REGULATION OF CEREBRAL BLOOD FLOW DURING TRANSIENT HYPERTENSION IN HUMANS

by

Kevin Wayne Wildfong

B.H.K., The University of British Columbia - Okanagan, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE COLLEGE OF GRADUATE STUDIES

(Interdisciplinary Studies)

THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

August 2016

© Kevin Wildfong, 2016
Thesis Committee

The undersigned certify that they have read, and recommend to the College of Graduate Studies for acceptance, a thesis entitled:

Regulation of cerebral blood flow during transient hypertension in humans

submitted by Kevin Wayne Wildfong in partial fulfilment of the requirements of

the degree of Master of Science.

Dr. Philip Ainslie – Faculty of Health and Social Development – UBC Okanagan
Supervisor, Professor (please print name and faculty/school above the line)

Dr. Neil Eves – Faculty of Health and Social Development – UBC Okanagan
Supervisory Committee Member, Professor (please print name and faculty/school in the line above)

Supervisory Committee Member, Professor (please print name and faculty/school in the line above)

Dr. Craig Steinback – Faculty of Physical Education and Recreation – U of Alberta
University Examiner, Professor (please print name and faculty/school in the line above)

External Examiner, Professor (please print name and university in the line above)

August 31, 2016

(Date Submitted to Grad Studies)

Additional Committee Members include:

(please print name and faculty/school in the line above)

(please print name and faculty/school in the line above)
Abstract
Although the role of sympathetic nervous activity (SNA) in cerebral blood flow (CBF) regulation is poorly understood in humans, animal studies have demonstrated that elevations in cerebral SNA may protect against cerebral hyper-perfusion during elevations in blood pressure (BP). We examined the hypothesis that alpha-1 receptor blockade (Prazosin) would augment increases in CBF during acute hypertension. In 15 healthy volunteers, beat-by-beat BP, extra-cranial artery blood flow (internal carotid artery (QICA) & vertebral artery (QVA)), intra-cranial artery velocity (middle cerebral artery (MCAv) & posterior cerebral artery (PCAv)), and end-tidal gases (PETO₂ & PETCO₂) were controlled before and 90 min following oral Prazosin (1mg/20kg) at rest and during transient hypertension. Hypertension was non-pharmacologically induced using 30% maximal voluntary contraction (MVC) handgrip exercise (HG), lower-body positive pressure (LBPP), and combined LBPP & HG (LBPP+HG). Following Prazosin administration, baseline PETCO₂ (-1 mmHg), MAP (-7 mmHg) & MCAv (-5 cm/sec) were all significantly reduced (P<0.05), while PCAv, QICA and QVA and respective diameter were all unchanged; VA velocity was reduced (P=0.03), while ICA velocity was unchanged (P=0.96). Following Prazosin, MAP during 30% MVC HG was unchanged while MAP was significantly reduced during LBPP (-29 ± 68%) and LBPP+HG (-40 ± 38%); despite these changes in MAP, MCAv, PCAv, QICA & QVA all remained unchanged (P>0.05). There was significant attenuation of absolute PCAv CVC following Prazosin during LBPP and LBPP+HG (Δ0.036 ± 0.032 vs. Δ0.007 ± 0.019 & Δ0.053 ± 0.04 vs. Δ0.024 ± 0.025 cm/sec/mmHg; P<0.05), but not HG (P=0.4). Although changes in QICA and MAP were unrelated (r²=0.07; P=0.16) during 30% MVC HG, LBPP & LBPP+HG, QVA was positively correlated (r²=0.14; P=0.02) with changes in MAP following Prazosin. This latter observation is consistent with changes in PCAv CVC, potentially indicative of vasodilation in the PCA or alternatively redistribution of QVA before communicating with the PCA, or both. While these findings are contrary to our hypothesis, these differential findings are interpreted to indicate that there is a disparity between the extra- and intra-cranial arteries and that SNA serves a functional role in the regulation of the posterior region of the brain during transient hypertension.
Preface

This thesis contains original data collected for partial fulfillment of the requirements for Master of Science degree. All protocols were approved by the University of British Columbia Clinical Research Ethics Board (ID: H13-03081) and conducted at the Center for Heart, Lung and Vascular Health at the University of British Columbia Okanagan. Prof. Ainslie and myself developed the research proposal and experimental design. The lower-body positive pressure (LBPP) chamber(s) were designed and constructed with assistance from Mike Tymko, with Prof. Ainslie supplying the necessary financial assistance for purchase of materials. Data collection was completed with the assistance of Dr. Anthony Bain, Dr. Kurt Smith, Ryan Hoiland and Dr. Nia Lewis; Dr. Brad Monteleone provided insight into the administration procedures of the drug intervention used in this thesis study. I completed all data analysis and wrote this thesis, which consists of four chapters: chapter 1 - literature review, chapter 2 – methodology, chapter 3 – results & chapter 4 – conclusions. Prof. Ainslie provided extensive feedback and critically reviewed the thesis for appropriate data interpretation and content. A modified version of this thesis is being prepared for submitted to the Journal of Hypertension for publication.
Table of Contents

Thesis Committee ........................................................................................................ i

Abstract ...................................................................................................................... iii

Preface ......................................................................................................................... iv

Table of Contents ....................................................................................................... v

List of Tables ............................................................................................................... vii

List of Figures ............................................................................................................ viii

List of Abbreviations ............................................................................................... xiii

Acknowledgements ................................................................................................. xv

Dedication ................................................................................................................... xvi

Chapter 1: Introduction ............................................................................................ 1
  1.1 Brief background and overview of literature review ........................................... 1
  1.2 Cerebral anatomy ............................................................................................... 2
  1.3 Arterial blood gases and the regulation of CBF ................................................. 3
    1.3.1 Changes in carbon dioxide (PaCO₂) ............................................................ 3
    1.3.2 Changes in oxygen (PaO₂) ........................................................................ 6
    1.3.3 End-tidal forcing ........................................................................................ 7
  1.4 Cerebral metabolism and the regulation of CBF ................................................. 8
  1.5 Cerebral autoregulation .................................................................................... 10
    1.5.1 Hysteresis effect of cerebral autoregulation .............................................. 13
    1.5.2 Myogenic response of cerebral autoregulation ......................................... 14
    1.5.3 Neurogenic influence on cerebral autoregulation .................................... 15
  1.6 Cerebral perfusion and CBF ........................................................................... 17
    1.6.1 Perfusion pressure ...................................................................................... 17
    1.6.2 Cardiac output ........................................................................................... 19
    1.6.3 Baroreflex ................................................................................................ 19
    1.6.4 Cerebrovascular response to handgrip exercise ...................................... 20
    1.6.5 Cerebrovasculature response to lower body positive pressure ............. 21
  1.7 Neurogenic regulation of CBF ......................................................................... 22
    1.7.1 Adrenergic receptors ................................................................................. 27
  1.8 Research questions and hypothesis .................................................................. 28

Chapter 2: Methodology .......................................................................................... 30
  2.1 Ethical approval ............................................................................................... 30
  2.2 Study participants ............................................................................................. 30
  2.3 Experimental protocol ...................................................................................... 30
  2.4 Handgrip exercise ............................................................................................ 32
2.5 Lower body positive pressure .............................................................. 32
2.6 Instrumentation & data collection ....................................................... 35
2.7 Cardio-respiratory measurement techniques ....................................... 35
2.8 End-tidal forcing .................................................................................. 35
2.9 Hemodynamic measures .................................................................... 36
2.9.1 Heart rate measurement .................................................................. 36
2.9.2 Blood pressure measurement ......................................................... 37
2.10 Cerebrovascular measures .................................................................. 38
2.10.1 Intra-cranial cerebrovascular measurement: Transcranial doppler ultrasound .......................................................... 38
2.10.2 Validity of Transcranial doppler ultrasound ..................................... 41
2.10.3 Extra-cranial cerebrovascular measurement: Duplex vascular ultrasound .............................................................. 44
2.11 Duplex ultrasound edge-detection analysis software ........................... 50
2.12 Quantification of CBF ......................................................................... 52
2.13 Data and statistical analysis ................................................................ 53

Chapter 3: Results .................................................................................... 54
3.1 Baseline measures following Prazosin administration ........................... 54
3.2 Relative changes during BP manipulations before and following Prazosin administration .......................................................... 55
3.3 Relationship between changes in extra-cranial blood flow and intra-cranial blood velocity during BP manipulations ......................................................... 62
3.4 Absolute changes in extra-cranial diameter or velocity with changes in BP manipulations ........................................................................ 62
3.5 Absolute changes in extra-cranial blood flow & velocity with absolute changes in intra-cranial velocity ......................................................... 63

Chapter 4: Conclusion ............................................................................. 70
4.1 Summary of main findings ................................................................. 70
4.2 Transient hypertension and CBF ......................................................... 70
4.3 Regional differences in CBF: Contralateral vs. Ipsilateral ...................... 72
4.4 Methodological considerations .......................................................... 75
4.4.1 Transcranial doppler ultrasound .................................................... 75
4.4.2 Adrenergic control of CBF and redundant pathways ......................... 77
4.4.3 Blood pressure manipulations following Prazosin administration .......... 77
4.4.4 Central venous pressure .................................................................. 78
4.5 Conclusion ......................................................................................... 79
4.5.1 Future directions ............................................................................. 80

Bibliography ........................................................................................... 81
List of Tables

Table 3.1 Absolute and relative changes in baseline values pre and post Prazosin...................... 55

Table 3.2 Summary of Trial 1 (30% MVC isometric HG) pre and post Prazosin......................... 60

Table 3.3 Summary of Trial 2 (LBPP, LBPP+30% MVC HG) pre and post Prazosin ................. 61
List of Figures

Figure 1.1 Major factors involved in the regulation of CBF: response to changes in metabolism, blood pressure, neurogenic (SNA), partial pressures of arterial CO₂ and O₂ and cardiac output. ................................................................................................................................. 2

Figure 1.2 Anatomical diagram of extra- and intra-cranial arteries: A. Anterior (i.e., internal Carotid Artery) and posterior (i.e., vertebral artery) blood supply of the extra-cranial cerebral vessels (Thomas et al., 2015). B. The circle of Willis and schematic of intracranial cerebral vessels (Reproduced from Drake et al., 2009). .................................................................................................................. 3

Figure 1.3 Regional differences in cerebrovascular reactivity during changes in Carbon Dioxide. The above figure outlines both intra- and extra-cranial reactivity to CO₂. A. Individual vessel relative reactivity to changes is PaCO₂ ranging from ~15mmHg to 65mmHg. B. Individual vessel relative reactivity calculated with linear regression in the hypercapnic range and hypocapnic range. Relative hypercapnic reactivity was not different between vessels, while the VA showed selectively higher reactivity than all other vessels during hypocapnia. * Significantly different from other vessels, P<0.05. ......................................................................................... 5

Figure 1.4 CBF and CDO₂ during acute hypoxemic hypoxia in humans. The mean lines for CBF and CDO₂ are calculated as the linear slope from the mean data of each study in proportion to sample size (Hoiland et al., 2015). ............................................................................................................................. 6

Figure 1.5 Regional differences in cerebrovascular reactivity to hypoxia. The above figure outlines both intra- and extra-cranial reactivity to hypoxia. There was a pronounced increase in VA flow during hypoxia compared to the other vessels. ........................................................................................................................................ 7

Figure 1.6 Theoretical illustration of the major cellular components that comprise the neurovascular unit. Vascular tone of cerebral parenchymal arterioles is modulated by transmission of signals from neurons and astrocytes that are attached to the vascular surface of the cerebral arterioles (Filosa & Iddings, 2013). ............................................................................................................................... 9

Figure 1.7 The left panel depicts the classic interpretation of the relationship between MAP and CBF (i.e., CA) with a plateau region ranging 50 to 150 mmHg (Lassen, 1959). The right panel represents the contemporary depiction of CA, with a smaller plateau region of 5 to 25 mmHg (Lucas et al., 2010; Tan, 2012). Furthermore, the right panel illustrates that the cerebrovasculature is more effective at buffering increases in MAP than decreases; hysteresis effect of CA ........................................................................................................................................... 11

Figure 1.8 Relationship between %MAP and %CBF during increases and decrease in MAP. Individual lines represent individual studies; the length of each line depicts the range of MAP in the corresponding experiment. ........................................................................................................................................ 13

Figure 1.9 Individual changes from baseline for mean MAP (left) and mean MCAv (right). Mean MCAv increased from baseline 5% during +20 mmHg LBPP, but conversely decreased by
2% from baseline values during +40 mmHg LBPP. Mean MAP increase 7 mmHg from baseline during +20 mmHg, and further increased 8 mmHg (+15 mmHg from baseline) during +40 mmHg LBPP.

Figure 1.10 Representational schematic of the network of the different cerebral perivascular nerves. The ‘extrinsic’ nerves to cerebral blood vessels at the surface of the brain come from the peripheral nervous system (PNS) and originate either in the superior cervical ganglion (SCG), sphenopalatine (SPG), or otic ganglion (OG) or trigeminal ganglion (TG). Blood vessels located within the brain parenchyma, or the microcirculation, are innervated by ‘intrinsic’ nerve pathways that find their origin in the central nervous system (CNS). Inset: schematic representation of the ‘neurovascular unit’ as seen at the electron microscopic level with the vascular [endothelium (medium gray) and smooth muscle or pericyte (dark gray)], astroglial (light gray), and neuronal (axon varicosities are highlighted) compartments (Hamel, 2006).

Figure 2.1 Schematic of experimental protocol. Trial 1, 1-min of baseline measures followed by 2-min of 30% MVC isometric handgrip (HG) exercise. Trial 2, 1-min of baseline measures, 6-min of supine LBPP (~+40 mmHg) followed by 2-min of LBPP & 30% MVC isometric handgrip exercise (LBPP+HG). Subjects were then orally administered Prazosin, and trials were repeated following 90 min.

Figure 2.2 Schematic of custom designed and constructed lower-body positive pressure (LBPP) chamber. A. Computer generated representation of LBPP chamber frame. B. Computer generated picture of completed LBPP chamber. C. Picture of complete LBPP chamber set-up with - Vacuum and variable transformer for LBPP power output.

Figure 2.3 Schematic diagram of the four principle temporal windows: Frontal, Anterior, Middle and Posterior windows. A. Sagittal view. B. Coronal section view.

Figure 2.4 In the image: Red stripped lines = vessel walls, red arrow = direction blood flow, blue dashed line = ultrasound beam, green bar = angle cursor, black dashed lines indicate percentage of ultrasound signal that is reflected and received by the receiver. The relationship between angle of insonation and cosine of the angle (Cos θ). The image demonstrates the progressive and disproportional reduction in the percentage of the transmitted signal reflected back to the ultrasound probe as the angle of insonation increases such that at 90 degrees no signal will be recorded.

Figure 2.5 Discrepancy between volumetric and velocity of MCA vasomotion during changes in CBF. Previously reported changes in middle cerebral artery (MCA) diameter (left y-axis) and their calculated impact on the discrepancy between flow and velocity measures (right y-axis) during changes in end-tidal PCO₂ (P_{ET}CO₂). To highlight the effects of MCA vasomotion we estimated the potential difference between CBF and velocity changes using the following. For example, cross-sectional area (CSA; cm²) * Velocity (cm/s) * 60 s = Flow (ml/min). Assuming a baseline MCA velocity of 60 cm/s (which was done for all studies to facilitate diameter effect comparisons) coupled with the observed alterations in CSA with hyper- or hypocapnia (Coverdale et al., 2014), we calculated a representative baseline MCA flow value:
5.6 mm² * 60 cm/s * 60 s = Flow; therefore, 0.056 cm² * 60 cm/s * 60 s = 201.6 ml/min. Assuming previously reported values of cerebrovascular reactivity (Willie et al., 2012), MCA velocity increases ~4%/mmHg increase in P_{ET}CO_2. Assuming this as vessel reactivity (for all studies), we can estimate the volumetric MCA flow during hypercapnia using the reported CSA: 6.5 mm² * 84 cm/s * 60 s = Flow; therefore, 0.065 cm² * 84 cm/s * 60 s = 327.6 ml/min. As such, the percent difference for flow between baseline and hypercapnia is [(327.6 – 201.6)/201.6]*100 = 62.5%. The percent difference in velocity between baseline and hypercapnia is [(84 – 60)/60]*100 = 40%, indicating that TCD would underestimate the increase in flow of the MCA during hypercapnia (+9 mmHg P_{ET}CO_2 from baseline) by >20%. However, if the percent difference is quantified via the magnitude of change in flow and velocity during hypercapnia, the increase in flow is ~50% greater than that of velocity! This can be calculated as %difference = [((%increase in flow - %increase in velocity)/%increase in velocity] * 100 and therefore [(62.5 – 40)/40]*100 = 56.25%. As such, we have conservatively represented the effect of changes in diameter on flow vs. velocity discrepancies. For hypocapnia we again assumed a baseline MCA velocity of 60 cm/s and used the prehypocapnia CSA reported (Coverdale et al., 2014) to calculate baseline flow (Portegies et al., 2014): 5.8 mm² * 60 cm/s * 60 s = Flow; therefore, 0.058 cm² * 60 cm/s * 60 s = 208.8 ml/min. Incorporating a 2% change in MCAv per mmHg reduction in P_{ET}CO_2 (Willie et al., 2012) and the associated change in CSA, we estimated volumetric MCA flow during hypocapnia (Serrador et al., 2000): 5.3 mm² * 46.8 cm/s * 60 s = Flow; therefore, 0.053 cm² * 46.8 cm/s * 60 s = 148.8 ml/min. As such, the percent change in flow between baseline and hypocapnia is [(148.8 – 208.8)/208.8]*100 = −28.7%. The percent difference in velocity between baseline and hypocapnia is [(46.8 – 60)/60]*100 = −22%, indicating that TCD would underestimate the decrease in flow of the MCA during hypocapnia (−13 mmHg P_{ET}CO_2 from baseline) by ~7%. Thus it is evident by these calculations and those seen above that small changes in MCA diameter are responsible for large discrepancies between flow and velocity measures. Data are collated from Coverdale et al., 2014 and Serrador et al., 2000; Valdueza et al., 1997; Verbree et al., 2014. As noted in the hypercapnic calculations, this graph represents the most conservative way to quantify the percent difference in flow and velocity changes, highlighting the large impact changes in MCA diameter has in quantifying CBF (Ainslie & Hoiland, 2014).

Figure 2.6 Example B-mode image of transverse view of the carotid bifurcation (Thomas et al., 2015).

Figure 2.7 Example B-mode image of the CCA and bifurcation branching into the ICA and ECA (Thomas et al., 2015).

Figure 2.8 Example Spectral Doppler trace of A. ICA and B. ECA (Thomas et al., 2015).

Figure 2.9 Example B-mode image of the VA insonated from between the cervical spinal cord sections (vertebral processes) (Thomas et al., 2015).

Figure 2.10 Steering angle trade-off between B-mode and Pulse Wave Doppler. In all images: black parallel lines = vessel walls, red arrow = direction of moving blood, blue dashed line = ultrasound beam, green bar = angle cursor. A. 90° angle between blood flow and ultrasound.
beam will generate a bi-directional and low magnitude Doppler shift and therefore the system is unable to accurately determine velocity. **B.** The ultrasound beam has been appropriately steered to reduce the angle to 60°. **C.** The transducer has been “heeled” to decrease the angle of the beam relative to the vessel to an acceptable 60°. **D.** Appropriate alignment of angle cursor parallel to the vessel walls at 60°. **E.** Inappropriate placement of angle cursor relative to the direction of blood flow. In this case the system will assume at 60° angle (between ultrasound beam and angle cursor) as opposed to the actual angle of 45° (between the ultrasound beam and the blood flow) and will therefore underestimate the velocity calculated by ~30% (Thomas et al., 2015).

**Figure 2.11** Duplex ultrasound image of the ICA: **A.** Image A corresponds to the anatomical area of insonation, highlighted in the red box, of the ICA on the human figure. The velocity measurement trace is directly inferior to the B-mode image of the insonated ICA vessel. **B.** Image B is a depiction of the B-mode image, as it would appear in the edge-detection blood flow analysis software. The yellow rectangles represent the regions of interest (ROI) for sampling concurrent ICA diameter and velocity; the dotted lines on the velocity trace depict the edge detection tracking by the analysis software.

**Figure 3.1** Relative changes (Δ%) from baseline for MCAv, PCAv and MAP. *Significantly different following Prazosin administration, P<0.05. Significant differences in MAP (LBPP, LBPP+HG, but not HG) while MCAv & PCAv were unchanged following Prazosin administration. MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; MAP, mean arterial pressure; HG, 30% MVC handgrip exercise; LBPP, lower-body positive pressure; LBPP+HG, lower-body positive pressure combined with handgrip exercise.

**Figure 3.2** Relative changes (Δ%) from baseline for mean diameter, velocity & flow for the ICA and VA. No significant differences were found following Prazosin administration. ICA, internal carotid artery; ICAv, internal carotid artery velocity; Q_{ICA}, internal carotid artery blood flow; VA, vertebral artery; VAv, vertebral artery velocity; Q_{VA}, vertebral artery blood flow; HG, 30% MVC handgrip exercise; LBPP, lower-body positive pressure; LBPP+HG, lower-body positive pressure combined with handgrip exercise.

**Figure 3.3** Absolute changes (Δ) from baseline for mean cerebrovascular conductance of MCAv, PCAv, ICA flow & VA flow. *Significantly different following Prazosin administration, P<0.05. Significant differences in PCAv CVC during LBPP and LBPP+HG, but not 30% MVC HG following Prazosin administration. Q_{ICA}, internal carotid artery blood flow; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; Q_{VA}, vertebral artery blood flow; CVC, cerebrovascular conductance.

**Figure 3.4.1** Relationship between absolute changes in extra-cranial blood flow (Q_{ICA} & Q_{VA}) and intra-cranial blood velocity (MCAv & PCAv) during changes in MAP, within subjects. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.
Figure 3.4 2 Absolute changes in extra-cranial (ICA & VA) diameter / velocity vs. absolute changes in MAP. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.

Figure 3.4.3 Absolute changes in extra-cranial (ICA & VA) blood flow / velocity vs. absolute changes in intra-cranial (MCA & PCA) blood velocity. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>Anterior carotid artery</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphosphate</td>
</tr>
<tr>
<td>BA</td>
<td>Basilar artery</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CA</td>
<td>Cerebral autoregulation</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Calcium</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CDO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Cerebral oxygen delivery</td>
</tr>
<tr>
<td>CMRO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Cerebral metabolic rate of oxygen</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>CVC</td>
<td>Cerebral vascular resistance</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>CVR</td>
<td>Cerebrovascular resistance</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>dCA</td>
<td>Dynamic cerebral autoregulation</td>
</tr>
<tr>
<td>ECA</td>
<td>External carotid artery</td>
</tr>
<tr>
<td>F&lt;sub&gt;B&lt;/sub&gt;</td>
<td>Breathing frequency</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilation</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>H&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>HG</td>
<td>Handgrip exercise</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>ICA</td>
<td>Internal carotid artery</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Potassium</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LBNP</td>
<td>Lower body negative pressure</td>
</tr>
<tr>
<td>LBPP</td>
<td>Lower body positive pressure</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>MCAv</td>
<td>Middle cerebral artery blood velocity</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal voluntary contraction</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NVC</td>
<td>Neurovascular coupling</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Partial pressure of arterial carbon dioxide</td>
</tr>
<tr>
<td>PaO₂</td>
<td>Partial pressure of arterial oxygen</td>
</tr>
<tr>
<td>PCA</td>
<td>Posterior cerebral artery</td>
</tr>
<tr>
<td>PCAv</td>
<td>Posterior cerebral artery blood velocity</td>
</tr>
<tr>
<td>PETCO₂</td>
<td>Partial pressure of end-tidal carbon dioxide</td>
</tr>
<tr>
<td>PETO₂</td>
<td>Partial pressure of end-tidal oxygen</td>
</tr>
<tr>
<td>PSNA</td>
<td>Parasympathetic nervous activation</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>sCA</td>
<td>Static cerebral autoregulation</td>
</tr>
<tr>
<td>SNA</td>
<td>Sympathetic nervous activation</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>TCCD</td>
<td>Transcranial color-coded Doppler ultrasound</td>
</tr>
<tr>
<td>TCD</td>
<td>Transcranial Doppler ultrasound</td>
</tr>
<tr>
<td>TFA</td>
<td>Transfer function analysis</td>
</tr>
<tr>
<td>VA</td>
<td>Vertebral artery</td>
</tr>
<tr>
<td>Vₑ</td>
<td>Expired volume</td>
</tr>
<tr>
<td>Vₜ</td>
<td>Tidal volume</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to acknowledge and extend my enduring gratitude to my supervisor, Dr. Philip Ainslie for his guidance during my tenure at UBC Okanagan. It was your lectures and teachings in HMKN 411 and HMKN 499 that fostered my interest in physiology and scientific research. I would like to thank the supervisory committee for their feedback on this thesis. I would also like to acknowledge past and present colleagues within the Centre for Heart, Lung and Vascular Health for their scientific teachings, insight and guidance – not to mention comic relief - during my graduate studies. Lastly, I would like to thank my parents and Grandma B for your enduring support throughout my academic endeavors, especially financially.
Dedication

For all of my teachers, both good and bad, during my scholastic studies.

“The function of education is to teach one to think intensively and to think critically. Intelligence plus character – that is the goal of true education.”

-Dr. Martin Luther King, Jr.
Chapter 1: Introduction

In this chapter, the main modulating elements in regulation of blood flow in the human brain will be briefly reviewed. This review is followed by the main objectives and hypotheses of the presented thesis.

1.1 Brief background and overview of literature review

The human brain is one of the most complex and highly energetic organs in the human body. Despite its small relatively small mass (~2% of total body weight; 1200-1400 grams), it requires 15-20% of the body’s total cardiac output and ~20% of the body’s total oxygen consumption (~3.5 ml of oxygen per 100 grams of brain mass) (Williams & Leggett, 1989; Clarke & Sokoloff, 1999; Rowell, 1993; Brown & Ransom, 2015), making it one of the most highly perfused organs in the body. Despite these enormous metabolic requirements, the brain has limited capacity to store such metabolic substrates (i.e., glycogen) and therefore is almost entirely dependent upon dynamic changes in blood flow in order to deliver the necessary substrates (glucose and oxygen) and to eliminate unnecessary bi-products of metabolism, such as CO₂ and hydrogen ions (H⁺) (Brown & Ransom, 2007). A reduction in blood flow to the brain even for a short duration of time will lead to decreased cognitive function and can eventually lead to unconsciousness (Lennox, Gibbs, & Gibbs, 1935; Van Lieshout et al., 2003). If blood flow is not restored, potentially irreversible brain damage and death occurs (Smith, Clayton, & Robertson, 2011). As illustrated in the figure below (Figure 1.1.), the main modulators of cerebral blood flow (CBF) involve changes in arterial blood gases (Wolff & Lennox, 1930; Kety & Schmidt, 1948; Ainslie & Duffin, 2009; Willie et al., 2012; Hoiland et al., 2015), cerebral metabolism (Roy & Sherrington, 1890; Attwell et al., 2010; Phillips et al., 2015), changes in perfusion pressure (Paulson, Strandgaard, & Edvinsson, 1990; Koller & Toth, 2012; Tzeng & Ainslie, 2014; Meng et al., 2015) and neurogenic innervation (Edvinsson, 1982; Sándor, 1999; Hamel, 2006; Ainslie & Brassard, 2014; Willie et al., 2014). Modulation of CBF involves an integration of these modulating influences as well as a myriad of redundant mechanisms in order to maintain adequate perfusion of blood flow.
Figure 1.1 Major factors involved in the regulation of CBF: response to changes in metabolism, blood pressure, neurogenic (SNA), partial pressures of arterial CO₂ and O₂ and cardiac output. Adapted from Ainslie & Duffin, 2009. Permission to reproduce this adapted image was not required by American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.

1.2 Cerebral anatomy

To meet the high metabolic demands, the brain receives blood flow from the larger bilateral extracranial arteries - the internal carotid arteries (ICA) and vertebral arteries (VA). The figure below (Figure 1.2) depicts the main intra-cranial circulatory networks of the brain, notably the circle of Willis, as well as main extra-cranial arteries (ICA & VA) supplying blood flow to the brain. The ICA and VA, respectively, contribute ~75% and ~25% of the total CBF during resting conditions (Schoning, Walter, & Scheel, 1994; Sato & Sadamoto, 2010; Sato et al., 2011; Sato et al., 2012). The supplying blood flow to the ICA is delivered from the common carotid arteries (CCA) – which branches off the brachiocephalic artery and bifurcates in the ICA and external carotid arteries (ECA). The ICA supplies blood flow to the anterior portion of the brain and makes up the anterior portion of the circle of Willis, while the ECA supplies blood flow to the facial area. The ICA further branches into the middle cerebral arteries (MCA), anterior cerebral arteries (ACA) and posterior cerebral arteries (PCA). The MCA supplies ~80% of the blood flow to the temporal, parietal and frontal regions of each hemisphere of the brain (Olufsen, Nadim, & Lipsitz, 2002; Alastruey et al., 2007). Blood flow to the posterior region of the brain is delivered through the VA’s, which is supplied from the subclavian arteries; with the right subclavian artery branching from the brachiocephalic artery. The VA’s merges from the spinal cord into the basilar artery to
from the vertebrobasilar arterial network supplying blood flow to the cerebellum and merging to the posterior region of the circle of Willis into the PCA (Alastruey et al., 2007).

**Figure 1.2** Anatomical diagram of extra- and intra-cranial arteries: A. Anterior (i.e., internal Carotid Artery) and posterior (i.e., vertebral artery) blood supply of the extra-cranial cerebral vessels (Thomas et al., 2015). B. The circle of Willis and schematic of intracranial cerebral vessels (Reproduced from Drake et al., 2009). Permission to reproduce panel A was not required by the American Journal of Physiology – Regulatory, Integrative and Comparative Physiology. Panel B reproduced, with ©permission, from (Drake et al., 2009).

### 1.3 Arterial blood gases and the regulation of CBF

The brain is highly sensitive to changes in the partial pressure of arterial dioxide (PaCO₂) and, to a lesser extent, the partial pressure of arterial oxygen (PaO₂).

#### 1.3.1 Changes in carbon dioxide (PaCO₂)

Changes in PaCO₂ in particularly elicit marked alterations in corresponding blood pH (Chesler, 2003), thus influencing cerebrovascular resistance and CBF (Kety & Schmidt, 1948; Wasserman & Patterson, 1961). Increases in PaCO₂ (hypercapnia) affect the cerebrovasculature by inducing vasodilation and a concurrent increase in CBF, while decreases in PaCO₂ (hypocapnia) provoke vasoconstriction and a decrease in CBF. The changes in cerebral vascular resistance to changes in PaCO₂ is dependent upon the changes in extracellular pH, as the concentration of hydrogen ions (H⁺) has a direct effect on the cerebrovascular smooth muscle; increased [H⁺] induces acidosis and
vasodilation while decreased [H+] induces alkalosis and vasoconstriction (Heistad & Kontos, 1983; Kontos, Raper, & Patterson, 1977).

The entire cerebrovascular network is impacted by changes in PaCO₂ - from the smallest parenchymal vessels (Binks et al., 2008; Mandell et al., 2008; Nöth et al., 2008; Piechnik, Chiarelli, & Jezzard, 2008) and pial vessels (Wolff & Lennox, 1930) to the largest intracranial (MCA, PCA & BA - Giller et al., 1993; Wilson et al., 2011; Willie et al., 2013) and extra-cranial vessels (ICA, ECA & VA - Willie et al., 2012; Sato et al., 2012). Moreover, differences in CO₂ reactivity of the cerebral microvasculature have also been reported by high magnetic resonance imaging studies (Mandell et al., 2008; Nöth et al., 2008), as grey matter (cortical) tissue was significantly higher (respectively, ~1.5% & 0.5% and 5.8 ± 0.9% per mmHg PaCO₂) than white matter (subcortical) tissue. The latter is less vascularized than the former (Reich & Rusinek, 1989; Ramsay et al., 1993).

In response to hypercapnia and increased CSF PCO₂, pial arterioles have the ability to dilate by ~40% (Wolff, 1930; Kontos et al., 1977). The responsiveness of pial arterioles is dependent upon the vessel size, as smaller arterioles are more reactive to hypercapnia than larger pial arterioles (Wei, Kontos, & Patterson, 1980). The pial arterioles dilate in order to modulate changes in regional CBF, while the larger extra-cranial arteries (ICA & VA) modulate changes in global brain perfusion, and are sensitive to changes in blood gases and perfusion pressure (Heistad, Marcus, & Abboud, 1978; Faraci, Heistad, & Mayhan, 1987; Willie et al., 2012). In humans, as depicted in Figure 1.3, the diameter of the ICA & VA has been reported to increase by ~20% through a range of 15-65 mmHg PaCO₂ (Willie et al., 2012; Hoiland & Ainslie, 2016).
Figure 1.3 Regional differences in cerebrovascular reactivity during changes in Carbon Dioxide. The above figure outlines both intra- and extra-cranial reactivity to CO$_2$. **A.** Individual vessel relative reactivity to changes is PaCO$_2$ ranging from ~15mmHg to 65mmHg. **B.** Individual vessel relative reactivity calculated with linear regression in the hypercapnic range and hypocapnic range. Relative hypercapnic reactivity was not different between vessels, while the VA showed selectively higher reactivity than all other vessels during hypocapnia. * Significantly different from other vessels, P<0.05. Figure adapted, with ©permission, from (Willie et al., 2012).

In contrast, hypocapnia does not appear to have a similar impact on the cerebral vasculature as hypercapnia does. For example, studies employing Duplex ultrasound measures of the VA have reported a lower reactivity (Sato et al., 2012) or greater reactivity (Willie et al., 2012) to hypocapnia. Thus, it seems that the posterior regional responses differently to hypocapnia than that of the anterior regions. Due to the impact of CO$_2$ on the cerebral vasculature, CBF changes proportionally to changes in PaCO$_2$. Multiple studies (MCA – Ide, Eliasziw, & Poulin, 2003; Battisti-Charbonney, Fisher, & Duffin, 2011; Hoiland et al., 2015); PCA & BA - Skow et al., 2013; PCA - Hoiland et al., 2015; ICA & VA - Sato et al., 2012; Willie et al., 2012) have reported an approximate ~3-6% increase in CBF per mmHg increase in PaCO$_2$, while a decrease in PaCO$_2$ yields a decrease in CBF of ~1-3% below baseline values. Studies employing high magnetic resonance imaging techniques have reported similar reactivity findings (Serrador et al., 2000; Kemna et al., 2001; Mandell et al., 2008; Coverdale et al., 2014; Verbree et al., 2014).
1.3.2 Changes in oxygen (PaO₂)

Reductions in PaO₂ (hypoxemia) causes an increase in CBF, but only upon reaching a threshold of ~50 mmHg in both animals (Noell & Schneider, 1942; Baboons – James et al., 1969; Hoff et al., 1977; Ponte & Purves, 1974; Dogs – McDowall & Harper, 1967; Kogure et al., 1970; Haggendal & Winso, 1975; Dogs - Trastman, Fitzgerald, & Loscutoff, 1978; Dogs & Rhesus Monkeys – Heistad et al., 1976; Cats – Bates & Sundt, 1976; Rats – Borgström, Jóhannsson, & Siesjö, 1975; Lambs – Jones et al., 1981) and humans (Kety & Schmidt, 1948; Severinghaus et al., 1966; Shapiro, Wasserman, & Patterson, 1966; Shapiro et al., 1970; Cohen et al., 1967; Ainslie & Ogoh, 2010; Rowell, 1993) in order to increase oxygen delivery to the cerebral tissue. Data from humans (Cohen et al., 1967; Shapiro et al., 1970; Jensen et al., 1996; Ainslie & Poulin, 2004; Querido, Godwin, & Sheel, 2008; Reichmuth et al., 2009; Willie et al., 2012; Hoiland et al., 2015) have reported hypoxic induced increases in CBF of approximately 0.5 – 2.5% increase in CBF per mmHg PaO₂ below the threshold of ~50 mmHg in order to maintain CDO₂ (Figure 1.4), while little or no change in CBF has been reported with hyperoxia (Willie et al., 2012).

**Figure 1.4** CBF and CDO₂ during acute hypoxemic hypoxia in humans. The mean lines for CBF and CDO₂ are calculated as the linear slope from the mean data of each study in proportion to sample size (Hoiland et al., 2015). Permission to reproduce this image was not required by American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.
Hypoxia induces vasodilation in the smallest pial vessels (Cats - Wolff & Lennox, 1930) to the larger intra-cranial (MCA – Imray et al., 2014; Willie et al., 2013; Wilson et al., 2011) and extra-cranial vessels (ICA & VA - Lewis et al., 2014). Similar to hypercapnia, hypoxic vasoreactivity has been shown (Heistad et al., 1976) to be greater in grey matter (cortical) tissue than white matter (subcortical) tissue; this has been confirmed with high resonance imaging studies (Dyer et al., 2008; Nöth et al., 2008). Hypoxia effects cerebrovascular smooth muscle tone by disrupting transmembrane Ca\(^{2+}\) flux (Vinall & Simeone, 1986; Pearce et al., 1992; Pearce, 1995) and via second messenger, can modulate changes in K\(^+\) channel activation/hyperpolarizing pathways (Bonnet et al., 1991; Gebremedhin, Yamaura, & Harder, 2008). The changes result in a reduction in cerebrovascular smooth muscle tone and dilation. There are a number of endogenous substances that mediate hypoxic vasodilation – these pathways have been recently described in more detail elsewhere (Hoiland et al., 2015). Vessel reactivity in response to hypoxic stimulus have been investigated by a number of studies (Willie et al., 2012; Ogoh et al., 2013; Lewis et al., 2014; Hoiland et al., 2015), with the VA demonstrating the greatest reactivity (1.3% per mmHg reduction in PaO\(_2\)) compared to ICA, MCA and PCA (all: 0.5 to 0.6 per mmHg) (Figure 1.5.). These studies demonstrate the heterogeneous nature of the cerebrovasculature to response to hypoxemia, especially in the posterior circulation.

![Figure 1.5](image)

**Figure 1.5** Regional differences in cerebrovascular reactivity to hypoxia. The above figure outlines both intra- and extra-cranial reactivity to hypoxia. There was a pronounced increase in VA flow during hypoxia compared to the other vessels. Figure adapted, with ©permission, from (Willie et al., 2012).

### 1.3.3 End-tidal forcing

End-tidal forcing is a feed-forward ventilatory control technique that was employed so as to control end-tidal gases (P\(_{ET}\)O\(_2\) & P\(_{ET}\)CO\(_2\)) independent of ventilation. This technique allows for greater
standardization of ventilatory stimulus magnitude irrespective of subject variability and ventilatory sensitivity. This technique also minimizes the gradient between $P_{ET}O_2$ and $P_{ET}CO_2$, thus reducing the associated risk of underestimating cerebrovascular reactivity when changing the $FiCO_2$. End-tidal forcing has been employed in a number of other studies with various experimental stressors (Foster et al., 2014; Querido et al., 2013; Smith et al., 2016; Hoiland et al., 2015; Willie et al., 2012; Tymko et al., 2015; Tymko et al., 2016; Bain et al., 2013; Lewis et al., 2014) and is an innovative technique to effectively and reliably manipulate blood gases. Dynamic end-tidal forcing was used to effectively control arterial blood gases to isocapnic levels in the present thesis study.

1.4 Cerebral metabolism and the regulation of CBF

The brain is a highly energetic organ and requires an enormous amount of nutrients (glucose and oxygen) in order to function. Cerebral metabolism and local cerebral perfusion are tightly regulated by the product of anatomical and metabolic relationship between neurons, astrocyte glial cells and cortical penetrating arterioles – which makes up the neurovascular unit (Figure 1.6.). This relationship between metabolic demand and supplying blood flow to active tissues is termed neurovascular coupling (NVC). In the neurovascular unit, neuronal activation increases extracellular glutamate thereby increasing calcium availability within the astrocyte (Howarth, 2014). This response yields a release of vasoactive signaling substances that elicit changes in vascular tone allowing for increases in CBF to meet increased metabolic demand to the desired brain region – termed functional hyperemia. This process is relatively slow and is sufficiently adequate to increase glucose delivery during low neuronal activation (i.e., 3-4s), but not during periods of high intensity neuronal activation (i.e., <1s) (Zonta et al., 2003; Mulligan & MacVicar, 2004; Rosenegger & Gordon, 2014). There are a number of excellent reviews that discuss the intricacies of the mechanisms of the neurovascular unit; these topics are beyond the scope of this thesis (Attwell et al., 2010; Iadecola, 2004; Iadecola & Nedergaard, 2007; Filosa & Iddings, 2013; Phillips et al., 2015; Jakovcevic & Harder, 2007; Petzold & Murthy, 2011; Muoio, Persson, & Sendeski, 2014).
Sufficient metabolic coupling for any biological tissue is characterized by the ability to supply oxygen and other substrates (i.e., glucose and lactate) to meet changing metabolic demands of the tissue. During hypoxia there is an increase energy demand and therefore a subsequent increase in CBF, though cerebral metabolic rate of oxygen (CMRO\textsubscript{2}) remains relatively constant during periods of isocapnic hypoxia (PaO\textsubscript{2} = 36 mmHg – Ainslie & Subudhi, 2014) and following 6-12 hours of moderate altitude (3700 m) (Severinghaus et al., 1966). During hypocapnia, CMRO\textsubscript{2} appears to remain unchanged during modest hypocapnia (P\textsubscript{ET}CO\textsubscript{2} of 20 mmHg), but was decreased during extreme hypocapnia (P\textsubscript{ET}CO\textsubscript{2} of 10 mmHg) (Alexander et al., 1968). During hypercapnia, the CMRO\textsubscript{2} response appears to be equivocal within the literature. For example, animal studies
have reported increases (+25% - Berntman, Dahlgren, & Siesjö, 1979), decreases (-10-40% - Artru & Michenfelder, 1980; Kliefoth, Grubb, & Raichle, 1979) or no change (Eklöf et al., 1973) in CMRO₂. Human studies have demonstrated similar equivocal findings yielding either no change (Chen & Pike, 2010; Jain et al., 2011) or a decrease (Xu et al., 2011) in CMRO₂. Though these findings are not definitive in establishing proportional changes in CMRO₂ during changes in blood gases, these findings demonstrate the steadiness of CMRO₂ during changes in CBF at rest. Glucose is the essential substrate for cerebral metabolism and is capable of maintaining whole brain functionality during rest and activation (Clarke & Sokoloff, 1999). Conversely, lactate differs from glucose in that lactate ATP production is less efficient, and may not be the compulsory substrate utilized during normal resting brain function (Knudsen, Paulson, & Hertz, 1991). Lactate utilization, however, may be used as a fuel source during periods of elevated cortical activation or when lactate concentration is high, such as during exercise (Ide, Horn, & Secher, 1999; Volianitis et al., 2011; Fisher et al., 2013; Larsen et al., 2008).

Human studies exploring NVC are limited but have employed various techniques including pharmacological agents (Nimodipine – Phillips et al., 2015), exercise (Willie et al., 2011), mathematical modelling (Maggio et al., 2013; Maggio et al., 2014; Moody et al., 2005) and visual and cognitive stimulation (Boms et al., 2010; Rosengarten et al., 2001; Rosengarten et al., 2003). The fundamental mechanism(s) of NVC in humans has yet to elucidated and requires further investigation.

1.5 Cerebral autoregulation

The concept of how the brain regulates its blood supply in face of changes in perfusion pressure is termed cerebral autoregulation (CA) (Paulson et al., 1990; Bain, Nybo & Ainslie, 2015). The pressure-passive nature of the cerebrovasculature was first described by Bayliss, Hill and Gulland (1895), as stated, “In all physiological conditions a rise in arterial pressure accelerates the flow of blood through the brain, and a fall slackens it” (Bayliss, Hill, & Gulland, 1895). Although on a continuum, CA is often characterized by being either static (sCA - changes in MAP and CBF relationship over several minutes to hours) or dynamic (dCA - acute changes in the MAP and CBF relationship over a few seconds). This rendition of CA, however, is an experimental distinction as opposed to physiological, as both static and dynamic CA could vary back and forth depending upon the metric and during changes in MAP. If CA is assumed to be a high-pass filter model,
higher frequency changes in MAP are linearly transferred to cerebral circulation than lower frequency changes in MAP, sCA and dCA could both represent the same mechanism, but data are limited and further investigation is required to determine the precise mechanism. Currently, there is no evidence to indicate that sCA and dCA are separate mechanistic entities (Tan & Taylor, 2014).

In 1959, Niels Lassen (Lassen, 1959) published the landmark review paper on CA by combining a review on multiple previous studies of differing subject populations to construct a plot of average MAP and CBF. This graph depicted a large plateau region of relatively constant CBF over a wide range of pressures (~50-150 mmHg) - static autoregulation (Figure 1.7. - left panel). Although this has been the prevailing model of CA in the literature and is still frequently cited, there are a number of confounding factors in the construction of the curve itself that is vulnerable to criticism. The key criticism of Lassen’s curve, as outlined by Heistad and Kontos (Heistad & Kontos, 1983), is that the curve was constructed from data points from multiple different studies. These studies involved various clinical populations, including study participants under anesthesia. Furthermore, the CA plateau region in the curve (~50 – 150 mmHg) is contentious based on mathematical models, of which require higher feedback gain than what is tolerable for a biological system in order to generate a replicable curve (Panerai, 1998).

![Figure 1.7](Figure 1.7 The left panel depicts the classic interpretation of the relationship between MAP and CBF (i.e., CA) with a plateau region ranging 50 to 150 mmHg; changes in CBF are therefore modulated via changes in cerebrovascular resistance (red line) (Lassen, 1959). The right panel represents the contemporary depiction of CA, with a smaller plateau region of 5 to 25 mmHg (Lucas et al., 2010; Tan, 2012). Furthermore, the right panel illustrates that the cerebrovasculature is more effective at buffering increases in MAP than decreases; hysteresis effect of CA. Reproduced, with ©permission, from (Willie et al., 2014).)
The current model of CA suggests that regulation of CBF is maintained over a much narrower range of pressures - 5 to 25 mmHg (Figure 1.7. – right panel) from resting values (Lucas et al., 2010; Tan, 2012; Numan et al., 2014) and for specific time scales of variation (e.g., 0.03 Hz; 30 secs) (Tan, 2012). The brain is more effective at buffering against slower changes (<0.2 Hz; 5 secs) in MAP compared to transient changes (>0.2 Hz) in MAP (Aaslid et al., 1989; Tzeng & Ainslie, 2014). Autoregulation becomes deficient when oscillations in MAP are greater than 0.20 Hz (5 secs), as such oscillations in MAP are transmitted into the cerebrovasculature (Claassen, Levine, & Zhang, 2009; Tzeng et al., 2012; Zhang, Zuckerman, & Levine, 1998). This frequency domain characteristic of CA is important since such changes in MAP can be as a result of a variety of perturbations, including:

(i) Orthostasis - (Sorond et al., 2009; supine to stand – Thomas et al., 2009; thigh-cuff – Aaslid et al., 1989; Aaslid et al., 2007; Lipsitz et al., 2000; Deegan et al., 2010)
(ii) Central hypovolemia - (Romero et al., 2011; Ogawa et al., 2013; Saleem et al., 2015)
(iii) Heat stress or hyperthermia - (Wilson et al., 2006); dCA – (Brothers et al., 2009; Doering et al., 1999; Low et al., 2009))
(iv) Exercise - (Ogoh & Ainslie, 2009; Smirl et al., 2016)
(v) Pharmacological - (Immink et al., 2010; Ogoh et al., 2011b; Stewart et al., 2013); Hypocapnia – (McCulloch, Boesel, & Lam, 2005); Sedation – (Ogawa et al., 2010; Tieks et al., 1995); Thigh-cuff & Dexmedetomidine – (Ogawa et al., 2008); Sevoflurane – (Ogawa et al., 2006); Phenylephrine – (Zhang, Behbehani, & Levine, 2009; Lucas et al., 2010); Nitroprusside & Phenylephrine – (Liu et al., 2013; Willie et al., 2013); Ganglion blockade – (Zhang et al., 2002)
(vi) Pathological - (Stroke – (Dawson et al., 2000); Intra-cranial hemorrhage – (Paulson et al., 1990); Traumatic brain injury – (Golding, Robertson, & Bryan, 1999; Golding, 2002); Angiotensin – (Krejcy et al., 1997).

The potential mechanisms for CA are multifactorial and controversial (reviewed in: Ainslie & Brassard, 2014), with integration of myogenic, metabolic, neurogenic and endothelial-mediated factors or the possibility of a combination of all these factors (Tan, 2012; Hamner & Tan,
1.5.1 Hysteresis effect of cerebral autoregulation

The brain possesses a hysteresis effect in regulating dynamic changes in MAP and CBF, in that changes in MAP during transient hypertension are more effectively regulated than during hypotension (Heistad & Kontos, 1983). This has been shown in animal studies (Bill & Linder, 1976; Cassaglia, Griffiths, & Walker, 2008; Cassaglia, Griffiths, & Walker, 2009) and human studies (Tzeng et al., 2010; Aaslid et al., 2007; Schmidt et al., 2009). These findings were further supported in a recent meta-analysis of 40 studies reporting a $0.82 \pm 0.77 \% \text{CBF}/\%\text{ΔMAP}$ in the hypotensive range, compared to a $0.21 \pm 0.47 \% \text{CBF}/\%\text{ΔMAP}$ in the hypertensive range (Figure 1.8.), thus indicating a more pressure passive relationship between CBF and MAP within-individuals during hypotension (Numan et al., 2014).

![Figure 1.8](image_url) Relationship between %MAP and %CBF during increases and decrease in MAP. Individual lines represent individual studies; the length of each line depicts the range of MAP in the corresponding experiment. Reproduced, with ©permission, from (Numan et al., 2014).
1.5.2 Myogenic response of cerebral autoregulation

The myogenic response in the regulation of CBF refers to the vasculatures intrinsic ability in responding to changes in either mechanical load or intravascular pressure. This response was first described in the early 20th century and was alternatively named the Bayliss effect (Bayliss, 1902). The myogenic response is an integral feature of the cerebrovasculature in the maintenance of vascular resistance. The smooth muscle vasculature of both large and small arteries and arterioles allow for the vasculature to dilate and constrict accordingly to changes in intravascular pressure. This serves to protect the smaller downstream arterioles and capillaries from potential damage as a result of transient changes in perfusion pressure, and additionally to maintain adequate tissue perfusion (Kontos et al., 1978; Mellander, 1989).

There are two critical mechanistic interactions that lead to increases in actin-myosin interactions eliciting constriction of the vascular smooth muscle cells, a Ca$^{2+}$-dependent and independent mechanism. The Ca$^{2+}$ dependent pathway for the myogenic autoregulatory response of CBF is due to changes in local vascular wall tension. This leads to membrane depolarization eliciting Ca$^{2+}$ influx via myosin light-chain kinase (MLCK) activation and phosphorylation of MLC$_{20}$ (Davis & Hill, 1999; Schubert & Mulvany, 1999; Hill et al., 2001). The Ca$^{2+}$ independent pathway involves protein kinase C, diacylglycerol, RhoA/Rho kinase and 20-HETE to regulate MLCP, which determines the phosphorylation state of MLC$_{20}$ and the sensitization of actin myosin to Ca$^{2+}$ (Somlyo & Somlyo, 2003; Pfitzer, 2001; Lagaud et al., 2002; Narayanan et al., 1994).

Evidence of myogenic control of CBF has been reported, although data are limited with equivocal findings. Ischaemic thigh cuff release following calcium channel blockade administration (Nicardipine) rendered an attenuated blood flow response (Endoh et al., 2000; Endoh et al., 2002), though these data were collected while subjects were under anaesthesia (Propofol) - an intervention that has a direct effect on cerebral autoregulation (Matta et al., 1995; Ederberg et al., 1998; Harrison, Girling, & Mahajan, 1999). Two studies employing oscillatory LBNP and calcium antagonist (Nimodipine) (Tzeng et al., 2011) and a calcium channel blockade (Nicardipine) (Tan et al., 2013) reported altered flow-pressure response only during low frequencies (~20-30 secs) using linear TFA metrics. These data depict minimal myogenic involvement in control of CBF, though the LBNP stimulus approaches large oscillations in MAP may exceed the autoregulatory capacity of the cerebrovasculature (Leung et al., 2013). In addition,
the use of linear TFA metrics may be insufficient to quantify the non-linear physiological characteristics in the regulation of CBF.

1.5.3 Neurogenic influence on cerebral autoregulation

1.5.3.1 Sympathetic nervous activation on cerebral autoregulation

Since the role of SNA innervation in the regulation CBF is unclear, it perhaps comes as no surprise that the SNA in CA is controversial (van Lieshout & Secher, 2008; Strandgaard & Sigurdsson, 2008). Animal data (Cats - Bill & Linder, 1976; Dog, Cat, Monkey - Heistad, Marcus, & Gross, 1978; Dogs & Cats - Busija et al., 1980; Sheep - Cassaglia et al., 2008; Cassaglia et al., 2009) denote that sympathetic nerves are excited during hypertension but not hypotension during stimulation of superior cervical ganglion. Conversely, autoregulatory capacity has been reportedly been preserved in cats following denervation of preganglionic fibres supplying the superior cervical ganglion in cats (Busija & Heistad, 1984a). Human studies have suggested sympathetic influence (Kimmerly et al., 2003; Zhang, Crandall, & Levine, 2004; Ogoh et al., 2008) and parasympathetic influence (Hamner et al., 2012) in CA. Moreover, studies involving sympathetic ganglion blockade (Trimethapahan) (Zhang et al., 2004) and alpha-adrenergic blockade (Phentolamine) (Kimmerly et al., 2003) reported greater increase in CBF per increase in MAP during a Valsalva maneuver or norepinephrine infusion, respectively. Collectively, these findings indicate that SNA actively modulates CBF during periods of transient hypertension, but not during periods of hypotension.

1.5.3.2 Parasympathetic nervous activation on cerebral autoregulation

In addition to the sympathetic component of the extrinsic innervation of the nervous system that is responsible for vasoconstriction, PSNA modulates vasodilation of the cerebral vessels (Boysen, Dragon, & Talman, 2009; Busija & Heistad, 1981; D’Aleyc & Rose, 1977). Parasympathetic nerves may also contribute a vasodilatory role during certain pathological conditions, such as ischemia/reperfusion injuries (Busija, 1996), Alzheimers (Geaney et al., 1990) and migraine headaches (Goadsby et al., 2002). Anatomical studies demonstrate that there are cholinergic nerve terminals throughout the intracranial vessels proximal to the Virchow-Robin spaces (Florence & Bevan, 1979; Heistad et al., 1980; Sato, Sato, & Uchida, 2001; Hamel, 2004), thus resulting in the potential for interaction of cholinergic nerve terminals with noradrenergic nerve terminals near
cerebrovascular smooth muscle and modifying neurotransmitter release. Whether the parasympathetic nervous system is involved in controlling CBF remains unknown. Evidence from animal studies appears to be species specific, akin to SNS control, as petrosal nerve resection or stimulation did not alter baseline CBF in cats, but did in dogs (D’Alecy & Rose, 1977) and rats (Pinard et al., 1979). Furthermore, activation of nicotinic receptors on SNS nerves attenuated stimulation-induced release of noradrenaline in feline pial arteries (Edvinsson, Falck, & Owman, 1977), and nicotinic agonists reduced sympathetic stimulation-induced constrictor effects on cerebral vessels in rabbits (Aubineau, Sercombe, & Seylaz, 1980).

There is limited human data indicating parasympathetic involvement in the regulation of CBF. One study (Hamner et al., 2012) employing oscillatory LBNP reported increased cross-spectral coherence and gain (via TFA analysis), indicative of impaired CA at higher frequencies (>0.05 Hz) following cholinergic blockade (Glycopyrrolate). Another study reported that elevations in MCAv were abolished during static handgrip and cycling exercise (Seifert et al., 2010), both following cholinergic blockade of Glycopyrrolate. However, a recent imaging study (Rokamp et al., 2014) employing blood oxygen level dependent fMRI reported no effect of Glycopyrrolate on regional CBF during a visual and handgrip motor task.

While these studies underline a frequency-dependent role of SNA and PSNA influence on CA, there are limitations involving vasoactive drugs on systemic vasculature as well as the use of transcranial Doppler (TCD) when assessing changes in CBF that need to be considered when interpreting the data from these studies. Ultimately further research is needed in order to delineate parasympathetic nervous system involvement in the regulation of CBF.

1.5.3.3 Other neurogenic pathways and cerebral autoregulation
In addition to the sympathetic and parasympathetic nervous system pathways, there is the trigeminovascular system pathway, which is responsible for providing sensory feedback information. Trigeminovascular nerves may play a role in the upper limit (i.e., hypertension) of regulation of CBF (May & Goadsby, 1999); however, this postulation is limited as current literature details the trigeminovascular system in modulating cerebrovascular dilation during post-ischemia reperfusion (Moskowitz et al., 1989; Macfarlane et al., 1991), post-seizure hyperemia, cortical spreading depression leading to migraine headaches (Colonna et al., 1994; Wahl et al., 1994; Mayberg et al., 1981; Goadsby & Edvinsson, 1994; Hadjikhani et al., 2001; Woods,
Iacoboni, & Mazziotta, 1994; Bolay et al., 2002), arterial hypotension (Hong et al., 1994), as well as a possible protective role of vasospasm un subarachnoid hemorrhage (Norregaard & Moskowitz, 1985). There is also evidence supporting the role of the trigeminovascular system in the mammalian diving reflex upon cold-water immersion as this reflex initiates release of the vasodilator, substance P (Moskowitz et al., 1979). Animal studies employing trigeminal stimulation rendered marked increases in CBF in rats (Atalay et al., 2002; Just, Petersen, & Gruetter, 2010), cats (Goadsby & Duckworth, 1987; Lambert et al., 1984) and pigs (Salar et al., 1992).

Limited studies in humans have also reported increases in CBF following stimulation of the trigeminal ganglion via local glycerol injection (Tran Dinh et al., 1991) as well as thermocoagulation (Tran Dinh et al., 1992). Though these studies have provided important insight and findings, caution should be exercised in extrapolating the findings of these two studies due to anaesthesia as well as known pathological disorders (i.e., idiopathic trigeminal neuralgia) of the subjects involved in these studies. Conversely, one study reported decreased MCAv during trigeminal ganglion electrical stimulation (Visocchi et al., 1996); however, these changes may be due to confounding changes in PaCO₂ (Visocchi et al., 1996) or changes in MCA diameter, or both. Further research is needed in determining the trigeminovascular systems exact role in the regulation of CBF.

There is also evidence that suggest other central nervous pathways could contribute to regulation of CBF, but these findings are conflicting. For instance, stimulation of the fastigal nucleus of the cerebellum activated cholinergic pathways resulting in dilation of cerebral vessels and an increase in CBF in cats and rats (Doba & Reis, 1972; Nakai, Iadecola, & Reis, 1982; Nakai et al., 1983; Mraovitch, Pinard, & Seylaz, 1986), while stimulation of the locus coeruleus rendered a decrease in CBF in rhesus monkeys (Raichle et al., 1975). Presently, evidence for the role of the trigeminovascular and central nervous systems in the regulation of CBF is inconclusive and requires further study.

1.6 Cerebral perfusion and CBF

1.6.1 Perfusion pressure

Cerebral blood flow is calculated by the change in cerebral perfusion pressure (CPP) and cerebrovascular resistance (CVR). Cerebral perfusion pressure is the difference between mean
arterial pressure (MAP) and intracranial pressure (ICP), with ICP being primarily influenced by cerebral venous pressure (CVP) and cerebral spinal fluid (CSF) pressure. See equation below:

\[
\text{Cerebral blood flow} = \frac{\text{CPP}}{\text{CVR}}
\]

Under normal resting conditions in humans, while in supine position, ICP is unperturbed within an approximate range of 7 to 15 mmHg (Albeck et al., 1991); therefore, assuming this relationship remains constant, changes in CPP would reflect changes in MAP. There are a number of pathologies and conditions that can perturb ICP and thus alter CPP (e.g., intracranial tumors (Rees, 2011)), traumatic brain injury and hemorrhage (Sheth et al., 2013), reduced CSF absorption or increased CSF production (Lyons & Meyer, 1990), increased intrathoracic pressure (Valsalava maneuver) (Porth et al., 1984), disruption of blood brain barrier (BBB) integrity (Gumerlock, York, & Durkis, 1994), heat stress (Chang et al., 2004; Lin & Lin, 1991; Shih, Lin, & Tsai, 1984). As such, any changes in CPP is mainly influenced by changes in MAP (Powers, 1991). Conversely if MAP is relatively constant, CBF is modulated by changes in vascular resistance.

Observation studies of pial vessels in the brain were the first to demonstrate that large and small pial arteries undergo autoregulatory caliber adjustments in response to changes in arterial blood pressure (Fog, 1939; Kontos et al., 1978). It was further reported that the larger pial arterioles constricted and smaller pial arterioles dilated during increases in blood pressure, while the opposite effect occurred during decreases in blood pressure with the larger arterioles dilating and the smaller arterioles also dilating at a much lower pressure threshold (Heistad et al., 1978); (Faraci, Heistad, et al., 1987). It has since been shown that the larger intra-cranial (MCA, PCA, ACA – (Busija & Heistad, 1984; Heistad et al., 1978) and extra-cranial neck vessels (ICA, VA – Rabbits & Dogs - (Mchedlishvili, 1964), Dogs – (Mchedlishvili, Mitagvaria, & Ormotsadze, 1973), Cats – (Faraci, Heistad, et al., 1987); (Kontos et al., 1978); Dogs – (Heistad et al., 1978) are more integral than previously thought in the regulation of CBF through modifying vascular resistance. Comparative responses have also been identified during changes in blood gases as shown in high resolution magnetic imaging studies (Coverdale et al., 2014; Verbree et al., 2014; Wilson et al., 2011) as well as other studies employing Duplex ultrasonography (Willie et al., 2012). Thus, it has been suggested that the larger extra-cranial arteries act as the first line of defense in regulating blood
flow during transient changes in perfusion pressure, while the smaller pial arterioles serve to modulate changes in regional blood flow.

1.6.2 Cardiac output
Cardiac output (CO) is a determinant of CBF that is normally considered independent of CPP (Ogoh et al., 2005); it is not typically considered a determinant of flow according to Poiseuille’s law. This detail has been considered in a number of studies in humans (Brown et al., 2003; Guo et al., 2006; Ogoh et al., 2005; Van Lieshout et al., 2001; Van Lieshout et al., 2003). Any experimental intervention that alters CO may also alter CBF via flow-mediated changes in vascular resistance (Koller & Toth, 2012). A recent review (Meng et al., 2015) indicates that an alteration in CO, either acutely or chronically, can lead to a change in CBF that is independent of other CBF-regulating parameters including MAP and PaCO$_2$; however, related to the current thesis, such relationships have not been explored in the context of controlled changes and manipulation of MAP and PaCO$_2$.

1.6.3 Baroreflex
Mediated by stretch receptors in the aortic arch and carotid sinus, the baroreflex is a homeostatic mechanism that attenuates changes in arterial pressure. Increases in arterial pressure elicit a baroreflex-mediated reduction in sympathetic outflow, thereby decreasing heart rate, contractility and peripheral vascular resistance yielding a reduction in MAP (Chen & Bonham, 2010; Macefield & Henderson, 2010). Conversely, a decrease in arterial pressure mediates an increase in sympathetic drive, thereby increasing heart rate, peripheral vascular resistance, thus increasing MAP (Levick & Michel, 2010). These alterations in heart rate and peripheral vasomotor tone act harmoniously to stabilize arterial pressure (Mancia et al., Fadel et al., 2003; Fadel & Raven, 2012). Normal baroreflex function limits the range of changes in BP without employing vasoactive drugs (i.e., Phenylephrine and/or Nitroprusside [the Oxford technique] – Ebert & Cowley, 1992; Lucas et al., 2010; Tzeng et al., 2010; Ogoh et al., 2011). Baroreflex function can also be challenged by changing central blood volume using LBNP (Stevens & Lamb, 1965; Lewis et al., 2014), LBPP (Shi, Foresman, & Raven, 1997; Fu et al., 1998; Perry et al., 2013; Perry et al., 2014) or by changing body position (Sit to stand and head-down tilt) (Zhang et al., 2009; Smirl et al., 2015; Tymko et al., 2015). In addition, thigh-cuff release (Ogoh et al., 2008; Ogoh et al., 2010), neck
suction (Ebert et al., 1984; Eckberg et al., 1975) or Valsalva maneuver (Smith et al., 1996) can experimentally alter baroreflex functionality. Thus, by extension of control of MAP, there is an important role of the baroreflex in the regulation of CBF.

1.6.4 Cerebrovascular response to handgrip exercise

Isometric handgrip exercise (HG) is a useful research tool for eliciting dynamic increases in BP that can challenge the cerebral autoregulatory capacity of the cerebral circulation. Initiation of dynamic resistance exercise increases SNA in order to increase CO and divert blood flow to the active musculature (Rowell, 1993). Similar to active peripheral skeletal muscle, increases in neuronal activity appear to be coupled with increases in cerebral perfusion pressure during exercise (Secher, Seifert, & Van Lieshout, 2008). Though SNA innervation of cerebral perfusion at rest and during exercise remains contentious in the literature (Strandgaard & Sigurdsson, 2008; Ogoh & Ainslie, 2009; Ainslie & Tzeng, 2010), it is unclear whether or not sympatho-excitation manoeuvres (i.e., HG) elicit cerebrovascular responses, as there are differences in the distribution of alpha-adrenergic receptors between the peripheral and cerebral vasculature (Faraci & Heistad, 1998). A study by Ainslie and co-authors (Ainslie et al., 2005) employed isometric HG and measured MCAV and blood flow in the femoral artery during isocapnia and hypercapnia. During isocapnia, MAP was significantly increased by 28%, while MCAV was elevated, albeit not significantly (Δ+5.6%, 60.2 ± 8.9 to 63.6 ± 11.2 cm/sec; P>0.05). In contrast, during severe hypercapnia (+10 mmHg CO₂) MAP increased by 34% (89.7 ± 9.8 to 120.3 ± 14.7 mmHg, P<0.05) and MCAV was elevated by 66% (60.2 ± 8.9 to 100.4 cm/sec, P<0.05). Cerebrovascular resistance (CVR) was significantly increased by 21% during isocapnia isometric HG (1.49 ± 0.23 vs. 1.80 ± 0.32 mmHg/cm/sec; P<0.05) and was significantly decreased during severe hypercapnia (+10 mmHg CO₂) by 19% (1.49 ± 0.23 vs. 1.20 ± 0.20 mmHg/cm/sec; P<0.05). The study concluded that cerebral vasculature is more sensitive to changes in PaCO₂ compared to the peripheral vasculature during isometric HG, as there were marked decreases in CVR during HG in hypercapnia (1.49 ± 0.23 to 1.20 ± 0.20 mmHg/cm/sec) whereas femoral vascular resistance increased (0.17 ± 0.09 to 0.21 ± 0.10 mmHg/ml/min). These findings highlight the differential vascular responsiveness between the peripheral and cerebral vasculature via the sympathetic nervous system. In addition, there have been studies employing sympathetic agonist drugs (Ephedrine, Dobutamine, Dopexamine – Moppett et al., 2004; Noradrenaline – Kimmerly et al.,
2003) known to have systemic effects on the peripheral vasculature that have reported no effects on the cerebrovasculature in humans. The use of HG in the present study was to further augment increases in hypertension with and without LBPP.

### 1.6.5 Cerebrovasculature response to lower body positive pressure

The use of lower-body positive pressure (LBPP) is an effective non-pharmacological experimental method of increasing in perfusion pressure. During LBPP there is a displacement of blood in the lower limbs into the upper torso (Hinghofer-Szalkay, Kravik, & Greenleaf, 1988; Nishiyasu et al., 1998), thereby increasing central venous pressure (CVP), cardiac filling and MAP (Bevegård, Castenfors, & Lindblad, 1977; Eiken & Bjurstedt, 1987; Nishiyasu et al., 1998; Shi et al., 1997; Fu et al., 1998). Despite this increase in CVP, both CO and HR remain unchanged due to increased afterload offsetting the elevated filling pressures (Rubal, Geer, & Bickell, 1989); however, likely due to some fluid translocation, there have been reported increases of 25% in CO during prolonged periods of LBPP (30 min) (Geelen et al., 1992). Data concerning the cerebrovascular response to LBPP is limited; one study (Cutuk et al., 2006) employing short bouts (~1 min) of LBPP applied to subjects in upright postural position reported no significant effect on MCAv, though MCAv was increased from baseline during +20 mmHg LBPP (75 to 81 cm/sec) and then decreased back to baseline levels at +40 mmHg LBPP (78 cm/sec). Despite these intriguing findings, Shi and co-authors (Shi et al., 1997) reported that physiological stability to LBPP requires several minutes (~4 min) in order to achieve steady state, thereby undermining the aforementioned non-significant data depicting MCAv change during different changes in LBPP. Thus, the initial increase in MAP from the transient LBPP could be counteracted by dynamic CA rendering a non-steady state response (Zhang et al., 2009). In addition, differences in posture – upright vs. supine – and variables changes in PaCO$_2$ on MCAv in response to LBPP have not been elucidated and require further investigation.

There have been two recent studies (Perry et al., 2013; Perry et al., 2014) that have investigated changes in MCAv during differential steady state supine LBPP. The first of these two studies (Perry et al., 2013) reported small but significant increases in MCAv from baseline to LBPP of +20 mmHg (74 to 77 cm/sec) which caused a 7mmHg increase in MAP. These MCAv values then decreased below baseline values during +40 mmHg LBPP (74 to 72 cm/sec) that provoked a 13 mmHg increase in MAP (Figure 1.9.). In the second study (Perry et al., 2014), LBPP in steady
state supine position was employed during eucapnia and hypercapnia (5% CO₂). Hypercapnia was employed in an attempt to compromise CA. In contrast to the previous study, supine LBPP of +20 and +40 mmHg in eucapnia yielded no significant changes in MCAv during +20 mmHg (62 to 61 cm/sec) or during +40 mmHg LBPP (62 to 60 cm/sec) despite respective concomitant increases in MAP of Δ6 ± 5 & Δ9 ± 3 mmHg during hypercapnia. MCAv was significantly increased from baseline and at +40 mmHg LBPP (85 to 92 cm/sec), with concurrent increases in MAP during both +20 (88 to 94 mmHg) and +40 mmHg LBPP (88 to 98 mmHg). Both studies postulated that cerebral sympathetic activation acted as a protective mechanism in controlling increases in blood flow to the brain (as indexed by MCAv) during transient elevations in MAP. Potential limitations of these studies were the lack of PaCO₂ control and the use of velocity as an index of CBF. These findings and related limitations contributed to the formation of the research question and experimental design of this thesis.

![Graph showing individual changes from baseline for mean MAP (left) and mean MCAv (right).](image)

**Figure 1.9** Individual changes from baseline for mean MAP (left) and mean MCAv (right). Mean MCAv increased from baseline 5% during +20 mmHg LBPP, but conversely decreased by 2% from baseline values during +40 mmHg LBPP. Mean MAP increase 7 mmHg from baseline during +20 mmHg, and further increased 8 mmHg (+15 mmHg from baseline) during +40 mmHg LBPP. Reproduced, with ©permission, from (Perry et al., 2013).

### 1.7 Neurogenic regulation of CBF

Perivascular nerve fibers in the anterior and posterior cerebral circulation were first identified and described by Sir Thomas Willis in 1664 (Willis, 1664). In contrast, the origin of sympathetic
innervation of major cerebral vessels at the brainstem and spinal cord of the brain was not described until a few hundred years later (Benedikt, 1874; Aronson, 1890; Huber, 1899). The extrinsic perivascular nerves, which innervate the extra-cerebral blood vessels (e.g., ICA & VA) and superficial pial vessels, originate in the superior cervical ganglion (adrenergic), the sphenopalatine & optic ganglia (both cholinergic) and trigeminal ganglion (sensory). As the peripheral extrinsic nerves dive into the brain parenchyma and the Virchow-Robin space, the nerves begin to receive neural input from within the brain itself (e.g., locus coeruleus, fastigal nucleus and dorsal raphe nucleus) and are therefore deemed the intrinsic neural component of the cerebrovasculature (Figure 1.10.) (Clark, 1929; Penfield, 1932; McNaughton, 1938; Sándor, 1999; Hamel, 2006; Willie et al., 2014). The extrinsic and intrinsic neural components have the ability to either dilate or constrict the cerebrovasculature in response to different released neurotransmitters: norepinephrine, neuropeptide Y, vasoactive intestinal peptide, acetylcholine, nitric oxide, synthase, peptide histidine isoleucine or methionine, calcitonin gene-related peptide, substance P, neurokinin A and pituitary adenylate-cyclase activating polypeptide (Sándor, 1999; Hamel, 2006). Though there has been a great deal of anatomical work establishing the neurogenic innervation of the cerebral circulation, whether or not the perivascular nerves are involved in moment-to-moment dynamic regulation of CBF remains controversial (Forbes, Henry & Cobb, 1938; Purves, 1972; Ponte & Purves, 1974; van Lieshout & Secher, 2008; Strandgaard & Sigurdsson, 2008).
Figure 1.10 Representational schematic of the network of the different cerebral perivascular nerves. The ‘extrinsic’ nerves to cerebral blood vessels at the surface of the brain come from the peripheral nervous system (PNS) and originate either in the superior cervical ganglion (SCG), sphenopalatine (SPG), or otic ganglion (OG) or trigeminal ganglion (TG). Blood vessels located within the brain parenchyma, or the microcirculation, are innervated by ‘intrinsic’ nerve pathways that find their origin in the central nervous system (CNS). Inset: schematic representation of the ‘neurovascular unit’ as seen at the electron microscopic level with the vascular [endothelium (medium gray) and smooth muscle or pericyte (dark gray)], astroglial (light gray), and neuronal (axon varicosities are highlighted) compartments (Hamel, 2006). Permission to reproduce this image was not required by the Journal of Applied Physiology.

It has been suggested that during steady-state physiological conditions, neurogenic innervation has minimal influence in regulating CBF (Edvinsson, MacKenzie, & McCulloch, 1993; Busija, 1996; Strandgaard & Sigurdsson, 2008; Ter Laan et al., 2013). Studies in animals (Fog, 1939; Eklöf et al., 1971; Waltz, Yamaguchi, & Regli, 1971) have employed alpha-adrenergic antagonists and reported unchanged pial arteriolar diameter in anesthetized cats, piglets and lambs.
(Kuschinsky & Wahl, 1975; Busija & Leffler, 1987; Wagerle, Kurth, & Roth, 1990). Consistent with these findings, studies report minimal or no change in CBF in awake dogs, cats, rabbits and lambs following either sympathetic nerve stimulation, denervation/ganglionectomy, or both (Mueller, Heistad, & Marcus, 1977; Heistad et al., 1977; Heistad, Marcus, & Gross, 1978; Marcus & Heistad, 1979; Busija, 1984; Sadoshima, Busija, & Heistad, 1983; Kurth, Wagerle, & Delivoria-Papadopoulos, 1988). In contrast, intra-arterial or intravenous administration of catecholamines yielded an increase in CBF in baboons and lambs (MacKenzie et al., 1979; Purves & James, 1969), a small decrease in goats (anesthetized) and dogs (Lluch, Reimann, & Glick, 1973; Oberdörster, Lang, & Zimmer, 1973), and was unchanged in humans (Olesen, 1972). Due to these contradictory findings the general conclusion is that during steady-state physiological conditions perivascular nerve innervation have little or no modulating capacity of CBF. However, there is evidence to suggest that perivascular nerves are involved during non-steady-state physiological conditions, especially hypertension (Bill & Linder, 1976; Edvinsson, Owman, & Siesjö, 1976; MacKenzie et al., 1976; Heistad & Marcus, 1979; Waldemar et al., 1989; Paulson et al., 1990), as well as during hypercapnia (Harper et al., 1972; Busija & Heistad, 1984a) and hypoxia (Wagerle et al., 1983).

Drawing from well-controlled animal model studies (Bill & Linder, 1976; Edvinsson et al., 1976; Heistad, Marcus, & Gross, 1978; Cassaglia et al., 2008a; Cassaglia, Griffiths, & Walker, 2008b), it has been proposed that the sympathetic nervous system modulates CBF during acute changes in blood pressure, thus acting as a protective buffer for the cerebral microcirculation against potential hyper-perfusion injury (Mayhan, Werber, & Heistad, 1987). This has been shown in animal studies involving stimulation of the superior cervical ganglion, in which CBF is reduced at baseline and is further attenuated during hypertension (Mayhan et al., 1987; Cassaglia et al., 2008a; Cassaglia et al., 2008b; Cassaglia et al., 2009). This protective buffering against hypertension from the SNS does seem to be a function of the larger cerebral arteries (rabbits & cats - Baumbach & Heistad, 1983), including the large pial arterioles (cats - (Wei et al., 1975), as opposed to the cerebral microcirculation. Unilateral resection of the superior cervical ganglion produced ipsilateral disruption of cortical vessel integrity during hypertension in baboons, dogs, cats and monkeys (Ponte & Purves, 1974; Heistad, Marcus, & Gross, 1978). A critique of studies involving denervation is that the ability for the larger vessels to maintain flow during changes in perfusion pressure is dependent on the innervation of the vessel, as the vessel becomes pressure passive once denervated. This may directly influence the autoregulatory capacity of the larger
vessels, due to the plausibility that these vessels are under neurogenic modulation (Mchedlishvili et al., 1973; Tamaki & Heistad, 1986). Although animal models provide insight into the plausibility and mechanisms of SNS regulation in cerebrovascular function, extrapolation to humans should be done cautiously as there are distinct differences between species (Heistad, Marcus, & Gross, 1978; Busija, Heistad, & Marcus, 1980) as well as heterogeneous distribution of sympathetic innervation (Edvinsson, 1982; Busija, 1996; Sândor, 1999) of the cerebrovasculature.

Studies in humans have employed various techniques, including local subcutaneous ganglion blockade or oral / IV pharmacological sympathetic blockades in healthy human subjects or disease populations that have received ganglionectomies. The key findings from these studies have been mixed. In the key clinical studies, CBF was reported to have increased by ~20% following ganglion excision (Shenkin, Cabieses, & van den Noordt, 1951); (Shenkin, 1969); (Suzuki, Iwabuchi, & Hori, 1975); (Jeng et al., 1999). Jeng and co-authors (Jeng et al., 1999) reported that ICA blood flow and MCA velocity were increased by 9% and 7.5%, respectively 2-4 weeks following T2 sympathectomy. In numerous studies, CBF has been reported to increase following local stellate ganglion blockade, most notably on the ipsilateral side of experimental intervention (Linden, 1955; Umeyama et al., 1995; Ide et al., 2000; Treggiari et al., 2003; Yokoyama, Kishida, & Sugiyama, 2004). However, three other studies observed no change in CBF following a chemical blockade of percutaneous infiltration of local anesthetic (Harmel et al., 1949; Scheinberg, 1950; Ohta, Hadeishi, & Suzuki, 1990). Moreover, another study observed a decrease in CBF (decrease of MRA signal intensity and unchanged intra-cranial arterial caliber) following stellate ganglion blockade (Kang et al., 2010). The lack of CBF change in a few of these aforementioned studies (Harmel et al., 1949; Scheinberg, 1950; Ohta et al., 1990) may be attributed to local anesthesia resulting in a partial or incomplete blockade. There is also the likely that the various pathological conditions or disorders (e.g., Parkinson’s, hemiplegia, traumatic brain injury, stroke, cirrhosis, syphilis, hypertension) that may have altered the true effect of CBF changes. Despite these pathologies the consensus of studies employing ganglionectomy (Shenkin, Cabieses, & van den Noordt, 1951; Shenkin, 1969; Suzuki et al., 1975; Jeng et al., 1999) yield comprehensive increases in CBF, providing evidence of sympathetic nervous system innervation of the cerebral circulation. However, these studies do not elucidate the functional role of SNA in the regulation of CBF.
To summarize, the cerebral circulation is highly innervated with sympathetic and parasympathetic perivascular nerves allowing for vasoconstriction and vasodilation of the cerebrovasculature mediated through neurotransmitter release. Sympathetic modulation in the regulation of CBF at rest remains controversial, but appears to be influential in eliciting cerebral vasoconstriction during transient hypertensive conditions. There are a number of confounding factors that contribute to the controversial findings in the literature for sympathetic modulation in the regulation of CBF, including: (1) redundancy of other autoregulatory mechanisms, (2) inappropriate or incomplete CBF measurements, (3) species-related differences, (4) duration and intensity of sympathetic stimulation, (5) heterogeneous distribution of sympathetic innervation, (6) metabolic restraint. These six factors are discussed in further depth elsewhere (Busija, 1996; Sándor, 1999; Ter Laan et al., 2013; Ainslie & Brassard, 2014). Further research is needed to fully understand and appreciate these factors in order to update our current controversial perspective of the role SNA serves in the regulation of CBF.

1.7.1 Adrenergic receptors
The cerebral vasculature is richly supplied by both adrenergic and cholinergic receptors of both extrinsic (i.e., cervical, sphenopalatine and trigeminal ganglia) and intrinsic (i.e., locus coeruleus, fastigial nucleus and dorsal raphe nucleus) origins (Lowe & Gilboe, 1971; Edvinsson et al., 1976; Edvinsson & Hamel, 2002). The large cerebral arteries are highly innervated mostly by adrenergic fibers originating from the ipsilateral superior cervical ganglion (Itakura et al., 1977). Smaller arteries or arterioles have much less adrenergic innervation, and are innervated mainly by secondary systems arising from the locus coeruleus (Nielsen & Owman, 1967; Itakura et al., 1977). Alpha-1 adrenergic receptors are the most abundant, as they are located on the post-synaptic cleft, alpha-2 adrenergic receptors are located on the pre-synaptic membrane of the nerve terminal and function primarily as a local negative feedback loop in regulating norepinephrine release (Charkoudian & Wallin, 2014). The alpha-1 adrenergic receptors are primarily responsible for vasoconstriction, while beta-adrenergic receptors are primarily responsible for mediating vasodilation (Edvinsson, 1982; Winquist, Webb, & Bohr, 1982; Wroblewska et al., 1984). Due to differences in distribution of alpha and beta-receptors, beta receptor-mediated vasodilatation occurs mainly in small cerebral vessels, while α receptor-mediated vasoconstriction preferentially affects large cerebral arteries (Fitch, MacKenzie, & Harper, 1975). Similar to alpha-adrenergic
receptors, beta-adrenergic receptors are located on the post-synaptic (beta-1) and pre-synaptic cleft (beta-2), with density ratio of beta-1 to beta-2 adrenergic receptors in human cerebral arteries being approximately 40:60 (Tsukahara et al., 1986). Thus, at least in experimental animals, sympathetic stimulation causes vasoconstriction in large vessels. However, as long as arterial pressure remains in the so-called autoregulatory range, there is little change in cerebral blood flow because of a concomitant decrease in pial vessel resistance (Baumbach & Heistad, 1983). This is likely caused by either autoregulatory or beta-adrenergic vasodilatation (Sohn, 1998), as it has been suggested that stimulation of the sympathetic nerves elicits vasoconstriction of the larger cerebral arteries, while the smaller vessels are dilated (Harper et al., 1972; Kuschinsky & Wahl, 1975; Wei et al., 1975; Wahlgren et al., 1992). Prazosin is a selective alpha-1 antagonist and effectively blocks ~70-80% of alpha-1 adrenergic receptors (Ogoh et al., 2008; Jaillon, 1980), making it an ideal research tool in comparison to a non-selective alpha antagonist.

1.8 Research questions and hypothesis
Sympathetic modulation of CBF in humans has been extensively debated, with reviews and investigations reporting that sympathetic modulation is more effective during hypertensive conditions than hypotensive conditions. Therefore, the purpose of this thesis is to investigate the functional role of SNA in the regulation of CBF during transient hypertension with and without pharmacological blockade.

Objectives and Hypothesis:
Objectives:
I. Examine changes in extra-cranial blood flow and intra-cranial velocity during non-pharmacologically induced transient hypertension.

II. Investigate effects of alpha-adrenergic antagonist (Prazosin) administration during transient hypertension.

Hypothesis:
I. Orally administered alpha-1 adrenergic antagonist (Prazosin) will augment increases in extra-cranial (ICA & VA) blood flow and intra-cranial (MCA & PCA) velocity during transient hypertension.
Chapter 2: Methodology

This chapter contains an overview of the experimental methodology that was used in this thesis. Furthermore, details are provided on participant background, pre-screening criteria, experimental protocols and design as well as collected variables and data and statistical analyses.

2.1 Ethical approval

This study received ethical approval from the Clinical Research Ethics Board at the University of British Columbia (H13-03081), and was abided by the Canadian Government Tri-Council Policy Statement for Integrity in Research. This thesis study abided by the Canadian Government Tri-Council Policy Statement for Integrity in Research and adhered to the principles of the Declaration of Helsinki and title 45, US code of Federal Regulations, Part 46, Protection of Human Subjects. All volunteer subjects provided written informed consent, and procedures were followed in accordance to institutional guidelines.

2.2 Study participants

Fifteen healthy young volunteers were recruited to participate in this study (22 ± 3 years, 9 male & 6 female, 173 ± 8 cm, 72 ± 16 kg). Inclusion criteria for this study included the following: non-smoking, non-obese (BMI <30 kg/m²) with no history of cardiovascular disease, diabetes, hypertension or respiratory disease (including asthma) as well as no known adverse reaction to Prazosin. The subjects were screened to ensure reliable ultrasound images of the extra-cranial (ICA & VA) and intra-cranial arteries (MCA & PCA) and were familiarized with the experimental protocol. Subjects were not on any medications, except for female subjects using oral contraceptives. Female subjects were tested in the early follicular phase (day 1-7) of menstrual cycle od during the menstruation and contraceptive withdrawal phase (day 2-7). All subjects were asked to abstain from caffeine and alcohol (12 hours) as well as vigorous exercise (24 hours) prior to study participation.

2.3 Experimental protocol

Study subjects visited the laboratory at the University of British Columbia-Okanagan on two separate occasions. The first visit included an introduction to the study, and was familiarized with
the equipment and experimental procedures that accompanied the study. This session was also used to screen the volunteer(s) for ultrasound image quality. The second visit consisted of the main experimental protocol, whereby the subjects lay at rest in a supine position, secured up to their iliac crest, inside the custom engineered LBPP chamber. During the resting period, subjects were instrumented with the TCD, Finometer, automated manual BP cuff and ECG electrodes. Subjects then breathed room air through the snorkel mouthpiece apparatus for approximately 5 minutes to collect resting room air end-tidal gases (P_{ET}O_2 & P_{ET}CO_2). These resting values were then inputted into the dynamic end-tidal forcing system (Airforce 4.7), where the subject’s end-tidal gases were effectively controlled to isocapnia levels for the duration of the experimental protocol. Baseline measures were collected once steady-state values were achieved. The experimental protocol is outlined in Figure 2.1. The experimental protocol consisted of two trials: 1) trial 1 consisted of a 1-minute baseline followed by 2-minutes of 30% MVC isometric HG exercise, 2) a 1-minute baseline followed by 6-minutes of ~+40 mmHg LBPP and then proceeded by a combination of ~+40 mmHg LBPP & 30% MVC isometric HG exercise for 2-minutes. Time was allocated between each trial to ensure that all physiological variables had returned to initial baseline levels (~5 minutes).

Following the completion of the experimental protocols, the participant was administered the alpha-1-adrenergic antagonist (Prazosin) drug-intervention via oral tablet(s) (1mg/20kg). Prazosin has been shown to reach peak activation between 1-2 hours (Lewis et al., 2014; Jones et al., 2011; Ogoh et al., 2008; Jaillon, 1980). A 90-minute break was allocated for the participant for the subject. Once re-instrumented, the experimental protocols were repeated for post drug-intervention comparison.
2.4 Handgrip exercise

Handgrip exercise was performed and measured via a pressure transducer (ADInstruments MLT004/ST, Colorado Springs, CO, USA) connected to a LabChart computer through Powerlab (Powerlab 16/30, ADInstruments, Colorado Springs, CO, USA), with real time pressure output displayed on a computer screen for the participant to view - pressure output was in Newtons (N). Subjects were required to perform the handgrip exercise isometrically, thereby maintaining 30% of the maximal voluntary contraction (MVC). This MVC was predetermined before commencing the study protocols (3 MVC’s performed with the highest pressure output selected for determination of 30%). The use of the isometric handgrip exercise was performed with and without the addition of LBPP. The purpose of the isometric handgrip exercise was used to further elevate BP.

2.5 Lower body positive pressure

Lower body positive pressure (LBPP) provided a means of increasing MAP without the utilization of vasoactive drugs. LBPP translocates blood from the lower limbs to upper torso by increasing CVP and MAP (Bevegård et al., 1977; Eiken & Bjurstedt, 1987; Nishiyasu et al., 1998; Shi, Crandall, & Raven, 1993). LBPP has been employed in clinical application during hypovolemia.
(Wayne & Macdonald, 1983) as well being applied to aviation pilots for maintaining central blood volume (Gaffney et al., 1981; Wood, 1987; Hinghofer-Szalkay, Kravik, & Greenleaf, 1988b; Geelen et al., 1992). Two recent studies (Perry et al., 2013; Perry et al., 2014) employed LBPP to investigate changes in MCAv during supine steady state of differential +20 mmHg and +40 mmHg LBPP. Little detail is given by Perry and co-authors in regards to the construction of their LBPP chamber besides describing how positive pressure was produced through the usage of two commercially available vacuum cleaners and pressure measured by a pressure transducer mounted within the chamber, which was titrated and controlled via a manual bleed valve. Based on this description and drawing inspiration from previous designs of lower body negative pressure (LBNP) chambers, a custom LBPP was specifically designed and engineered for the purpose of this thesis (Figure 2.2.).
The LBPP chamber was designed with the capability of both positive and negative pressures, and as such adopted a triangular design with the frame constructed out of steel tubing. Wooden panels were inserted within the frame and fastened to the steel frame and sealed to make the chamber airtight. A kayak neoprene skirt and additional weightlifting belts were used to provide an airtight seal around the volunteer and subjects were secured up to their iliac crest within the LBPP chamber in a supine position. An adjustable bike seat was also added into the chamber so as to provide support during negative pressures. Positive pressure for the LBPP was produced by employing a commercially available vacuum cleaner (Shop-Vac Wet/Dry 15 Gallon (68L) 6.5 HP, 120 V/60Hz/12A, Shop-Vac Canada, Burlington, Ontario, Canada) and power output was controlled by a 120 V input, 0-140V output variable transformer (Variac, 3PN1510B, ISE Inc., Cleveland, OH, USA), with positive pressure inside the LBPP monitored by a pressure manometer (DigiMano 1000, 200-200IN, Netech Corporation, Farmingdale, NY, USA) measured in millimeters of mercury (mmHg). The LBPP trial was 6 minutes in length, with the final 2 minutes used for data analysis. Previous reports indicate that 4 minutes is required to research steady-state (Shi et al., 1997).
2.6 Instrumentation & data collection

Data acquisition of cardio-respiratory (e.g. respiration, blood pressure and heart rate) measures was collected via a continuous data collection device at 200 Hz (Powerlab/16SP ML 880; ADInstruments, Colorado, USA). This device was connected to a computer software program, LabChart Pro (version 7.3.7, ADInstruments, Colorado Springs, CO) in which all transmitted data from the collection device were displayed as digital computerized signals.

2.7 Cardio-respiratory measurement techniques

Respiratory variables (e.g., breathing frequency, tidal volume and ventilation) were quantified using the flow signal from a two-way pneumotach (Model 800 L, Hans-Rudolph, Shawnee, KS, USA) connected to the PowerLab acquisition device (Powerlab/16SP ML 880; ADInstruments, Colorado, USA). The pneumotach was calibrated using a 3L syringe (Model 5530, Hans Rudolph, Shawnee, KS, USA) in order to define the oscillatory pressure changes received by the spirometer pod (pressure amplifier connected with pneumotach) during breathing. The spirometer pod was attached to the data acquisition device and used to calculate continuous tidal volume, breathing frequency and $V_E$. End-tidal gases (end-tidal $PO_2$ ($P_{ETO_2}$) and $PCO_2$ ($P_{ETCO_2}$)) was measured on a breath-by-breath basis using a calibrated online gas analyzer (Model ML206, AD Instruments, Colorado Springs, CO, USA) which was connected to the PowerLab device and LabChart software program for real-time data observation. Daily atmospheric pressure was used to correct for BTPS in millimeters of mercury (mmHg) in order to calculate the partial pressure of end-tidal gases ($P_{ETCO_2}$ and $P_{ETO_2}$).

All data were collected continuously using an analogue-to-digital converter at 200 Hz (Powerlab Model 880 ADInstruments, Colorado Springs, CO, USA) and was interfaced with LabChart Pro (version 7.3.7; ADInstruments) software, which was displayed in real time during the entire study on a computer screen. Data were stored on a portable hard-drive for off-line analysis using the same LabChart Pro software (version 7.3.7; ADInstruments).

2.8 End-tidal forcing

End-tidal forcing is a feed-forward ventilatory control technique that was employed so as to control end-tidal gases ($P_{ETO_2}$ & $P_{ETCO_2}$) independent of ventilation. This was performed on a breath-by-breath basis in which feedback data of $P_{ETO_2}$ and $P_{ETCO_2}$, $V_T$, $F_B$, and $V_E$ (inspired and expired)
were analyzed through custom developed software (Airforce V. 4.7) programmed in Labview (Version 13.0, National Instruments, Austin, TX, USA) and a validated error reduction algorithm (Tymko et al., 2015). Based on these feedback data, the end-tidal forcing system dynamically adjusted the subsequent required inspiratory volumes of O₂, CO₂ and N₂ (from medical grade gas bottles) using independent gas solenoid valves in order to achieve desired end-tidal gases. The gases were mixed in a humidified mixing chamber before being delivered to the subject via a 6L reservoir bag attached to the inspiratory port on the Hans-Rudolph pneumotach. Blood gases were effectively controlled to isocapnic levels for the duration of the experimental protocols.

2.9 Hemodynamic measures

Study participants were instrumented for measures of heart rate via a standard electrocardiogram (HR, beats/min; ADI bioamp ML132), beat-by-beat blood pressure (Finometer Pro, Finapress Medical System, Amsterdam, Netherlands) as well as manual blood pressure (SunTech Tango³, SunTech Medical Inc. Morrisville, NC, USA). Finometer blood pressure recordings were calibrated to brachial blood pressure. Stroke volume (SV) and cardiac output (CO) was calculated from the BP waveform obtained from the Finometer Pro finger photoplethysmography using the Modelflow method, incorporating age, sex, height and weight (BeatScope 1.0 software; TNO TPD; Biomedical Instruments, Amsterdam, Netherlands) (Wesseling et al., 1993).

2.9.1 Heart rate measurement

To collect heart rate, a 3-lead electrocardiography (ECG) transducer (ML 132, ADInstruments BIO Amp, Colorado Springs, CO, USA) was connected to the PowerLab data acquisition device. The 3-lead ECG electrodes are placed in a triangular pattern around the heart utilizing Einthoven’s triangle to detect the direction and magnitude of the hearts electrical rhythms. The electrical rhythms from the echoing from the heart are a product of neuro-muscular electrical signals from the contraction (depolarization) and relaxation (repolarization) phases of the cardiac tissue via neural synapses intrinsic to the cardiac tissue. The direct and magnitude of the electrical signals detected by the 3-lead ECG electrodes form a specialized pattern with 5 distinct shapes and waveforms corresponding to each of the cardiac cycle stages. Continuous beat-to-beat changes in heart rate are calculated by the determination of the time between consecutive R-waves (identified by largest peak in the QRS complex waveform).
2.9.2 Blood pressure measurement

Continuous assessment of blood pressure was performed using photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, Netherlands). This technique provides a non-invasive means to estimate beat-to-beat systolic and diastolic blood pressure using the finger as a surrogate for pulse-pressure. The small arteries in the fingers do not change diameter during changes in artery volume (Guelen et al., 2003; Guelen et al., 2008). The photoplethysmography device utilizes infrared light to illuminate the red blood cells moving through the small non-compliant arteries in the finger and then quantifies the amount of infrared light absorbed by the vessels (Nijboer, Dorlas, & Mahieu, 1981). The finger is used to index blood pressure and is then corrected using the difference between the finger and the brachial artery pressure to engineer a continuous intra-arterial waveform (Guelen et al., 2003).

While this technique is useful during resting experiments, the desired waveform is not always attainable in some subjects. Differences in circulation of the hand and fingers as well as temperature can influence the waveform outcome. When photoplethysmography was discontinuous or intermittent, blood pressure was measured using an automated device at the brachial artery (SunTech Tango+, SunTech Medical Inc., Morrisville, NC, USA). This device has been well validated as an index of intra-arterial blood pressure (Guelen et al., 2003; Guelen et al., 2008; Elvan-Taspinar et al., 2003). Calculation of MAP is as follows:

\[
\text{MAP} = \frac{1}{3} \times \text{SBP} + \frac{2}{3} \times \text{DBP}
\]

Stroke volume (SV) and cardiac output (CO) was calculated from the Finometer BP waveform using the Modelflow method, which incorporates age, sex, height and weight (BeatScope 1.0 software, TNO TPD, Biomedical Instruments, Amsterdam, Netherlands) (Wesseling et al., 1993). Cerebrovascular conductance was calculated for ICA, VA, MCAv and PCAv (e.g., CVC = QICA/MAP). Due to the experimental protocol and associated neck movement, acquisition of optimal image quality was not universally achieved in all participants.
2.10 Cerebrovascular measures

2.10.1 Intra-cranial cerebrovascular measurement: Transcranial doppler ultrasound

Transcranial Doppler ultrasound (TCD) was used to measure intracranial blood velocity of the MCA and PCA on a beat-to-beat basis (MCAv & PCAv; cm/sec). The TCD ultrasound probes were secured in position using a commercially available fixated head frame (Marc 600 Head frame, Spencer Technologies, Seattle, WA, USA). The Doppler probe emits sound waves which are reflected off of the red blood cells circulating in the insonated target vessel and are detected by the transducer. The difference between the frequency of the transmitted signal and the resultant received signal if the Doppler-shift and is proportional to the velocity of the circulating blood (Aaslid, 1986; DeWitt & Wechsler, 1988; Newell & Aaslid, 1992; Purkayastha & Sorond, 2012; Willie et al., 2011. The calculation of the Doppler-shift is depicted below:

\[
\text{Doppler shift} = 2 \times \text{Ft} \times \text{V} \times \cos\theta / C
\]

Where: Ft: transmitted frequency (i.e., 2 MHz); V: velocity of red blood cells; Cos\(\theta\): correction factor based on insonation angle; C: speed of sound (1540 m/sec)

This Doppler-shift is then converted from a frequency domain into a time domain using Fast Fourier transformation, and maximum or mean velocity is then calculated from the respective maximum or intensity weighted mean signal (Lohmann, Ringelstein, & Knecht, 2006; Aaslid, 1986). This changes the ultrasound waves into a velocity trace or pulse-wave velocity and is exhibited onto the TCD screen. This pulse-wave velocity measure received by the Doppler probe is used as an index of blood velocity and is readily available for further analysis. The insonation angle of the TCD probe in relation to the target intracranial vessel is critical in attaining an accurate and reliable signal. The TCD employs a lower ultrasound frequency (1.5-2 MHz) in order to penetrate the cranium (DeWitt & Wechsler, 1988; Aaslid, Markwalder, & Nornes, 1982), as where Duplex vascular ultrasound employs a much higher frequency (3-12 MHz). This is dependent upon the acoustic window of the subject as well the operator level of competency as well as anatomical knowledge of the cerebral circulation. The choice of acoustic window and target vessel can dramatically affect the type of TCD recording, as there are three temporal windows: anterior window, middle window and posterior window.
Insonation of the MCA can be achieved through the anterior window and offers an optimal insonation angle and thus more accurate velocity measurements (Figure 2.3.). The posterior window is feasible to attain MCA insonation; however, due to the change in insonation angle the velocity measures are less accurate. An insonation angle between 0 and 30 degrees is the optimal angle for accurate measures of velocity, while accurate cerebral blood velocity can be achieved up to 60 degrees (Wintermark et al., 2005). The insonation angle (Figure 2.4.) required for optimal insonation of the PCA is more difficult to attain with the desired acoustic window and is more variable between subjects. In some subjects the insonation angle is higher than the MCA at ~30 degrees with the insonation window allowing for only a 90 degree insonation angle. It is also noted - because of the depth and tortuosity of the MCA - that a further 10-20% of the general population do not possess a desirable acoustic window for experimental study (Aaslid et al., 1982).

The depth of the target intracranial vessel can also play a factor into the accuracy of cerebral velocity measurement. The MCA is relatively easy to landmark at a depth of 25 to 50 mm, however the MCA can be confused for the middle meningeal artery (Aaslid, Huber, & Nornes, 1986). Blood flow should be towards the probe until the MCA and ACA bifurcate and blood flow flows both to and away from the probe. This anatomical landmark is useful in standardizing TCD measures, as
the bifurcation can easily visually distinguished on the TCD spectral screen. In order to confirm insonation of the MCA vessel an occlusion of the CCA is performed by the operator while monitoring the waveform, as occlusion of the correct insonation of the MCA will yield a marked decrease in the spectral waveform.

The PCA is more difficult to insonate as it lays much deeper relative to the MCA - at a depth of 60 to 70 mm. It is optimal to insonate the PCA by aiming the probe from the anterior window posteriorly and the using fine manipulations of the TCD probe to attain the most optimal signal. The PCA will always exhibit a much smaller waveform than the MCA due to the increased perfusion of blood from the supplying ICA. In order to confirm correct insonation, occlusion of the CCA and subsequent ipsilateral MCA will either yield an increase in the ipsilateral PCA waveform or remain unchanged. In addition to this test, a visual test is administered to determine occipital cortical activation. The subject is instructed to close their eyes for a period of ~10 seconds and the open their eyes for ~10 seconds, and then to follow the operators finger while moving to different spatial positions. During the eyes open and visual spatial exercise, the PCA should increase velocity by ~20% with only a marginal response (~5%) from in MCA velocity (Willie et al., 2011).
Figure 2.4 In the image: Red stripped lines = vessel walls, red arrow = direction blood flow, blue dashed line = ultrasound beam, green bar = angle cursor, black dashed lines indicate percentage of ultrasound signal that is reflected and received by the receiver. The relationship between angle of insonation and cosine of the angle (Cos $\theta$). The image demonstrates the progressive and disproportional reduction in the percentage of the transmitted signal reflected back to the ultrasound probe as the angle of insonation increases such that at 90 degrees no signal will be recorded. Reproduced, with ©permission, from (Smith, 2015).

2.10.2 Validity of Transcranial doppler ultrasound

There are a number of advantages to the utility of the TCD in experimental studies, as this portable lightweight device is relatively low cost in comparison to other imaging technologies (~$20 – 50K USD) and has the ability to noninvasively capture continuous blood-flow velocity through the major intracranial blood vessels (i.e., ACA, MCA, PCA, BA). The TCD is relatively easy to learn, though in order to produce high quality images does require practice as well as knowledge of the cerebral anatomy in order to locate the desired target vessel. Another advantage of the TCD is that assessment of integrative cerebrovascular function (e.g., CA, NVC, CVR, orthostatic stress, exercise) is possible without the usage of tracers and/or radiation in order to obtain CBF signals.
There are, however, a number of disadvantages to the utility of TCD that must be addressed. The minor disadvantages of the utility of TCD include the limitations of measuring only one vessel per hemisphere at one time and only being able to assess the larger arteries and not the smaller arteries. In addition, the quality of Doppler signal is operator dependent and can be complicated by subject differences in anatomy. The major hindrance of TCD utility is that TCD only measures cerebral blood velocity, while the diameter of the insonated vessel is unknown and is assumed not to change. Poiseuille’s law understates the impact of changing the vessel diameter when assessing measurement of flow, as the law states that flow within a vessel is proportional to the fourth power of the radius. Thus, any small change in diameter will directly affect the magnitude of cerebral blood flow (Figure 2.5.). This assumption has been supported by studies demonstrating constant diameter (Bishop et al., 1986; Giller et al., 1993; Newell et al., 1994; Nuttall et al., 1996; Valdueza et al., 1997; ter Minassian et al., 1998; Serrador et al., 2000). However, there are two recent high resonance-imaging studies that have challenged the assumption of constant vessel diameter (3T - Coverdale et al., 2014; 7T - Verbree et al., 2014). Coverdale and co-authors used 3 Tesla MRI to measure MCA diameter during hypercapnia (+9 mmHg $P_{ET\text{CO}_2}$) and hypocapnia (-13 mmHg $P_{ET\text{CO}_2}$) and reported significant increases (~8%) and decreases (~4%) during hypercapnia and hypocapnia, respectively. Verbree and co-authors used 7 Tesla MRI to measures MCA diameter during mild (+7.5 mmHg $P_{ET\text{CO}_2}$) and severe (+15 mmHg $P_{ET\text{CO}_2}$) hypercapnia and during hypocapnia (-7.5 mmHg $P_{ET\text{CO}_2}$). An elevation of ~1.5% in MCA diameter was observed during mild hypercapnia, while during severe hypercapnia MCA diameter was significantly increased by ~7%. During the hypocapnia, a ~1% reduction in MCA diameter was observed, however it was not significantly different from baseline MCA diameter. These data highlight that though there is much utility in TCD for use in experimental protocols and clinical settings, however parsimonious interpretation of changes in intra-cranial cerebral blood velocity should be exercised when extrapolating to blood flow quantification as a result of alterations in MCA diameter from changes in arterial blood gases.
Figure 2.5 Discrepancy between volumetric and velocity of MCA vasomotion during changes in CBF. Previously reported changes in middle cerebral artery (MCA) diameter (left y-axis) and their calculated impact on the discrepancy between flow and velocity measures (right y-axis) during changes in end-tidal PCO$_2$ (P$_{ET}$CO$_2$). To highlight the effects of MCA vasomotion we estimated the potential difference between CBF and velocity changes using the following. For example, cross-sectional area (CSA; cm$^2$) * Velocity (cm/s) * 60 s = Flow (ml/min). Assuming a baseline MCA velocity of 60 cm/s (which was done for all studies to facilitate diameter effect comparisons) coupled with the observed alterations in CSA with hyper- or hypocapnia (Coverdale et al., 2014), we calculated a representative baseline MCA flow value: 5.6 mm$^2$ * 60 cm/s * 60 s = Flow; therefore, 0.056 cm$^2$ * 60 cm/s * 60 s = 201.6 ml/min. Assuming previously reported values of cerebrovascular reactivity (Willie et al., 2012), MCA velocity increases ~4%/mmHg increase in P$_{ET}$CO$_2$. Assuming this as vessel reactivity (for all studies), we can estimate the volumetric MCA flow during hypercapnia using the reported CSA: 6.5 mm$^2$ * 84 cm/s * 60 s = Flow; therefore, 0.065 cm$^2$ * 84 cm/s * 60 s = 327.6 ml/min. As such, the percent difference for flow between baseline and hypercapnia is [(327.6 - 201.6)/201.6]*100 = 62.5%. The percent difference in velocity between baseline and hypercapnia is [(84 - 60)/60]*100 = 40%, indicating that TCD would underestimate the increase in flow of the MCA during hypercapnia (+9 mmHg P$_{ET}$CO$_2$ from baseline) by >20%. However, if the percent difference is quantified via the magnitude of change in flow and velocity during hypercapnia, the increase in flow is ~50% greater than that of velocity! This can be calculated as %difference = [(%increase in flow - %increase in velocity)/%increase in velocity] * 100 and therefore [(62.5 - 40)/40]*100 = 56.25%. As such, we have conservatively represented the effect of changes in diameter on flow vs. velocity discrepancies. For hypocapnia we again assumed a baseline MCA velocity of 60 cm/s and used the prehypocapnia CSA reported (Coverdale et al., 2014) to calculate baseline flow (Portegies et al., 2014): 5.8 mm$^2$ * 60 cm/s * 60 s = Flow; therefore, 0.058 cm$^2$ * 60 cm/s * 60 s = 208.8 ml/min. Incorporating a 2% change in MCAv per mmHg reduction in P$_{ET}$CO$_2$ (Willie et al., 2012) and the associated change in CSA, we estimated volumetric MCA flow during hypocapnia (Serrador et al., 2000): 5.3 mm$^2$ * 46.8 cm/s
* 60 s = Flow; therefore, \(0.053 \text{ cm}^2 \times 46.8 \text{ cm/s} \times 60 \text{ s} = 148.8 \text{ ml/min.}\) As such, the percent change in flow between baseline and hypocapnia is \(\left(\frac{148.8 - 208.8}{208.8}\right) \times 100 = -28.7\%\). The percent difference in velocity between baseline and hypocapnia is \(\left(\frac{46.8 - 60}{60}\right) \times 100 = -22\%\), indicating that TCD would underestimate the decrease in flow of the MCA during hypocapnia \((-13 \text{ mmHg P}_{\text{ET}}\text{CO}_2 \text{ from baseline})\) by \(~7\%\). Thus it is evident by these calculations and those seen above that small changes in MCA diameter are responsible for large discrepancies between flow and velocity measures. Data are collated from Coverdale et al., 2014 and Serrador et al., 2000; Valdueza et al., 1997; Verbree et al., 2014. As noted in the hypercapnic calculations, this graph represents the most conservative way to quantify the percent difference in flow and velocity changes, highlighting the large impact changes in MCA diameter has in quantifying CBF (Ainslie & Hoiland, 2014). Permission to reproduce this image was not required by the Journal of Applied Physiology.

### 2.10.3 Extra-cranial cerebrovascular measurement: Duplex vascular ultrasound

Duplex ultrasound was used to measure extra-cranial blood flow in the contralateral ICA and ipsilateral VA using a 10 Mhz multi frequency linear array vascular ultrasound device (Terason T3200, Teratech, Burlington, MA, USA) coupled with a Macbook Pro computer. Measurements of ICA diameter and velocity were acquired no less than 2 cm distal to the bifurcation of the common carotid artery, while avoiding turbulent or retrograde flow during the video recording for analysis. Diameter and velocity of the VA were acquired between segments of either the C4-C5 and C5-C6 or alternatively proximal to entry into the vertebral column, where the best possible image could be attained.

Duplex ultrasound (concurrent 2-dimensional B-mode and pulse-wave velocity) is used primarily on extra-cranial vascular ultrasound for measuring both vessel luminal diameter and blood velocity, and therefore volumetric blood flow. Duplex ultrasound is dissimilar to TCD in that it utilizes both temporal and spatial resolution in order to quantify both blood velocity as well as cross-sectional area of the insonated target vessel (i.e., CCA, ICA, ECA, VA) in determining beat-to-beat changes in flow. The spatial resolution provides data from the returning ultrasound wave in regarding longitudinal and lateral as well as position of echogenic tissues and is then computed as a graphical representation of the underlying structures. The graphical representation is deemed the B-mode or brightness mode and is represented as a 2-dimensional grey scale image. The duplex ultrasound also employs a much higher ultrasound frequency than TCD (9-12 Mhz vs. 1.5-2 Mhz), which allows for an increase in the axial resolution or longitudinal resolution of the 2-dimensional grey scale representation allowing for the requisite identification of the target vessel wall.
Identification of the target vessel requires knowledge of the anatomy of the neck in beginning duplex ultrasonography. In order to insonate the ICA, the CCA is first insonated in a cross-sectional view – the internal jugular vein can be seen lateral to the CCA as well as the thyroid medially to the CCA (Figure 2.6). Next, the transducer is tracked upward from the base of the neck until a B-mode image of the bulb and bifurcation of the CCA appears. By turning the ultrasound transducer 90 degrees clockwise whilst maintaining transducer position a longitudinal B-mode image of bulb and bifurcation of the CCA will appear (Figure 2.7.) as well as the branching ICA and external carotid artery (ECA) (Figure 2.7.) (Thomas et al., 2015).

Figure 2.6 Example B-mode image of transverse view of the carotid bifurcation (Thomas et al., 2015). Permission to reproduce this image was not required by the American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.
In order to properly differentiate between these two arteries there a number of ways to do this (Thomas et al., 2015): i) the ICA typically branches upward while the ECA branches off downward – a slight pivot of the transducer or ‘sweeping motion’ will alternate the visualization of each respective vessels longitudinal section ii) the diameter of the ICA is typically larger (~6 mm) than that of the ECA (~3-4 mm) and thus the ICA should appear larger on the B-mode image iii) the ICA has no discernable extra-cranial branches, whereas the ECA has eight of them (Primozich, 2002) which supply blood to the thyroid and facial area and scalp iv) the spectral Doppler waveform for the ICA and ECA are noticeably different, with the ECA demonstrating a narrow systolic peak and low end-diastolic velocity while the ICA has a broader systolic peak and a higher end-diastolic velocity (Figure 2.8.).
A. Spectral Doppler trace of ICA.

B. Spectral Doppler trace of ECA.

Figure 2.8 Example Spectral Doppler trace of A. ICA and B. ECA (Thomas et al., 2015). Permission to reproduce this image was not required by the American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.
Insonation of the VA is difficult to achieve in some individuals as it lies deeper and laterally to the CCA and internal jugular vein and is only observable in between its supplying subclavian artery and transverse process of the C4 vertebrae. Akin to the procedure of insonating the ICA, a longitudinal view of the CCA is done first and the transducer is tracked laterally towards the ipsilateral ear (Figure 2.9).

**Figure 2.9** Example B-mode image of the VA insonated from between the cervical spinal cord sections (vertebral processes) (Thomas et al., 2015). Permission to reproduce this image was not required by the American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.

Duplex ultrasound utilizes the same principles as TCD when insonating the target vessel as the angle with which the ultrasound probe must be at no greater than 60 degrees in order to avoid measurement error. Like TCD, duplex ultrasound is operator dependent and requires many hours of practice and reliability measures in order to become proficient, though duplex ultrasound is more difficult to begin to learn. As such, Duplex ultrasound is easily confounded by operator error as a one-degree error in angle of insonation yields a 0.1 mm error in diameter measurement.
and a one cm/sec error in mean blood velocity equating to a 4% error in measurement of blood flow. This can easily compound and produce significant errors in measurement data, as for every one-degree error in insonation angle a resultant ~3% error in velocity and flow can occur (Schoning et al., 1994). In contrast, measurement error of luminal diameter has marked ramifications due to the diameter calculation being squared to the luminal area, whereas velocity and flow are linearly related. One tool that the operator has with Duplex ultrasound that TCD lacks is that of the angle correction feature. This angle correction cursor (Figure 2.10.), when executed correctly, allows for quantification of pulse-wave velocity in vessels that are difficult to insonate (as a result of plaque or tortuous nature of vessel, i.e., VA). This angle correction feature must be aligned parallel with blood flow within the vessel; incorrect angle correction will yield inaccurate calculation of pulse-wave velocity. There are also a number of features and technical maneuvers that the operator can use to their advantage in attaining reliable images which can be found in this detailed review by Thomas and co-authors (Thomas et al., 2015).

![Figure 2.10](image)

**Figure 2.10** Steering angle trade-off between B-mode and Pulse Wave Doppler. In all images: black parallel lines = vessel walls, red arrow = direction of moving blood, blue dashed line = ultrasound beam, green bar = angle cursor. **A.** 90° angle between blood flow and ultrasound beam, **B.** 60° angle between blood flow and ultrasound beam, **C.** Incorrect 60° angle correction, **D.** Correct 45° angle correction.
will generate a bi-directional and low magnitude Doppler shift and therefore the system is unable to accurately determine velocity. B. The ultrasound beam has been appropriately steered to reduce the angle to 60°. C. The transducer has been “heeled” to decrease the angle of the beam relative to the vessel to an acceptable 60°. D. Appropriate alignment of angle cursor parallel to the vessel walls at 60°. E. Inappropriate placement of angle cursor relative to the direction of blood flow. In this case the system will assume at 60° angle (between ultrasound beam and angle cursor) as opposed to the actual angle of 45° (between the ultrasound beam and the blood flow) and will therefore underestimate the velocity calculated by ~30% (Thomas et al., 2015). Permission to reproduce this image was not required by the American Journal of Physiology – Regulatory, Integrative and Comparative Physiology.

The appeal of Duplex ultrasound is that it is relatively low cost and can be used in conjuncture with TCD to compare blood flow magnitude changes in the proximal intracranial vessels to provide insight into whether or not diameter in the intracranial target vessel is remaining constant. The coupling of these two methods can provide valuable new insight into the mechanisms of cerebral blood flow regulation.

2.11 Duplex ultrasound edge-detection analysis software

To analyze the Duplex ultrasound video files, an automated software program employing computational vessel wall edge-detection (FMD/BloodFlow Software Version 4.0 – LabView 10.0) has been shown to be highly effective and valid in reliably assessing and determining arterial luminal wall responsiveness to respective blood flow responses (Woodman et al., 2001; Black et al., 2008). Though this software is simple to learn and only requires a few steps to quantify CBF. For example, the use of this software does require a familiarity with the Duplex ultrasound technique and anatomy of the insonated target vessel in order to properly set-up the automated software prior to analysis.

During experimental protocols, Duplex ultrasound images were recorded using a video recording software (Camtasia) and then appropriately labelled and saved for off-line analysis. When ready to analyze, the videos were loaded into the edge-detection software and regions of interest (ROI) are selected by the operator in order to calibrate for both vessel diameter and blood velocity – as placing ROI over a known length (i.e., B-mode depth scale or pulse-wave mode velocity scale) allows for calibration of both diameter and velocity values, therefore an ROI is selected over the entire waveform (Figure 2.11.). Though the software is automated, it is critical that the operator view the entire video before and during analysis to ensure that the ROI is selected
to the most stable section of the vessel and to take note of any movement artefacts that may result in erroneous CBF values. Should the operator deem the bulk of the recorded videos to be stable enough for automated analysis, the operator may choose to select the ‘batch analysis’ feature, whereby each video recording ROI’s are set-up and selected and then are analyzed continuously in a pre-determined order.

Though diameter and velocity of the insonated target vessels are assessed by the edge-detection software concurrently, each are calculated differently. For velocity, the peak velocity waveform is automatically detected as a result of the contrast within the velocity ROI (*Yellow contrast in velocity profile in panel B* - Figure 2.11.). For diameter, two signals are sent out from the middle of the ROI – one up and one down – which detect the first pixel of contrast and the resultant distance between the two points is calculated as a diameter value. From this process, many points are calculated at the same time at a rate of 30Hz.

**Figure 2.11** Duplex ultrasound image of the ICA: **A.** Image A corresponds to the anatomical area of insonation, highlighted in the red box, of the ICA on the human figure. The velocity measurement trace is directly inferior to the B-mode image of the insonated ICA vessel. **B.** Image B is a depiction of the B-mode image, as it would appear in the edge-detection blood flow analysis software. The yellow rectangles represent the regions of interest (ROI) for sampling concurrent
ICA diameter and velocity; the dotted lines on the velocity trace depict the edge detection tracking by the analysis software. Reproduced, with ©permission, from (Hoiland, 2015).

This technique of edge-detection analysis differs greatly from previous methods employing the use of calipers to manually measure one luminal vessel wall to the other. Techniques such as these do not offer the ability to definitely observe changes in diameter from a respective stimulus. Changes in arterial response from manipulating blood gases or shear stress would not be observable due to inter-individual variability when only taking a few measures at a time. Furthermore, the pulsatile nature of the arterial vessels would cause disproportionate results of diameter responsiveness if employing the caliper measurement method, with some studies accounting for this by measuring systolic and diastolic diameters and then calculating the mean diameter ratio (Sato, Sato, & Uchida, 2001; Sato & Sadamoto, 2010) while other studies have reported data without taking pulsatile changes all through the cardiac cycle into consideration (Scheel, Ruge, & Schöning, 2000; Scheel et al., 2000; Schöning & Hartig, 1996; Schöning et al., 2005; Schoning et al., 1994).

2.12 Quantification of CBF
In using duplex ultrasound, diameter and velocity measurements are simultaneously collected via B-mode and Doppler signal acquisition. By collecting these measures, volumetric flow can be determined by multiplying the velocity of the blood flow circulating through the insonated target vessel by the cross sectional area of the target vessel. The equation to determine cross sectional area is as follows:

\[
\text{Cross sectional area} = \pi r^2
\]

Where ‘r’ represents the vessel radius. Using Duplex ultrasound, the formula becomes:

\[
\text{Cross sectional area} = \pi (0.5 \cdot d)^2
\]

Where ‘d’ represents the vessel diameter, as measured by duplex ultrasound B-mode imaging. If blood flowing through a vessel is laminar, blood velocity is parabolic across the vessel lumen and
therefore can be accurately calculated as one half of the peak blood velocity through the vessel. Volumetric blood flow can then be calculated using the following calculation (Evans, 1985):

\[
\text{Volumetric blood flow} = (\pi (0.5 \cdot d)^2 \cdot (1/2)(V_{\text{max}}))
\]

Where \(V_{\text{max}}\) represents the maximum blood velocity through the target vessel. The calculation of blood flow in this thesis study included no less than 10 consecutive stable cardiac cycles, and included entire averaging periods where possible. Other parameters applicable to vascular function can be determined with duplex ultrasound such as shear rate, flow mediated dilation and vessel compliance.

### 2.13 Data and statistical analysis

Baseline data for breath-by-breath \(\text{P}_{\text{ETCO}_2}\) & \(\text{P}_{\text{ETO}_2}\), blood velocity (MCA and PCA), CBF (ICA and VA), mean arterial pressure (MAP), HR (beats per min) were acquired and quantified with the subject breathing simulated room air, both before and after administration of Prazosin drug intervention. All baseline data were averaged over the 2-min baseline periods immediately prior to initiation of experimental protocols. Steady-state data for the LBPP protocol was acquired and exported from LabChart in 30-sec bins and averaged across the final 2-min of the 6-min protocol, as previous reports indicate that a minimum of 4-min is required in order to achieve steady-state measures (Shi et al., 1997). Handgrip data were acquired in the final minute of the 2-min protocol and was exported in 30-sec bins and an average was determined between the two exported values.

Following confirmation of data normality, student’s paired \(t\) tests were used to compare baseline variables before and following Prazosin administration within-subjects. Paired \(t\) tests were also used to compare differences in relative MAP, extra- and intra-cranial variables during the BP manipulations (HG, LBPP, LBPP+HG) before and following Prazosin administration within-subjects. Paired \(t\) tests were used to compare differences in absolute differences in extra- and intra-cranial CVC before and following Prazosin administration within-subjects. Pearson R correlations were used to assess the relationship between absolute and relative changes in extra- and intra-cranial variables during changes in MAP & respective intra-cranial blood velocity. Significance was assumed at \(P < 0.05\).
Chapter 3: Results

To be described herein are absolute and relative changes of baseline hemodynamics (MAP & HR), respective contralateral and ipsilateral extra-cranial (ICA & VA) blood flow and intra-cranial (MCA & PCA) velocity as well as end-tidal gases ($P_{ET}O_2$ & $P_{ET}CO_2$). These variables were measured before and 90 minutes following Prazosin administration (Table 3.1). End-tidal respiratory gases ($P_{ET}O_2$ & $P_{ET}CO_2$) were effectively controlled at isocapnic values using dynamic end-tidal forcing (Table 3.1). Relative changes in MAP, contralateral and ipsilateral intra-cranial blood velocity (MCAv & PCAv) and extra-cranial (ICA & VA) diameter, velocity & blood flow from baseline, before and following Prazosin administration during the various BP manipulations (30% MVC HG, LBPP & LBPP+HG) are illustrated in Figures 3.1 & 3.2. Changes in absolute CVC for contralateral and ipsilateral intra- and extra-cranial arteries are displayed in Figure 3.3. Finally, correlational analyses are presented in Figure 3.4.(1-3) to depict the relationship between absolute changes in contralateral and ipsilateral extra-cranial blood flow ($Q_{ICA}$ & $Q_{VA}$) and intra-cranial blood velocity (MCA & PCA) during changes in MAP. Additionally, there were no differences between males and females in MAP, extra-cranial blood flow or intra-cranial velocity responses to the BP manipulations both prior to or following Prazosin administration.

3.1 Baseline measures following Prazosin administration

Following Prazosin administration, baseline measures (Table 3.1) of $P_{ET}CO_2$, MAP and contralateral MCAv (n=13) were significantly reduced by -2.8 ± 2.3%, 7.5 ± 10.9% and 7.9 ± 8.5%, respectively (both: $P<0.05$ vs. Pre-Prazosin). Although ipsilateral PCAv (n=14) was unchanged ($P>0.3$), velocity in the ipsilateral VA was significantly reduced by 11 ± 18%, and both diameter and flow was unchanged (both: $P>0.05$). Diameter, velocity and flow in the contralateral ICA were all unchanged following Prazosin administration (all: $P>0.05$). Cerebrovascular conductance was similarly unchanged following Prazosin for contralateral ICA, MCAv and ipsilateral VA & PCAv (all: $P>0.05$).
Table 3.1 Absolute and relative changes in baseline values pre and post Prazosin

<table>
<thead>
<tr>
<th></th>
<th>Pre-Prazosin Baseline</th>
<th>Post-Prazosin Baseline</th>
<th>Absolute (Δ)</th>
<th>Relative (%Δ)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pstitial O$_2$ (mmHg)</td>
<td>98.3 ± 1.9</td>
<td>99.1 ± 1.2</td>
<td>0.8 ± 2.5</td>
<td>0.8 ± 2.7%</td>
<td>P=0.26</td>
</tr>
<tr>
<td>Pstitial CO$_2$ (mmHg)</td>
<td>37.9 ± 3.0</td>
<td>36.8 ± 2.5*</td>
<td>-1.1 ± 0.9</td>
<td>-2.8 ± 2.3%</td>
<td>P=0.0002</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>93 ± 8</td>
<td>86 ± 10*</td>
<td>-7.3 ± 10</td>
<td>-7.5 ± 10.9%</td>
<td>P=0.013</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 15</td>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats / min)</td>
<td>68.8 ± 11.2</td>
<td>79.2 ± 16.5*</td>
<td>10.4 ± 10.9</td>
<td>15.1 ± 16.0%</td>
<td>P=0.002</td>
</tr>
<tr>
<td>QICA (ml/min)</td>
<td>246 ± 57.8</td>
<td>256.2 ± 68.2</td>
<td>10.3 ± 43.6</td>
<td>5 ± 18%</td>
<td>P=0.5</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.49 ± 0.04</td>
<td>0.51 ± 0.04</td>
<td>0.01 ± 0.02</td>
<td>2.7 ± 5.1%</td>
<td>P=0.12</td>
</tr>
<tr>
<td>Velocity (cm/sec)</td>
<td>42.7 ± 7.2</td>
<td>42.8 ± 12.1</td>
<td>0.1 ± 7.6</td>
<td>-0.5 ± 15.3%</td>
<td>P=0.96</td>
</tr>
<tr>
<td>CVC (ml/min/mmHg)</td>
<td>2.66 ± 0.64</td>
<td>3.01 ± 0.93</td>
<td>0.36 ± 0.65</td>
<td>14 ± 24%</td>
<td>P=0.1</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QIVA (ml/min)</td>
<td>76.5 ± 27.3</td>
<td>74.4 ± 37.4</td>
<td>-2.1 ± 24.4</td>
<td>-3 ± 31%</td>
<td>P=0.75</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>0.37 ± 0.05</td>
<td>0.38 ± 0.06</td>
<td>0.01 ± 0.03</td>
<td>3.5 ± 7.0%</td>
<td>P=0.1</td>
</tr>
<tr>
<td>Velocity (cm/sec)</td>
<td>23.2 ± 5.2</td>
<td>20.5 ± 6.1*</td>
<td>-2.7 ± 4.0</td>
<td>-11 ± 18%</td>
<td>P=0.03</td>
</tr>
<tr>
<td>CVC (ml/min/mmHg)</td>
<td>0.83 ± 0.32</td>
<td>0.89 ± 0.48</td>
<td>0.06 ± 0.34</td>
<td>7 ± 40%</td>
<td>P=0.5</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 13</td>
<td>n = 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCAv (cm/sec)</td>
<td>63.2 ± 10.2</td>
<td>58.0 ± 9.3*</td>
<td>-5.2 ± 5.7</td>
<td>-7.8 ± 8.6%</td>
<td>P=0.006</td>
</tr>
<tr>
<td>CVC (cm/sec/mmHg)</td>
<td>0.68 ± 0.11</td>
<td>0.68 ± 0.08</td>
<td>-0.003 ± 0.09</td>
<td>1.0 ± 13.1%</td>
<td>P=0.9</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 13</td>
<td>n = 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCAv (cm/sec)</td>
<td>43.2 ± 14.4</td>
<td>40.0 ± 10.7</td>
<td>-3.2 ± 11.1</td>
<td>-2.2 ± 27.9%</td>
<td>P=0.3</td>
</tr>
<tr>
<td>CVC (cm/sec/mmHg)</td>
<td>0.46 ± 0.14</td>
<td>0.47 ± 0.14</td>
<td>0.008 ± 0.114</td>
<td>6.2 ± 29.3%</td>
<td>P=0.8</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 14</td>
<td>n = 14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different following Prazosin, P<0.05. Pstitial O$_2$, partial pressure of end-tidal oxygen; Pstitial CO$_2$, partial pressure of end-tidal carbon dioxide; MAP, mean arterial pressure; HR, heart rate; QICA, volumetric internal carotid artery blood flow; QIVA, volumetric vertebral artery blood flow; CVC, cerebrovascular conductance; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity.

3.2 Relative changes during BP manipulations before and following Prazosin administration

Relative changes in MAP during 30% MVC HG, before and following Prazosin administration were similar (both: P>0.05 - Figure 3.1). In contrast, relative changes in MAP during LBPP and LBPP+HG following Prazosin administration were significantly reduced (+5.4 ± 4.1% vs. +10.0 ± 7.5% & +15.7 ± 9.1% vs. +23.6 ± 10.7%; P<0.05 – Figure 3.1), respectively. Relative changes in contralateral ICA and ipsilateral VA diameter, velocity & blood flow and respective MCAv & PCAv, were similar during 30% MVC HG, LBPP and LBPP+HG, before and following Prazosin administration (all: P>0.05 – Figure 3.2).
Absolute changes in CVC during 30% MVC HG, LBPP & LBPP+HG are displayed in Figure 3.3. Although absolute changes in ipsilateral PCAv conductance was unchanged (P=0.4) during 30% MVC HG, absolute changes in PCAv conductance during LBPP and LBPP+HG were significantly attenuated following Prazosin administration (P=0.024 & P=0.04, respectively). Conductance for contralateral ICA, MCAv and ipsilateral VA were all unchanged following Prazosin administration across all BP manipulation trials (30% MVC HG, LBPP & LBPP+HG; all: P>0.05).

**Figure 3.1** Relative changes (Δ%) from baseline for MCAv, PCAv and MAP. *Significantly different following Prazosin administration, P<0.05. Significant differences in MAP (LBPP, LBPP+HG, but not HG) while MCAv & PCAv were unchanged following Prazosin administration. MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity;
MAP, mean arterial pressure; HG, 30% MVC handgrip exercise; LBPP, lower-body positive pressure; LBPP+HG, lower-body positive pressure combined with handgrip exercise.
Figure 3.2 Relative changes (Δ%) from baseline for mean diameter, velocity & flow for the ICA and VA. No significant differences were found following Prazosin administration. ICA, internal carotid artery; ICAT, internal carotid artery velocity; QICA, internal carotid artery blood flow; VA, vertebral artery; VAV, vertebral artery velocity; QVA, vertebral artery blood flow; HG, 30% MVC handgrip exercise; LBPP, lower-body positive pressure; LBPP+HG, lower-body positive pressure combined handgrip exercise.
**Figure 3.3** Absolute changes (Δ) from baseline for mean cerebrovascular conductance of MCAv, PCAv, ICA flow & VA flow. *Significantly different following Prazosin administration, P<0.05. Significant differences in PCAv CVC during LBPP and LBPP+HG, but not 30% MVC HG following Prazosin administration. QICA, internal carotid artery blood flow; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; QVA, vertebral artery blood flow; CVC, cerebrovascular conductance.
<table>
<thead>
<tr>
<th></th>
<th>Condition</th>
<th>Baseline</th>
<th>30% MVC HG</th>
<th>Delta (Δ)</th>
<th>Relative (Δ%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>Pre</td>
<td>93 ± 8</td>
<td>108 ± 13*</td>
<td>15 ± 10</td>
<td>16 ± 11%</td>
<td>P=0.00006</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>86 ± 10</td>
<td>96 ± 12*</td>
<td>10 ± 6†</td>
<td>11 ± 7%</td>
<td>P=0.00002</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>Pre</td>
<td>71 ± 15</td>
<td>80 ± 12*</td>
<td>9 ± 11</td>
<td>14 ± 14%</td>
<td>P=0.008</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>81 ± 18</td>
<td>102 ± 19*</td>
<td>21 ± 11†</td>
<td>28 ± 15%†</td>
<td>P=0.000002</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>QICA (ml/min)</strong></td>
<td>Pre</td>
<td>256.3 ± 49.2</td>
<td>267.5 ± 66.4</td>
<td>11.2 ± 53.7</td>
<td>4.8 ± 19.4%</td>
<td>P=0.5</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>265.9 ± 63.3</td>
<td>264.7 ± 82.4</td>
<td>-1.3 ± 28.6</td>
<td>-1.7 ± 11.0%</td>
<td>P=0.9</td>
</tr>
<tr>
<td><strong>Diameter (mm)</strong></td>
<td>Pre</td>
<td>0.50 ± 0.04</td>
<td>0.49 ± 0.04</td>
<td>-0.01 ± 0.02</td>
<td>-0.9 ± 4.6%</td>
<td>P=0.5</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>0.51 ± 0.04</td>
<td>0.49 ± 0.04*</td>
<td>-0.02 ± 0.02</td>
<td>-3.2 ± 3.0%</td>
<td>P=0.01</td>
</tr>
<tr>
<td><strong>Velocity (cm/sec)</strong></td>
<td>Pre</td>
<td>44.2 ± 5.5</td>
<td>47.6 ± 13.4</td>
<td>3.5 ± 9.8</td>
<td>6.9 ± 18.8%</td>
<td>P=0.29</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>44.2 ± 11.8</td>
<td>47.2 ± 17.1</td>
<td>3.0 ± 6.4</td>
<td>5.2 ± 11.8%</td>
<td>P=0.2</td>
</tr>
<tr>
<td><strong>CVC (ml/min/mmHg)</strong></td>
<td>Pre</td>
<td>2.66 ± 0.49</td>
<td>2.55 ± 0.88</td>
<td>-0.12 ± 0.56</td>
<td>-5.6 ± 17.9%</td>
<td>P=0.6</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>2.90 ± 0.82</td>
<td>2.63 ± 0.97*</td>
<td>-0.27 ± 0.26</td>
<td>-10.9 ± 10.3%</td>
<td>P=0.02</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>QVA (ml/min)</strong></td>
<td>Pre</td>
<td>77.5 ± 28.2</td>
<td>87.4 ± 34.4*</td>
<td>9.94 ± 13.3</td>
<td>12.1 ± 16.5%</td>
<td>P=0.02</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>76.7 ± 38.0</td>
<td>82.7 ± 41.2</td>
<td>6.01 ± 10.4</td>
<td>7.8 ± 12.8%</td>
<td>P=0.06</td>
</tr>
<tr>
<td><strong>Diameter (mm)</strong></td>
<td>Pre</td>
<td>0.37 ± 0.05</td>
<td>0.37 ± 0.05</td>
<td>0.00 ± 0.02</td>
<td>0.9 ± 5.0%</td>
<td>P=0.6</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>0.38 ± 0.06</td>
<td>0.38 ± 0.06</td>
<td>0.00 ± 0.01</td>
<td>-0.9 ± 2.8%</td>
<td>P=0.3</td>
</tr>
<tr>
<td><strong>Velocity (cm/sec)</strong></td>
<td>Pre</td>
<td>23.4 ± 5.3</td>
<td>25.9 ± 7.3*</td>
<td>2.5 ± 3.4</td>
<td>9.9 ± 13.0%</td>
<td>P=0.02</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>20.9 ± 6.2</td>
<td>23.04 ± 7.6*</td>
<td>2.2 ± 3.0</td>
<td>9.9 ± 12.7%</td>
<td>P=0.02</td>
</tr>
<tr>
<td><strong>CVC (ml/min/mmHg)</strong></td>
<td>Pre</td>
<td>0.88 ± 0.37</td>
<td>0.84 ± 0.38</td>
<td>-0.04 ± 0.04</td>
<td>-6.3 ± 7.6%</td>
<td>P=0.8</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>1.03 ± 0.56</td>
<td>0.99 ± 0.51</td>
<td>-0.04 ± 0.13</td>
<td>-2.8 ± 11.6%</td>
<td>P=0.3</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCAv (cm/sec)</strong></td>
<td>Pre</td>
<td>63.2 ± 10.2</td>
<td>69.5 ± 10.9*</td>
<td>6.3 ± 5.2</td>
<td>10.5 ± 9.7%</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>58.0 ± 9.3</td>
<td>62.6 ± 9.4*</td>
<td>4.6 ± 5.4</td>
<td>8.4 ± 10.2%</td>
<td>P=0.01</td>
</tr>
<tr>
<td><strong>CVC (cm/sec/mmHg)</strong></td>
<td>Pre</td>
<td>0.68 ± 0.1</td>
<td>0.65 ± 0.13</td>
<td>-0.03 ± 0.06</td>
<td>-5.2 ± 9.1%</td>
<td>P=0.08</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>0.68 ± 0.07</td>
<td>0.65 ± 0.07</td>
<td>-0.02 ± 0.05</td>
<td>-2.8 ± 7.3%</td>
<td>P=0.2</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCAv (cm/sec)</strong></td>
<td>Pre</td>
<td>43.2 ± 14.4</td>
<td>46.2 ± 14.5*</td>
<td>2.98 ± 5.1</td>
<td>8.1 ± 14.3%</td>
<td>P=0.05</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>40.1 ± 10.7</td>
<td>42.4 ± 13.1</td>
<td>2.30 ± 4.19</td>
<td>5.2 ± 10.1%</td>
<td>P=0.06</td>
</tr>
<tr>
<td><strong>CVC (cm/sec/mmHg)</strong></td>
<td>Pre</td>
<td>0.46 ± 0.14</td>
<td>0.43 ± 0.13*</td>
<td>-0.03 ± 0.04</td>
<td>-7.2 ± 8.7%</td>
<td>P=0.01</td>
</tr>
<tr>
<td></td>
<td>Post-Prazosin</td>
<td>0.47 ± 0.15</td>
<td>0.44 ± 0.15*</td>
<td>-0.02 ± 0.03</td>
<td>-5.7 ± 7.9%</td>
<td>P=0.02</td>
</tr>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from baseline, P<0.05. †Statistically different following Prazosin, P<0.05. MAP, mean arterial pressure; HR, heart rate; QICA, volumetric internal carotid artery blood flow; QVA, volumetric vertebral artery blood flow; CVC, cerebrovascular conductance; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>LBPP</th>
<th>Delta (Δ)</th>
<th>Relative (Δ%)</th>
<th>P-value</th>
<th>LBPP+HG</th>
<th>Delta (Δ)</th>
<th>Relative (Δ%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg) Pre</td>
<td>94 ± 7</td>
<td>104 ± 7</td>
<td>9.3 ± 6.7*</td>
<td>(10.1 ± 7.5%)</td>
<td>P=0.0001</td>
<td>117 ± 9</td>
<td>22 ± 10*</td>
<td>(24.3 ± 11.7%)</td>
<td>P=0.0000005</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats / min) Pre</td>
<td>69 ± 14</td>
<td>65 ± 10</td>
<td>-4 ± 8</td>
<td>(-4.6 ± 8.7%)</td>
<td>P=0.09</td>
<td>77 ± 11</td>
<td>9 ± 9*</td>
<td>(14.7 ± 13.6%)</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QICA (ml/min) Pre</td>
<td>233.4 ± 54.6</td>
<td>232.9 ± 56.7</td>
<td>-0.4 ± 31.6</td>
<td>(0.5 ± 14.6%)</td>
<td>P=0.96</td>
<td>242.3 ± 52.3</td>
<td>9.0 ± 26.0</td>
<td>(4.6 ± 10.9%)</td>
<td>P=0.3</td>
</tr>
<tr>
<td>Diameter (mm) Pre</td>
<td>0.49 ± 0.04</td>
<td>0.48 ± 0.04</td>
<td>-0.01 ± 0.03</td>
<td>(-2.2 ± 6.7%)</td>
<td>P=0.3</td>
<td>0.48 ± 0.04</td>
<td>-0.01 ± 0.03</td>
<td>(-2.9 ± 5.6%)</td>
<td>P=0.1</td>
</tr>
<tr>
<td>Velocity (cm/sec) Pre</td>
<td>40.8 ± 9.3</td>
<td>42.5 ± 8.0</td>
<td>1.6 ± 2.8</td>
<td>(4.9 ± 7.5%)</td>
<td>P=0.1</td>
<td>44.7 ± 7.6</td>
<td>3.9 ± 4.3*</td>
<td>(11.1 ± 11.4%)</td>
<td>P=0.02</td>
</tr>
<tr>
<td>CVC (ml/min/mmHg) Pre</td>
<td>2.9 ± 0.71</td>
<td>2.7 ± 0.73</td>
<td>-0.18 ± 0.31</td>
<td>(-6.0 ± 11.4%)</td>
<td>P=0.15</td>
<td>2.5 ± 0.76</td>
<td>-0.37 ± 0.31*</td>
<td>(-13.2 ± 12.6%)</td>
<td>P=0.012</td>
</tr>
<tr>
<td>QVA (ml/min) Pre</td>
<td>73.8 ± 21.9</td>
<td>74.5 ± 22.4</td>
<td>0.7 ± 9.6</td>
<td>(2.1 ± 14.4%)</td>
<td>P=0.8</td>
<td>86.1 ± 30.5</td>
<td>12.2 ± 14.6*</td>
<td>(16.6 ± 17.0%)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Diameter (mm) Pre</td>
<td>0.37 ± 0.04</td>
<td>0.37 ± 0.04</td>
<td>-0.003 ± 0.01</td>
<td>(-0.7 ± 2.3%)</td>
<td>P=0.2</td>
<td>0.37 ± 0.04</td>
<td>-0.002 ± 0.013</td>
<td>(-0.4 ± 3.4%)</td>
<td>P=0.6</td>
</tr>
<tr>
<td>Velocity (cm/sec) Pre</td>
<td>22.2 ± 4.1</td>
<td>22.6 ± 3.5</td>
<td>-0.5 ± 3.2</td>
<td>(3.6 ± 14.4%)</td>
<td>P=0.6</td>
<td>26.0 ± 5.4</td>
<td>3.8 ± 3.2*</td>
<td>(17.5 ± 14.0%)</td>
<td>P=0.002</td>
</tr>
<tr>
<td>CVC (ml/min/mmHg) Pre</td>
<td>20.2 ± 5.7</td>
<td>21.5 ± 6.8</td>
<td>1.3 ± 1.9*</td>
<td>(6.2 ± 7.9%)</td>
<td>P=0.04</td>
<td>23.4 ± 7.3</td>
<td>3.1 ± 3.0*</td>
<td>(15.4 ± 14.5%)</td>
<td>P=0.004</td>
</tr>
<tr>
<td>MCAv (cm/sec) Pre</td>
<td>63.6 ± 10.2</td>
<td>65.5 ± 10.2</td>
<td>1.1 ± 3.2</td>
<td>(1.8 ± 4.9%)</td>
<td>P=0.3</td>
<td>70.1 ± 10.1</td>
<td>5.7 ± 3.9*</td>
<td>(9.3 ± 6.3%)</td>
<td>P=0.0002</td>
</tr>
<tr>
<td>CVC (cm/sec/mmHg) Pre</td>
<td>0.68 ± 0.11</td>
<td>0.62 ± 0.10</td>
<td>-0.05 ± 0.05*</td>
<td>(-7.6 ± 5.8%)</td>
<td>P=0.0001</td>
<td>0.60 ± 0.10</td>
<td>-0.08 ± 0.06*</td>
<td>(-11.2 ± 7.5%)</td>
<td>P=0.0004</td>
</tr>
<tr>
<td>PCAv (cm/sec) Pre</td>
<td>43.7 ± 13.1</td>
<td>44.4 ± 12.5</td>
<td>0.5 ± 2.9</td>
<td>(2.2 ± 7.8%)</td>
<td>P=0.4</td>
<td>48.2 ± 4.0</td>
<td>4.5 ± 4.0*</td>
<td>(10.8 ± 9.1%)</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVC (cm/sec/mmHg) Pre</td>
<td>0.47 ± 0.14</td>
<td>0.43 ± 0.13</td>
<td>-0.036 ± 0.03*</td>
<td>(-7.2 ± 5.1%)</td>
<td>P=0.001</td>
<td>0.41 ± 0.13</td>
<td>-0.053 ± 0.04*</td>
<td>(-11.5 ± 7.6%)</td>
<td>P=0.0002</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically different from baseline, P<0.05. †Statistically different following Prazosin, P<0.05. MAP, mean arterial pressure; HR, heart rate; QICA, volumetric internal carotid artery blood flow; QVA, volumetric vertebral artery blood flow; CVC, cerebrovascular conductance; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity.
3.3 Relationship between changes in extra-cranial blood flow and intra-cranial blood velocity during BP manipulations

*Pre-Prazosin administration:* Absolute changes in contralateral Q_{ICA} ($r^2=0.003; P=0.78$) and ipsilateral Q_{VA} ($r^2=0.03; P=0.3$) were not significantly correlated with changes in MAP prior to Prazosin administration. Conversely, intra-cranially, there was a positive correlation between changes in contralateral MCAv ($r^2=0.2; P=0.005$) and ipsilateral PCAv ($r^2=0.37; P<0.0001$) with increases in MAP.

*Post-Prazosin administration:* Similar to pre-blockade, there was a non-significant correlation between changes in contralateral Q_{ICA} and MAP ($r^2=0.07; P=0.16$) following Prazosin. In contrast, following blockade, there was a positive correlation between changes in ipsilateral Q_{VA} and MAP ($r^2=0.14; P=0.02$). The positive relationships between changes in contralateral MCAv ($r^2=0.27; P=0.0007$) and ipsilateral PCAv ($r^2=0.23; P=0.0012$) and MAP remained statistically different following Prazosin administration; however, based on comparisons of individual regressions, the relationship and slope did not differ significantly between pre and post-Prazosin comparison.

3.4 Absolute changes in extra-cranial diameter & velocity with changes in BP manipulations

*Pre-Prazosin administration:* There was a small, albeit insignificant, correlation between absolute changes in contralateral ICA diameter and MAP ($r^2=0.09; P=0.1$). Likewise, absolute changes in ipsilateral VA diameter during changes in MAP were also not correlated ($r^2=0.006; P=0.6$). Although absolute changes in contralateral ICA velocity and MAP were also not significantly correlated ($r^2=0.02; P=0.4$), there was a small positive correlation between changes in ipsilateral VA velocity and MAP ($r^2=0.11; P=0.04$).

*Post-Prazosin administration:* Following Prazosin administration, there was a weak negative correlation between absolute changes in contralateral ICA diameter with changes in MAP ($r^2=0.05; P=0.3$). Similarly, there was also small – albeit not significant - negative correlation between changes in ipsilateral VA diameter with the changes in MAP ($r^2=0.1; P=0.06$). There was a positive correlation between absolute changes in contralateral ICA velocity with MAP ($r^2=0.13; P=0.05$).
Likewise, changes in ipsilateral VA velocity with changes MAP ($r^2=0.29$; $P=0.0006$) were also modestly correlated following Prazosin administration.

### 3.5 Absolute changes in extra-cranial blood flow & velocity with absolute changes in intra-cranial velocity

**Pre-Prazosin administration:** There was no correlation between the absolute changes in contralateral $Q_{ICA}$ with MCAv ($r^2=0.03$; $P=0.4$); however, there was a modest correlation between the changes in contralateral ICA velocity with MCAv ($r^2=0.31$; $P=0.005$). There was no correlations between changes in ipsilateral $Q_{VA}$ with PCAv ($r^2=0.05$; $P=0.2$). Similarly, there was no significant relationship between changes in ipsilateral VA velocity with PCAv ($r^2=0.003$; $P=0.7$).

**Post-Prazosin administration:** Following Prazosin administration there was a small, albeit not significant, positive correlation between the changes in contralateral $Q_{ICA}$ with MCAv ($r^2=0.12$; $P=0.08$). The relationship between changes in contralateral ICA velocity with MCAv was also not significantly correlated following Prazosin ($r^2=0.1$; $P=0.1$). There were no significant correlations between changes in ipsilateral $Q_{VA}$ with PCAv ($r^2=0.15$; $P=0.22$), nor with changes in ipsilateral VA velocity with PCAv were ($r^2=0.09$; $P=0.08$) following Prazosin administration.
Pre-Prazosin - $\Delta$ MCAv v. $\Delta$ MAP

- $r^2 = 0.2$; $P = 0.005$

Post-Prazosin - $\Delta$ MCAv v. $\Delta$ MAP

- $r^2 = 0.07$; $P = 0.16$

Pre-Prazosin - $\Delta$ QICA v. $\Delta$ MAP

- $r^2 = 0.003$; $P = 0.8$

Post-Prazosin - $\Delta$ QICA v. $\Delta$ MAP

- $r^2 = 0.07$; $P = 0.16$

Post-Prazosin - $\Delta$ MCAv v. $\Delta$ MAP

- $r^2 = 0.27$; $P = 0.0007$
Figure 3.4.1 Relationship between absolute changes in extra-cranial blood flow (QICA & QVA) and intra-cranial blood velocity (MCAv & PCAv) during changes in MAP, within subjects. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.
Negative correlation, indicative of vasoconstriction of the ICA during transient hypertension...
Figure 3.4.2 Absolute changes in extra-cranial (ICA & VA) diameter / velocity vs. absolute changes in MAP. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.
Pre-Prazosin:

\[ \Delta \text{ICA Velocity (cm/sec)} \]

\[ \Delta \text{MCAv (cm/sec)} \]

\[ r^2 = 0.1; P = 0.1037 \]

\[ R^2 = 0.1024 \]

Post-Prazosin:

\[ \Delta \text{ICA Flow (ml/min)} \]

\[ \Delta \text{MCAv (cm/sec)} \]

\[ r^2 = 0.12; P = 0.0774 \]

\[ R^2 = 0.1195 \]

Pre-Prazosin:

\[ \Delta \text{ICA (ml/min)} \]

\[ \Delta \text{MCAv (cm/sec)} \]

\[ r^2 = 0.31; P = 0.005 \]

\[ R^2 = 0.3059 \]

\[ \Delta \text{ICAv (cm/sec)} \]

\[ \Delta \text{MCAv (cm/sec)} \]

\[ r^2 = 0.03; P = 0.37 \]

\[ R^2 = 0.03191 \]

\[ \Delta \text{MCAv (cm/sec)} \]

\[ r^2 = 0.12; P = 0.08 \]

\[ R^2 = 0.1195 \]
Figure 3.4.3 Absolute changes in extra-cranial (ICA & VA) blood flow / velocity vs. absolute changes in intra-cranial (MCA & PCA) blood velocity. Data are from the pooled 30% MVC HG, LBPP and LBPP+HG interventions.
Chapter 4: Conclusion

4.1 Summary of main findings

The purpose of this study was to investigate the role of SNA in the regulation of CBF during transient hypertension. Hypertension was induced, non-pharmacologically, by 30% MVC HG exercise, LBPP and LBPP+HG before and following a pharmacological alpha-1 adrenergic receptor antagonist (Prazosin). The hypothesis was that the alpha-1 adrenergic antagonist would cause augmentation in both contralateral and ipsilateral extra-cranial artery blood flow (QICA & QVA) and intra-cranial blood velocity (MCAv & PCAv), thus demonstrating that SNA serves as a protective function during transient hypertension. Partly contrary to our hypothesis, however, at the group average level there were no significant relative increases in contralateral QICA or conductance, nor significant relative changes in contralateral MCAv or conductance during 30% MVC HG following Prazosin administration. Additionally, there were no significant increases in contralateral QICA or MCAv (conductance as well for both) during LBPP or LBPP+HG exercise following Prazosin administration. Similarly, there were no significant relative changes in ipsilateral QVA or conductance. However, upon exploring the intra-individual variability on these changes in intra-cranial blood velocity and extra-cranial blood flow during manipulation of BP, although changes in contralateral QICA and BP were unrelated, the changes in ipsilateral QVA and BP were positively correlated following Prazosin administration. Consistent with these regional differences, changes in ipsilateral PCAv CVC were significantly attenuated during LBPP and LBPP+HG following Prazosin. These discrepancies between ipsilateral VA and PCAv CVC may reflect dilation of the PCA or some distribution of blood flow from VA before it reaches the PCA, or both. While these findings are contrary to our hypothesis, these differential findings are interpreted to indicate that there is a disparity between the extra- and intra-cranial arteries and that there is a small, but functional, role of SNA in the regulation of the posterior region of the brain during transient hypertension.

4.2 Transient hypertension and CBF

During normotensive conditions, animal studies suggest that isolated cervical ganglia stimulation yields no change in cerebrovascular tone (Mayhan et al., 1987; Cassaglia et al., 2008a; Cassaglia et al., 2008b), reviewed in (Ainslie, 2009; Ter Laan et al., 2013; Edvinsson et al., 1993; Busija,
However, studies indicate that SNA modulates CBF regulation during acute changes in blood pressure, thus acting as a protective buffer for the cerebral microcirculation against potential hyper-perfusion injury (Mayhan et al., 1987). For example, it has been reported that sympathetic nerves become excited during hypertension, but not hypotension, during stimulation of the superior cervical ganglion (Bill & Linder, 1976; Heistad, Marcus, & Gross, 1978; Busija et al., 1980; Cassaglia et al., 2008a; Cassaglia et al., 2009). Conversely, autoregulatory capacity (in cats) has been reported to be preserved following denervation of preganglionic fibres supplying the superior cervical ganglion (Busija & Heistad, 1984a). This protective buffering capacity of the SNA against transient hypertension seems to be a function of the larger cerebral arteries and large pial arterioles, as opposed to the cerebral microcirculation (Baumbach & Heistad, 1983; Wei et al., 1975). Thus, it seems that sympathetic control of the cerebral circulation exhibits little more than tonic activity during normo- and hypotension, but is particularly important to buffer transient increases in blood pressure (Bill & Linder, 1976; Edvinsson et al., 1976; MacKenzie et al., 1976; Heistad & Marcus, 1979; Waldemar et al., 1989; Paulson et al., 1990).

Human studies have similarly suggested a SNA influence during transient hypertension (Kimmerly et al., 2003; Zhang et al., 2004). Two studies have reported greater increases in MCAv per increase in MAP during Valsalva maneuver (Zhang et al., 2004) and norepinephrine infusion (Kimmerly et al., 2003) following sympathetic ganglion blockade (Trimethapahan) and alpha-adrenergic blockade (Phentolamine), respectively. Two other studies used LBPP to non-pharmacologically increase BP (Perry et al., 2013; Perry et al., 2014). In the first study, the authors reported that during differential LBPP, there was an increase in MCAv during +20 mmHg LBPP, but then decreased at higher LBPP (~+40 mmHg) (Perry et al., 2013). In the second of these studies (Perry et al., 2014), a similar decrease in MCAv during +40 mmHg LBPP was observed. In addition, extra-cranial blood flow was measured in the ICA in a small subset of subjects (n=5) and a decrease in CVC was observed at +20 mmHg LBPP and then increased close to baseline values during +40 mmHg. The authors speculated from these two studies that an increase in cerebral SNA was responsible for the decreases in MCAv and ICA CVC. Consistent with this notion, well controlled animal studies also indicate that the extra-cranial arteries (ICA & VA) act as the first line of defense in regulating perfusion pressure to the brain (Heistad, Marcus, & Abboud, 1978; Faraci, Heistad, et al., 1987; Faraci, Mayhan, Werber, & Heistad, 1987).
Based on these aforementioned studies we expected that there would be a vasoconstriction of the extra-cranial arteries during transient hypertension, so as to limit cerebral perfusion. However, based on the lack of change in group averages (Figure 3.2) and correlations (Figure 3.4.(1-3)) following Prazosin administration, our findings do not support the notion that extra-cranial arteries at least in the anterior region of the brain play a modulatory role in limiting cerebral perfusion during transient hypertension; therefore, the modulatory control of cerebral vascular resistance to transient hypertension occurs farther upstream at the pial arterioles (Girouard & Iadecola, 2006; Hamel, 2006); however, in our experimental model, this response is not affected by alpha-1 adrenergic receptor antagonist. Despite this finding, as illustrated in Figure 3.4.(1-3), upon exploring the intra-individual variability on these changes in intra-cranial blood velocity and extra-cranial blood flow during manipulation of BP, although changes in $Q_{ICA}$ and BP were unrelated, the changes in $Q_{VA}$ and BP were positively correlated following Prazosin administration. Consistent with these regional differences, absolute changes in PCAv CVC during LBPP and LBPP+HG were attenuated following Prazosin. In the conditions of the current experimental manipulations, these differential findings are interpreted to indicate that there is a disparity between the extra- and intra-cranial arteries that seem to be specific to a small but functional role of SNA in the regulation of the posterior region of the brain during transient hypertension.

4.3 Regional differences in CBF: Contralateral vs. Ipsilateral

Exercise induces increases in cerebral neuronal activity (Seifert & Secher, 2011); therefore, it is possible that SNA modulation of resting cerebrovascular tone may become blunted during muscular contractions (Gross, Marcus, & Heistad, 1980). It has been shown that unilateral HG increases neuronal activity in the contralateral primary motor cortex (Sander, Macefield, & Henderson, 2010) as well as being coupled with cortical representation of the exercising muscle group due to regional increases in perfusion (Linkis et al., 1995). This contralateral increase in blood velocity has been demonstrated in studies using rhythmic HG (Jørgensen, Perko, & Secher, 1992) and dynamic exercise of the hand and foot (rhythmic hand squeezing of rubber ball and foot flexion and extension (Linkis et al., 1995). In this former study, MCAv was elevated by 20% on the left side and 24% on the right side from respective contralateral rhythmic HG (Jørgensen et al., 1992). In the latter study, dynamic hand and foot exercise elevated contralateral MCAv by 19%
and anterior cerebral artery mean velocity by 23% (Linkis et al., 1995). In a recent study (Fernandes et al., 2016), concurrent contralateral and ipsilateral \( Q_{ICA} \) and conductance was measured during unilateral isometric HG before and following oral Prazosin. There was a significant increase in contralateral \( Q_{ICA} \) during isometric HG exercise prior to Prazosin, while ipsilateral \( Q_{ICA} \) was reduced, albeit not significantly, and conductance were significantly reduced; a finding indicative of SNA regulation of vascular resistance. Following Prazosin administration contralateral \( Q_{ICA} \) and ipsilateral \( Q_{ICA} \) were similarly increased during isometric HG exercise. In comparison to the Fernandes study, we only measured contralateral \( Q_{ICA} \) and observed no significant changes in ICA diameter, blood flow or CVC during 30% MVC HG, LBPP or LBPP+HG prior to Prazosin administration. We also measured contralateral MCAv and observed significant increases during HG and LBPP+HG, but not during LBPP prior to blockade. Following Prazosin administration, we did not observe any significant changes in MCAv or conductance during HG, LBPP or LBPP+HG. Similarly, correlational analyses rendered no significant relationships between changes in contralateral ICA blood flow, velocity & diameter during changes in MAP following Prazosin administration. However, there was a significant relationship between changes in MCAv and MAP following Prazosin administration. Although there was no relationship between changes in contralateral \( Q_{ICA} \) and MCAv –both prior to and following Prazosin administration, there was a significant relationship between ICA velocity and MCAv prior to Prazosin; however, this relationship was abolished following Prazosin administration. These findings could be indicative of changes in the distal contralateral MCA tone following Prazosin administration, as the corresponding proximal ICA vessel diameter remained unchanged.

Additionally, we measured ipsilateral blood flow in the VA. Following Prazosin administration, relative changes in ipsilateral VA blood flow, velocity & diameter were similar to pre-Prazosin values during 30% MVC HG, LBPP and LBPP+HG. However, we did observe a significant decrease in ipsilateral PCAv CVC during LBPP and LBPP+HG, but interestingly not during HG. Intra-individual comparisons of changes in ipsilateral VA blood flow, velocity and diameter during changes in MAP rendered a significant relationship following Prazosin administration in blood flow and diameter; moreover, while the relationship between changes in VA velocity and MAP was further strengthened following Prazosin administration. Similarly, there was a significant positive relationship between changes in PCAv and MAP following Prazosin; however, the relationship was weakened following Prazosin administration. This
observation is likely related to the changes in PCAv CVC following Prazosin in the group-mean observations. Comparisons of ipsilateral $Q_{VA}$ and velocity during changes in PCAv were both not significantly correlated, prior to and following Prazosin administration. This disparity between the changes in ipsilateral VA blood flow & velocity during changes in PCAv would suggest the VA and PCA do not share uniform autoregulatory capacities during changes in MAP. For example, there is evidence that in some physiological conditions the posterior circulation has less effective autoregulation than the anterior circulation (Haubrich et al., 2004); however, this is not a universal finding (Park et al., 2003; Deegan et al., 2010). It should be noted, however, none of these studies assessed CA during hypertension. Moreover, Prazosin has been reported to attenuate dynamic CA in MCAv at rest or during transient hypotension (Purkayastha et al., 2013; Ogoh et al., 2008), head-up tilt (Lewis et al., 2012; Lewis et al., 2013). In contrast to these reports, we found no functional consequences of blockade during transient hypertension in the anterior circulation, while the autoregulatory capacity of the posterior circulation was impaired.

The apparent differences between VA and PCAv during BP elevations following Prazosin administration could be either a technical or physiological discussion point: i) technical being that the difference between flow and index of flow using velocity from the TCD (i.e., possibility of dilation of the PCA – see Methodological considerations below), and ii) physiological differential regulation between the VA and PCA (i.e., changes in the relative distribution of blood in the various territories distal to the vertebral artery). For example, both VA’s feed a number of other vessels in addition to the PCA, which in regards to the vertebrobasilar system, has a higher degree of complexity than the anterior circulation. There are numerous anastomoses with the VA both extra- and intra-cranially - the VA communicates with branches of the deep cervical artery, and inferior thyroid artery extra-cranially, 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., 2011). Unfortunately, from our data set, we cannot differentiate between these potential technical or physiological – or both – explanations of our findings. Currently, the mechanisms underpinning these regional differences are unknown. A possible reason for the disparity in regulation between anterior and posterior circulation is that the VA distributes blood flow to the regions of the brain
responsible for the regulation of homeostatic functions (e.g., medulla oblongata, cerebellum, hypothalamus, thalamus & brainstem (Tatu et al., 1996) – this could be a potential intrinsic protective mechanism to maintain consciousness. Evidence persists that the extra-cranial vessels constrict during hypotension, as increases in MSNA have been reported (Cooke et al., 2009); given that the ICA & VA are highly innervated with perivascular bundles (Bleys et al., 1996; Borodulya & Pletchkova, 1973; Borodulya & Pletchkova, 1976), it is plausible that elevated SNA inhibits dilation of the larger extra-cerebral arteries during hypertension.

The potential of the extra-cranial arteries (ICA and VA) acting as ‘proximal resistors’ has been supported in humans during changes in arterial blood gases (Willie et al., 2012; Sato et al., 2012; Hoiland et al., 2016) and, in animals, during changes in BP (Heistad, Marcus, & Abboud, 1978; Faraci, Heistad, et al., 1987; Faraci, Mayhan, et al., 1987). To the best of our knowledge, this is the first time that there is evidence of these arteries playing a role in buffering changes in BP during transient hypertension. The correlations were notably mild, explaining statistically ~15% of the statistical variance between the QVA during changes in BP. Nevertheless, in a biological system under multiple and complex non-linear regulatory control mechanisms (i.e., myogenic, metabolic, intrinsic and extrinsic SNA control, parasympathetic, including alpha-1, 2 and beta-1, 2 receptors), these findings deserve future exploration (see Adrenergic control of CBF and redundant pathways below). It is also possible that the small effect observed at the extra-cranial arteries during transient hypertension may be due to the modest - yet physiological - non-pharmacological manipulations of BP not being marked enough to elicit a cerebral SNA response.

4.4 Methodological considerations

4.4.1 Transcranial doppler ultrasound

A methodological consideration that needs to be further discussed in the current study is the utility of TCD in indexing blood flow from blood velocity, as TCD operates on the assumption that the insonated vessel remains at a constant diameter. Older studies have partially corroborated that under various stimuli (e.g., orthostasis), MCAv accurately reflected the magnitude of changes in MCA blood flow as diameter remained unchanged (Giller et al., 1993; Valdueza et al., 1997; Serrador et al., 2000); however, recent high resonance imaging studies (Coverdale et al., 2014; Verbree et al., 2014; Wilson et al., 2011) have challenged this assumption of constant vessel diameter during marked changes in PaCO₂ or PaO₂. However, as recently reviewed (Hoiland &
Ainslie, 2016), it is not known if the MCA (or PCA) diameter changes during elevations in BP. Interestingly, during changes in PaCO$_2$ or PaO$_2$, the result changes in blood flow in the extra-cranial circulation is normally highly related to those in the intra-cranial vessels (Willie et al., 2012; Sato et al., 2012). For example, previous studies (Hoiland et al., 2016) have reported a strong relationship between ICA blood velocity with changes in MCA v during changes in PaCO$_2$ ($r^2$=0.88). A similar relationship is observed during changes in PaCO$_2$ in the posterior circulation - i.e., blood flow in the VA and PCA v are strongly correlated ($r^2$=0.88) under similar conditions (Hoiland et al., 2016). During progressive hypotension with controlled PaCO$_2$ levels, a recent study (Lewis et al., 2015) reported an absence of relationship between reductions in ICA blood flow with MCA v $r$=0.09 (P=0.75). In contrast, and broadly consistent with the current findings, was a selective relationship ($r^2$=0.47; P=0.07) during hypotension with reductions in VA blood flow and PCA v (Lewis et al., 2015). To the best of our knowledge, this is the first study to compare extra-cranial blood flow vs. intra-cranial blood flow velocity during transient hypertension using a multimodal approach. Contrary to what has been observed during changes in arterial blood gases (Hoiland et al., 2016) or hypotension (Lewis et al., 2015), after pooling our data, there were no clear correlations in either the anterior or posterior regions between changes in extra-cranial flows with intra-cranial velocities during transient hypertension. Such findings highlight a differentiating relationship between extra-cranial blood flow vs. intra-cranial velocities during transient hypertension. The extent to which these differences might be explained by changes in intra-cranial vessel diameter, or simply due to intrinsic differences in the regulation of BP remains to be established. For example, it has been well documented that during changes in BP the majority of changes in vascular resistance occurs at the level of the small pial vessels (Ngai & Winn, 1995; Iadecola et al., 1997; Girouard & Iadecola, 2006; Hamel, 2006) as well as (in cats) the larger extra-cranial vessels (Mchedlishvili, 1964; Mchedlishvili et al., 1973; Heistad, Marcus, & Abboud, 1978; Faraci, Heistad, et al., 1987; Faraci, Mayhan, et al., 1987; Faraci & Heistad, 1990). How such combined changes might impact the linear relationship between extra-cranial blood flow and intra-cranial velocities are unclear, but likely reflective of multiple complex and non-linear factors. Regardless, however, the disparity between extra-cranial blood flow and intra-cranial velocities during transient hypertension is an important observation that has implications for the design and interpretation of future studies in this field.
4.4.2 Adrenergic control of CBF and redundant pathways

The cerebral vasculature is richly supplied by both adrenergic and cholinergic receptors of both extrinsic (i.e., cervical, sphenopalatine and trigeminal ganglia) and intrinsic (i.e., locus coeruleus, fastigial nucleus and dorsal raphe nucleus) origins (Lowe & Gilboe, 1971; Edvinsson et al., 1976; Edvinsson & Hamel, 2002). The large cerebral arteries are highly innervated mostly by adrenergic fibers originating from the ipsilateral superior cervical ganglion (Itakura et al., 1977). Prazosin is a selective alpha-1 antagonist and effectively blocks ~70-80% of alpha-1 adrenergic receptors (Ogoh et al., 2008; Jaillon, 1980), making it an ideal research tool preferred to a non-selective alpha antagonist. While Prazosin is an effective alpha-1 antagonist, the remaining ~20% of unblocked alpha-1 receptors cannot be excluded from the discussion. Moreover, the possibility of an up-regulation of alpha-2 adrenergic receptors could be masking any major physiological changes during transient hypertension (Ferrari-DiLeo & Potter, 1985). Likewise, beta-receptors in the brain can cause vasodilation (McCulloch & Edvinsson, 1984) or constriction depending upon the size of the cerebral vessels. Though the results of our study support only a minor role of SNA in regulating extra-cranial CBF in the posterior circulation during transient hypertension it has been reported that when alpha-adrenergic receptors are blocked by an alpha-antagonist, there is a dose dependent vasodilatory response that has been observed in humans, cats and dog cerebral arteries (Tsukahara et al., 1986). Whether blockades of different pathways (alpha-2 or beta, etc.) would reveal additional changes in CBF during transient hypertension remains to be explored.

4.4.3 Blood pressure manipulations following Prazosin administration

Isometric HG is a useful research tool, as this stimulus increases efferent sympathetic pathways yielding non-pharmacological increases in HR and MAP (Riendl et al., 1977; Hietanen, 1984). In the present study following Prazosin administration, MAP response to HG was reduced by 16 %, albeit not reaching statistical significance. In contrast, changes in MAP were significantly decreased respectively by 29% and 40% following Prazosin administration during LBPP and LBPP combined HG. In a recent study (Fernandes et al., 2016), isometric HG at 30% of MVC elicited an increase in MAP of +32 mmHg (73 to 105 mmHg) prior to Prazosin administration; however, following Prazosin administration, the MAP response was significantly reduced by ~38% (~10 mmHg). In comparison to this study, our findings were comparable but our MAP response to HG was not as pronounced both prior to and following Prazosin administration. The
reasons for this discrepant influence of HG on MAP is not clear but could be related to differences in the very low baseline MAP (73 mmHg) and related subject demographics (e.g., fitness levels) as these factors have been shown to influence BP response to exercise (Bouchard & Rankinen, 2001).

An alternative methodology for non-pharmacologically increasing MAP is that of LBPP. LBPP translocates blood to the upper torso, thereby increasing central venous pressure and elevating MAP (Shi et al., 1993; Shi et al., 1997; Fu et al., 1998) in the face of unchanged muscle SNA. Two recent studies (Perry et al., 2013; Perry et al., 2014) increased MAP using different LBPP steps of +20 mmHg and +40 mmHg, thereby increasing MAP respectively by +7 ± 6 mmHg (+8 ± 7%) and +13 ± 7 mmHg (+19 ± 11%). These increases in MAP are similar to the increases in MAP in our present study prior to Prazosin administration, with MAP significantly increased further when combining LBPP with HG. Following Prazosin administration we observed a significantly reduced response in MAP to LBPP (+9.3 vs. +5 mmHg) as well as during the combined LBPP and HG (+22 vs. +14 mmHg). To our knowledge, this is the first study to employ an alpha-1 antagonist during LBPP.

4.4.4 Central venous pressure

Another potential methodological consideration is the potential change in central venous pressure induced by the application of LBPP, and consequently increases in right arterial pressure (Shi et al., 1993; Shi et al., 1997; Nishiyasu et al., 1998). This augmentation in central venous pressure, if present, may result in mechanical restriction of cerebral venous outflow and subsequent elevations in ICP (Nakagawa, Tsuru, & Yada, 1974; Piechnik et al., 2001). Since CPP is the difference between MAP and ICP, under normal resting conditions in humans, if ICP did change this would lead to changes in perfusion pressure. Whether this is advantageous (by helping limit over-perfusion) or deleterious (by caused brain edema) is unknown. Likewise, we do not know of any evidence that such changes in ICP do indeed occur. However, although there is evidence that raised intravascular pressure may compromise the integrity of the blood-brain barrier resulting in “leak through” of intravascular catecholamines (leading to brain edema and elevations in ICP), the evidence for this is based on animals studies conducted under conditions that are vastly different from those of the current study, that is, prolonged (hours) periods of extreme hypertension (e.g., MAP >180 mm Hg) (MacKenzie et al., 1976). It would seem unlikely that small mechanically
induced (i.e., LBPP) restriction of cerebral venous outflow and/or SNA blockade would raise ICP substantially.

4.5 Conclusion

The modulation of CBF involves the integration of a myriad of modulating influences (e.g., metabolic, BP, CO, arterial blood gases (PaCO₂ & PaO₂) and neurogenic activation (i.e., SNA)) as well as redundant mechanisms in order to maintain a continuous perfusion of blood flow. Despite the extensive anatomical work describing the neurogenic innervation of the cerebral circulation, the role of SNA innervation on the dynamic regulation of CBF remains controversial. The brain appears to possess an intrinsic ability to buffer against dynamic changes in MAP and CBF – hysteresis effect (Heistad & Kontos, 1983; Numan et al., 2014). This intrinsic buffering capacity appears to be most effective during transient hypertension than hypotension - which has been suggested to be primarily modulated by an increase in SNA. The purpose of this study was to investigate this role of SNA in the regulation of CBF during transient hypertension – of which was non-pharmacologically induced by HG, LBPP, or a combination of both, before and following a pharmacological alpha-1 adrenergic receptor antagonist (Prazosin). We expected that there would be an augmentation of both extra-cranial blood flow and intra-cranial blood velocity and thus demonstrating that SNA serves as a protective mechanism of the cerebral circulation during transient hypertension. However, contrary to our hypothesis, at the group mean level there were no such significant augmentation of extra-cranial blood flow or intra-cranial blood velocity during our non-pharmacological BP interventions following Prazosin administration. Upon exploring intra-individual variability following Prazosin administration however, there appeared to be a disparity between extra-cranial blood flow and intra-cranial blood velocity in the posterior circulation (i.e., VA & PCA) during transient changes in BP. This is consistent with an observed reduction in PCA v CVC in the group mean data, which may reflect vasodilation or a redistribution of blood flow from the VA before communicating with the PCA, or both. Currently, the mechanisms underlying these regional differences are unknown. While these findings are contrary to our hypothesis, these differential findings are interpreted to indicate that there is a disparity between the extra- and intra-cranial arteries and that there is a small, but functional, role of SNA in the regulation of CBF of the posterior region of the brain during periods of transient hypertension.
4.5.1 Future directions

Future studies should consider employment of high resolution imaging (e.g., MRI) approaches to measure the diameter of the intra-cranial arteries. This approach would provide insight upon whether or not the intra-cranial artery (i.e., MCA or PCA) diameter is indeed remaining constant or changing due to the particular experimental manipulation being performed. In addition, future studies should consider using other adrenergic blockades (alpha-2) or a combination (alpha-1 & alpha-2, beta-blockades) to investigate the role of SNA in regulating CBF.
Bibliography


Brain Research Reviews, 38(3), 377–388.


Harmel, M. H., Hafkenschiel, J. H., Austin, G. M., Crumpton, C. W., & Kety, S. S. (1949). The


blood flow and to anoxemia, 44.


Mchedlishvili, G. I. (1964). Vascular Mechanisms Pertaining To the Intrinsic Regulation of the


Sato, K., & Sadamoto, T. (2010). Different blood flow responses to dynamic exercise between


Smirl, J. D., Hoffman, K., Tzeng, Y.-C., Hansen, A., & Ainslie, P. N. (2016). Relationship between


Stimulation in Humans Increases Middle Cerebral Artery Blood Flow Velocity. *Cerebrovascular Diseases, 2*(6), 359–364.


