10%), whereas ICA flow only increased during isocapnic hypoxia, suggesting that sensitivity to hypocapnia was greater in the ICA than VA. This study was confounded, however, by mild hypoxemia (SpO\(_2\) > ~89%) below that typically required to elicit an increase in CBF (Cohen et al., 1967; Mardimae et al., 2012; Shapiro et al., 1970). It is therefore difficult to interpret these findings, as this apparent sensitivity to such mild hypoxemia does not conform to known norms from our laboratory (Willie et al., 2012) or the literature (Cohen et al., 1967; Mardimae et al., 2012; Shapiro et al., 1970).

Notwithstanding the findings of Ogoh et al (2013), the above studies can be interpreted to collectively indicate greater sensitivity of the VA than ICA to acute (hours) poikilocapnic or isocapnic hypoxia. The mechanisms mediating regionally different sensitivity are unknown, but could be related to differences in density of sympathetic innervation (Edvinsson & Hamel, 2002), adenosine receptor concentrations (Jarvis & Williams, 1989), and/or nitric oxide synthesis (Pelligrino et al., 1993). Both the data shown in Chapter 5, and that of Subudhi et al (2014) indicate that following prolonged (>12 days) or gradual exposure to
hypoxia the CBF response is regionally similar across the brain. It is important to recall that brain tissue perfusion is additionally modulated to local metabolic demands by the neurovascular unit (reviewed in Chapter 2.1.3); as such, similar ICA and VA flow patterns do not indicate tissue perfusion is identical across the entire brain. Indeed, the perfusion response to changes in CO$_2$ is greater in gray than white matter (Binks et al., 2008) and transient local hypoxia following increased local metabolism is the basis for BOLD MRI imaging (Offenhauser et al., 2005; Thompson et al., 2003; Vanzetta & Grinvald, 1999). Certainly future research should strive to integrate these in vivo measurements of regional CBF with more sophisticated metrics capable of quantifying local tissue perfusion.

8.2.2 Interaction between arterial blood gases and cerebral autoregulation.

Traditional tests to assess cerebrovascular CO$_2$ reactivity or cerebral autoregulation treat these concepts as separate entities. Clearly they are not; there are persuasive data that both CA and CO$_2$ responses use the same vascular reserve. In a landmark study, Harper & Glass (1965) examined the brain vascular reactivity to changes in PaCO$_2$ in dogs at various blood pressures (see Figure 2.4). Severely hypotensive animals (approximately −60% MAP) showed no change in cerebral vessel diameter in response to increases or decreases in PaCO$_2$ (i.e. CBF reactivity was abolished), because the vessels were already maximally dilated (Harper & Glass, 1965). The reciprocal instance was also demonstrated where, in hypocapnia, CBF was well maintained in the face of controlled haemorrhage-induced hypotension; whereas with hypercapnia CBF fell linearly with MAP (Iwabuchi et al., 1973).

Such a study to examine CBF regulation of concurrent changes in blood pressure and arterial blood gases in humans has not yet been completed. Our understanding of the effects of simultaneous changes in MAP and arterial blood gases on CBF comes principally from the chemoreflex driven changes in MAP incurred during hypoxia or hypercapnia. This has consequently made it difficult to differentiate the effects of changes in perfusion pressure from the effects of changes in blood gases per se. Although the present data suffer from this same methodological confounder, the increase in MAP was always significantly correlated ($R^2 \approx 0.8-0.9$) with the increase in CBF during hypercapnia. In Chapter 4 (Figure 4.6), Chapter 6 (Figure 6.4), and Chapter 7 (Figure 7.3) all show that the potent chemoreflex response to hypercapnia and hypoxia manifest in large increases in MAP, particularly to
hypercapnia the response to which is augmented further at high altitude.

The chemoreflex induced increase in MAP – termed the pressor response – originates from peripheral chemoreceptors located at both the aortic arch and carotid bodies and the central chemoreceptors located in the retrotrapezoid nucleus (Guyenet et al., 2013). Traditionally the peripheral chemoreceptors are attributed the primary role of sensing hypoxia, however, the type I glomus cells of the carotid body are also sensitive to hypercapnia (Lahiri & Forster, 2003). Indeed, the carotid body responds to hypoxia and CO₂ in a multiplicative manner, such that, for example, the afferent chemosensory response to hypercapnia is steeper and the threshold for activation decreased with concomitant hypoxia (Mohan & Duffin, 1997; Lahiri & DeLaney, 1975; Hornbein et al., 1961). This feature of the peripheral chemoreceptors translates to the in vivo ventilatory and pressor responses (Halliwill et al., 2003). As can be seen in the present data (Chapter 6), the chemosensory response to hypercapnia is augmented with chronic hypoxia, an effect immediately ameliorated with acute restoration of normoxia at high altitude. Cerebral autoregulation remains intact during such extreme changes in arterial blood gases, an effect best exhibited by the data of elite breath hold divers shown in Chapter 7. Figure 7.2 clearly shows that despite the much greater increase in MAP during apnea than during clamp (between which arterial blood gases were very similar), the increase in CBF was nearly identical.

The arterial baroreflex originates from stretch receptors in close proximity to the peripheral chemoreceptors at the carotid bifurcation and aortic arch the afferents of which may course in the same nerve and project to similar areas of the medulla (reviewed in Fadel, 2008; Persson, 1996). These two reflexes interact. For example, baroreflex activation inhibits chemoreceptor inputs to the brain stem in anesthetized cats (Mifflin, 1993). In humans the response of muscle sympathetic nervous activity to hypoxia is attenuated by baroreflex activation, whereas the response to hypercapnia is not (Somers et al., 1991) due to the additional input of the central chemoreceptor. This provides a mechanistic explanation for the much larger pressor response observed with hypercapnia than with hypoxia (Chapters 4 and 6). Ventilation also serves an inhibitory role in the chemoreflex, via inhibitory thoracic stretch receptors (Somers et al., 1989a; Somers et al., 1989b) which is why sympathetic activity is much higher during apnea than during breathing for the same arterial blood gas.
partial pressures (Steinback et al., 2010). The enhanced MAP response observed during apnea in elite breath hold divers (Chapter 7) is due to this augmentation of sympathetic activity and consequent increases in peripheral resistance (Steinback et al., 2010). Thus, during prolonged apnea, hypercapnia and hypoxia not only cause cerebrovascular dilation directly, but also increase perfusion pressure (MAP) via peripheral and central chemoreflex mediated sympathetic activation (Somers et al., 1991). Normally through the action of inhibitory thoracic stretch receptors, ventilation inhibits sympathetic outflow, but during apnea this ventilatory restraint is removed and MAP increases further (Figure 7.2). Nevertheless, CBF exhibited a similar increase during apnea and clamped breathing (Chapter 7, Figure 7.2) indicating efficacious autoregulation.

8.2.3 Mechanisms of CBF regulation: pH versus PCO$_2$

Altered alveolar gas exchange (either by changes in alveolar ventilation and/or metabolic CO$_2$ production) elicits concomitant changes in both PaCO$_2$ and pH. The latter is thus influenced by both respiratory alkalosis or acidosis in the short term and over longer periods by the degree of renal compensation. However, a long-standing question has been which of these (PaCO$_2$ or pH), or both, are responsible for the resultant change in CBF. Direct manipulation of arterial pH does not alter CBF under conditions of maintained PaCO$_2$ (Harper & Bell, 1963; Lambertsen et al., 1961). In contrast, manipulation of extravascular pH induces changes in arteriolar diameter, but extravascular manipulation of HCO$_3^-$ or molecular CO$_2$ elicit no vasoactive effect (Kontos et al., 1977a; Kontos et al., 1977b; Wahl et al., 1970). These observations suggest the CO$_2$ mechanism is independent of arterial pH, and therefore likely to be dependent on the diffusion of non-polar CO$_2$ molecules across the cerebrovascular blood-brain barrier that can induce a change in pH in the extracellular space of the vessel (Lambertsen et al., 1961; Lassen, 1968). This model is supported by animal work utilizing in vivo cranial windows and superficial application of solutions with varying PCO$_2$ and pH (Kontos et al., 1977b; Wolff & Lennox, 1930).

That CBF reactivity to changes in CO$_2$ is similar between chronic normocapnia (~40 mmHg) and partially corrected respiratory alkalosis (~25 mmHg) likewise supports this model where extravascular pH rather than arterial PCO$_2$ or pH drives prevailing CBF (Chapter 6). Following 8-10 days at 5050m subjects were hypocapnic (PaCO$_2$ ~25 mmHg) and still
mildly alkalotic (+0.07 pH units), yet baseline CBF and cerebral oxygen delivery had returned to near sea level values. During acute respiratory alkalosis with exposure to hypoxia, CBF increases indicating that hypoxic cerebral vasodilation supercedes the constrictory effects of hypocapnia and low arterial pH (Severinghaus et al., 1966). During ascent and early acclimatization to 5050m, SpO_2 explained ~40% of the statistical variation in CBF (Chapter 5). The importance of hypoxic vasodilation is further exemplified by the immediate 18% CBF decrease at HA when PaO_2 is returned to 100 mmHg in isocapnia (Chapter 6). Exactly how hypoxia causes cerebral vasodilation is not well understood, but appears to be facilitated by the coordinated interaction of 1) a retrograde stimulus arising from the neurovascular unit following decreases in local tissue oxygenation (Gordon et al., 2008; Pelligrino et al., 1995; Thompson et al., 2003); 2) local extracellular acidosis due to anaerobic metabolite production (Kogure et al., 1970; Nolan et al., 1982); and, 3) direct vascular mechanisms involving adenosine (Meno et al., 1993; Winn et al., 1981), and/or nitric oxide (Van Mil et al., 2002).

While the human data presented in this thesis cannot elucidate molecular mechanisms underlying CBF regulation, they collectively indicate that: 1) cerebral oxygen delivery is prioritized acutely (e.g., prolonged apnea) and over time (e.g., at high altitude) 2) that with chronic hypocapnia cerebral vasomotor control is reset to lower prevailing PCO_2. Parenthetically, cerebrovascular reactivity appears to be similar between normocapnic and hypercapnic COPD patients (Van de Ven et al., 2001), suggesting resetting occurs with chronic hypercapnia as well as chronic hypocapnia. Altitude is thus characterized by a cerebrovascular adaptation to lower PaCO_2, lower bicarbonate concentration, and higher pH during the first few weeks, that allows the prioritization of oxygen delivery despite hypocapnia. During this time the brain retains its sensitivity to changes in CO_2 (relative to the new eupnic PaCO_2) and to oxygen, with CBF regulated to conserve cerebral oxygen delivery. Given that metabolic compensation eventually reestablishes normal systemic pH to both respiratory alkalosis (e.g., high altitude) and respiratory acidosis (e.g., CO_2 retention), it seems probable the resetting of cerebrovascular reactivity is a function of changes in acid base balance, however, this has not be explicitly demonstrated.
8.3 Future directions

This thesis describes the role of arterial blood gases in regulating CBF. Future research should focus on a number of themes that, particularly in humans, have not been thoroughly addressed experimentally.

1. Is cerebral blood flow in hypoxia regulated by PaO$_2$ or oxygen content?

The CBF response to oxygen appears to be determined by oxygen content rather than PaO$_2$ per se, as reduced oxygen content with carbon monoxide exposure, acute or chronic anemia, and hemodilution produces increased CBF (Brown et al., 1985; Gottesman et al., 2012; Hare, 2004; Metry et al., 1999; Paulson et al., 1973; Todd et al., 1994). Indeed, the limited data available suggest the inverse relationship between blood hematocrit (ergo viscosity) and CBF (Metry et al., 1999; Muizelaar et al., 1986) is a function of oxygen delivery rather than viscosity per se (Todd et al., 1994; Tomiyama et al., 1999), but this remains to be explicitly studied in humans.

2. What is the mechanism of cerebral hypoxic vasodilation?

The cellular mechanisms responsible for the cerebrovascular response to changes in PaCO$_2$ and PaO$_2$ have been subject of countless animal studies (see Faraci & Heistad, 1998; Yoon et al., 2012). Yet, the resulting diversity of conclusions serves largely to demonstrate a mechanistic redundancy inherent to the precise cerebrovascular regulation by arterial blood gases (see Chapter 2.1.2.1). In humans, however, there have been surprisingly few studies that have attempted to delineate potential mechanisms by which hypoxia leads to cerebral vasodilation. The few studies assessing the role of adenosine on the human cerebrovasculature have reported a 20-30% decrease in CBF in normoxia but that the cerebral hypoxic-vasodilatory response in fact remains intact (Wechsler et al., 1950; Magnussen & Hoedt-Rasmussen, 1977; Gottstein & Paulson, 1972; Bowton et al., 1988; Nishimura et al., 1993; Nishimura et al., 1992). The role of nitric oxide in cerebral hypoxic vessel dilation also remains equivocal: only two studies have assessed its role and reported opposite findings (Ide et al., 2007; Van Mil et al., 2002). Gaining mechanistic insight into the cerebral response to hypoxia is within reach given the advanced imaging
techniques available today, but will require careful study design that accounts for PaCO₂ and MAP, and provides accurate measures of cerebral vessel vasomotion.

3. **Is the mechanism of cerebrovascular resetting to chronic changes in PCO₂ a function of CO₂ or pH?**

   Following manipulation of metabolic acid-base balance by ingestion of organic acid or base for five days, Fencl *et al.*, (1969) demonstrated in four healthy humans that CBF varied inversely with pH for a given PaCO₂. The effect of acute changes in PCO₂/acid-base balance could equally be assessed by NaHCO₃ infusion following respiratory acidosis. Such an elegant study design integrating modern measurement techniques to assess the response of cerebral vessels would elucidate if cerebrovascular resetting is due to systemic pH correction or local cerebrovascular mechanisms. This experimental approach could also be used to probe if related changes in acid-base status impact on the cerebrovascular response to hypoxemia.

4. **What is the precise nature of the stimulatory synergism and regulatory interdependence of arterial blood gases and blood pressure on CBF regulation?**

   The study of cerebrovascular regulation needs to consider this relationship because if MAP and PaCO₂ or PaO₂ change simultaneously, interpretation of the relative impact of each becomes difficult. The relationship between MAP and cerebrovascular reactivity to PaCO₂ furthermore remains unknown in the hypertensive range. Whether the neck arteries participate in buffering increasing perfusion pressure can be assessed using vascular ultrasound, and advanced imaging techniques should facilitate study of the effects of vascular reactivity and resistance heterogeneity in the cerebral vascular bed on the distribution of CBF in response to vasoactive stimuli.

5. **How and under what circumstances is neurogenic control of the cerebral vasculature an important player in autoregulatory function?**

   There are numerous studies yet to be completed in humans to determine precisely the capacity for sympathetic and cholinergic nervous input on the cerebrovasculature. Future studies in healthy humans should not rely solely on transcranial Doppler
ultrasound in assessing these questions. Newer imaging modalities, and direct modification of cerebral SNS outflow, rather than global perturbation of SNS receptors, will help to elucidate the role of the autonomic nervous system in cerebrovascular control. Particularly during transient bouts of hypertension – such as that commonly experienced during activity, strain (defecation, lifting, etc.), pathology (autonomic dysreflexia, subarachnoid hemorrhage, etc.) and during rapid-eye movement sleep – sympathetically mediated buffering of global CBF, and constriction of large cerebral arteries should be assessed. One methodological possibility to this end would be to use a centrally acting α₂-adrenoreceptor agonist (e.g., clonidine) to diminish SNS outflow whilst quantifying regional (cerebral) noradrenaline spillover (Mitchell et al., 2009) during non-pharmacologically induced changes in MAP. Another approach would be assessing CBF and large artery vasomotion (by ultrasound or MRI) during transient hypotension and hypertension before and following cervical ganglion block.

8.4 Conclusion and significance

The brain is a highly metabolically active organ incapable of surviving even brief shortages of oxygen supply. It therefore possesses an effective and redundant array of regulatory mechanisms that prioritize the delivery of oxygen. Cerebral blood flow is principally regulated by tissue metabolism, perfusion pressure, autonomic nervous activity, and PaO₂ and PaCO₂ – an integrative process thus involving the marked influence of pulmonary gas exchange and cardiovascular function, in addition to intracranial mediators of cerebrovascular resistance. This thesis explicated the roles of PaO₂ and PaCO₂ in human regulation of regional CBF during acute conditions of independently manipulated changes in arterial blood gases, during ascent and acclimatization to high altitude, and during profound hypoxemia during prolonged apnea. The cerebral circulation is extremely sensitive to acute changes in PaCO₂; indeed, the only paradigm in which a significant reduction in cerebral oxygen delivery was observed was during profound hypocapnia at least 10mmHg below eupnic PaCO₂. During acute isocapnic hypoxia at sea level, isocapnic or hypercapnic hypoxia at high altitude, and during maximum apnea in elite breath hold divers oxygen delivery was maintained. These findings collectively inform our understanding of human cerebrovascular
physiology, a field lagging behind other systems of the body in large part because of the
difficulty in studying an organ bound within the skull.

The methods used herein provide tremendous utility to investigate cerebrovascular function. While advanced MRI-based imaging modalities are undoubtedly necessary to elucidate certain cerebrovascular regulatory mechanisms, in particular those describing discrete regulation in localized regions of the brain, the utility of neck vascular ultrasound as a tool to understand cerebrovascular function is by no means exhausted; each of the five areas outlined above can be readily explored using vascular ultrasound. This thesis provides much insight into the functional capacity of arterial blood gases to regulate cerebral blood flow, laying the groundwork for future studies specifically designed to elucidate the mechanisms of arterial blood gas modulation of CBF. These efforts to describe and then explicate fundamental mechanisms of cerebrovascular regulation in healthy humans forms the foundation for addressing the role of CBF regulatory impairment in myriad cerebrovascular diseases ranging from Alzheimer’s and vascular dementia (Hebert et al., 2004; Qiu et al., 2003a) to neurogenic hypertension (Waki et al., 2011).
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