Magnetic Resonance Mapping of Cerebrovascular Reserve: 
Steal Phenomena in Normal and Abnormal Brain

by

Daniel Michael Mandell

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy
Institute of Medical Science
University of Toronto

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2013

Abstract

Blood oxygen level-dependent (BOLD) magnetic resonance (MR) imaging enables non-invasive spatial mapping of changes in cerebral blood flow (CBF). By applying a vasodilatory stimulus (such as inhaled CO₂) during BOLD MR imaging, one can measure cerebral vasodilatory capacity. “Cerebrovascular reactivity” (CVR) is defined as the change in CBF per unit of vasodilatory stimulus. Vasodilatory capacity is clinically important as vasodilatation is a mechanism by which the brain maintains constant CBF despite reductions in cerebral perfusion pressure.
Patients with arterial narrowing commonly demonstrate a paradoxical response: vasodilatory stimulus-induced reduction of BOLD MR signal. BOLD MR depends on CBF but on other factors too. Does a reduction of BOLD MR signal indicate a decrease in flow? Does BOLD MR CVR correlate with CVR measured using arterial spin labeling (ASL) MR? I studied thirty-eight patients with stenosis of brain-supplying arteries and found that the BOLD CVR and ASL CVR results correlate strongly ($R=0.83$, $P<0.0001$ for cerebral hemispheric gray matter).

The second study aimed to determine whether preoperative CVR predicts the hemodynamic effect of extracranial-intracranial bypass surgery. Whereas prior studies relied on right-left interhemispheric CVR asymmetry indices, this study used “absolute” CVR from each hemisphere. I studied twenty-five patients with intracranial arterial stenosis. I found that the group with normal pre-operative CVR showed no change in CVR following bypass surgery (0.22% ± 0.05% to 0.22% ± 0.01% (mean ± SD)(P=0.881)), the group with reduced pre-operative CVR demonstrated an improvement (0.08% ± 0.05% to 0.21 ± 0.08% (mean ± SD)(P<0.001)), and the group with paradoxical pre-operative CVR demonstrated the greatest improvement (-0.04% ± 0.03% to 0.27% ± 0.03% (P=0.028)).

The third study arose from an unexpected observation: paradoxical reactivity in the white matter of young healthy subjects. I evaluated healthy subjects using BOLD CVR and ASL CVR, transformed all CVR maps into a common brain space, and generated composite maps of CVR. Composite maps confirmed regions of significant paradoxical
reactivity in the white matter. These regions may represent the physiological correlate of previously anatomically defined border-zones (watershed zones). The regions match the locations where elderly patients develop white matter rarefaction, so-called “leukoaraiosis.”
Acknowledgments

I am grateful to those who inspired and enabled my research training. In particular, I thank:

Dr. Walter Kucharczyk who discussed magnetic resonance imaging with me before I knew anything about MRI. He sparked my interest in developing a deeper understanding, and that led to everything else.

Drs. Walter Montanera and Walter Kucharczyk who encouraged me to pursue research training during my radiology residency. They created a path for me to move from clinical training into full-time graduate studies, and provided great advice along the way.

Drs. Alan Fox and Manohar Shroff who suggested that I extend my graduate studies into my neuroradiology fellowship, and then found ways to make it work.

Dr. Karel ter Brugge who warmly welcomed me into the neuroradiology group at the Toronto Western Hospital, provided unwavering administrative support, and taught me much about cerebrovascular disease.

Jay Han who spent many hours with me acquiring cerebrovascular reserve data, and for a period of time, kept me company driving to and from a course at McMaster University.
Julien Poublanc who introduced me to quantitative image analysis, and was always helpful when I got stuck.

Dr. Adrian Crawley who listened to problems, thought about them thoroughly, and returned with detailed analysis and suggestions.

Dr. Simon Graham who always provided superb advice, and particularly helpful critique of my thesis.

Anne Battisti, Jorn Fierstra, David MacLean, Olivia Pucci, and Kevin Sam, a bright and engaged group of graduate students who passed through the research lab at various points during my graduate work.

The Toronto Western Hospital MRI technologists, particularly Garry Detzler, David Johnstone, Eugen Hlasny, and Keith Ta who shared their wealth of experience.

Dr. Howard Mount for his administrative guidance at several points during my graduate studies.

Dr. Alan Moody for his mentorship and helpful feedback during my final program advisory committee meeting.
Dr. Howard Yonas for his thoughtful appraisal of my thesis.

Dr. James Duffin who was an all-star pinch hitter in the bottom of the ninth.

Drs. Frank Silver and Michael Tymianski who enthusiastically promoted this research endeavor and were always generous with their time and expertise.

Dr. Joseph Fisher for encouraging me to follow new ideas, for providing detailed and incisive comments on my manuscripts, and above all else, for his unbridled enthusiasm for the process of scientific discovery. He is an outstanding role model.

I cannot sufficiently thank my graduate supervisor, Dr. David Mikulis. He inspired my pursuit of a career in neuroradiology, and outside of my family, he is the person who has had the greatest influence on my life.

I thank the Mandell and Libman families for their support and encouragement. Without them, this would not have been possible. Maya and Simone, thank you for your patience when daddy needed some time to finish this thesis! I hope that my happiness with my career path inspires you to pursue your interests with passion. Caroline, you have been my closest advisor and supporter. I could not have found a better companion for my research journey or for the broader journey of life.
Contributions

Dr. Joseph Fisher invented the Respiract™, the device used to control arterial blood gas concentrations.

Jay Han contributed much time and expertise to achieving the gas manipulations during acquisition of cerebrovascular reserve data. Anne Battisti and Olivia Pucci contributed to this as well.

Julien Poublanc oversaw scanning of the healthy subjects in Experiment 1 of Chapter 3.

Dr. George Tomlinson provided advice on using a mixed model to calculate correlation between BOLD and ASL measurements when there are repeated measurements in each subject (Chapter t3).

Dr. James Duffin generated a LabVIEW simulation of a collateral flow circuit I had drawn, and I used his LabVIEW program to generate Figure 6.6.

Dr. Tilak Das contributed to the literature review for section 8.4
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<th>Description</th>
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<tbody>
<tr>
<td>A1</td>
<td>First segment of the anterior cerebral artery</td>
</tr>
<tr>
<td>A2</td>
<td>Second segment of the anterior cerebral artery</td>
</tr>
<tr>
<td>ACA</td>
<td>Anterior cerebral artery</td>
</tr>
<tr>
<td>ASL</td>
<td>Arterial spin labeling</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygen level-dependent</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<tr>
<td>CBV</td>
<td>Cerebral blood volume</td>
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<tr>
<td>CMRO₂</td>
<td>Cerebral metabolic rate of oxygen consumption</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CVR</td>
<td>Cerebrovascular reactivity</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>EC-IC bypass</td>
<td>Extracranial-intracranial bypass</td>
</tr>
<tr>
<td>FiCO₂</td>
<td>Inspiratory partial pressure of CO₂</td>
</tr>
<tr>
<td>FAIR</td>
<td>Flow-sensitive alternating inversion recovery</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid attenuated inversion recovery</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>mD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>MRA</td>
<td>Magnetic resonance angiography</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NF1</td>
<td>Neurofibromatosis type 1</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
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<tr>
<td>O2</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OEF</td>
<td>Oxygen extraction fraction</td>
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<tr>
<td>P</td>
<td>Pressure</td>
</tr>
<tr>
<td>PaO2</td>
<td>Arterial partial pressure of oxygen</td>
</tr>
<tr>
<td>PaCO2</td>
<td>Arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PETCO2</td>
<td>End-tidal partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PETO2</td>
<td>End-tidal partial pressure of oxygen</td>
</tr>
<tr>
<td>Q</td>
<td>Flow</td>
</tr>
<tr>
<td>R</td>
<td>Resistance</td>
</tr>
<tr>
<td>ROC curve</td>
<td>Receiver operating characteristic curve</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photo emission computed tomography</td>
</tr>
<tr>
<td>STA</td>
<td>Superficial temporal artery</td>
</tr>
<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient ischemic attack</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>Xe-CT</td>
<td>Stable-xenon inhalation computed tomography</td>
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Chapter 1 – Background

1.1 Cerebral Blood Flow

The human brain accounts for only 2% of total body mass, but it receives 15% of cardiac output and consumes 20% of total body energy when the body is in a resting state. (reviewed in Magistretti & Pellerin, 1996) This energy is predominantly used to maintain the intracellular-extracellular ion gradients that underlie neuronal action potentials and synaptic potentials, and is also used for the synthesis and transportation of cell constituents and neurotransmitters. (reviewed in Attwell et al., 2010) Brain cells primarily derive energy from adenosine triphosphate (ATP) generated by aerobic metabolism of glucose. As the brain has limited capacity to store energy substrates, it depends on a continuous inflow of oxygen in arterial blood. Total cerebral blood flow (CBF) in healthy young adults is approximately 50 ml per 100 mg brain tissue per minute. (Madsen, Holm, Herning, & Lassen, 1993) The brain can compensate for some reduction in CBF by increasing the amount of oxygen extracted per volume of arterial blood, but greater reduction in blood flow can lead to ischemia and infarction.

The body has several means of maintaining CBF within a narrow range. The most important of these are collateral pathways of blood supply to the brain, and cerebral autoregulation.
Collateral Pathways of Arterial Supply

Redundant or “collateral” pathways of arterial supply to the brain can compensate for narrowing or occlusion of one or more brain-supplying arteries in the head and neck. These pathways include extracranial-to-intracranial arterial communications, circle of Willis communications, and leptomeningeal arterial anastomoses. (reviewed in Liebeskind, 2003) The extracranial-to-intracranial communications are numerous and include, for example, flow from the external carotid artery into the superficial temporal artery, through the supraorbital artery, retrograde through the ophthalmic artery, and then into the supraclinoid segment of the internal carotid artery. The circle of Willis is a coalescence of four major brain-supplying arteries into a common structure that can redistribute blood flow between cerebral hemispheres or between the anterior and posterior circulation if there is narrowing of one of the supplying arteries. Anatomic variation in the circle of Willis is common, and in any particular individual, some communications may be hypoplastic or absent. (Waaijer et al., 2007) Leptomeningeal collaterals are small arteriolar end-to-end or end-to-side anastomoses between arteries on the surface of the brain, providing collateral flow both within and between large artery vascular territories. An example of the latter is the anastomoses between the pericallosal branches of the anterior cerebral arteries and the splenial branches of the posterior cerebral arteries. Leptomeningeal anastomoses may be anatomically present, but with reduction in cerebral perfusion pressure, compensatory flow through these collaterals likely requires days to weeks to reach its maximal state, related to gradual increase in luminal diameter. (Coyle & Heistad, 1991) In contrast with the substantial interconnections among the leptomeningeal arteries, the penetrating parenchymal arteries
of the brain are largely devoid of anastomoses. (N. Nishimura, Schaffer, Friedman, Lyden, & Kleinfeld, 2007)

**Blood Flow – Electrical Current Analogy**

Before proceeding to a discussion of cerebral autoregulation, it is instructive to consider a simple model of total CBF. If we imagine fluid flowing at a constant velocity with a laminar profile through a long cylindrical pipe, then flow between two points along the pipe is equal to the difference in pressure between the points, divided by flow resistance along that segment of pipe. This is analogous to Ohm’s law for electrical flow in which current is equal to voltage drop divided by resistance. In the absence of obstruction to cerebral venous outflow, intracranial venous backpressure is typically negligible, so total CBF reflects systemic arterial blood pressure divided by cerebral vascular resistance. (Panerai, 2009) As described in the Hagen-Poiseuille law, cerebral vascular resistance varies linearly with blood viscosity and vessel length, but with the fourth power of vessel radius. Thus, a change in vessel radius may have a marked effect on cerebral vascular resistance, and CBF.

**1.2 Autoregulation of Cerebral Blood Flow**

In 1902, Bayliss observed that a decrease in perfusion pressure elicited arterial dilatation in canine hind limbs. (Bayliss, 1902) Fog observed a similar dilatation in the leptomeningeal vessels of cats, thought this otherwise paradoxical vessel caliber change was involved in the regulation of CBF, and questioned the accepted view that the cerebral
circulation is passive to changes in pressure. (Fog, 1937) In 1959, Lassen established the term “autoregulation” to describe the process by which the brain alters arterial and arteriolar smooth muscle tone, to modulate cerebrovascular resistance, and thus maintain relatively constant CBF despite variation in cerebral perfusion pressure. (Lassen & Lassen, 1959) Consolidating previous studies of CBF during hypotension in humans, Lassen plotted a curve showing that CBF is constant over a wide range of system arterial blood pressures, with pressure below this range resulting in a decrease in flow. The upper blood pressure limit of autoregulation was subsequently documented in the literature by Skinhoj and Strandgaard. (Skinhoj & Strandgaard, 1973) The human autoregulatory curve has a lower limit of autoregulation at approximately 50-70 mm Hg mean arterial blood pressure and upper limit at approximately 150-160 mm Hg. These are not precise limits, but inflection points at which the flow-pressure relationship changes considerably. Furthermore, the CBF plateau is not quite flat, and may increase from 80% to 120% of baseline between these inflection points. (van Beek, Claassen, Rikkert, & Jansen, 2008; Banaji, Tachtsidis, Delpy, & Baigent, 2005; Rosenblum, 1995)

Throughout most of the body, large and small arteries are low-resistance conduit vessels and it is predominantly arterioles (particularly those less than 100 µm in diameter) that determine vascular resistance of an organ. However, this is not true for the brain. (Faraci & Heistad, 1990) Pressure measurements with micropipettes in different segments of the brain-supplying arterial tree in cats, dogs, and monkeys have shown that the internal carotid and vertebral arteries and intracranial leptomeningeal arteries account for approximately half of the total cerebral vascular resistance. (Faraci & Heistad, 1990;
Shapiro, Stromberg, Lee, & Wiederhielm, 1971) and alteration of muscular tone in these large and small brain-supplying arteries contributes significantly to the autoregulatory response. (Heistad, Marcus, & Abboud, 1978) Although less often discussed, autoregulation may also directly involve the capillaries. Pericytes are small cells that wrap around the endothelial cells of capillaries. Pericytes express contractile proteins, (reviewed in Attwell et al., 2010) and recent studies have shown that substances such as arachidonic acid derivatives and neurotransmitters can contract and relax pericytes, potentially altering capillary resistance. (Hamilton, Attwell, & Hall, 2010)

**Mechanism of Cerebral Autoregulation**

The mechanisms underlying cerebral autoregulation are incompletely understood. There are three main theories, which are not mutually exclusive, so-called *myogenic, metabolic*, and *neurogenic* hypotheses.

The myogenic hypothesis (Folkow, 1964) posits that arterial wall stretch is a link between arterial blood pressure and compensatory (autoregulatory) change in arterial smooth muscle tone. For example, a decrease in blood pressure results in reduced stretch of the arterial wall, triggering arterial smooth muscle relaxation, which will reduce vascular resistance and tend to maintain blood flow. The myogenic response has been demonstrated in arteries and arterioles denuded of endothelium. (Busija & Heistad, 1984) that is, it is truly *myogenic*, but endothelial factors and perivascular neuronal-glial factors may modify the response. For example, it is well known that the endothelium is sensitive to the properties of blood flow along its surface, and endothelium synthesizes several
vasoactive molecules (such as nitric oxide) that diffuse into the muscular layer. (Furchgott, 1983) Despite evidence of a myogenic response in vitro, the myogenic hypothesis fails to explain findings in vivo. For example, the myogenic hypothesis predicts that an increase in arterial pressure will elicit a vasoconstrictive response, yet in subjects with raised arterial blood pressure due to venous outflow obstruction, the arteries dilate rather than constrict. (McPherson, Koehler, & Traystman, 1988; Wei & Kontos, 1982)

The metabolic hypothesis (Kuschinsky & Wahl, 1978) suggests that active neurons generate a metabolic signal, for example a decrease in tissue partial pressure of oxygen or concentration of glucose or an increase in tissue partial pressure of carbon dioxide, which is detected by the vessels, resulting in a change in vascular smooth muscle tone. The observation that CBF locally increases in regions of increased neuronal activity supports this hypothesis. However, CBF may increase out of proportion to metabolic demands, may increase without significant change in local metabolism, and may increase much faster than the accumulation of metabolic end-products, and evidence supporting a particular metabolic signal is lacking. (Sandor, 1999)

Finally, the neurogenic hypothesis suggests that the nervous system is involved in controlling and mediating cerebral autoregulation. The brain-supplying arteries in the neck, at the skull base, and in the basal cisterns, and the leptomeningeal arteries contain a network of nerves within their adventitial layer. (Hamel, 2006) This network arises mainly from the superior cervical, sphenopalatine, otic, and trigeminal ganglia. (Hamel,
that is, the peripheral nervous system. Numerous studies have shown that autoregulation is preserved in sympathetically and parasympathetically denervated animals, (Busija & Heistad, 1984) suggesting that this pathway alone is not responsible for cerebral autoregulation. In contrast with the innervation of leptomeningeal arteries, vessels within the brain parenchyma do not contain an adventitial nerve network from the peripheral nervous system. While direct contact between parenchymal vessels and neurons is relatively minimal, central nervous system neurons are closely associated with astrocytes. Astrocytic end-foot processes envelope the parenchymal arterioles, (Cohen, Molinatti, & Hamel, 1997) and recent studies have shown that neurons may activate astrocytes to release vasoactive agents such as glutamate which alter arteriolar smooth muscle tone. (Attwell et al., 2010) This astrocyte and neurotransmitter mediated pathway likely has a major role in the regulation of CBF.

Exhaustion of Cerebral Autoregulation

Powers (1991) developed a framework to describe the successive changes in cerebral perfusion parameters that occur with progressive worsening of cerebral perfusion pressure. His framework consists of 3 stages. In stage 1, cerebral perfusion pressure (CPP) is within the range of cerebral autoregulation. Within this range, decreasing CPP is countered by compensatory cerebral vasodilatation that reduces cerebral vascular resistance, and maintains CBF near normal. Compensatory vasodilatation results in increased cerebral blood volume (CBV), so there is progressive rise in CBV as CPP decreases throughout this stage. Stage 2 begins when the capacity for compensatory vasodilatation is exhausted. Throughout stage 2, decreasing CPP is associated with
progressive reduction in CBF. This reduction in CBF prolongs the transit time of oxygen through the capillaries, yielding an increase in the amount of oxygen the brain extracts from a given volume blood (OEF) (Buxton & Frank, 1997) so cerebral oxygen metabolism (CMRO$_2$) is maintained near normal. The combination of reduced CBF and increased OEF has been called “misery-perfusion” as increased OEF indicates that perfusion is inadequate relative to the oxygen demands of the tissues (Baron et al., 1981). Stage 3 begins when capillary transit time is sufficiently prolonged that there is equilibration between capillary pO$_2$ and tissue oxygen concentration, that is, the capacity to increase OEF is exhausted. Throughout stage 3, decreasing CPP is associated with progressive reduction in CMRO$_2$, and ischemia or infarction may occur. There is inadequate data to conclude whether the state of maximal CBV is maintained throughout all of stages 2 and 3, and whether the state of maximal OEF is maintained throughout all of stage 3.

These sequential stages are a framework for understanding pathophysiology, but the boundaries between the stages are not truly discreet. For example, CBF falls slightly as CPP decreases throughout the autoregulatory range, leading to a mild increase in OEF prior to exhaustion of autoregulatory capacity (Derdeyn et al., 2002). As well, once ischemic injury has occurred, the normal mechanisms of cerebrovascular control may no longer operate, even when CPP has returned to normal, so the relationships between CBF, CBV, OEF, and CMRO$_2$ may remain abnormal. For example, acutely infarcted brain parenchyma demonstrates a decrease in CBF and a reduction rather than increase in CBV.
1.3 Measuring Cerebrovascular Reactivity

I have discussed two compensatory responses to reduced cerebral perfusion pressure: cerebral autoregulation and increase in oxygen extraction fraction. Both of these responses can be measured:

The best-validated technique for performing regional measurements of OEF uses oxygen-15 (O-15) positron emission tomography (PET). Limited availability, high cost, and the requirement for an on-site cyclotron to produce the very short-lived radiopharmaceutical O-15 (half-life 2 minutes) has thus far limited clinical translation of this technique. As well, quantitative measurement of OEF is possible, but few PET facilities are equipped to do this,(Carlson, Yonas, Chang, & Nemoto, 2011) and arterial catheterization is also required. Thus, OEF is typically calculated as a right-left hemispheric ratio, which limits applicability in patients with bilateral disease.

One may measure cerebral autoregulatory reserve directly by inducing a transient decrease in systemic arterial blood pressure while measuring the cerebral blood flow response. However, it is technically challenging to induce accurate changes in systemic arterial blood pressure in vivo, and it is potentially unsafe. Autoregulatory vasodilatation in response to a reduction in CPP impairs the ability of the brain-supplying arteries to respond to other vasodilatory stimuli such as inhaled carbon dioxide.(Harper & Glass, 1965) Thus, one may indirectly evaluate autoregulatory capacity by measuring the change in cerebral blood flow per unit of applied vasodilatory stimulus, that is, “cerebrovascular reactivity” (CVR).
Vasodilatory Stimuli

Commonly used cerebral vasodilatory stimuli are intravenous injection of acetazolamide (Diamox) and hypercapnia induced by breath-holding or inhalation of carbon dioxide (CO₂).

The mechanism underlying acetazolamide-induced vasodilatation is unclear. Acetazolamide is a carbonic anhydrase inhibitor, and one possibility is that the drug results in carbonic acidosis, with decreased pH triggering arterial smooth muscle dilatation. However, it is also plausible that the drug exerts a more direct effect on arterial smooth muscle. Acetazolamide is typically administered as an intravenous bolus, with a standard dose of 1g. Maximal augmentation of cerebral blood flow occurs 10-15 minutes after injection, with flow increasing 30-60% in healthy subjects. Systemic blood pressure, arterial CO₂, and CMRO₂ are unaffected. (Vagal, Leach, Fernandez-Ulloa, & Zuccarello, 2009) Acetazolamide is widely available and easily administered. A limitation is that variability in pharmacokinetics (and pharmacodynamics) within subjects over time, and between subjects, results in unmeasured variation in magnitude of the vasodilatory stimulus. With a higher dose of acetazolamide, it may be possible to maximally augment cerebral blood flow in most subjects and overcome this limitation, but increased frequency of adverse events at higher doses limits clinical applicability. (Grossmann & Koeberle, 2000) Even at standard dosing, a prospective study found that 63% of patients developed transient symptoms (such as extremity numbness or motor weakness, generalized malaise, headache, nausea), lasting a mean of 7.9 hours, related to intravenous administration of acetazolamide. (Saito et al., 2011)
The mechanism of carbon dioxide-induced vasodilatation is also unclear. (Brian, 1998) It is well known that carbon dioxide triggers vasodilatation in vitro. (You et al., 1994) indicating that extravascular substances are not required, but the specific target of CO₂ within the vessel wall is not known. Kontos et al. (Kontos, Raper, & Patterson, 1977) applied artificial cerebrospinal fluid topically to the cerebral cortex of cats, and found that the diameter of cerebral arterioles responded to changes in pH, regardless of fluid partial pressure of CO₂, suggesting that pH may play an intermediary role. Experiments in humans have shown that CMRO₂ is stable across the range of CO₂ values relevant for measurement of CVR. (Chen & Pike, 2010) However, CO₂ has a positive inotropic effect on the heart, and most studies using hypercapnia to measure CVR have found that hypercapnia induces a mild increase in systemic arterial blood pressure. This increase in blood pressure is in the order of 7-12 mm Hg. (Markwalder, Grolimund, Seiler, Roth, & Aaslid, 1984; Norrving, Nilsson, & Risberg, 1982; Tominaga, Strandgaard, Uemura, Ito, & Kutsuzawa, 1976; Widder, Paulat, Hackspacher, & Mayr, 1986) A more systemic study of the confounding effect of blood pressure found that 83% of hypercapnia-induced augmentation of CBF was directly attributable to the increase in CO₂ and the other 17% was due to the associated increase in blood pressure. (Yamamoto, Meyer, Sakai, & Yamaguchi, 1980) Induction of hypercapnia through breath holding yields a constantly changing blood CO₂ level making it difficult to quantify the magnitude of vasodilatory stimulus. I have used a re-breathing device called the RespirAct™. (Slessarev et al., 2007) This device is capable of controlling end-tidal CO₂ and O₂ precisely and independently, with transitions between a stable baseline state and stable hypercapnic state occurring within three breaths. (Slessarev et al., 2007) End-tidal
PCO₂ measured with this device accurately reflects arterial PCO₂ measured from arterial blood sampling. (Ito et al., 2008)

**Imaging Techniques**

The most commonly used techniques for measuring vasodilator-induced changes in cerebral blood flow have been transcranial Doppler ultrasound (TCD), single photon emission computed tomography (SPECT), and stable-xenon inhalation computed tomography (Xe-CT).

The TCD approach relies on measurement of blood velocity in the middle cerebral artery (or other large intracranial artery) as a surrogate for blood flow. It is unique among the CVR imaging techniques in that it is relatively quick and inexpensive, non-invasive, and free of ionizing radiation. A major limitation is that TCD yields only a single measurement of CVR for the middle cerebral artery territory on each side rather than a spatial map of CVR for the brain. This measurement does not include flow which reaches the MCA territory via leptomeningeal collateral supply. As well, regions of reduced CVR due to prior ischemic injury will be averaged together with regions of reduced CVR in tissue that is intact but at hemodynamic risk, potentially confounding the results. Similarly regions of impaired CVR may be missed when averaged together with regions that are more normally perfused. There are several other technical limitations particular to TCD, for example, derivation of CBF from TCD measurement of velocity assumes that changes in the diameter of the insonated vessel with changes in CBF are negligible, but there is evidence to the contrary. (Lunt, Jenkinson, & Kerr, 2000)
SPECT, using radiopharmaceuticals such as $^{99m}$Tc-HMPAO, $^{99m}$Tc-ECD, and $^{123}$I-IMP, has had the most widespread use for spatial mapping of CVR. This approach is based on the principle that these radiopharmaceuticals are extracted by the brain in proportion to local CBF. A disadvantage of SPECT is that the pre- and post-vasodilatory stimulus scans are typically performed on different days to allow clearing of radioactivity. It is possible to perform the study over a single day, but this requires much higher activity for the second scan, increasing radiation dose to the patient. (Vagal et al., 2009)

Xe-CT relies on stable xenon, a radiopaque diffusible gas that accumulates in tissues based on blood flow. The technique is technically robust with high accuracy and reproducibility, but it is not widely available, and inhalation of stable xenon can have undesirable side effects such as a decrease in respiratory rate, headache, nausea, vomiting, and convulsions. (Pindzola, Balzer, Nemoto, Goldstein, & Yonas, 2001)

More recently, several groups have used dynamic contrast bolus MRI (Guckel et al., 1996; Ma et al., 2007; Schreiber et al., 1998) and CT (Rim, Kim, Shin, & Kim, 2008) to map CVR. These techniques use MRI or CT to track the passage of an intravascular non-diffusible tracer through the cerebral circulation, with indicator-dilution theory and a mathematical deconvolution operation used to derive maps of CBF. These techniques are widely available. A limitation of the CT technique is the ionizing radiation from two (pre and post vasodilator) perfusion scans. A disadvantage of the MR technique is a more complex relationship between the tracer (typically, a gadolinium chelate) and MR signal intensity. Both methods require more sophisticated post-processing than TCD, SPECT, or
Xe-CT, and standardized methods of post-processing are lacking.

Another MR technique for measuring CVR is based on arterial spin labeling (ASL). In its most basic form, arterial spin labeling (ASL) MRI uses radiofrequency pulses to magnetically label water protons that then flow into a selected imaging slice in the brain. These labeled protons act as an endogenous contrast agent causing an MRI signal change that is directly proportional to CBF. There are many technical variations on this basic approach. Advantages include lack of ionizing radiation, no need for intravenous injection, and ability to obtain anatomical MRI and MRA in the same imaging session. A disadvantage of ASL is the relatively low signal-noise ratio. Another disadvantage is that the degree of proton labeling (labeling efficiency) depends on flow velocity, so measurements of flow are confounded by changes in flow. ASL MRI CVR in a cerebrovascular disease population has been demonstrated (Arbab et al., 2002; Detre et al., 1999) but further validation is needed prior to widespread clinical implementation.

**BOLD MR Mapping of CVR**

Blood oxygen level-dependent (BOLD) MR refers to a MRI pulse sequence that is sensitive to the concentration of deoxyhemoglobin in blood. Arterial blood is normally 95-100% saturated with oxygen, and venous blood is normally 60-70% saturated (Gibbs, Lennox, Nims, & Gibbs, 1942) An increase in CBF, during stable CMRO₂, results in dilution or “wash-out” of deoxyhemoglobin in the microcirculation. Deoxyhemoglobin is a paramagnetic substance, that is, in the presence of an external magnetic field, the molecule tends to orient itself parallel to the applied field, increasing the local magnetic
field. Paramagnetic substances constrained by boundaries such as red blood cell membranes or blood vessel walls yield magnetic field inhomogeneities that result in loss of phase coherence and therefore a reduction in MR signal. Therefore, an increase in CBF elicits a decrease in deoxyhemoglobin concentration in the microcirculation, yielding an increase in MR signal. Through this mechanism, it is possible to measure change in CBF indirectly. The relationship has been modeled using the following equation:

\[
\frac{\Delta \text{BOLD}}{\text{BOLD}_0} \approx M \cdot [\text{dHb}]_v^\beta \left(1 - \left(\frac{\text{CBV}}{\text{CBV}_0}\right)\left(\frac{[\text{dHb}]_v}{[\text{dHb}]_{v0}}\right)^\beta\right)
\]

where “0” denotes the baseline state, \([\text{dHb}]_v\) is the concentration of deoxyhemoglobin in venous blood, \(\beta\) is a constant, and “M” is the fractional BOLD signal attenuation attributable to the presence of dHb at baseline. M, and therefore reflects the hypothetical maximum BOLD response that could occur. M is determined by both MR scanner factors such as magnetic field strength and echo time, and patient factors such as baseline CBV and baseline concentration of deoxyhemoglobin in venous blood. CBF is reflected in the \([\text{dHb}]_v\) terms.

The BOLD MR technique shares many of the advantages of ASL MR: wide availability of the MR pulse sequence, no requirement for intravenous injection, no ionizing radiation, and the ability to acquire routine MR imaging and MR angiography in the same imaging session. In addition, the technique is particularly well suited to the use of a \(\text{CO}_2\) vasodilatory stimulus: unlike the sustained effect of acetazolamide, arterial partial pressure of \(\text{CO}_2\) is rapidly responsive to changes in the inhaled concentration of \(\text{CO}_2\). The
BOLD MR pulse sequence, which is based on rapid echoplanar imaging, can acquire a complete data set covering the entire brain in 2 seconds. This is repeated several hundred times over the course of several minutes in order to provide statistical power for the data analysis, while correcting for any baseline drift in CBF or MR signal. The BOLD MR sequence also has higher signal-noise ratio than ASL MR.

Figure 1.1 shows P\textsubscript{ET}CO\textsubscript{2} (in green) and whole brain BOLD MR signal (in yellow) as a function of time for a normal subject. Notice how each increase in P\textsubscript{ET}CO\textsubscript{2} is accompanied by a corresponding increase in BOLD MR signal. The BOLD MRI signal time course is then regressed (least squares) against the P\textsubscript{ET}CO\textsubscript{2} waveform on a voxel-by-voxel basis. The slope of the regression, expressed as $\%\Delta$BOLD MR signal per mm Hg $\Delta$P\textsubscript{ET}CO\textsubscript{2}, is CVR. The CVR maps are color-coded using a two-color continuous spectrum ranging from red for positive CVR, to blue for negative CVR, and overlaid on anatomical images in Figures 1.2 and 1.3.

Figure 1.1  P\textsubscript{ET}CO\textsubscript{2} (in green) and whole brain BOLD MR signal (in yellow) as a function of time for a normal subject. This was an 8 minute 20 second CVR acquisition.
Figure 1.2  BOLD CO$_2$ CVR map (%ΔBOLD MR signal per mm Hg ΔPETCO$_2$) overlaid on T1-weighted anatomical images (left) and a magnified view of a single slice (right). There is reduced CVR in the left MCA territory in this man with severe stenosis of the left middle cerebral artery.

Figure 1.3  BOLD CO$_2$ CVR map (%ΔBOLD MR signal per mm Hg ΔPETCO$_2$) overlaid on T1-weighted anatomical images in a man with severe stenosis at the termination of the left internal carotid artery. There is negative reactivity throughout the left cerebral hemisphere. The patient had a fetal-type left posterior cerebral artery (not shown), explaining why the negative reactivity extends into the left posterior cerebral artery territory.
1.4 Intracranial Steal Phenomena

The Meaning of “Steal”

The anatomist and physician William Hunter may have been the first to describe vascular steal. In 1764, describing an arteriovenous fistula which developed following antecubital phlebotomy, Hunter wrote that “the artery... will become larger in the arm... [but] smaller at the wrist, than it was in the natural state.” (Sekhon & Morgan, 2002) An arteriovenous fistula provides a low resistance path, and blood will preferentially flow from the artery through the fistula into the vein, at the expense of flow further along the artery in the forearm. Nearly two hundred years later, from catheter angiography of arteriovenous malformations, this phenomenon was described in the brain. (Norlen, 1949)

In 1961, Contorni reported that proximal stenosis of the subclavian artery can result in retrograde flow in the ipsilateral vertebral artery. (Toole & McGraw, 1975) This concept of “subclavian steal” was popularized by a case series published the following year in the New England Journal of Medicine. (Reivich, Holling, Roberts, & Toole, 1961) The authors described two patients with ischemic brain symptoms and proximal stenosis of the left subclavian artery, with reversed flow in the ipsilateral vertebral artery supplying the subclavian artery beyond the stenosis. This artery-to-artery shunt, with blood at the cranial termination of the right vertebral artery flowing retrograde down the left vertebral artery rather than up into the basilar artery, is analogous to the arteriovenous fistula described by Hunter, with one major difference: in the subclavian steal syndrome, one territory benefits at the expense of another, whereas Hunter’s description lacks a territory
that explicitly benefits. Several other evocative terms were applied to the subclavian steal syndrome such as “the Robinhood syndrome” (Lassen & Palvolgyi, 1968) and “vertebral grand larceny.” (Daves & Treger, 1964)

Whereas an arteriovenous shunt bypasses the capillary bed, an artery-to-artery connection has a capillary bed distal to the connection. The latter arrangement adds complexity as the capillary bed has the capacity to alter its vascular resistance and influence the degree of flow across the artery-to-artery connection. For example, in a patient with subclavian steal, active exercise of the ipsilateral upper extremity will trigger vasodilatation in the upper extremity and greater steal of blood flow from the brain. It is this latter (dynamic) steal phenomenon that Symon (Symon, 1969) is discussing in a 1969 review paper that defines steal as the “reduction of blood flow within an area of already imperfectly perfused brain, [due to]... the influence of vasodilatation in neighboring zones.” This vasodilatation may be physiological (as with upper extremity exercise) or induced pharmacologically using cerebral vasodilatory agents such as inhaled carbon dioxide or intravenously injected acetazolamide (Diamox). Indeed, pharmacological vasodilator-induced steal has been demonstrated with several different imaging techniques, including positron emission tomography (PET), (Nariai et al., 1998; S. Nishimura et al., 1999) single photon emission computed tomography (SPECT), (Kawaguchi, Sakaki, & Uranishi, 1999; Shiino et al., 2003) stable-xenon computed tomography (Xe-CT) (Smith, Thompson-Dobkin, Yonas, & Flint, 1994; Webster et al., 1995), and transcranial Doppler ultrasound (TCD). (Baumgartner & Baumgartner, 1998; Ringelstein, Sievers, Ecker, Schneider, & Otis, 1988)
1.5 Clinical Implications of Reduced and Paradoxical Cerebrovascular Reactivity in Patients with Stenosis of Brain-Supplying Arteries

In a healthy subject, a cerebral vasodilator induces an increase in total CBF.(Grubb et al., 1974; Yamauchi, Okazawa, Kishibe, Sugimoto, & Takahashi, 2003) In patients with stenosis of brain-supplying arteries and insufficient collateral supply, CBF augmentation is reduced in magnitude or even absent in the brain territory distal to the stenosis.(Ringelstein et al., 1988) In patients with greater stenosis and or even less collateral supply, CBF augmentation is often not only reduced or absent, but a vasodilatory stimulus actually induces a decrease in CBF in the brain territory distal to the stenosis. This paradoxical response is attributed to the steal phenomenon described in the previous section:(Nariai et al., 1998) If there is relatively preserved CVR in one region of brain, and exhausted CVR in a second region, vasodilatation in the first region results in increased flow to that first region at the expense of flow to the second region. Consider a patient with severe stenosis of the right middle cerebral artery (MCA), and a normal right internal carotid artery (ICA) and right anterior cerebral artery (ACA). A vasodilatory stimulus will result in dilatation of the arterial vessels of the right ACA territory, and a corresponding decrease in vascular resistance in this territory. The right MCA territory arterial vessels are already maximally dilated at rest, and will not dilate any further. Following application of a vasodilatory stimulus, the decrease in vascular resistance in the ACA territory relative to the MCA territory results in redistribution of blood flow from the MCA to the ACA. In this way, the ACA territory steals from the MCA territory.
Impairment of CVR is a strong and independent predictor of future ischemic events in patients with stenosis or occlusion of brain-supplying arteries, and paradoxical CVR indicates particularly severe impairment. (Webster et al., 1995; Yonas, Smith, Durham, Pentheny, & Johnson, 1993) This has been shown in both asymptomatic and symptomatic populations. For example, Silvestrini et al. (Silvestrini et al., 2000) studied 94 patients with asymptomatic severe (>70%) carotid artery stenosis. Patients were categorized as having normal versus impaired CVR using hypercapnia and TCD, and were followed prospectively for a median of 2.4 years. The annual rate of ipsilateral ischemic events was 4% in patients with normal CVR at entry, compared with 14% in patients with impaired CVR. Ogasawara et al. (Ogasawara, Ogawa, & Yoshimoto, 2002) studied 70 patients with symptomatic unilateral ICA or MCA occlusion. Patients were categorized as having normal versus impaired CVR using acetazolamide and Xe-133 SPECT, and were followed prospectively for 2 years. Recurrent stroke rate was 6% in patients with normal CVR at entry, compared with 35% in patients with impaired CVR (significant difference, \( P=0.03 \)). Markus et al. (Markus & Cullinane, 2001) studied 107 patients with symptomatic carotid artery stenosis or carotid occlusion. Patients were categorized as having normal versus impaired CVR using \( \text{CO}_2 \) and TCD, and were prospectively followed for a mean of 4.4 years. Controlling for patient demographics, vascular risk factors, prior infarct, and degree of stenosis, impaired CVR was an independent predictor of ipsilateral TIA or stroke (odds ratio 14.4, 95% CI 2.63 – 78.74, \( P = 0.002 \)). From these studies, and others with similar results, (Blaser et al., 2002; Kleiser, Widder, Kleiser, & Widder, 1992) it is well known that CVR can identify a subgroup of steno-occlusive disease patients at higher risk of stroke.
Chapter 2 – Aims and Hypotheses

2.1 Overview and Aims

This thesis was precipitated by a question from Dr. David Mikulis as to whether negative cerebrovascular reactivity (CVR) on BOLD MR maps of CVR truly represents vasodilator-induced reduction of cerebral blood flow, that is, a steal phenomenon. The concern was that BOLD MR signal depends on CBF, but also on other factors such as CBV, CMRO$_2$, PaO$_2$, and hematocrit. There was empirical evidence that, in healthy subjects, the BOLD MR signal response to changes in PETCO$_2$ is dominated by CBF effects,(Shiino et al., 2003; Ziyeh et al., 2005) but limited literature on this relationship in patients with cerebrovascular disease. The question is important from a clinical perspective as studies using other imaging techniques have demonstrated that steal phenomenon has prognostic and therapeutic implications. I decided to broaden the initial question and investigate whether cerebral hemispheric BOLD MR CVR expressed as absolute an value (%ΔBOLD MR signal intensity per mm Hg ΔPETCO$_2$) would correlate with CVR measured using arterial spin labelling (ASL), a more direct measure of CBF, in patients with stenosis or occlusion of brain-supplying arteries. The steal phenomenon question was retained as a sub-question within the larger study. This initial work gave rise to two additional studies, which I will now summarize.

In the BOLD-ASL comparison study, I measured cerebral hemispheric CVR in units of
%ΔBOLD MR signal intensity per mm Hg Δ PETCO₂. This approach differs from that of most other groups who used the contralateral hemisphere as an internal standard, and reported right-left interhemispheric asymmetry indices. I avoided using the contralateral hemisphere as a reference, as many patients with cerebrovascular disease have bilateral involvement. After demonstrating the validity of this absolute measure in a cerebrovascular patient population, I sought to begin determining its clinical utility. This led to the second study in this thesis, an investigation to determine whether preoperative cerebral hemispheric CVR values would predict the hemodynamic effect of surgical revascularization using an extracranial-intracranial (EC-IC) bypass procedure.

The third study in this thesis arose from a detail I noticed early in the BOLD-ASL comparison project. When reviewing BOLD CVR maps, we had been concentrating on CVR in the cortex and deep grey matter, with the much noisier CVR signal in the white matter filtered out of the final CVR maps. However, reviewing the unfiltered CVR maps in a group of healthy subjects, I noticed that there was a noisy but consistent pattern of paradoxical CVR bilaterally in the periventricular and deep white matter. The spatial distribution of this paradoxical CVR seemed to match those regions of the brain where people develop age-related white matter changes (leukoaraiosis). I conducted a study to define the spatial extent of these zones.

2.2 Hypotheses

1. In patients with stenosis or occlusion of brain-supply arteries, the BOLD MR signal response to hypercapnia is directly related to changes in CBF as measured
using ASL MR.

2. Preoperative cerebral hemispheric CO₂ BOLD CVR, expressed as %ΔBOLD MR signal intensity per mm Hg Δ PETCO₂) predicts the hemodynamic effect of surgical bypass in patients with intracranial arterial stenosis or occlusion.

3. There are regions of negative cerebrovascular CVR in the white matter of young healthy subjects.
Chapter 3 – Mapping Cerebrovascular Reactivity Using BOLD MRI in Patients with Arterial Steno-Occlusive Disease: Validation using Arterial Spin Labeling

This chapter is adapted with permission from the following publication:


3.1 Abstract

Blood oxygen level-dependent MRI (BOLD MRI) of hypercapnia-induced changes in cerebral blood flow is an emerging technique for mapping cerebrovascular reactivity (CVR). BOLD MRI signal reflects cerebral blood flow, but also depends on cerebral blood volume, cerebral metabolic rate, arterial oxygenation, and hematocrit. The purpose of this study was to determine whether, in patients with stenosis or occlusion of brain-supplying arteries, the BOLD MRI signal response to hypercapnia is directly related to changes in cerebral blood flow. Thirty-eight patients with steno-occlusive disease
underwent mapping of CVR by both BOLD MRI and arterial spin labeling MRI. The latter technique was used as a reference standard for measurement of cerebral blood flow changes. Hemispheric CVR measured by BOLD MRI was significantly correlated with that measured by arterial spin labeling MRI for both gray matter ($R=0.83$, $P<0.0001$) and white matter ($R=0.80$, $P<0.0001$). Diagnostic accuracy (area under receiver operating characteristic curve) for BOLD MRI discrimination between normal and abnormal hemispheric CVR was 0.90 (95% CI=0.81 to 0.98; $P<0.001$) for gray matter and 0.82 (95% CI=0.70 to 0.94; $P<0.001$) for white matter. Regions of paradoxical CVR on BOLD MRI had a moderate predictive value (14 of 19 hemispheres) for spatially corresponding paradoxical CVR on arterial spin labeling MRI. Complete absence of paradoxical CVR on BOLD MRI had a high predictive value (31 of 31 hemispheres) for corresponding non-paradoxical CVR on arterial spin labeling MRI. Arterial spin labeling MRI confirms that, even in patients with arterial steno-occlusive disease, the BOLD MRI signal response to hypercapnia predominantly reflects changes in cerebral blood flow.

3.2 Introduction

An emerging technique for mapping CVR is blood oxygen level-dependent MRI (BOLD MRI) (Ogawa, Lee, Kay, & Tank, 1990) of changes in cerebral blood flow (CBF) during controlled oscillation of end-tidal partial pressure of carbon dioxide (P\text{ETCO}_2). (Rostrup et al., 1994) This method is clinically attractive because it uses a pulse sequence routinely available on MR scanners, maps the entire brain with high spatial resolution, requires less than 10 minutes to perform, and generates quantitative results rather than simply an interhemispheric difference. The major concern (Eskey & Sanelli, 2005; Zaharchuk et al.,
1999) with this technique has been that BOLD MRI signal depends on CBF, but also on other factors: cerebral blood volume (CBV), cerebral metabolic rate of oxygen consumption, arterial partial pressure of oxygen (P$aO_2$), and hematocrit. (Ogawa et al., 1993) There is empirical evidence that, in healthy subjects, the BOLD MRI signal response to changes in PET$CO_2$ is dominated by CBF effects, (Shiino et al., 2003; Ziyeh et al., 2005) but there is limited literature on this relationship in patients with cerebrovascular disease.

The purpose of this study was to determine whether, in patients with stenosis or occlusion of the brain supplying arteries, the BOLD MRI signal response to hypercapnia is directly related to changes in CBF. I used arterial spin labeling (ASL) MRI as a reference standard for measurement of CBF changes. In its most basic form, ASL MRI uses radiofrequency pulses to magnetically label the water protons in blood flowing into the imaging plane in the brain. These modified protons then act as an endogenous contrast agent causing an MRI signal change that is proportional to CBF. Notably, ASL MRI has a much weaker dependence on factors such as CBV, cerebral metabolic rate of oxygen consumption, P$aO_2$, and hematocrit. (Hoge et al., 1999; Petersen et al., 2006) Also, ASL MRI offers sufficient temporal resolution to allow direct comparison with the echoplanar imaging-based BOLD MRI protocol.

### 3.3 Methods

#### Patients

The local ethics review board approved the study protocol. A total of 38 patients with
steno-occlusive cerebrovascular disease, referred from the neurology and neurosurgery services at the Toronto Western Hospital, were recruited into this study. All patients provided written informed consent.

Vasodilatory Stimuli

Patients were fitted with a sequential gas delivery mask (Hi-Ox-80; Viasys HealthCare, Yorba Linda, California) with a re-breathing bag on the expiratory limb. To provide an airtight seal, the mask was taped to the face (3 mol/L; Tegaderm, St. Paul, Minn). Gas flow to the mask was controlled by a gas blender (RespirAct; Thornhill Research, Toronto, Canada) programmed to provide the flow and blend of O₂, N₂, and CO₂ needed to attain the target PETCO₂ and PETO₂. The gas sequence is described in Table 3.1. Tidal pCO₂ and pO₂ were monitored continuously (RespirAct), digitized, and recorded (LabView; National Instruments Corporation, Austin, Texas). The apparatus and technique are described in greater detail elsewhere.(Slessarev et al., 2007)
Table 3.1  PETCO₂ and PETO₂ Targets

<table>
<thead>
<tr>
<th>Stage</th>
<th>Target PETCO₂ mm Hg</th>
<th>Target PETO₂ mm Hg</th>
<th>Duration Seconds</th>
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MRI

MRI was performed on a 3.0-Tesla scanner (Signa; GE Healthcare, Milwaukee, WI) with an 8-channel phased array head coil. T1-weighted anatomic images were acquired using a 3-dimensional spoiled gradient echo pulse sequence (whole brain coverage; matrix: 256x256; slice thickness: 2.2 mm; no inter-slice gap). BOLD MRI data were acquired with a T2* -weighted single-shot gradient echo pulse sequence with echoplanar readout (field of view: 24x24 cm; matrix: 64x64; TR: 2000 ms; TE: 30 ms; flip angle: 85°; slice thickness: 5.0 mm; inter-slice gap: 2.0 mm, number of frames: 254). ASL MRI data were then acquired using a 2-dimensional spin-echo flow-sensitive alternating inversion recovery (FAIR) sequence (Kim, 1995) with echoplanar readout (field of view: 24x24 cm; matrix: 64x64; TR: 2000 ms; TE: 22.7 ms; TI: 1000 ms; flip angle: 85°; slice thickness: 5.0 mm; inter-slice gap: 2.0 mm, number of frames: 254). The FAIR sequence applies alternating slice-selective and nonselective 180° pulses to acquire alternating flow-
encoded and non-flow-encoded images. The FAIR sequence has limited z-axis spatial coverage. I acquired 5 axial ASL MRI slices with the middle slice centered on the bodies of the lateral ventricles. The BOLD MRI and ASL MRI acquisitions were prescribed with matching slice locations.

In addition to the CVR data acquired for this study, all patients had previously undergone routine clinical imaging of the neck vessels and circle of Willis. This was by catheter angiography (10 of 25), MR angiography (9 of 25), CT angiography (4 of 25), or Doppler ultrasound (2 of 25).

**Data Processing**

$P_{ETCO_2}$ and $P_{ETO_2}$ values were selected automatically (LabView) from the continuous $p_{CO_2}$ and $p_{O_2}$ waveforms as the highest and lowest values, respectively, during exhalation. All values were confirmed by visual inspection. MRI and $P_{ETCO_2}$ data were then imported into the software AFNI (Analysis of Functional NeuroImages, NIH, Bethesda, USA; http://afni.nimh.nih.gov/afni). (Cox, 1996) A plot of head position versus time for each ASL MRI data set was used to exclude those patients (13 of 38) with head displacement of one half voxel width or greater along any of 3 orthogonal axes. Each patient’s BOLD MRI data set was temporally shifted to the point of maximum statistical correlation with the patient’s $P_{ETCO_2}$ waveform. The BOLD MRI signal time course underwent least squares fitting to the $P_{ETCO_2}$ waveform on a voxel-by-voxel basis, and 2 parameters were generated: $BOLD_\Delta=\Delta BOLD$ MRI signal per $\Delta P_{ETCO_2}$ and $BOLD_{Baseline}$. Anatomic images were manually segmented into right and left hemispheres and then automatically segmented into gray matter and white matter (SPM5; Wellcome
Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK). These anatomic masks were used to segment the BOLD and ASL MRI data sets. \( BOLD_\Delta \) and \( BOLD_{Baseline} \) were summed for each segment, and CVR was calculated for each segment as \( (100 \times \text{total } BOLD_\Delta) / \text{total } BOLD_{Baseline} \). Analysis of the ASL MRI data was identical with one additional step: for each pair of slice-selective and non-slice-selective images, the signal difference between the FAIR MRI images was calculated on a voxel-wise basis, and it was the resulting difference map that was then fit to the PETCO\(_2\) waveform to calculate CVR. (Kim, 1995)

**Statistical Analysis**

I generated a scatterplot of hemispheric CVR measured using BOLD MRI versus ASL MRI. To quantify the correlation between the BOLD and ASL measurements, while accounting for repeated (2) measurements on each subject, we used a random effects model. First, we fitted a model with only a random intercept for each subject and then fitted a model that also included a fixed effect for ASL. Using the residual variances from these 2 models, we computed a pseudo-\( R^2 \), which represents the proportional reduction in residual variance in BOLD that can be explained by ASL.

To establish a threshold for differentiating between normal and abnormal on the reference standard (ASL MRI), I calculated mean hemispheric CVR on ASL MRI across all hemispheres with ipsilateral normal angiography, and defined abnormal as 2 or more standard deviations below the mean. Using this threshold and statistical software (SPSS for Windows, Version 15.0. Chicago, SPSS Inc.) I generated receiver operating
characteristic curves for BOLD MRI measurement of hemispheric CVR in the gray matter and white matter.

Because paradoxical CVR (hypercapnia-induced decrease in CBF) has particularly strong prognostic implications,(Webster et al., 1995; Yonas et al., 1993) I sought to determine whether regions of paradoxical CVR on BOLD MRI correspond with paradoxical CVR on ASL MRI. Due to image noise, there are inevitably a small number of voxels with paradoxical CVR even in normal subjects. A threshold was therefore established to differentiate a small number of paradoxical voxels due to image noise from truly paradoxical CVR. For all hemispheres with ipsilateral normal angiography, I counted the number of paradoxical voxels in each hemisphere on BOLD MRI as a proportion of the total number of voxels in the hemisphere, and calculate the mean and standard deviation across all of these hemispheres for this “proportion” parameter. I defined a threshold for “significant paradoxical CVR” as 2 or more standard deviations above this mean. Mean CVR on ASL MRI was calculated for the region corresponding with paradoxical CVR on BOLD MRI for each hemisphere. Similarly, mean CVR on ASL MRI was calculated for the region corresponding with non-paradoxical CVR on BOLD MRI. In addition, differences in the spatial extent of paradoxical CVR on BOLD MRI versus ASL MRI were assessed by performing a paired t test of the proportion of voxels with a paradoxical response in each hemisphere for the 2 techniques.

3.4 Results

There were 15 women and 10 men. Median age was 41 years (interquartile range, 24
years). Patient demographics and diagnoses are listed in Table 3.2. There were no study-related adverse events.

### Table 3.2    Patient Characteristics and Results

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, years</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Angiographic Findings</th>
<th>Cerebrovascular Reactivity</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grey Matter</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>BOLD*</td>
</tr>
<tr>
<td>1</td>
<td>76</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Bilateral ICA stenosis</td>
<td>R 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.17</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Right ICA occlusion</td>
<td>R 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.28</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>M</td>
<td>Moyamoya phenomenon</td>
<td>Bilateral ICA bifurcation stenosis</td>
<td>R 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(sickle cell disease)</td>
<td></td>
<td>L 0.23</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>F</td>
<td>Moyamoya disease</td>
<td>Bilateral ICA bifurcation stenosis</td>
<td>R 0.04</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.07</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Right ICA occlusion</td>
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<td></td>
<td></td>
<td></td>
<td>L 0.20</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
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<td>Moyamoya disease</td>
<td>Bilateral ICA bifurcation stenosis</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.14</td>
</tr>
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<td>7</td>
<td>47</td>
<td>M</td>
<td>Intracranial stenosis of unknown etiology</td>
<td>Left MCA occlusion</td>
<td>R 0.32</td>
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<td></td>
<td>L 0.29</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
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<td>Carotid atherosclerosis</td>
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</tr>
<tr>
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<td></td>
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<td>L 0.27</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>M</td>
<td>Moyamoya disease</td>
<td>Bilateral ICA bifurcation stenosis</td>
<td>R 0.18</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.19</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>M</td>
<td>Subclavian steal</td>
<td>Normal</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.52</td>
</tr>
<tr>
<td>11</td>
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<td>M</td>
<td>Moyamoya disease</td>
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<td>R 0.06</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L 0.16</td>
</tr>
<tr>
<td>12</td>
<td>53</td>
<td>M</td>
<td>Carotid atherosclerosis</td>
<td>Left ICA stenosis</td>
<td>R 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>13</td>
<td>41</td>
<td>F</td>
<td>Moyamoya disease</td>
<td>Bilateral ICA bifurcation stenosis</td>
<td>L</td>
</tr>
<tr>
<td>14</td>
<td>35</td>
<td>F</td>
<td>Intracranial stenosis of unknown etiology</td>
<td>Left MCA occlusion</td>
<td>R</td>
</tr>
<tr>
<td>15</td>
<td>69</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Right ICA stenosis</td>
<td>R</td>
</tr>
<tr>
<td>16</td>
<td>70</td>
<td>M</td>
<td>Carotid atherosclerosis</td>
<td>Right ICA stenosis, Left ICA occlusion</td>
<td>R</td>
</tr>
<tr>
<td>17</td>
<td>70</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Bilateral ICA stenosis</td>
<td>R</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>F</td>
<td>Moyamoya disease</td>
<td>Bilateral ICA bifurcation stenosis</td>
<td>L</td>
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<tr>
<td>19</td>
<td>25</td>
<td>M</td>
<td>Moyamoya phenomenon (NF1)</td>
<td>Right ICA bifurcation stenosis</td>
<td>R</td>
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<tr>
<td>20</td>
<td>57</td>
<td>M</td>
<td>Carotid atherosclerosis</td>
<td>ICA stenosis, Left ICA occlusion</td>
<td>R</td>
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<tr>
<td>21</td>
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<td>F</td>
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<td>R</td>
</tr>
<tr>
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<td>35</td>
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<td>Moyamoya disease</td>
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</tr>
<tr>
<td>24</td>
<td>28</td>
<td>F</td>
<td>Moyamoya phenomenon (aplastic anemia)</td>
<td>Right MCA occlusion</td>
<td>R</td>
</tr>
<tr>
<td>25</td>
<td>72</td>
<td>F</td>
<td>Carotid atherosclerosis</td>
<td>Left ICA stenosis</td>
<td>R</td>
</tr>
</tbody>
</table>

*Units are %\(\Delta BOLD\) MR signal/mm \(\Delta P_{ETCO_2}\)

**Units are %\(\Delta CBF\)/mm \(\Delta P_{ETCO_2}\)

F indicates female; M, male; ICA, internal carotid artery; R, right; L, left; MCA, middle cerebral artery
All PetCO₂ stages were attained with standard deviation of 2 mm Hg or less, and PetO₂ was maintained with standard deviation of 3 mm Hg over all gas stages. There was no significant difference in end-tidal gas concentrations for the BOLD MRI versus ASL MRI acquisitions (Table 3.3).

### Table 3.3 PetCO₂ and PetO₂ Achieved

<table>
<thead>
<tr>
<th>Stage</th>
<th>PetCO₂ Mean ± SD</th>
<th>PetO₂ Mean ± SD</th>
<th>Paired t Test*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage</td>
<td>BOLD</td>
<td>ASL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>40.3 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2</td>
<td>48.9 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3</td>
<td>40.8 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>4</td>
<td>49.5 ± 1.1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>5</td>
<td>41.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>PetO₂ Mean ± SD</td>
<td>1-5</td>
<td>103.0 ± 2.7</td>
</tr>
<tr>
<td>mm Hg</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Two-sided paired t-test comparing BOLD MRI and ASL MRI acquisitions.

### Correlation between BOLD MRI and ASL MRI Measurement of Hemispheric CVR

Hemispheric CVR measured by BOLD MRI was significantly correlated with that...
measured by ASL MRI for both gray matter ($R=0.83$, $P<0.0001$; Figure 3.1) and white matter ($R=0.80$, $P<0.0001$; Figure 3.2). There was only a single hemisphere (case 19) with paradoxical hemispheric gray matter CVR on BOLD MRI, whereas 7 hemispheres (cases 4, 6, 12, 13, and 24) had paradoxical hemispheric gray matter CVR on ASL MRI.

**Figure 3.1** Correlation of gray matter CVR measured by BOLD MRI with gray matter CVR measured by ASL MRI for 50 hemispheres in 25 patients. $R=0.83$; $P<0.0001$. 
Figure 3.2  Correlation of white matter CVR measured by BOLD MRI with white matter CVR measured by ASL MRI for 50 hemispheres in 25 patients. R=0.80; P<0.0001.

Accuracy of BOLD MRI Diagnosis of Impaired Hemispheric CVR

For the 11 hemispheres with ipsilateral normal angiography, mean hemispheric CVR on ASL MRI (%ΔCBF/mm ΔPETCO₂) was 5.70% (95% CI=2.67 to 8.73) for gray matter and 5.48% (95% CI=1.16 to 9.81) for white matter. Defining 2 or more standard deviations below the mean as abnormal resulted in 23 of 50 and 15 of 50 hemispheres categorized as having reduced CVR on ASL MRI for gray and white matter, respectively. Figures 3.3 and 3.4 show receiver operating characteristic plots for BOLD MRI diagnosis of impaired CVR in the gray and white matter, respectively, using ASL MRI CVR as a reference standard. The areas under the curves are 0.90 (95% CI=0.81 to 0.98; P<0.001) for gray matter and 0.82 (95% CI=0.70 to 0.94; P<0.001) for white matter.
Figure 3.3  Receiver operating characteristic (ROC) curve for hemispheric gray matter CVR measured by BOLD MRI for 50 hemispheres in 25 patients using ASL MRI as the reference standard.
Figure 3.4  Receiver operating characteristic (ROC) curves for hemispheric white matter CVR measured by BOLD MRI for 50 hemispheres in 25 patients using ASL MRI as the reference standard.

Region-of-Interest Analysis for Paradoxical Cerebrovascular Reactivity

For the 11 hemispheres with ipsilateral normal angiography, the mean proportion of voxels with a paradoxical response on BOLD MRI was 2% (95% CI=0% to 6%). Defining significant paradoxical CVR as 2 or more standard deviations above this mean (that is proportion of paradoxical voxels > 6%) yielded 19 of 50 hemispheres containing a region of significant paradoxical CVR on BOLD MRI. For these regions of paradoxical CVR, corresponding regions on ASL MRI showed paradoxical CVR in 14 of 19 of the hemispheres. For the regions of non-paradoxical CVR on BOLD MRI in these same 19 hemispheres, corresponding regions on ASL MRI showed non-paradoxical CVR in 15 of
19 of the hemispheres. There were 31 of 50 hemispheres with no regions of paradoxical CVR on BOLD MRI, and ASL MRI was non-paradoxical in all of these 31 hemispheres.

Of the 7 patients with paradoxical hemispheric CVR on ASL MRI, none had net paradoxical hemispheric CVR on BOLD MRI. However, 7 of 7 had subhemispheric foci of significant paradoxical CVR on BOLD MRI. Similarly, the one patient with paradoxical hemispheric CVR on BOLD MRI had non-paradoxical hemispheric CVR on ASL MRI but did have a region of significant paradoxical CVR on ASL MRI.

**Spatial Extent of Paradoxical CVR on BOLD MRI versus ASL MRI**

For the 11 hemispheres with normal angiography, the proportion of voxels with a paradoxical response on BOLD MRI (mean=8%, SD=4%) was not significantly different from that on ASL MRI (mean=11%, SD=8%; paired t test, $P=0.282$). For the remaining 39 hemispheres, the mean proportion of voxels with a paradoxical response on BOLD MRI (mean=23%, SD=15%) was significantly less than that on ASL MRI (mean=54%, SD=51%; paired t test, $P<0.001$). Figure 3.5 is a representative case (case 24) illustrating this difference.
Figure 3.5  Comparison of BOLD and ASL maps of CVR for corresponding slices from a patient with right middle cerebral artery occlusion and Moyamoya phenomenon secondary to aplastic anemia. BOLD MRI shows paradoxical CVR (depicted in blue) mainly in the right MCA territory. ASL MRI shows a corresponding, but more extensive, region of paradoxical CVR. Units are %ΔBOLD MR signal per mm Hg ΔPetCO₂ and %ΔCBF per mm Hg ΔPetCO₂, respectively.

3.5 Discussion

The results demonstrate a strong correlation between hemispheric CVR measured using BOLD MRI and ASL MRI. This suggests that even in patients with stenosis or occlusion of brain-supplying arteries, the BOLD MRI signal response to hypercapnia is directly related to changes in CBF. Previous studies did not allow this conclusion. Shiino et al. studied 10 patients with cerebrovascular disease and found a significant correlation ($R=0.698$, $P<0.0001$) between CVR measured by BOLD MRI (with breath-holding as the vasodilatory stimulus) and I-123 single photon emission CT (with acetazolamide as the
vasodilatory stimulus). However, the BOLD and single photon emission CT measurements are difficult to compare because breath-holding and acetazolamide injection are physiologically different stimuli, and also, the 2 measurements were performed at different times. In contrast, I have used identical vasodilatory stimuli for both BOLD and ASL MRI, and the 2 types of imaging were performed in the same MRI session, only 10 minutes apart. Lythgoe et al (Lythgoe, Williams, Cullinane, & Markus, 1999) studied 16 patients with unilateral carotid artery stenosis or occlusion and found no significant correlation between BOLD MRI and transcranial Doppler ultrasound responses to CO$_2$ provocation. However, Ziyeh et al (Ziyeh et al., 2005) later performed a similar comparison of BOLD MRI and transcranial Doppler in 27 patients and reported a significant correlation ($R=0.71$, $P<0.001$).

Furthermore, the findings support the hypothesis that BOLD MRI can accurately discriminate between normal and abnormal hemispheric CVR. Diagnostic accuracy was reflected in the large areas under the receiver operating characteristic curves for both gray matter (Figure 3.3) and white matter (Figure 3.4). I found that regions of paradoxical CVR on BOLD MRI had a moderate positive predictive value (73%) for true paradoxical CVR. Importantly, complete lack of paradoxical CVR on BOLD MRI had a high negative predictive value (100%).

The results might be criticized on the grounds that ASL MRI is not as well established as other techniques for measuring CBF. The decision to use ASL MRI arose from a need to map CBF with comparable spatial and temporal resolution to the BOLD MRI technique.
Although transcranial Doppler is clinically the most commonly used method for measuring CVR, it offers little spatial discrimination and only assesses the middle cerebral artery territory. Also, it has limited diagnostic accuracy. (Pindzola et al., 2001) H$_2^{15}$O positron emission tomography is considered the most accurate method of measuring CBF \textit{in vivo}. However, H$_2^{15}$O has a 123-second half-life necessitating a 10- to 15-minute delay between PETCO$_2$ states to allow for decay of the radiopharmaceutical between measurements of CBF. This precludes direct comparison with the BOLD MRI protocol that involves relatively rapid changes in PETCO$_2$ (to minimize study duration). Single photon emission CT also requires a delay between measurements. In contrast with these other techniques, ASL MRI has comparable spatial and temporal resolution to BOLD MRI. FAIR MRI measurement of CBF is accurate when compared with positron emission tomography, (Ye et al., 2000) and in the functional MRI literature, FAIR has been used as a reference standard for measurement of CBF. (Hoge et al., 1999) FAIR has also been validated for measuring CVR in normal subjects (Yen et al., 2002) and in patients with arterial steno-occlusive disease. (Arbab et al., 2002) ASL MRI has lower signal-to-noise ratio than BOLD MRI, but this should be less of a problem for this study in which mean CVR is calculated over large regions-of-interest.

The study revealed several limitations of using BOLD MRI signal to map CVR. First, the predictive value of paradoxical CVR on BOLD MRI was only moderate. Second, BOLD MRI underestimated the spatial extent of paradoxical CVR. We may gain insight into these findings by considering the nature of BOLD MRI signal. BOLD MRI signal depends on the intravoxel concentration of deoxyhemoglobin. This concentration is
determined by the fraction of the voxel occupied by blood (CBV), the concentration of hemoglobin in blood (hematocrit), the concentration of dissolved oxygen in blood (PaO₂), the rate of inflow of fresh blood and outflow of deoxygenated blood (CBF), and the rate of oxygen use by tissues (cerebral metabolic rate of oxygen consumption).

The influence of arterial blood volume on the BOLD MR signal is likely insignificant since arterial blood is a small fraction (25%) of total CBV and it contains relatively negligible amounts of deoxyhemoglobin. The more important CBV factor is therefore the passive inflation of venous vessels secondary to increased venous blood pressure when arterial resistance is lowered. (Hoge et al., 1999)

In normal subjects, hypercapnia induces an increase in both CBF and CBV. The relationship between ΔCBF and ΔCBV was determined empirically by Grubb (Grubb et al., 1974): ΔCBV/CBV₀=(ΔCBF/CBF₀)⁰.³⁸. Through inflow of fresh blood and washout of deoxygenated blood, increased CBF reduces the concentration of deoxyhemoglobin in blood, increasing BOLD MRI signal. Conversely, an elevation in CBV increases the fraction of the imaging voxel occupied by blood, reducing BOLD MRI signal. Despite these opposing influences, hypercapnia (Hoge et al., 1999) and neuronal activation (Zhu et al., 1998) each induce an increase in BOLD MR signal that is linearly related to the change in CBF. This suggests that in normal subjects, the BOLD MRI signal response to hypercapnia is dominated by flow effects and not blood volume effects.

In patients with cerebrovascular disease, the relationship between hypercapnia and CBF
is well known. However, there is little literature on the relationships between hypercapnia and CBV and hypercapnia and BOLD MRI signal. Sabatini et al (Sabatini, Celsis, Viallard, Rascol, & Marc-Vergnes, 1991) found that acetazolamide-induced $\Delta$CBV/$\Delta$CBF was greater in those with carotid occlusion than in healthy volunteers, suggesting that Grubb’s relationship is not valid in this patient population. Okazawa et al (Okazawa, Yamauchi, Sugimoto, & Takahashi, 2003) reproduced these findings and, of particular relevance, also described the acetazolamide-induced $\Delta$CBV and $\Delta$CBF for a subset of patients (4 of 16) with paradoxical CVR. These latter patients showed an acetazolamide-induced increase in CBV and decrease in CBF. Because an increase in CBV and a decrease in CBF both reduce BOLD MR signal, these changes do not explain why BOLD MRI underestimated the spatial extent of paradoxical CVR in my study. However, the Okazawa results are derived from only 4 patients, and only mean changes in CBV and CBF for entire hemispheres were measured. I anticipate that future studies that map hypercapnia-induced changes in both CBF and CBV in patients with steno-occlusive disease will help resolve these issues.

We have considered changes in CBV as a possible confounder in BOLD MRI mapping of CVR. $P_{aO_2}$, hematocrit, and cerebral metabolic rate of oxygen consumption are additional confounders to consider. When a hypercapnic stimulus is applied, the resulting hyperventilation results in a tandem increase in arterial $pO_2$, reducing deoxyhemoglobin concentration, causing an increase in BOLD MR signal independent of any change in CBF. This method maintains $P_{ETO_2}$ at normoxia within very narrow limits (mean=100 mm Hg, SD 2 mm Hg), (Slessarev et al., 2007) independent of minute ventilation and the
target PETCO₂, and thus removes variation in arterial pO₂ as a confounding variable. Hematocrit is also an unlikely confounder because one would not expect a hypercapnia-induced change in hematocrit during the course of a CVR acquisition. Similarly, hypercapnia does not affect cerebral metabolic rate of oxygen consumption. (Alberti et al., 1975; Rhodes, Lenzi, Frackowiak, Jones, & Pozzilli, 1981)

Another potential confounder is the time delay between change in partial pressure of carbon dioxide in the pulmonary capillaries and arrival of this change in a given voxel in the brain. This time delay is spatially heterogeneous. (van der Zande, Hofman, & Backes, 2005) If the relative difference in time delay between 2 regions of brain was large enough, then theoretically, the changes in arterial pCO₂ in one region of brain could be 180° out of phase with the changes elsewhere, mimicking a steal phenomenon. (Naganawa et al., 2002) I have used a non-periodic PETCO₂ stimulus to avoid this possibility.

3.6 Conclusion

ASL MRI confirms that even in patients with stenosis or occlusion of brain-supplying arteries, the BOLD MRI signal response to hypercapnia predominantly reflects changes in CBF. This result contributes to the validation of BOLD MR mapping of CVR.
Chapter 4 – BOLD MR Measurement of CVR in Patients with Intracranial Steno-occlusive Disease: Preoperative Values Predict the Hemodynamic Effect of EC-IC Bypass Surgery

This chapter is adapted with permission from the following publication:


4.1 Abstract

Cerebrovascular reactivity (CVR) is a measure of cerebral hemodynamic impairment. A recently validated technique quantifies CVR using a precise CO₂ (carbon dioxide) vasodilatory stimulus and BOLD (blood oxygen level-dependent) MRI. Our aim was to determine whether pre-operative CO₂ BOLD CVR predicts the hemodynamic effect of
extracranial-intracranial (EC-IC) bypass surgery in patients with intracranial steno-occlusive disease. In particular, some studies have relied on right-left interhemispheric CVR asymmetry indices, which are problematic in patients with bilateral disease, and the intention of this study was to use “absolute” CVR from each hemisphere in units of %ΔBOLD MR signal intensity per mm Hg ΔPetCO₂. Twenty-five patients undergoing EC-IC bypass surgery for treatment of intracranial stenosis or occlusion were recruited. CVR was measured pre-operatively and post-operatively, and expressed as %ΔBOLD MR signal intensity per mm Hg Δ end-tidal partial pressure of CO₂. Using normative data from healthy subjects, patients were stratified based on pre-operative CVR into 3 groups: normal CVR, reduced CVR, and negative (“paradoxical”) CVR. Wilcoxon two sample tests (two-sided, α= 0.05) were used to determine whether the 3 groups differed with respect to change in CVR following bypass surgery. The group with normal pre-operative CVR demonstrated no significant change in CVR following bypass surgery (0.22% ± 0.05% to 0.22% ± 0.01% (mean ± SD)(P=0.881)). The group with reduced pre-operative CVR demonstrated a significant improvement following bypass surgery (0.08% ± 0.05% to 0.21 ± 0.08% (mean ± SD)(P<0.001)), and the group with paradoxical pre-operative CVR demonstrated the greatest improvement (-0.04% ± 0.03% to 0.27% ± 0.03% (mean change ± SD)(P=0.028)). These results suggest that pre-operative measurement of CVR using CO₂ BOLD MRI may predict the hemodynamic effect of EC-IC bypass in patients with intracranial steno-occlusive disease. The technique is potentially useful for selecting patients for surgical revascularization.
4.2 Introduction

A large population-based study (White et al., 2005) in the United States found that 9-15% of ischemic strokes are due to intracranial stenosis or occlusion. The proportion is even higher in Asian populations. (Suri & Johnston, 2009) Management of intracranial steno-occlusive disease is a challenge as most etiologies lack effective medical therapy. (Marc I. Chimowitz et al., 2005; Kuroda & Houkin, 2008) Surgical treatment, traditionally by extracranial-intracranial (EC-IC) arterial bypass, was popular until the EC-IC Bypass Study (1985) found no difference in stroke incidence between patients randomized to medical therapy versus surgical bypass. A critique of the EC-IC Bypass Study was that patients were included based on degree of angiographic stenosis, without specifically assessing for cerebral hemodynamic compromise. Assessment of cerebral hemodynamic compromise is important: for a given degree of angiographic stenosis, some patients have adequate collateral supply while others lack sufficient collateral flow. Subsequent advances in brain imaging have improved our ability to identify the latter group.

A recently validated (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008) technique for measuring CVR employs precise changes in end-tidal partial pressure of carbon dioxide (PETCO2) as a vasodilatory stimulus, and blood oxygen level-dependent (BOLD) MRI to map the stimulus-induced changes in cerebral blood flow. This technique uses a MR pulse sequence that is standard on clinical MR systems, and it can be performed at the same session as routine MRI and MRA. Some groups (Goode, Altaf, Auer, & MacSweeney, 2009; Goode, Krishan, Alexakis, Mahajan, & Auer, 2009; Haller et al., 2008) have measured CO2 BOLD CVR using the contralateral hemisphere as an internal
standard, and reported “quantitative” right-left interhemispheric asymmetry indices. This kind of relative measure is necessarily problematic in patients with bilateral disease. It has previously been shown (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008) that expression of hemispheric CVR in units of $%\Delta \text{BOLD} \ MR$ signal intensity per mm Hg $\Delta P_{\text{ETCO}_2}$, rather than as an interhemispheric asymmetry index, correlates well with CVR measured using arterial spin labeling MRI.

Our aim was to determine whether pre-operative $CO_2$ BOLD CVR, expressed in units of $%\Delta \text{BOLD} \ MR$ signal intensity per mm Hg $\Delta P_{\text{ETCO}_2}$ predicts the hemodynamic effect of surgical bypass in patients with intracranial steno-occlusive disease. I hypothesized that patients with normal pre-operative CVR would not improve in CVR following bypass, those with reduced pre-operative CVR would improve, and those with markedly reduced CVR (that is, negative or “paradoxical” CVR) would have the greatest improvement.

### 4.3 Methods

**Patients**

The institutional ethics committee approved the study protocol and all patients provided written informed consent. There were no study-related adverse events. Patients scheduled for EC-IC bypass surgery for treatment of intracranial arterial stenosis or occlusion at the Toronto Western Hospital (Toronto, Ontario, Canada) were recruited. Twenty-five consecutive patients (16 women, 9 men; mean age, 39.6 years ± 14.5 [standard deviation]) were recruited between August 2006 and January 2010. Seven patients had
bilateral bypass procedures during the study period, resulting in a total of 32 EC-IC bypass procedures. Patient demographics, clinical presentations, and diagnoses are listed in Table 1.

**Surgical Procedure**

The most common surgical procedure (31/32) was direct EC-IC bypass by end-side anastomosis of the superficial temporal artery (STA) to a middle cerebral artery (MCA) branch. In patients with small recipient MCA branches, this direct bypass was supplemented (7 procedures) with pial synangiosis. Pial synangiosis is an indirect bypass, performed by opening the arachnoid layer of the leptomeninges and attaching a branch of the STA to the pia. Two procedures consisted of indirect bypass only: one was a pial synangiosis and one was an encephalodural arterial synangiosis.

**Table 4.1  Patient characteristics**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, years</th>
<th>Sex</th>
<th>Clinical Presentation</th>
<th>Diagnosis</th>
<th>Procedure(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>F</td>
<td>TIA</td>
<td>Atherosclerosis</td>
<td>R STA-MCA direct bypass</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>F</td>
<td>TIA</td>
<td>Trisomy 21</td>
<td>L encephalodural arterial synangiosis</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>F</td>
<td>TIA</td>
<td>Moyamoya disease</td>
<td>R STA-MCA direct bypass</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>F</td>
<td>Hemorrhage</td>
<td>Moyamoya disease</td>
<td>L STA-MCA direct bypass</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>Infarct</td>
<td>Vasculitis</td>
<td>R STA-MCA direct bypass</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>M</td>
<td>Asymptomatic</td>
<td>Neurofibromatosis type 1</td>
<td>R STA-MCA direct bypass</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>M</td>
<td>Cognitive</td>
<td>Sickle cell disease</td>
<td>R STA-MCA direct bypass</td>
</tr>
<tr>
<td>Patient</td>
<td>Age</td>
<td>Gender</td>
<td>Diagnosis</td>
<td>Intervention</td>
<td></td>
</tr>
<tr>
<td>---------</td>
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<td>-----------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>F</td>
<td>Hemorrhage</td>
<td>Chronic dissection, R STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>F</td>
<td>Infarct</td>
<td>Radiation, L STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>M</td>
<td>Hemorrhage</td>
<td>Moyamoya disease, R STA-MCA direct bypass and pial synangiosis</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>F</td>
<td>TIA</td>
<td>Stenosis of unclear etiology, L STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>M</td>
<td>Worsening</td>
<td>Moyamoya disease, L STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>38</td>
<td>F</td>
<td>TIA</td>
<td>Moyamoya disease, L STA-MCA direct bypass; R STA-MCA direct and indirect bypass</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>35</td>
<td>F</td>
<td>Infarct</td>
<td>Mixed connective tissue disease, R STA-MCA direct bypass; L STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>65</td>
<td>F</td>
<td>Hemorrhage</td>
<td>Moyamoya disease, R STA-MCA direct bypass; L STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>33</td>
<td>F</td>
<td>Infarct</td>
<td>Moyamoya disease, R STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>36</td>
<td>F</td>
<td>Infarct</td>
<td>Moyamoya disease, R STA-MCA direct bypass; L STA-MCA indirect bypass</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>42</td>
<td>M</td>
<td>Infarct</td>
<td>Moyamoya disease, L STA-MCA direct bypass; R STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>42</td>
<td>F</td>
<td>Hemorrhage</td>
<td>Moyamoya disease, L STA-MCA direct bypass; R STA-MCA direct and indirect bypass</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>26</td>
<td>F</td>
<td>Hemorrhage</td>
<td>Cocaine use, L STA-MCA direct bypass; R STA-MCA direct bypass</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>43</td>
<td>F</td>
<td>TIA</td>
<td>Moyamoya disease, L STA-MCA direct and indirect bypass</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>50</td>
<td>F</td>
<td>TIA</td>
<td>Moyamoya disease, L STA-MCA indirect bypass</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>46</td>
<td>M</td>
<td>TIA</td>
<td>Stenosis of unclear etiology, L STA-MCA direct bypass</td>
<td></td>
</tr>
</tbody>
</table>
Vasodilatory Stimulus

$\text{PETCO}_2$ and end-tidal partial pressure of $\text{O}_2$ ($\text{PETO}_2$) were controlled using model-based prospective end-tidal gas targeting. (Slessarev et al., 2007) These algorithms are incorporated into a device (RespirAct™, Thornhill Research Inc., Toronto, Canada) that consists of a computer-controlled gas blender, sensors for $\text{PCO}_2$ and $\text{PO}_2$, and a sequential re-breathing circuit applied to the patient. The user enters the target end-tidal gas values and their durations. The RespirAct™ system then uses the algorithm (Slessarev et al., 2007) to calculate the various gas concentrations and flows it will administer to the breathing circuit in order to attain the target end-tidal gas partial pressures. The RespirAct™ system is capable of inducing precise square wave changes in $\text{PETCO}_2$ and $\text{PETO}_2$ independently of each other over a wide range of minute ventilation and breathing patterns. The gas sequence for CVR was 8 minutes 20 seconds in duration. Iso-oxia (target $\text{PETO}_2$ of 100 mmHg) was maintained throughout. The target $\text{PETCO}_2$ was (a) 40 mmHg for 60 s (normocapnia), (b) 50 mmHg for 60 s (hypercapnia), (c) normocapnia for 100 s, (d) hypercapnia for 160 s, and (e) normocapnia for 120 s. (Mandell, Han, Poublanc, Crawley, Kassner, et al., 2008) Tidal $\text{PCO}_2$ and $\text{PO}_2$ were monitored continuously, digitized, and recorded (RespirAct™).
Imaging

MR imaging was performed on a 3.0-T whole body scanner (Signa HDx; GE Healthcare, Milwaukee, WI), with an eight-channel phased-array head coil for signal reception. Each patient was imaged with an identical CO₂ BOLD CVR protocol before and after the bypass procedure. Each CVR session included routine clinical pulse sequences (sagittal T1-weighted, axial T2-weighted, axial T2-weighted FLAIR, and diffusion-weighted), an axial T1-weighted three-dimensional spoiled gradient echo sequence (matrix size, 256 x 256; slice thickness, 2.2 mm; inter-slice gap, 0) for anatomical co-registration, and an axial T2*-weighted single-shot gradient echo echo-planar BOLD sequence (flip angle, 85°; repetition time, 2000 ms; echo time, 30 ms; field of view, 24 x 24 cm; matrix size, 64 x 64; slice thickness, 5 mm; inter-slice gap, 2 mm, number of frames, 255) during controlled changes in PETCO₂. Median time from baseline CVR measurement to bypass procedure was 14.6 weeks (interquartile range 13.5 weeks). Median time from bypass procedure to follow-up CVR measurement was 21.9 weeks (interquartile range 9.5 weeks).

As part of their routine clinical care, all patients with direct EC-IC bypasses also underwent post-operative imaging to assess bypass patency. This imaging was a combination of CT angiography (23/30 bypasses), MR angiography (12/30), and catheter angiography (10/30). Imaging to assess bypass patency was performed on the same day as post-operative CVR for 9/30 bypasses, and both before and after the post-operative CVR for 13/30 bypasses. For the latter cases, one may infer bypass patency at the time of CVR if the bypass was patent in both the pre CVR and post CVR vascular studies. In the
remaining 8/30 cases, patency was only assessed prior (4.0 ± 1.7 months (mean ± SD)) to the post-operative CVR. Mean time from assessment of vessel patency to post-operative CVR was 4.0 ± 1.7 months (mean ± SD) for these 8 cases. There was no suspicion of bypass non-patency on clinical follow-up in any of these 8 cases.

Data Analysis

MRI and PETCO₂ data were imported into the software AFNI (Analysis of Functional NeuroImages, NIH, Bethesda, USA; http://afni.nimh.nih.gov/afni). (Cox, 1996) An AFNI algorithm was used to calculate head motion for each BOLD MRI acquisition. Bypass procedures (4/32) with greater than 1 voxel width head motion on either the pre-operative or post-operative CVR study were excluded from further analysis. Each patient’s whole brain BOLD MR signal dataset was temporally shifted to the point of maximum correlation with the patient’s PETCO₂ waveform. The BOLD MR signal-time waveform then underwent least squares fitting to the PETCO₂-time waveform on a voxel-by-voxel basis, and CVR was calculated as %ΔBOLD MR signal intensity per mm Hg ΔPETCO₂. Anatomical images were automatically segmented into grey matter and white matter, and transformed into Montreal Neurological Institute space using the software SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK; http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Anatomic images were further segmented into anterior cerebral artery (ACA), middle cerebral artery (MCA), and posterior circulation (including cerebellum) using masks created from the atlas of Kretschmann and Weinrich. (Kretschmann & Weinrich, 2004) Mean grey matter CVR was calculated for each of these segments, for each CVR study.
The pre-operative and post-operative routine T1 and T2-weighted images were reviewed to identify regions of parenchymal infarction or hemorrhage. Using AFNI, a mask of each region of parenchymal abnormality was created, and a second set of CVR maps was generated with these regions segmented out. Clinical reports from post-operative CTA, MRA, and DSA were also reviewed, and each bypass was categorized as patent or non-patent.

**Statistical Analysis**

Bypass procedures excluded from the analysis included those associated with excessive head motion on a CVR study (4/32), non-patent bypass (6/32), and a single case with a moderate size post-operative subdural hematoma. The details of the 6 non-patent bypasses are as follows: One bypass (Case 15) had already been excluded from the analysis due to head motion. One bypass (Case 1) was indeterminate for patency as the bypass was patent on an immediate post-operative CTA, the post-operative CVR study was performed 2 months later, and the bypass was non-patent on a CTA 2 months following the CVR. One bypass (Case 17) showed extremely delayed passage of contrast through the STA on DSA, with severe disease of the MCA M2 and M3 branches resulting in lack of retrograde flow into the MCA. Bilateral bypasses in a single patient (Case 13) showed significant stenosis at the arterial anastomoses. One bypass (Case 11) was occluded at the time of follow-up CVR.

To determine whether the EC-IC bypass procedures had a significant effect on CVR, I performed a Wilcoxon two sample test (two-sided, $\alpha=0.05$) comparing pre-operative and
post-operative CVR values for each of the six vascular territories. Then, to determine
whether pre-operative stratification of patients by absolute CO₂ BOLD CVR values
predicts the hemodynamic effect of bypass surgery, I categorized patients based on pre-
operative CVR in the territory of planned surgical bypass. Patients were categorized into
three groups: I. normal CVR, II. reduced CVR, III. paradoxical CVR (that is, negative
CVR). In a previous study, (Han et al., 2009) an identical CO₂ BOLD CVR technique was
used to study a group of 10 healthy subjects, age 30.0 years ± 8.2 (mean ± SD). In that
study, CVR in the MCA territory was 0.30 ± 0.07 (mean ± SD) [%ΔBOLD MR signal
intensity per mm Hg PETCO₂]. As this data was approximately age-matched with the
current study population, I used two standard deviations below the mean CVR from the
healthy subjects as a threshold for reduced CVR. The lower threshold CVR value was
0.16% ΔBOLD MR signal intensity per mmHg ΔPETCO₂. I performed Wilcoxon two
sample tests (two-sided, α= 0.05) to determine whether the change in CVR following
EC-IC bypass was significant for each of the 3 groups, and also tested whether the
change in CVR following EC-IC bypass differed significantly between the normal versus
reduced CVR groups and reduced versus paradoxical CVR groups. All statistical analyses
were then repeated for the CVR maps with regions of infarction or intraparenchymal
hemorrhage removed. I also performed a Wilcoxon two sample test to test for a
difference in pre-operative CVR between those subjects who had direct bypass only
versus those who received direct and indirect bypass.

4.4 Results

There was no significant difference in pre-operative CVR between subjects who received
direct EC-IC bypass only versus those who received direct and indirect bypass (0.09% ± 0.10% versus 0.09% ± 0.08% respectively (mean ± SD)(P=0.857).

Effect of EC-IC Bypass on CO₂ BOLD MRI CVR

CVR in the vascular territory of the surgical bypasses improved significantly following treatment (0.09% ± 0.10% pre-bypass to 0.22% ± 0.08% post-bypass (mean ± SD)(two-sided P<0.001)). CVR in the other vascular territories did not change significantly. Figure 4.1 shows these summary results and Figure 4.2 is a representative case (Case 6). Repeating the analysis with regions of infarction or hemorrhage removed from the CVR maps did not significantly alter these results.

![CO₂ BOLD CVR](image)

**Figure 4.1** Effect of EC-IC bypass on CO₂ BOLD CVR in grey matter vascular territories ipsilateral and contralateral to bypass. Whiskers mark the 10th and 90th percentiles, and boxes are bounded by the 25th and 75th percentiles.

Pre-operative CVR as a Predictor of the Hemodynamic Effect of EC-IC Bypass
Defining “reduced CVR” as two standard deviations below the mean CVR of approximately age-matched healthy subjects, resulted in 5 patients with normal CVR, 12 patients with reduced (but positive) CVR, and 4 patients with paradoxical (negative) CVR. The group with normal pre-operative CVR demonstrated no significant change in CVR following bypass surgery (0.22% ± 0.05% to 0.22% ± 0.01% (mean ± SD)(P=0.881)). The group with reduced pre-operative CVR demonstrated a significant improvement following bypass surgery (0.08% ± 0.05% to 0.21 ± 0.08% (mean ± SD)(P<0.001)), as did those with paradoxical pre-operative CVR (-0.04% ± 0.03% to 0.27% ± 0.03% (mean change ± SD)(P=0.028)) (Figure 4.3). The differences in CVR improvement between the normal versus reduced pre-operative CVR groups, and between the reduced and paradoxical pre-operative CVR groups were both significant ((P=0.012 and P<0.001 respectively). Repeating the analysis with regions of infarction removed from the CVR maps yielded no significant change in the results.

Figure 4.2  Grey matter CVR map overlaid on anatomical T1-weighted images for a representative patient (Case 24). Top row is before bypass and bottom row is after bypass surgery. CVR units are %ΔBOLD MR signal intensity per mm Hg ΔPETCO2. Images demonstrate decreased, and in fact paradoxical (negative), CVR in the left MCA territory cortex and deep grey matter prior to bypass, nd marked improvement post bypass surgery.
Figure 4.3  Boxplot showing the change in ipsilateral MCA territory CVR following EC-IC bypass surgery for 3 groups: normal pre-operative CVR, reduced pre-operative CVR, and paradoxical pre-operative CVR. Units of CVR are \( \% \Delta \text{BOLD MR signal intensity per mm Hg } \Delta \text{PETCO}_2 \).

4.5 Discussion

In this study, I found that CO\(_2\) BOLD CVR, expressed in absolute values, predicted the hemodynamic effect of EC-IC bypass. Patients with normal pre-operative CVR did not improve in CVR following bypass, those with reduced pre-operative CVR did improve, and those with paradoxical pre-operative CVR had the greatest improvement. These results have two main implications. First, they further validate the use of absolute CO\(_2\) BOLD CVR values for the measurement of cerebral hemodynamic compromise. Second, they suggest that absolute values from CO\(_2\) BOLD CVR are potentially of use in determining which patients with intracranial steno-occlusive disease should undergo surgical revascularization.
Quantitative Assessment of Hemodynamic Impairment Using CO$_2$ BOLD CVR

I have expressed CVR in absolute values of %ΔBOLD MR signal intensity per mm Hg ΔPETCO$_2$. This approach was validated in a study (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008) that compared BOLD MR and arterial spin labeling MR measurement of CVR in 25 patients with steno-occlusive disease. This differs from much of the published CO$_2$ BOLD CVR work (Goode, Altaf, et al., 2009; Goode, Krishan, et al., 2009; Haller et al., 2008) that has used the contralateral hemisphere as an internal standard, and reported CVR as a right-left interhemispheric asymmetry index. Use of an interhemispheric asymmetry index is problematic as patients commonly have bilateral disease, that is, no normal hemisphere for normalization. I will briefly discuss a few methodological details that may be important for obtaining absolute values of CO$_2$ BOLD CVR.

First, is it necessary to normalize %ΔBOLD MR signal to the magnitude of the vasodilatory stimulus? Goode et al. (Goode, Krishan, et al., 2009) suggest that use of a strong CO$_2$ challenge yields near maximal vasodilatation, and removes the need for normalization. Hypothetically, a CO$_2$ stimulus sufficiently strong to cause near maximal vasodilatory capacity in all patients could enable measurement of cerebrovascular reserve. However, the 40-50 mm Hg PETCO$_2$ range typical of a CVR study is unlikely to consistently result in complete exhaustion of vasodilatory reserve. Cerebral blood flow (CBF) continues to increase in response to incremental increases in PETCO$_2$ well above 60 mm Hg in many subjects. (Grubb et al., 1974) In those patients in whom the CO$_2$
stimulus does not result in complete exhaustion of vasodilatory capacity, %ΔBOLD MR signal intensity depends on the magnitude of the CO₂ stimulus, and thus CVR must be expressed as a slope: ΔBOLD MR signal intensity / Δ PETCO₂.

Second, how does one measure the magnitude of the CO₂ stimulus? The independent variable affecting CBF is the arterial partial pressure of CO₂ (PaCO₂), but measurement of PaCO₂ requires arterial puncture, which is uncomfortable for the patient. Instead, I have non-invasively measured its surrogate, (Crosby, Robbins, Crosby, & Robbins, 2003) end-tidal CO₂ (PETCO₂). With the end-tidal gas targeting system I have used, PETCO₂ correlates well with PaCO₂ (mean difference 0.5 ± 1.7 mm Hg (P=0.53;95% CI –2.8, 38 mm Hg)).(Ito et al., 2008) Some have used the inspiratory partial pressure of CO₂ (FiCO₂) as the independent variable, but the ventilatory response to hypercapnia varies between subjects so FiCO₂ does not have a consistent relationship with PaCO₂.(Mark et al., 2010)

Third, if one normalizes %ΔBOLD MR signal intensity to the magnitude of the vasodilatory stimulus, is it necessary to maintain a consistent magnitude of vasodilatory stimulus for all subjects? The relationship between CBF and PaCO₂ is sigmoidal. If only 2 points on the curve are taken (PaCO₂ at 2 levels), then the slope (that is, the CVR) will depend on both the initial PaCO₂, and the magnitude of the change in PaCO₂.(Han et al., 2009; Ide et al., 2003) Therefore, to have comparable CVR measurements in one subject over time, or between subjects, it is necessary to establish a consistent baseline PaCO₂ and a consistent magnitude of change.
Consider the “BOLD MR signal” term. BOLD MR is an indirect measure of changes in CBF. Increased CBF results in dilution of intravascular deoxyhemoglobin, generating increased signal on T2*-weighted (BOLD) images. BOLD MR signal intensity also depends on cerebral blood volume, cerebral metabolic rate of oxygen consumption, arterial partial pressure of oxygen (PaO₂), and hematocrit,(Ogawa et al., 1993) on pulse sequence parameters such as echo time and voxel size, and on hardware factors such as magnetic field strength. Despite the many influences on absolute BOLD MR signal intensity, it has been shown empirically that, using a tightly controlled CO₂ stimulus and a single MR scanner with consistent sequence parameters, hemispheric CVR measured in absolute values of %ΔBOLD MR signal intensity per mm Hg ΔPETO₂ is both precise(Kassner, Winter, Poublanc, Mikulis, & Crawley, 2010) and accurate.(Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008) The technique does not provide a measure of CVR in the gold-standard cerebral blood flow units of ml / 100g / min, but it does enable quantitative intra and inter-subject comparisons.

Is %ΔBOLD MR signal intensity a reproducible measure? Scanning subjects on a single 1.5 Tesla scanner, CVR measured as %ΔBOLD MR signal intensity per mm Hg ΔPETO₂ had excellent within-day and between-day reproducibility.(Kassner et al., 2010) Similarly, in subjects who performed a finger-tapping fMRI paradigm on each of several MR scanners (of the same model type), only 8.3% of total variance observed in the % Δ BOLD MR signal changes was due to between-scanner variability, and there was no evidence that scanners introduced a statistically significant variability in the results.(Costafreda et al., 2007) Another approach is to use a multi-echo sequence and
Absolute Values of BOLD CVR to Select Patients for Surgical Revascularization

To determine which patients with intracranial arterial steno-occlusive disease should undergo surgical revascularization, one may aim to identify the subset of patients with cerebral hemodynamic impairment. Our results suggest that absolute values of CO₂ BOLD CVR are potentially useful in identifying a subset of patients with intracranial steno-occlusive disease who are most likely to have a hemodynamic improvement following surgical revascularization. The CO₂ BOLD MRI technique is quick, non-ionizing, and it can be performed at the same session as routine MRI and MRA. The technique may also enable accurate follow-up of patients who are not surgically revascularized, and measurement of treatment response in those who are revascularized.

Limitations

Several bypass procedures were excluded from the quantitative analysis: procedures with excessive head motion (4/32), non-patent bypass (6/32), and one patient with a moderate-large post-operative subdural hematoma. I have found that patient head motion during BOLD MRI acquisition is the most common reason for a failed CO₂ BOLD CVR exam. In this study, I used an automated tool to exclude BOLD acquisitions with excessive head motion. Our analysis for head motion was performed after the MR imaging was complete, but it is possible to analyze BOLD MRI data in real-time. Analysis of head motion while the patient is in the MR scanner would allow one to repeat those CVR...
acquisitions with head motion, potentially reducing the number of failed exams. Non-patent bypasses were excluded as I wanted to study the relationship between CO$_2$ BOLD CVR and EC-IC bypass, and non-patent bypasses would confound the results. Future randomized controlled trials using CO$_2$ BOLD CVR as a means of selecting patients for randomization will likely require an intention-to-treat analysis, with patients in the surgical arm included whether or not the bypass procedure is technically successful.

An inclusion criterion for the study was presence of intracranial stenosis or occlusion. I did not have a sufficient number of subjects to perform subgroup analyses for each particular cause of stenosis or occlusion. There was also some variability in surgical treatment, with direct surgical bypass in 31/32 procedures and indirect bypass in 8/32. This could confound the study results if the subjects with lower pre-operative CVR had a greater likelihood of receiving more extensive revascularization. However, there was no significant difference in pre-operative CVR between those who received direct bypass only versus direct and indirect bypass.

A limitation was the assessment of EC-IC bypass patency using a clinically dictated schedule of CTA, MRA, and DSA. In the majority of cases (22/30 direct bypass procedures), patency was assessed on the same day as the post-operative CVR, or both before and after the post-operative CVR, enabling one to infer vessel patency if patent on both studies. In a minority of cases (8/30 direct bypasses), patency was only assessed prior to the CVR study. All 8 of these latter cases had patent bypasses on imaging, and none had clinical suspicion of bypass non-patency, but I cannot completely exclude the
possibility of bypass occlusion between the time of patency assessment and the post-operative CVR study.

I have used an atlas-based segmentation of vascular territories into ACA, MCA, and posterior distributions. A limitation of this approach is that vascular territories may have altered size and geometry in patients with intracranial stenosis or occlusion. The atlas-based approach does still allow assessment of CVR near the region of the MCA bypass, versus further from the bypass on the ipsilateral side, versus contralateral to the bypass.

4.6 Conclusion

CVR measured using CO\textsubscript{2} BOLD MRI identifies those patients with intracranial steno-occlusive disease who are more likely to have a hemodynamic improvement following EC-IC bypass surgery. The CO\textsubscript{2} BOLD MRI technique is quick, non-ionizing, and it can be performed at the same session as routine MRI and MRA.
Chapter 5 – Selective Reduction of Blood Flow to White Matter during Hypercapnia Corresponds with Leukoaraiosis

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5.1 Abstract

Age-related white matter disease (leukoaraiosis) clusters in bands in the deep white matter above the lateral ventricles, around the occipital and frontal horns of the lateral ventricles, in the corpus callosum and internal capsule. Cerebrovascular anatomy suggests that some of these locations represent border-zones between arterial supply territories. I hypothesized that there are zones of reduced cerebrovascular reserve (susceptible to selective reductions in blood flow, that is, steal phenomenon) in the white matter of young healthy subjects – the physiological correlate of these anatomically-defined border-zones. Furthermore, I hypothesized that these zones spatially correspond with the regions where the elderly develop leukoaraiosis. 28 healthy volunteers
underwent functional MR mapping of the cerebrovascular response to hypercapnia. I studied 18 subjects by blood oxygen level dependent (BOLD) MR, and 10 subjects by arterial spin labeling (ASL) MR. I controlled both end-tidal pCO$_2$ and pO$_2$. I registered all functional data in Montreal Neurological Institute space and generated composite BOLD MR and ASL MR maps of cerebrovascular reserve. I compared these maps with frequency maps of leukoaraiosis published previously. Composite maps demonstrated significant (90% confidence interval excluding the value zero) steal phenomenon in the white matter. This steal was induced by relatively small changes in end-tidal pCO$_2$. It occurred precisely in those locations where elderly patients develop leukoaraiosis. This steal phenomenon likely represents the physiological correlate of the previously anatomically-defined internal border-zones. Spatial concordance with white matter changes in the elderly raises the possibility that this steal phenomenon may have a pathogenetic role.

5.2 Introduction

Since the advent of CT, physicians and researchers have noted the prevalence of abnormality in the white matter of elderly human brain. Characterized by patchy or diffuse low density on CT images, and corresponding hyperintensity on T2-weighted MRIs, this abnormality histopathologically represents rarefaction of myelin, loss of axons and oligodendrocytes, dilatation of perivascular spaces, and mild gliosis.(D. G. Munoz et al., 1993) It is simply called white matter disease, or leukoaraiosis,(Hachinski, Potter, & Merskey, 1986) literally meaning diminution of white matter density. Leukoaraiosis clusters in several locations: cigar-shaped bands in the deep white matter above the lateral
ventricles,(DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2005; Sachdev, Wen, Chen, & Brodaty, 2007) in the white matter around the occipital and frontal horns of the lateral ventricles,(DeCarli et al., 2005; Sachdev et al., 2007; Yoshita et al., 2006) in the genu and splenium of the corpus callosum,(DeCarli et al., 2005; Sachdev et al., 2007; Yoshita et al., 2006) and in the posterior limb of the internal capsule.(Sachdev et al., 2007) Prevalence increases with age with some degree of leukoaraiosis in more than half of those older than 60 years of age.(Launer, 2004) It was initially considered a benign age-related change, but more recent studies suggest it may be associated with cognitive dysfunction(de Groot et al., 2001) and the development of dementia.(Prins et al., 2004)

Despite growing appreciation of its clinical significance, the pathogenesis of leukoaraiosis is poorly understood.(Pantoni & Garcia, 1997) Evidence suggests an ischemic process,(David G. Munoz, 2006) but what causes the ischemia?

One theory is based on a concept of "internal border zones." Studying the patterns of white matter injury in gross pathological specimens of the human brain, Zulch(Wodarz, 1980) hypothesized that the borders between arterial supply territories are particularly vulnerable to injury. He described a border zone in the deep white matter above the lateral ventricles and one in the deep white matter below this level. Later studies(D. M. Moody et al., 1990) added a third border zone, in the periventricular white matter. Supplied by the longest arteries and arterioles, these zones may have relatively low perfusion pressure, yielding vulnerability to episodic reduction in systemic arterial blood pressure.(Pantoni & Pantoni, 2002) An argument against this theory is that, by
autoregulation of arterial and arteriole smooth muscle tone, the brain is able to maintain relatively constant cerebral blood flow (CBF) over a wide range of blood pressures (mean arterial blood pressure 50 to 150 mm Hg). (McHedlishvili, 1980)

A more widely accepted theory is that ischemia occurs secondary to (that is, requires) disease of the arterioles supplying the white matter, so-called "small vessel disease." This theory is based on an observed association between leukoaraiosis and small vessel disease. (van Swieten et al., 1991) It was initially hypothesized that arteriole stenosis causes chronic under-perfusion of downstream parenchyma, resulting in white matter injury. (van Gijn, 2000) More recent studies suggest that pathological stiffening of arterioles reduces their capacity for autoregulatory vasodilatation, making the brain vulnerable to episodic reductions in systemic arterial blood pressure. (Heistad, Mayhan, Coyle, & Baumbach, 1990) It is unknown whether small vessel disease temporally precedes leukoaraiosis. Also, only the minority of white matter lesions has spatially concordant small vessel disease. (M. I. Chimowitz et al., 1992; D. G. Munoz et al., 1993; Thomas et al., 2002)

Although it is true that the brain can autoregulate to keep flow constant despite reduction of blood pressure, autoregulatory capacity is not infinite. Once the cerebrovascular reserve is exhausted, further lowering of arterial blood pressure leads to a reduction of blood flow and risk of ischemia. (Powers, 1991) In normal subjects, if the arterial border zones of the white matter are continuously compensating for low perfusion pressure, then one might expect these regions to have reduced or exhausted cerebrovascular reserve.
Furthermore, even complete exhaustion of the cerebrovascular reserve is not the most hemodynamically vulnerable condition. Spatial heterogeneity of cerebrovascular reserve adds yet additional risk; an episode of hypotension will cause not only a direct reduction of blood flow in the region of exhausted reserve, but also autoregulatory vasodilatation in those parts of the brain with preserved reserve will cause redistribution of blood flow away from the region of exhausted reserve. (Furst et al., 1994) This is aptly called a steal phenomenon.

I hypothesized that there are regions of reduced cerebrovascular reserve, susceptible to steal phenomenon, in the white matter of young, healthy subjects, the physiological correlate of the anatomically defined internal border zones. Furthermore, I hypothesized that these zones spatially correspond with the regions where the elderly develop leukoaraiosis. I assessed cerebrovascular reserve by imaging the CBF response to a vasodilatory stimulus. I used blood oxygen level-dependent (BOLD) MR and arterial spin labeling MR to image blood flow and inhaled carbon dioxide (CO₂) as a vasodilatory stimulus. This approach has been described elsewhere. (Li, Kastrup, Takahashi, & Moseley, 1999)

5.3 Methods

Experiment 1

Subjects

There were 18 healthy volunteers who had no history of cardiovascular or neurological
disease. Age range was 22 to 42 years. There were 10 males and 8 females. The study was approved by the Ethics Review Board at the University Health Network, Toronto, and subjects consented to participating.

Magnetic Resonance Imaging

MRI was performed on a GE Signa 1.5 T scanner (GE Healthcare, Milwaukee, WI) with a single-channel head coil. I acquired T1-weighted anatomic images through the entire brain using a 3-dimensional spoiled gradient echo pulse sequence (slice thickness 2.2 mm, matrix size 256x256), then acquired BOLD MR data for the entire brain using a T2*-weighted single shot gradient echo sequence with spiral readout (TR 2240 ms, TE 40 ms, flip angle 85°, slice thickness 4.5 mm, field of view 20x20 cm, matrix size 64x64, 320 frames). Four identical BOLD MR acquisitions were performed for each subject.

End-tidal $pCO_2$ and $pO_2$ Manipulation

During the BOLD MR acquisitions, we alternated between high and low end-tidal partial pressure of carbon dioxide ($PETCO_2$) states using an automated gas sequencer (Gas Flow Sequencer Model GS01; Voltek Enterprises, Toronto, Canada), rebreathing circuit, mouthpiece, and nose clip. The gas sequence was: 8 periods of hypercapnia (45 seconds at $PETCO_2$=45 mm Hg, SD=1 mm Hg) interspersed with 8 periods of hypocapnia (45 seconds at $PETCO_2$=35 mm Hg, SD=1 mm Hg). Tidal $pCO_2$ was monitored continuously (Capnomac Ultima; Datex Corporation, Madison, WI), digitized, and recorded (LabView; National Instruments Corporation, Austin, Texas). The apparatus and technique are described in detail elsewhere. (Vesely et al., 2001)
**Data Analysis**

I imported the MR and PETCO$_2$ data into the software AFNI. (Cox, 1996) I viewed the first raw image of each BOLD MR acquisition and excluded those acquisitions (9 of 72) in which there was appreciable change in head position between the anatomic acquisition and the BOLD MR acquisition. Each BOLD MR acquisition was then temporally shifted to the point of maximum correlation with the subject’s PETCO$_2$ waveform. One can define a parameter called "cerebrovascular reactivity" (CVR) as the change in CBF per unit change in vasodilatory stimulus. I performed least squares fitting of the BOLD MR signal waveform to the PETCO$_2$ waveform on a voxel-by-voxel basis, and from the fitted data, I calculated percentage MR signal change per mm Hg PETCO$_2$ change on a voxel-by-voxel basis, that is, CVR. I then segmented the anatomic images into gray matter and white matter (SPM5; Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK), generated CVR maps containing only the brain parenchyma, and transformed these maps into Montreal Neurological Institute space. I generated mean intrasubject maps of CVR, then calculated the mean and SD of CVR across all 18 subjects on a voxel-wise basis. Using a t-distribution, I generated a 90% CI for the mean on a voxel-wise basis. Voxels with a 90% CI that did not include the value zero were deemed statistically significant for the directionality of BOLD MR signal change, that is, steal versus no steal. I plotted these composite maps of significant CVR as well as composite maps showing all voxels. I also calculated mean cerebrovascular reactivity for all gray matter and for all white matter.

**Experiment 2**
Theoretically, a negative BOLD MR signal response to hypercapnia could arise from decreased CBF, increased cerebral blood volume, increased cerebral metabolic rate of oxygen consumption, decreased arterial pO2, or spatial heterogeneity in the arrival of a pCO2 change at the brain. (Naganawa et al., 2002; Ogawa et al., 1993) Although empirical evidence suggests that the BOLD MR signal response to changes in pETCO2 is dominated by the CBF effect, (Shiino et al., 2003; Ziyeh et al., 2005) we felt it prudent to confirm these findings using a technique that is less dependent on these confounders.

I excluded arterial pO2 as a confounder by using a gas sequencer that can prospectively target and control PETO2 within a very narrow range (100 mm Hg, SD=2 mm Hg). (Slessarev et al., 2007)

The time delay between change in pCO2 in the pulmonary capillaries and arrival of this change in the cerebral circulation is spatially heterogeneous. If this relative difference in delay was large enough, then theoretically, the changes in arterial pCO2 in one region of the brain could be 180° out of phase with the changes elsewhere, mimicking a steal phenomenon. (Naganawa et al., 2002) I overcame this potential confounder by using non-periodic oscillation of the carbon dioxide stimulus (Figure 5.1).

Subjects

I studied an additional 10 healthy volunteers (age range, 29 to 40 years; 9 males).

MRI
MR imaging was performed on a Signa 3.0 T scanner (GE Healthcare, Milwaukee, WI) with eight channel phased array head coil. I acquired anatomical images as for the first experiment, and then acquired CVR data using an arterial spin labeling MR pulse sequence, specifically, a two-dimensional spin-echo flow-sensitive alternating inversion recovery (FAIR) sequence (Kim, 1995) with echo-planar readout (TR 2000 ms, TE 22.7 ms, TI 1000 ms, 5 axial slices, slice thickness 5.0 mm with 2.0 mm inter-slice gap, FOV 24 x 24 cm, matrix size 64 x 64, 345 frames). The FAIR technique has limited z-axis spatial coverage. I chose to acquire the FAIR slices through the white matter above the level of the lateral ventricles.

**Gas Manipulation**

During the FAIR MR acquisitions, I alternated between high and low PETCO₂ states using a more advanced version of the breathing apparatus used in the first experiment. Specifically, I used a computer-controlled gas blender (RespirAct; Thornhill Research, Toronto, Canada), sequential gas delivery mask (Hi-Ox-80 Viasys HealthCare, Yorba Linda, California), and rebreathing circuit. The gas sequence was: normocapnia normoxia (60 seconds at PETCO₂=40 mm Hg, SD=1 mm; PETO₂=100 mm Hg, SD=2 mm), hypercapnia normoxia (60 seconds at PETCO₂=50 mm Hg, SD=1 mm; PETO₂=100 mm Hg, SD=2 mm), normocapnia normoxia (100 seconds), hypercapnia normoxia (180 seconds), and normocapnia normoxia (110 seconds). Tidal pCO₂ and pO₂ were monitored continuously (RespirAct), digitized, and recorded (LabView; National Instruments Corporation). The apparatus and technique are described in detail elsewhere.(Slessarev et al., 2007)
Figure 5.1  End-tidal pCO₂ (green) and raw MR signal intensity (yellow) as a function of time for a representative single voxel with negative reactivity. The figure shows how use of non-periodic MR and pCO₂ waveforms enables confirmation that the observed reciprocal relationship between MR signal and pCO₂ is not simply due to a half-cycle temporal shift of the MR signal intensity waveform.

Data Analysis

Data analysis was identical to that for the first experiment with one exception: FAIR MR raw images underwent a preprocessing step. Specifically, I calculated the difference between each pair of FAIR MRIs on a voxel-wise basis and fit the resultant difference maps to the PETCO₂ waveform to calculate CVR.(Kim, 1995) One subject was excluded because of head motion.

5.4 Results

Experiment 1

Mean reactivity for all gray matter was positive: 0.12% SD 0.03% (percentage change in
BOLD MR signal intensity per mm Hg change in PETCO₂. Mean reactivity for all white matter was positive: 0.05% SD 0.01% (units as previously).

The composite BOLD MR CVR map (Figure 5.2A) showed several bilaterally symmetrical regions of negative reactivity (depicted in blue). There was negative reactivity in cigar-shaped bands in the deep white matter above the level of the lateral ventricles, in the white matter around the occipital horns of the lateral ventricles and to a lesser extent frontal horns, in the genu and splenium of the corpus callosum, and in the posterior limb of the internal capsule. The composite map of only significant reactivity (Figure 5.1B) showed persisting, although less marked, negative reactivity in the deep white matter, periventricular white matter, corpus callosum, and internal capsule (Figure 5.2B).

Experiment 2

The composite arterial spin labeling MR CVR map (Figure 5.3A) showed negative reactivity in the deep white matter above the level of the lateral ventricles, periventricular white matter, and corpus callosum—the same distribution as seen in the first experiment. The internal capsule could not be assessed because the arterial spin labeling MR pulse sequence has limited coverage in the craniocaudal dimension. Mean gray matter and white matter reactivity were both positive: 1.5% (SD, 0.4%) and 0.5% (SD, 0.2%), respectively (percentage change in CBF per mm Hg change in PETCO₂). The composite map of only significant CVR (Figure 5.3B) showed much less dramatic negative reactivity but still small foci of persisting negative reactivity in the deep white matter,
periventricular white matter, and corpus callosum (Figure 5.3B).

Figure 5.2 Composite BOLD MR map of cerebrovascular reactivity in young, healthy subjects (percentage change in MR signal intensity per mm Hg change in \( \text{PETCO}_2 \)) overlaid on anatomic images. The maps show all voxels (A) and only significant voxels (B). Negative reactivity is depicted in blue.
Figure 5.3 Composite arterial spin labeling MR map of cerebrovascular reactivity in young, healthy subjects (percentage FAIR MR signal change per mm Hg change in PETCO₂) overlaid on anatomic images. The maps show all voxels (A) and only significant voxels (B). Negative reactivity is depicted in blue. Note: absence of cerebrovascular reactivity data overlay for the posterior portion of the lower slices reflects the limited spatial coverage of the imaging technique rather than evidence of absent reactivity.

5.5 Discussion

Our results provide several new insights. First, I have identified regions of hemodynamic vulnerability in the white matter of young, healthy human subjects. The cerebrovascular response to hypercapnia (or potentially hypotension) may reduce blood flow to these regions in its attempt to maintain flow elsewhere in the brain. Second, I have shown that the observed steal phenomenon occurs with changes in pCO₂ and total CBF that are well within the range of total CBF autoregulation.(Grubb et al., 1974) That is, hemodynamic vulnerability may exist even within the range of blood pressure and arterial pCO₂ that is typically considered harmless. Third, I have shown that this steal phenomenon is clustered in those regions where elderly patients most frequently develop leukoaraiosis. Figure 4
compares the observed steal phenomenon (Figure 5.4A) with a composite map of leukoaraiosis published by Sachdev et al. (Sachdev et al., 2007) (Figure 5.4B). The map of leukoaraiosis was generated by imaging 55 community-dwelling elderly volunteers at an initial time and 3 years later and plotting regions that show significant increase in white matter hyperintensity over time.

Our results strengthen the idea that repeated episodes of mild hypercapnia, or potentially hypotension, may have a role in the pathogenesis of leukoaraiosis. Although I have used inhaled carbon dioxide as a research tool, hypercapnia occurs naturally as well. Arterial pCO$_2$ of healthy subjects varies by 4-10 mm Hg during waking hours (Crosby et al., 2003) and arterial pCO$_2$ increases by approximately 4 mm Hg during sleep (Shea & Shea, 1997). Among healthy adults, poorer respiratory function in midlife is associated with greater leukoaraiosis in later life (Shea & Shea, 1997). Similarly, the blood pressure of healthy subjects varies throughout the day (van Dijk et al., 2004) and postprandial hypotension (Kohara et al., 1999) orthostatic hypotension (Longstreth et al., 1996) and greater nocturnal fall in blood pressure (Kohara et al., 1999) are each associated with greater leukoaraiosis.

As well, hypercapnia and reduction of blood pressure are features of several disease states. Chronic obstructive pulmonary disease results in chronic elevation of pCO$_2$. It is associated with leukoaraiosis (Shea & Shea, 1997) and with cognitive deficit (Shim et al., 2001). Hypertensive disease deserves particular mention. Large randomized trials on the prevention of stroke, myocardial infarction, and other vascular events have led to a
widespread view that blood pressure should be kept as low as possible. However, some have cautioned that

![Composite BOLD MR map of cerebrovascular reactivity in young, healthy subjects (percentage change in BOLD MR signal per mm Hg change in PETCO2) (A) compared with composite map of leukoaraiosis in elderly subjects published by Sachdev et al(Sachdev et al., 2007) (B).](image)

Figure 5.4

antihypertensive medication may reduce CBF and lead to cognitive impairment.(Goodwin, 2003) This has led to hundreds of studies on the effects of antihypertensive medications on CBF. Notably, only a few of these studies report a medication-induced decrease in CBF.(Fagan, Payne, & Houtekier, 1989) However, my results suggest that this lack of evidence may reflect methodology rather than physiology. The majority of these studies assess total CBF. Some assess particular regions of brain, but these regions are nearly always large and contain both gray matter and white matter.
Our results define specific, relatively focal regions of white matter where one would expect to observe an antihypertensive medication-induced decrease in CBF.

Our results also have implications for BOLD MR mapping of CVR as a diagnostic tool. The transcranial Doppler, Xe-CT, single photon emission CT, and positron emission tomography literature shows that reduced cerebrovascular reserve, and especially steal phenomenon, is a strong and independent risk factor for ischemic stroke. Most of the stroke risk studies calculate mean reactivity for the entire middle cerebral artery territory (transcranial Doppler) or for each hemisphere (Xe-CT, single photon emission CT, positron emission tomography). The greater spatial resolution of BOLD MR offers the potential to identify more focal regions of steal phenomenon, but this also introduces the difficulty of differentiating normal white matter steal phenomenon from pathological steal.

Our results might be criticized on the grounds that both experiments demonstrated striking negative reactivity in the white matter, but the spatial extent of statistically significant negative reactivity was limited. This is a reasonable observation, but it should not affect the conclusions we draw from the study. First, the statistically significant negative reactivity was specifically clustered in those regions hypothesized to be negative a priori. Second, although I did transform all MR data into a standardized coordinate space, there is inter-subject variability in vascular anatomy and arterial border zones, (van der Zwan, Hillen, Tulleken, Dujovny, & Dragovic, 1992) for which I did not account. This would tend to blur relatively smaller regions of negative reactivity into the more abundant positive reactivity. It is thus likely that any observed common focus of
significant negative reactivity is an underestimation of the true spatial extent.

To date, there are only a few reports related to a white matter steal phenomenon. In 1977, a group studying the CBF response to hypotension in dogs unexpectedly observed a selective reduction of blood flow in their single white matter region of interest located in the deep white matter. (Mueller, Heistad, & Marcus, 1977) Five years later, a similar study revealed selective reduction of blood flow in 3 of 5 white matter regions assessed: a periventricular region, a deep white matter region, and a visual radiation region. (Young, Hernandez, & Yagel, 1982) There is one study (Naganawa et al., 2002) that provides evidence of a steal phenomenon in the white matter of normal human subjects. Measuring the CBF response to hyperventilation in several regions of interest in 6 normal subjects, the authors observed a selective increase in blood flow in their deep white matter region of interest. Given that hyperventilation is a vasoconstrictive rather than vasodilatory stimulus, one may consider their result a "reverse" steal phenomenon.

5.6 Conclusion

I have mapped a selective reduction of blood flow to white matter during mild hypercapnia in young, healthy human subjects. This steal phenomenon was induced by relatively small changes in PETCO₂. It occurred precisely in those locations where elderly patients develop leukoaraiosis. These results strengthen the evidence for a mechanism whereby repeated episodes of mild hypercapnia or hypotension may have a role in the pathogenesis of leukoaraiosis.
Chapter 6 – General Discussion

6.1 Summary

This thesis was precipitated by the question of whether a negative BOLD MR signal response to a CO\textsubscript{2} vasodilatory stimulus truly represents vasodilator-induced reduction of cerebral blood flow, that is, a steal phenomenon. I found that paradoxical reactivity on BOLD MRI CVR had a moderate predictive value for corresponding paradoxical reactivity on ASL MRI, and more generally, BOLD MRI CVR correlated well with ASL MRI CVR. These findings suggest that even in patients with steno-occlusive disease, the BOLD MR signal response to hypercapnia does predominantly reflect changes in CBF.

A second study tested the idea that BOLD MRI CVR expressed in absolute values provides clinically meaningful information. Specifically, I hypothesized that preoperative cerebral hemispheric CVR expressed in absolute values would predict the hemodynamic effect of EC-IC bypass surgery. The study involved twenty-five patients with intracranial arterial stenosis or occlusion. I found that patients with normal preoperative cerebral hemispheric CVR demonstrated no significant change in CVR following bypass surgery, those with reduced preoperative CVR demonstrated a significant improvement following bypass surgery, and those with paradoxical preoperative CVR demonstrated the greatest improvement. The results suggest that the CO\textsubscript{2} BOLD CVR technique is potentially useful for selecting patients for surgical revascularization.
The third study evaluated a noisy but consistent pattern of paradoxical reactivity I had noticed in the white matter of young healthy subjects. I had thought that these regions might be the physiological correlate of the anatomically defined white matter border-zones (watershed zones), and might spatially correspond with the regions where the elderly develop leukoaraiosis. I studied 18 healthy subjects using BOLD MRI CVR and 10 using ASL MRI CVR, transformed all CVR maps into a common brain space, and generated composite BOLD and ASL MRI maps of CVR. Composite maps demonstrated significant steal phenomenon in the white matter. This steal was induced by relatively small changes in end-tidal pCO₂. It occurred precisely in those locations where elderly patients develop leukoaraiosis. These results strengthen the evidence for a mechanism whereby repeated episodes of mild hypercapnia or hypotension may have a role in the pathogenesis of leukoaraiosis.

6.2 Broader Implications

The discussion sections of chapters three through five comprise the main discussion of results from this research, but there are a few broader implications:

What is Steal?

*Pressure, Resistance, and Flow at an Arterial Bifurcation*

Where an artery bifurcates, a difference in resistance between the branches will result in greater flow in the branch with lower resistance than the branch with higher resistance. This is analogous to electrical flow in a circuit. Ohm’s law states that ΔV = IR, where
ΔV is voltage drop, I is current, and R is resistance. Kirchhoff’s voltage law states that the sum of voltage drops around a closed circuit is zero. Combining these two laws:

\[(Q_{\text{branch}})(R_{\text{branch}}) = (Q_{\text{branch}}')(R_{\text{branch}}')\]

where Q is flow, R is resistance, and the subscripts “1” and “2” indicate the two different branches of a bifurcating artery (Figure 6.1). A change in resistance of one branch results in a redistribution of flow. This may manifest as redistribution of flow toward a low resistance lesion such as an arteriovenous malformation, or redistribution of flow away from a high resistance lesion such as an arterial stenosis. In addition to redistribution of blood flow between branches, a low or high resistance arterial lesion will also affect total blood flow through the supplying artery.

**Figure 6.1** Modeling an arterial bifurcation. There is a supplying artery (“1”) and two branches (“2” and “3”). Flow is denoted as Q and resistance as R. The pressure drop across this total system is labeled “delta pressure”.

Imagine a baseline condition of flow and resistance in the various components, and a second condition in which the branch labeled “2” has either a stenotic lesion or an arteriovenous shunt. I will denote this second condition by adding an asterisks to the flow
Using Ohm’s law and Kirchhoff’s law, one can derive the equations for flow in the supplying artery and branch arteries as a function of resistance in each of these arteries. Specifically, by simply rearranging these equations, one finds that:

\[
Q_1 = Q_1 \left( \frac{1}{R_1 + \left( \frac{1}{\frac{1}{R_2} + \frac{1}{R_3}} \right)} \right)
\]

\[
Q_2 = Q_2 \left( 1 + \frac{R_2}{R_3} \right) \left( \frac{1}{R_1 + \left( \frac{1}{\frac{1}{R_2} + \frac{1}{R_3}} \right)} \right) \left( \frac{1}{1 + \frac{R_3}{R_3}} \right)
\]

\[
Q_3 = Q_3 \left( 1 + \frac{R_3}{R_2} \right) \left( \frac{1}{R_1 + \left( \frac{1}{\frac{1}{R_2} + \frac{1}{R_3}} \right)} \right) \left( \frac{1}{1 + \frac{R_3}{R_2}} \right)
\]

These equations demonstrate that arteriovenous shunting (that is, decreased resistance) in a branch yields increased flow in the supplying artery (that is, increased total flow),
increased flow in the branch with arteriovenous shunting, and decreased flow in the other branch. Arterial stenosis in a branch will result in the opposite changes. These relationships are illustrated in Figure 6.2. In this figure, the intersection of the yellow and blue curves is the point at which the two branch have equal resistance, and therefore equal flow.

An important point is that resistance in the supplying artery is necessary for stenosis- or shunt-induced redistribution of flow. With a resistance of zero in the supplying artery, a change in the resistance of a branch affects flow through that branch and through the supplying artery, but does not affect flow in the other branch. This is shown in Figure 6.3.

**Figure 6.2** Flow proximal and distal to an arterial bifurcation as a function of varying resistance in one of the branches.
Figure 6.3  Flow proximal and distal to an arterial bifurcation as a function of varying resistance in one of the branches, for supplying artery resistance of zero.

Let us now add in the compensatory (autoregulatory) response that may accompany a shunting or stenotic lesion. For an arterial bifurcation with a shunting lesion in one branch, the reduction of flow in the other branch may elicit compensatory vasodilatation downstream from that branch, reducing resistance. According to Ohm’s law (Appendix, 9.1), this will lessen the shunt-induced redistribution of flow between the branches, reduce flow in the shunting branch, increase flow in the non-shunting branch, and overall increase flow, that is, increase flow in the supplying artery. For an arterial bifurcation with a stenotic lesion in one branch, the reduction in flow distal to the stenosis may elicit compensatory vasodilatation downstream from the stenosis, reducing resistance. This will lessen the stenosis-induced redistribution of flow between the branches, increase flow in the stenotic branch, reduce flow in the non-stenotic branch, and increase flow in the supplying artery.
Finally, consider the possibility of autoregulatory changes in the supplying artery. For an arterial bifurcation with stenosis in one branch, a compensatory decrease in resistance in the supplying artery will increase flow in the stenotic branch (and in the non-stenotic branch as well). For an arterial bifurcation with an arteriovenous shunting lesion, a compensatory decrease in resistance in the supplying artery will augment flow in the non-shunt branch, but also exacerbate the high flow in the shunting branch. Does such autoregulation exist? It has been hypothesized that regulation of resistance of the proximal brain-supplying arteries may have a role in reducing steal effects that would otherwise arise from normal neuronal activity-induced local vasodilatation in the brain. (Faraci & Heistad, 1990; Harel, Lee, Nagaoka, Kim, & Kim, 2002; Raichle, 1998)

The mechanisms via which distal changes in hemodynamics might result in compensatory changes in upstream arteries are incompletely understood. (Segal & Duling, 1986a, 1986b)

I have assumed that venous backpressure is negligible, and this is often true. However, patients with arteriovenous shunting lesions may develop venous hypertension, reducing the pressure drop across the arterial vasculature, and also influencing steal effects.

**Collateral Flow**

So-called subclavian steal is characterized by proximal stenosis of the subclavian artery, with reversed flow in the ipsilateral vertebral artery supplying the subclavian artery beyond the stenosis. This arrangement is reasonably described as a combination of an arterial stenosis and a collateral pathway of blood supply. Collateral pathways are parallel
supply systems which can replace one another in the event of a deficiency of blood supply. (Mosmans & Jonkman, 1980) Both anatomically and pathophysiologically, this configuration differs from the arterial bifurcation I have considered thus far. Anatomically, an example of collateral supply is a carotid artery occlusion with the ipsilateral middle and anterior cerebral arteries supplied from the contralateral carotid artery via the anterior communicating artery. Or considering only intracranial arteries, consider an anterior cerebral artery A1 segment occlusion with the ipsilateral A2 segment supplied via the anterior communicating artery from the left anterior cerebral artery A1 segment. This anatomical arrangement is schematized in Figure 6.4. The arrangement corresponds with the electrical circuit known as a Wheatstone bridge circuit.

Figure 6.4  Collateral supply. This simple example uses the normal anatomy of the anterior cerebral arteries to illustrate what is meant by a collateral supply pathway. Using the standard nomenclature. The proximal segment of each anterior cerebral artery is denoted as A1, the anterior cerebral arteries are joined by a bridging anterior communicating artery (ACOM), and the segment of each anterior cerebral artery distal to the ACOM is denoted as A2.

Again using Ohm’s law and Kirchhoff’s law, one can model flow in this circuit as a
function of resistance in the various components. We simulated this circuit (Figure 6.5) using the software LabVIEW (National Instruments, Quebec, Canada) and found that a stenotic lesion in one of the supplying arteries will result in a redistribution of flow from the tissue that is normally supplied via the collateral pathway toward the tissue that is downstream from the stenosis. There will be an increase in flow in the non-stenotic supplying artery, and a net decrease in flow in both of the branch arteries (Figure 6.6).

Figure 6.5  Modeling a collateral flow pathway. Two pathways (“1, 3” and “2, 4”) are connected by a bridge (“5”). As before, flow is noted as Q, resistance as R, and pressure drop across this total system is labeled “delta pressure”.
Figure 6.6  Flow in two supplying arteries (e.g. right and left A1 segments of the anterior cerebral arteries), bridge (e.g. anterior communicating artery), and two branch arteries (e.g. right and left A2 segments of the anterior cerebral arteries) as a function of increasing resistance in one of the supplying arteries. Direction of flow in the bridge artery is defined negative for flow from the side of supplying artery 1 to the side of supplying artery 2.

Symon (Symon, 1969) differentiates between “steal” and “collateral circulation” noting that in the latter, for example following occlusion of a major artery in the neck or at the level of the circle of Willis, flow will normally pass from the other cerebral vessels to fill the territory of the damaged artery. He states that there is no evidence in these circumstances that any portion of the brain is deprived of perfusion as a result of this collateral flow, and suggests that this hemodynamic readjustment of blood in contiguous vascular territories is more appropriately referred to as collateral circulation than as intracerebral steal. This interpretation is incorrect. As discussed above, “steal” is a hemodynamic concept and “collateral flow” is an anatomical (geometric) concept. These phenomena can exist together and they can each exist on their own.
**Asymptomatic Steal**

Some have suggested that steal should be defined as symptomatic vasodilator-induced reduction in blood flow. (Symon, 1969) How does one define symptomatic? Does one only include obvious symptoms of acute ischemia? It is possible that apparently asymptomatic steal is causing ischemia, but in a region of the brain (such as the parietal association areas) (Toole & McGraw, 1975) for which the ischemia may be unapparent on routine neurological examination. Similarly, chronic progressive tissue loss due to milder or smaller ischemic events may manifest as chronic symptoms such as dementia that will not manifest during the course of a brief vasodilatory challenge. Even more importantly, the presence or absence of vasodilator-induced symptoms depends on the magnitude of reduction in blood flow, which depends on the magnitude of the vasodilatory stimulus, for which there is no standard. For these reasons, it is problematic to limit the definition of steal to symptomatic vasodilator-induced reduction in blood flow.

**Victims and Thieves**

In Hunter’s original description of steal related to an antecubital arteriovenous fistula, (Pretnar-Oblak, Zaletel, Zvan, Sabovic, & Pogacnik, 2006) the patient’s forearm is a blood flow-deprived “victim”, but no single tissue bed is a clearly identifiable “thief”. In essence, the entire body is the thief. In the subclavian steal syndrome, the territory of the brain supplied by the posterior circulation is the victim, and the upper extremity ipsilateral to the stenosis is the thief.

The idea of steal occurring secondary to pharmacological vasodilatation complicates the
designation of a victim and a thief. Consider a brain arteriovenous malformation. In the resting state, blood flow is shunted from artery to vein. This shunted blood flow bypasses the brain parenchyma downstream from the supplying artery, but no neighboring region of parenchyma benefits from this shunt. That is, in the resting state, there is no identifiable thief. Let us now introduce a pharmacological vasodilator. The arterioles in the parenchyma downstream of the arteriovenous malformation may not dilate in response to the pharmacological vasodilatory stimulus as they have already exhausted their dilatory reserve, yet the global vasodilatory stimulus may induce vasodilatation in neighboring parenchyma and steal flow from the region of already poorly perfused brain. That is, in this case, an identifiable thief only emerges during pharmacological vasodilatation. This Symon-type pharmacologically induced steal is anatomically and pathophysiologically distinct from the co-existing Hunter-type steal directly attributed to the arteriovenous shunt.

Towards a Broader Definition of Steal

These various flow phenomena all reflect redistribution of flow at an arterial bifurcation due to a difference in vascular resistance between the branches. We cannot reasonably call some of these phenomena “steal” and others not based on a fundamental physiological difference. Rather, the difference between one type of steal and another arises from the broader context of the steal. For example, steal in a patient with a brain arteriovenous malformation likely differs in clinical significance from the deep white matter steal induced by inhalation of carbon dioxide in a healthy subject.
As the resistance of the brain-supplying arteries is distributed throughout the intracranial arteries and arteries of the neck, it follows that there are steal effects at each of the numerous bifurcations along the path from the aortic arch to the microcirculation of the brain. Every arterial bifurcation is point of potential redistribution of flow from one branch to the other. Each arterial segment that dilates or constricts more than some other segment causes a redistribution of blood flow. While some branch points may be greater redistributors than others, each bit of the brain is either a thief or victim relative to every other bit of the brain. Extending this to MRI, every voxel of the brain is a victim and a thief.

The Anatomical Basis of Spatially Heterogeneous Vascular Reserve

That the brains of healthy subjects have regions of higher and lower cerebrovascular reactivity has been well known. This thesis defines these regions with considerably higher spatial resolution than previously described, and adds the insight that some regions have not only lower reactivity, but also negative reactivity. What is the pathophysiological basis of spatial variation in cerebrovascular reactivity? The relationship between CVR and CBF is given by:

$$CVR = \frac{CBF_{\text{hypercapnia}} - CBF_{\text{normocapnia}}}{CBF_{\text{normocapnia}}}$$

The CBF terms depend on cerebral perfusion pressure and vascular resistance. Vascular resistance depends on vessel radius, which is not fixed, and on parameters such as vascular density and vessel length which are relatively fixed. Regions of the brain that are
further from the supplying arteries will have lower perfusion pressure, and might maintain local flow by reducing resistance. Resistance could be decreased by increasing vascular density in these regions, by vasodilatation, or by an anatomic or metabolic adjustment in these regions such that vessel radius is increased but vasodilatory reserve is preserved. Although the first and last options may seem intuitively likely as they avoid exhaustion of vasodilatory reserve, the finding that vascular reserve is exhausted in regions such as the deep white matter suggest that vasodilatation may be an important mechanism for maintaining flow in the so-called watershed zones of the brain. That is, spatial heterogeneity of CVR may reflect spatial heterogeneity in perfusion pressure. This could be explored further.

A Broader Comment on the Pathogenesis of Leukoaraiosis

I would like to briefly note a paradox that I believe is not yet mentioned in the literature. We may hypothesize that small vessel stenosis or stiffening results in hemodynamic impairment that leads to leukoaraiosis. However, several groups have demonstrated that there is no association, (Adachi, Takagi, Hoshino, & Inafuku, 1997; Altaf et al., 2008; Streifler et al., 1995) or only a weak association, (Saba, Sanfilippo, Pascalis, Montisci, & Mallarini, 2009) between carotid artery stenosis and leukoaraiosis. If small vessel hemodynamic impairment were important in the pathogenesis of leukoaraiosis, wouldn’t one expect that further hemodynamic impairment arising from the large arteries would make the leukoaraiosis worse?

Rethinking “Watershed” Ischemia
The word “watershed” is derived from the German phrase “die letzten wiesen.” This was an agricultural term used to describe a region of land dividing two areas drained by different water systems. The literal translation into English is “the last field,” the last region to drain. In medicine, the term has come to have a slightly different meaning, referring to the regions furthest from the supply of blood rather than furthest from the point of drainage. It is generally understood in medicine that a “watershed” zone is the area with the lowest perfusion pressure, so most vulnerable to a further reduction in perfusion pressure. However, this widely understood concept oversimplifies the underlying pathophysiology. It is not simply that the most distal zones of the brain have the lowest baseline perfusion pressure and therefore exhaust their vasodilatory capacity first, and reach their ischemic threshold first. Rather, even in healthy brain, these zones start with the lowest perfusion pressure, and in response to a further reduction in perfusion pressure, their reduced vascular reserve may make these regions susceptible to steal from regions of the brain with previously intact vasodilatory capacity. So-called watershed ischemia is likely a dynamic phenomenon in which low perfusion pressure in the resting state is exacerbated by dynamic steal effects that occur when perfusion pressure is further reduced by factors such as systemic arterial hypotension or thromboembolic arterial occlusion.
Chapter 7 – Conclusions

7.1 Conclusions

This thesis provides several new insights. First, in patients with stenosis or occlusion of brain-supplying arteries, the BOLD MRI signal response to hypercapnia predominantly reflects changes in CBF. This result contributes to the validation of BOLD MR mapping of CVR. Second, CVR measured in absolute values using CO$_2$ BOLD MRI identifies those patients with intracranial steno-occlusive disease who are more likely to have a hemodynamic improvement following surgical revascularization. Third, and perhaps most importantly, I have demonstrated that there are zones of negative cerebrovascular reactivity in the white matter of young, healthy human subjects. This steal phenomenon may be induced by relatively small changes in PETCO$_2$. It occurs precisely in those locations where elderly patients develop leukoaraiosis.
Chapter 8 – Future Directions

A major focus of this thesis is the application of BOLD MR measurement of CVR in patients with steno-occlusive disease of the brain-supplying arteries (Chapters 3 and 4). I intend to continue working on the clinical implementation of BOLD MRI CVR, and sections 8.1 and 8.2 describe the next steps. The component of this thesis on steal phenomenon in healthy subjects (Chapter 5) has piqued my interest in determining the pathogenesis of leukoaraiosis. I will investigate the time-course of individual lesions in leukoaraiosis using MRI techniques, and section 8.3 describes my plan. In parallel with my thesis work on cerebrovascular reserve, I have been studying intracranial arterial disease using high-resolution contrast-enhanced vessel wall MRI. I intend to carry this work forward with a project on arterial wall MRI in patients with migraine. This is described in section 8.4.

8.1 Clinical Implementation of CO2 BOLD MRI CVR

As discussed in chapter 1, there are several prospective studies that have demonstrated that CVR stratifies patients with both asymptomatic and symptomatic carotid artery disease into those at high versus low risk of future ischemic events. However, there is a lack of randomized controlled trials demonstrating that the use of CVR in therapeutic decision-making yields better outcomes. The closest to a clinical trial of CVR is the Carotid Occlusion Surgery Study (COSS), which used PET measurement of oxygen extraction fracture to assess hemodynamic impairment. The study aimed to determine whether the addition of EC-IC bypass surgery to medical therapy reduces the risk of
ipsilateral stroke in patients with a recent ischemic stroke, ipsilateral carotid occlusion, and increased oxygen extraction fraction. The study was stopped early (on June 24, 2010) due to a lack of difference in outcome between the two patient groups. Even if a technique is effective at identifying a high-risk subgroup of patients, one needs a treatment that adequately reduces the risk in this subgroup.

The CO₂ BOLD MR CVR technique is promising as it uses an MR pulse sequence that is widely available, the scan time is short, the study is safe, and one can acquire routine MR imaging and MR angiography in the same imaging session. Trial data is needed to establish the clinical role of the technique, but there are several other important tasks remaining such as:

- Determining whether a CO₂ stimulus normalized to the subject’s resting end-tidal pCO₂ provides a more meaningful measure of CVR than using the same CO₂ stimulus for all subjects

- Optimizing the CO₂ stimulus waveform to maximize tolerability while still achieving an adequate magnitude of vasodilatation.

- Determining the reproducibility of CO₂ BOLD MR CVR across different scanners and institutes. Does T2*- mapping improve reproducibility compared with T2*-weighted imaging?

- Characterizing the potential for CO₂-induced systemic arterial hypertension as a confounding factor in measurement of CVR

- Characterizing the relationship between head motion and measured CVR
• Reviewing our CVR database to characterize the safety, tolerability, and feasibility of CO₂ BOLD MR CVR

8.2 Risk Stratification in Patients with Asymptomatic Carotid Artery Stenosis Using BOLD MR Measurement of Cerebrovascular Reserve

Background and Significance

Ischemic stroke is the third most common cause of death in industrialized nations, and carotid artery atherosclerosis accounts for 15-20% of all ischemic strokes. The majority of these strokes occur without warning, that is, without premonitory transient ischemic attacks (TIA). However, nearly all of these patients had a detectable asymptomatic carotid lesion prior to presentation with an ischemic stroke. Large randomized controlled trials have shown that carotid endarterectomy results in a significant reduction in stroke risk in patients with asymptomatic carotid artery stenosis. ("Endarterectomy for asymptomatic carotid artery stenosis. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study," 1995; Halliday et al., 2004) However, given the large number of patients needed to treat (NNT) to prevent 1 event, ("Endarterectomy for asymptomatic carotid artery stenosis. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study," 1995; Halliday et al., 2004) the cost-effectiveness of this approach has been questioned. (Naylor, Gaines, & Rothwell, 2009) The high NNT reflects the low (1-2%) annual risk of ipsilateral stroke in patients with asymptomatic carotid...
artery stenosis. (Abbott, 2009; Marquardt, Geraghty, Mehta, & Rothwell, 2010; Spence et al., 2010) The cost-effectiveness and risk-to-benefit ratio of carotid endarterectomy would be greatly improved it were possible to identify the subset of patients with asymptomatic carotid artery stenosis who are at highest risk of stroke.

Current standard-of-care characterization of carotid artery stenosis is NASCET (Fox, 1993)-type measurement of the degree of luminal narrowing. Luminal narrowing is somewhat related to the volume of atherosclerotic plaque in the arterial wall. Larger plaque is more likely to have features, such as intraplaque hemorrhage, which are associated with increased risk of plaque rupture and embolization. Luminal narrowing can also cause a reduction in cerebral perfusion pressure, that is, exert a hemodynamic effect. There is evidence that embolic and hemodynamic factors interact in the pathogenesis of ischemic stroke, (Caplan, Hennerici, Caplan, & Hennerici, 1998) suggesting that evaluation of both factors may be useful; however, percentage luminal narrowing is not particularly indicative of either factor. Atherosclerotic plaque causes preferential expansion of the outer surface of the carotid artery wall, so luminal narrowing is a poor indicator of plaque volume. (Glagov, Weisenberg, Zarins, Stankunavicius, & Kolettis, 1987) Similarly, there is marked variability in collateral blood supply to the brain, and an identical degree of carotid stenosis in two different patients can have dramatically different effects on cerebral hemodynamics.

Measurement of cerebrovascular reactivity may enable risk stratification in patients with asymptomatic carotid artery stenosis. Using an inhaled carbon dioxide stimulus and
transcranial Doppler (TCD) ultrasound to measure changes in cerebral blood flow, Silvestrini et al. (Silvestrini et al., 2000) categorized 94 patients with asymptomatic carotid artery stenosis as having normal versus reduced CVR, and then followed the patients clinically for 2 years. The annual rate of ipsilateral TIA or stroke was 4% in patients with normal CVR at entry, compared with 14% in patients with reduced CVR. Using a similar technique, Markus et al. (Markus & Cullinane, 2001) reproduced these results. Using an intravenous acetazolamide (Diamox) vasodilatory stimulus, Gur et al. (Gur, Bova, & Bornstein, 1996) had similar findings. The TCD CVR technique is inexpensive and non-invasive, but it also highly operator dependent, and diagnostic accuracy may be limited. (Pindzola et al., 2001)

The primary aim of this study is to evaluate the possibility of using CO$_2$ BOLD CVR to identify a high-risk subset of patients with asymptomatic carotid artery stenosis. Specifically, I hypothesize that patients with asymptomatic 50-99% carotid artery stenosis and reduced CVR in the ipsilateral cerebral hemisphere are more likely to have subsequent ipsilateral ischemic events than patients with normal cerebrovascular reactivity.

The secondary aim of this study pertains to characterization of the underlying atherosclerotic plaque. Takaya et al. (Takaya et al., 2006) conducted a prospective cohort study of carotid vessel wall MRI using a dedicated surface coil in asymptomatic patients with 50-79% carotid artery stenosis. The study found that thin or ruptured fibrous caps, intraplaque hemorrhage, and larger maximum ratio of lipid rich component of the plaque
to the necrotic core were associated with increased risk of subsequent ipsilateral cerebral ischemic events. Singh et al. (Singh et al., 2009) performed a retrospective analysis of asymptomatic patients with carotid artery stenosis who had undergone carotid vessel wall imaging using a standard neurovascular coil and a single quick MR sequence to detect intraplaque hemorrhage. This study similarly found that presence of intraplaque hemorrhage was associated with subsequent ipsilateral ischemic events (hazard ratio, 3.59; 95% confidence interval: 2.38, 4.71; P<.001). Does the high-risk population identified using vessel wall MRI correspond with the high-risk population identified by measuring cerebrovascular reserve?

The proposed investigation is unique. We will conduct a prospective cohort study of asymptomatic patients with carotid artery stenosis to determine whether CO₂ BOLD CVR can identify a subgroup with increased risk of ischemic stroke. Additionally, I will determine whether the subgroup of patients with reduced CVR includes the same patients as the subgroup with high-risk plaque identified using vessel wall MRI. Results from this study may enable a randomized controlled trial comparing carotid endarterectomy to medial therapy alone in patients with asymptomatic carotid artery stenosis, using CO₂ BOLD CVR and or vessel wall imaging to guide patient selection.

In preparation for this prospective trial, I have recently performed a retrospective cohort study to determine whether CO₂ BOLD CVR differs between asymptomatic and symptomatic patients with carotid artery stenosis. I analyzed 51 consecutive patients with carotid artery stenosis or occlusion who underwent CO₂ BOLD MR mapping of
CVR between January 2006 and November 2011. Carotid stenosis was categorized as mild (<50%), moderate (50-70%), severe (>70%), near-occlusion, or occlusion. Cerebral hemispheres were categorized as symptomatic if there was clinical or MRI evidence of a TIA or stroke occurring in the 6 months preceding the CVR study. CVR was calculated as \( \% \Delta \text{BOLD} \) MR signal intensity per mm Hg \( \Delta \text{PETCO}_2 \) on a voxel-wise basis. I segmented the middle cerebral artery territory cortex using an automated algorithm, and calculated mean CVR for this segment in each cerebral hemisphere. There was a trend toward lower CVR in symptomatic patients relative to asymptomatic patients in the severe stenosis (0.15 versus 0.21; \( P=0.077 \)) and near occlusion (0.11 versus 0.18; \( P=0.093 \)) groups. There was insufficient data to compare symptomatic and asymptomatic patients in the 50-69% stenosis category. There was significantly lower CVR in symptomatic patients relative to asymptomatic patients in the carotid occlusion group (0.03 versus 0.10; \( P=0.018 \)). This pilot data is limited by relatively small sample size in each stenosis category; however, the relatively low \( P \) values in both the severe and near-occlusion categories suggest that using CO\(_2\) BOLD CVR may be useful for prognostication in asymptomatic patients with >50% carotid artery stenosis.
Figure 8.1  Relationship between CO2 BOLD CVR and degree of carotid artery stenosis for asymptomatic versus symptomatic patients. Boxes are bounded by the 25th and 75th percentiles, and whiskers mark the 10th and 90th percentiles. (Unpublished pilot data)

MR Detection of Carotid Intragraft Hemorrhage

Our team has extensive clinical experience with carotid vessel wall MRI. We routinely evaluate for carotid intraplaque hemorrhage using the three-dimensional fat-suppressed T1-weighted MR pulse sequence described in the asymptomatic carotid artery stenosis study by Singh et al. (Singh et al., 2009) This sequence is quick (4 minutes) and the three-dimensional nature enables multiplanar reformatting of the image data. Images are interpreted as positive or negative for evidence of intraplaque T1-hyperintensity that is, intraplaque hemorrhage. The relationship between intraplaque T1-hyperintensity and hemorrhage has been validated histopathologically. (Bitar et al., 2008) A representative case from our center is shown in Figure 6.
Figure 8.2  Coronal 3D fat suppressed T1-weighted vessel wall sequence using a standard neurovascular coil demonstrates hyperintensity in the arterial wall at the right common carotid artery bifurcation (long arrow) consistent with intraplaque hemorrhage. There is no evidence of intraplaque hemorrhage on the left side (short arrow).

Specific Aims

Long-term objectives are

(i) to clinically implement an MR tool for the identification of patients with asymptomatic carotid artery stenosis who have a high risk of ischemic stroke

(ii) to understand the relationship between embolic and hemodynamic factors in the pathogenesis of carotid atherosclerosis-related ischemic stroke

Specific aims are

(1st) to determine whether cerebrovascular reactivity measured using a precisely controlled hypercapnic stimulus and blood oxygen level-dependent (BOLD) MR identifies a higher risk subgroup among patients with asymptomatic carotid artery stenosis
to determine whether carotid vessel wall imaging and cerebrovascular identify the same or different high risk groups among patients with asymptomatic carotid artery stenosis

Hypotheses:

(I) Patients with asymptomatic 50-99% carotid artery stenosis and reduced cerebrovascular reactivity in the ipsilateral cerebral hemisphere are more likely to have subsequent ipsilateral ischemic events than patients with normal cerebrovascular reactivity

(II) The subgroup of asymptomatic carotid artery stenosis patients who have reduced cerebrovascular reactivity will be significantly different in composition from the subgroup of patients with vessel wall MRI evidence of carotid intraplaque hemorrhage

Methods

Experimental Design: This is a prospective cohort study of asymptomatic patients with moderate or severe carotid artery stenosis. At entry, we will categorize subjects as having normal versus reduced CO\textsubscript{2} BOLD CVR, and presence versus absence of vessel wall MRI evidence of intraplaque hemorrhage. We will follow the subjects clinically for 2 years for a primary outcome of TIA or stroke.

Patient Recruitment: A research coordinator will work with University Health Network
ultrasound clinic staff to identify patients who meet the following inclusion criteria: (i) 50-99% carotid artery stenosis, (ii) asymptomatic for at least 6 months. Patients with prior ipsilateral carotid endarterectomy or stenting will be excluded.

_Clinical Evaluation:_ All subjects will be seen by a research coordinator and physician in the hospital stroke clinic at the time of recruitment. Subject age, sex, neurological and cardiovascular history, vascular risk factors, and medication history will be recorded. The physician will perform a cardiovascular and neurological physical exam. Routine blood work including lipid profile will be obtained. All subjects will receive optimized medical management according to current guidelines.

_MRI Imaging:_ MRI will be performed on a GE 3-Tesla MR system (Signa HDx; GE Healthcare, Milwaukee, WI) using a neurovascular phased array for signal reception. The MR protocol will include: (i) routine T1 and T2-weighted, gradient echo, and diffusion-weighted brain sequences, (ii) T1-weighted three-dimensional spoiled gradient echo sequence to acquire high-resolution anatomical images for co-registration with the CVR map, (iii) T2*-weighted (“BOLD”) single-shot gradient echo echo-planar acquisition during controlled changes in PETCO₂ over 8 minutes for collection of CVR data, (iv) three-dimensional fat-suppressed T1-weighted spoiled gradient echo neck sequence optimized for detection of intraplaque hemorrhage, and (v) a gadolinium-bolus MR angiogram from the aortic arch through the circle of Willis to confirm the degree of stenosis observed in the ultrasound clinic. The vasodilatory stimulus(Slessarev et al., 2007, BOLD MR sequence(#1325), and vessel wall sequence(A. R. Moody et al., 2003)
are described in detail elsewhere.

**MR Post-processing:** MRI and PETCO$_2$ data is imported into the functional neuroimaging software package AFNI,(Cox, 1996) Total BOLD MR signal intensity is calculated for each whole brain volume, yielding a whole brain signal intensity waveform with 240 time points and a total duration of 480 seconds. This waveform is temporally shifted to the point of maximum statistical correlation with the PETCO$_2$ waveform. The BOLD MR waveform is then regressed (least squares) against the PETCO$_2$ waveform on a voxel-by-voxel basis. The slope of the regression, expressed as $\%\Delta$BOLD MR signal per mm Hg $\Delta$PETCO$_2$, is CVR. This analysis is described in detail elsewhere.(Mandell et al., 2011) Anatomic images are automatically segmented into grey matter and white matter, and then segmented further into anterior, middle, and posterior cerebral artery territories using an atlas-based automated algorithm. Mean CVR is calculated for the grey matter ipsilateral to the carotid artery stenosis. Using normative data,(Han et al., 2009) and defining reduced CVR as two standard deviations below the mean for healthy subjects, our thresholds for reduced CVR is $< 0.16\% \Delta$MR signal intensity per mm Hg $\Delta$PETCO$_2$. Our research group is experienced at acquisition and post-processing of imaging data, and none of the software used for data analysis is proprietary or to be developed. The vessel wall MR images will be interpreted as positive or negative for intraplaque hemorrhage,(Bitar et al., 2008) by an experienced neuroradiologist who is blinded to all other clinical and imaging data.

**Clinical Follow-up:** All subjects will be followed-up by telephone every 3 months by a
research coordinator who is blinded to the side and degree of stenosis, and blinded to the imaging results. Subjects will be seen in clinic every 6 months by a physician who is similarly blinded. Clinical follow-up will continue for 2 years after the initial 1-year subject accrual period.

Statistical Analysis: The annual rate of end points will be calculated using the person-year method. The Kaplan-Meier method will be used to plot and compare cumulative hazards for two CVR subgroups and the two vessel wall MRI subgroups, and Cox regression analysis will be performed to identify which factors could be considered independent predictors of ipsilateral ischemic events. Hazard ratios and 95% confidence intervals will be reported to indicate effect size. A Cohen’s Kappa test will be performed to evaluate for agreement between the high-risk subgroups identified using cerebrovascular reactivity versus vessel wall imaging.

Sample Size Calculation: In a prospective study of asymptomatic patients with severe (>70%) carotid artery stenosis using TCD, Silvestrini et al. (Silvestrini et al., 2000) found that 42.6% of patients had reduced CVR, and the annualized event rate was 13.9% among those with reduced CVR compared with 4.1% among those with normal reserve. We are including patients with moderate (50-69%) and severe (70-99%) stenosis, so the proportion of subjects with reduced CVR will likely be lower. Using an estimate of 25% of subjects in the reduced CVR group, event rates similar to Silvestrini et al. (Silvestrini et al., 2000), and subject accrual during the first year of the study, a one-sided logrank test with an overall sample size of 86 subjects (64 in the control group and 22 in the
treatment group) achieves 80.1% power at a 0.050 significance level. Using a more conservative total annualized event rate of 4%, (Abbott, 2009) with corresponding distribution of events between the normal and reduced CVR groups, a one-sided logrank test with an overall sample size of 158 subjects (90 in the control group and 68 in the treatment group) achieves 80.0% power at a 0.050 significance level.

Potential Pitfalls and Alternative Approaches

**Difficulty recruiting subjects**

The ultrasound clinic at the University Health Network performs carotid Doppler ultrasound screening of a high volume of patients with peripheral vascular disease. Based on the high prevalence of 50-99% carotid artery stenosis in this patient population, it is likely that enrolment will not be a problem. As well, our radiology group has a collegial relationship with the ultrasound divisions at two other large academic hospitals in Toronto. In the unlikely event that the recruitment rate at the University Health Network is lower than expected, expansion of recruitment to these additional sites is feasible.

**Lower than expected event rate**

I based the first sample size calculation on data from Silvestrini et al. (Silvestrini et al., 2000) This study only recruited patients with severe (>70%) stenosis. The 5-year stroke rate in patients with asymptomatic carotid artery stenosis varies with degree of stenosis: 12.9% for 50-59% stenosis, 14.8% for 60-74% stenosis, 18.5% for 75-95% stenosis, 14.7% for 95-99% stenosis. (Inzitari et al., 2000) Based on these results, we may expect a slightly lower event rate than Silvestrini et al. (Silvestrini et al., 2000) I therefore
performed a second sample size calculation based on a much more conservative event rate, and we will recruit based on this latter sample size. In the event that the event rate is still much lower than estimated, resulting in inadequate power, we will still be able to analyze the relationship between degree of luminal narrowing, presence versus absence of carotid intraplaque hemorrhage, and cerebrovascular reserve. This may provide new insight into the pathogenesis of ischemic stroke.

8.3 Understanding the Pathogenesis of Leukoaraiosis

Background and Significance

Despite growing appreciation of its clinical significance, the pathogenesis of leukoaraiosis is poorly understood. (Pantoni & Garcia, 1997) I intend to better understand the cause of leukoaraiosis by performing an in vivo detailed examination of the evolution of the individual lesions making up leukoaraiosis.

It has been suggested that a reduction in cerebral autoregulatory capacity, reflecting penetrating artery disease with arterial stenosis or stiffening, is the most likely cause of leukoaraiosis. (Pantoni & Pantoni, 2002) One can assess autoregulatory capacity at the tissue level by measuring CVR, the change in cerebral blood flow induced by an exogenous vasodilatory stimulus. Using BOLD MRI to map CVR, I have recently shown that regions of lowest CVR in young healthy adults spatially correspond with the locations where leukoaraiosis most frequently occurs in the elderly. (Mandell, Han, Poublanc, Crawley, Kassner, et al., 2008) In individual subjects, does regional exhaustion
of CVR predict subsequent development of leukoaraiosis?

Transverse relaxation time (T2) is a MRI parameter that primarily reflects concentration of free water in tissue. Leukoaraiosis is associated with increased free water content, and so quantitative mapping of T2 can provide an index of white matter injury for each voxel of the brain over serial MRI studies. Population studies have described the progression of leukoaraiosis between two time point years apart; however, the more detailed time scale of development of single lesions making up leukoaraiosis is unknown. (D. G. Munoz et al., 1993) Do these lesions develop over hours-days as one expects for an acute ischemic injury, or over months? Is there temporal clustering of the development of new lesions to suggest some systemic factor (such as transient hypotension or hypercoagulable state) is important? Or do single lesions arise randomly over time, suggesting a pathogenesis such as isolated independent microvascular events?

T2 mapping will provide detailed examination of time course of development of new lesions. Diffusion tensor imaging (DTI) will potentially improve pathological specificity. A transient focal reduction in mean diffusivity (mD) is seen with acute ischemic injury. Occasionally, clinical imaging reveals a focus of reduced diffusion in the white matter of a patient with leukoaraiosis. However, evidence of a focal ischemic injury at one point in time on clinical studies is insufficient to conclude that a more extensive white matter abnormality is of similar etiology. Reduction of diffusivity persists for 7-10 days after an acute ischemic event. Serial MRI with a time interval of 1 week between studies will enable identification of acute ischemic injuries that correspond temporally and spatially
with development of new white matter lesions on T2 maps.

The proposed study is unique. I will apply both well-known (T2 relaxometry, DTI) and novel (cerebrovascular reactivity) functional MR techniques to investigate the pathogenesis of leukoaraiosis. Furthermore, I will exploit the non-invasive and non-ionizing nature of MRI to investigate this disease process in vivo on a time scale not previously explored. Studies in the last decade have shown that leukoaraiosis is far from an incidental and harmless consequence of aging. The association with cognitive dysfunction has been convincingly demonstrated, yet the pathogenesis remains unknown. The proposed study has potential to truly advance our understanding of leukoaraiosis and dementia.

**Specific Aims**

The aims of the this project are to determine

(i) if regional exhaustion of autoregulation in normal-appearing white matter predicts subsequent development of leukoaraiosis.

(ii) the temporal evolution of individual white matter lesions in leukoaraiosis.

(iii) if development of new white matter lesions corresponds temporally and spatially with acute ischemic events on diffusion tensor imaging.

Hypotheses:

(I) Regions of normal-appearing white matter but with reduced
cerebrovascular reactivity (CVR) will be more likely to develop leukoaraiosis than regions of white matter with normal CVR.

(Ia) Individual white matter lesions develop over one week or less, rather than over months or years.

(Ib) White matter lesions arise in temporal clusters rather than as temporally random events.

(III) New white matter lesions correspond temporally and spatially with transient reduction in mean diffusivity.

Research Design

I plan to recruit subjects over the age of 60 who are being followed clinically for non-disabling ischemic events and have had MRI and either MRA or CTA work-up. All subjects will undergo baseline MR mapping of cerebrovascular reactivity and T2. A subset of subjects will undergo weekly mapping of T2 and mean diffusivity for the next 16 consecutive weeks. Close spacing of the MR sessions with mean diffusivity measurements is required as reduction in diffusivity persists for only 7-10 days after an acute ischemic event. All subjects will undergo follow-up T2 mapping at 1 year. Image data from the multiple time points will be spatially co-registered for each subject. I will perform automated segmentation of leukoaraiosis lesions on the initial and final scans to identify normal white matter that converts to abnormal white matter. Feed backward data analysis will determine the 16-week time course of changes in T2 and mean diffusivity for each new lesion, and feed forward analysis will determine the fate of regions of lowest baseline white matter CVR.
**Patient Recruitment:** A research coordinator will work with Neurology clinic staff to identify patients who meet the following criteria: Inclusion: over the age of 60, recent (6 months) MRA/CTA, MRI white matter disease burden > Fazekas grade 2. Exclusion: Unsafe for 3T MRI, cortical infarct > 2 cm, cardioembolic disease, dissection, pulmonary disease, carotid artery stenosis > 70%.

**Imaging:** Studies will be performed on a GE 3T MRI system. At the first imaging session, I will record patient demographics, cerebrovascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, smoking), and medications. The initial MRI session will include BOLD MRI mapping of CVR, 3D SPGR sequence for co-registration and segmentation, DTI sequence (23 directions) for mean diffusivity measurement, multi-echo (6) T2 sequence and T2-weighted images for automated segmentation of white matter lesions and for T2 relaxometry. The subset of subjects undergoing 16 consecutive weeks of imaging will have 3D SPGR, DTI, and multi-echo T2 sequences weekly, and these same sequences will be performed for all subjects at the 1-year follow-up session. Total imaging time will be 20:36 minutes per session, with an additional 7 minute acquisition at the first session. For quality assurance, a water phantom will be scanned using the same DTI sequence every week, and we will calculate the mean value of mD each week to assess for drift over time.

**Data Analysis:** Data from the initial imaging session will be processed to yield a whole brain CVR map. Gray matter/white matter/CSF segmentation and masking will provide a
white matter map of CVR. The multi-echo T2 series will be used to generate mono-exponential T2 relaxation maps. DTI data will be processed to provide mean diffusivity maps. Software used for these analyses include AFNI (http://afni.nimh.nih.gov/afni) and FSL (http://www.fmrib.ox.ac.uk/fsl/) operating in a Linux environment.

The first phase of the analysis will assess conversion of pre-existing normal white matter to abnormal white matter, testing the hypothesis (hypothesis 1) that the conversion occurs much more frequently in regions with reduced CVR on the initial MRI exam. The positive and negative voxels on the initial MRI study will serve as reference for observing this conversion through measurement of changes in T2 calculated from spatially co-registered baseline and final MRI exams. The possible parametric association between degree of CVR impairment and ∆T2 will also be tested to determine if conversion occurs more frequently in areas of more severely impaired CVR.

The second phase of the analysis will assess rate of development of individual white matter lesions, testing the hypothesis (hypothesis 2a) that conversion occurs over a time course of one week or shorter. For each new lesion that arises, I will calculate total change in T2 in this region between initial and final MRI studies, and then calculate the time span over which the central 90% of this signal change occurred. I will also test the hypothesis (hypothesis 3a) that white matter lesions arise in temporal clusters by calculation of the total number of new lesions per 16 weeks for each patient, and determining whether the number of new lesions in any individual week exceeds statistical expectation for random distribution over time.
Finally, I will test the hypothesis (hypothesis 3) that new white matter lesions correspond temporally and spatially with transient reduction in mean diffusivity. This will involve calculating mean diffusivity for each new lesion on the week at which it first appeared and the week prior, and determining whether either of these values is significantly lower than the mean ‘mean diffusivity’ for this region of white matter across all earlier time points.

Sample Size Considerations: In longitudinal studies, Taylor et al. (Taylor et al., 2003) found a WMH volume of 4.91 (±7.01) cc per subject at baseline (mean age 69.1 years) and 6.42 (±9.53) cc at 2-year follow-up. This increase in lesion load of 0.76 cc/yr represents the lower end of the spectrum. At the high end is Sachdev’s work, (Sachdev et al., 2007) in healthy elderly subjects with a mean age of 71 years, demonstrating a yearly increase in the volume of abnormal white matter by 2.17 cc. For the purposes of sample size calculation, I will assume that our subjects, pre-selected for entry with Fazekas score 2 or higher, will have a higher rate of progression at a rate of 3 cc per year. Three cc of new lesion developing over 12 months corresponds with about 83 CVR size voxels (3 x 3 x 4 mm). Previously acquired CVR data indicates that CVR measurements in white matter are noisy, due to the fact that CVR is 2-3 times lower in white matter than gray matter, and due to signal artifacts from motion near the ventricles. The estimated number of new abnormal voxels per subject on the 16 week T2/DTI portion of the study is similar as the T2/DTI voxels are ¼ the size of the CVR voxels. A similarly structured serial MR study (Werring et al., 2000) of T2 and mean diffusivity in patients with multiple sclerosis included 12 serial time points (where we have included 16) and found statistically
significant and meaningful results from a sample size of 5 subjects.

Feasibility: A challenge in a serial imaging project of this nature is recruitment and retention. Total MR imaging time for each subject is up to 5 hour 30 minutes. This is well within the range of total MR imaging time for patients who undergo serial follow-up imaging for a variety of neurological disorders. Imaging time is also similar to other serial MRI studies in human subjects such as a study of diffusion-tensor imaging in multiple sclerosis.(Werring et al., 2000)

8.4 Arterial Wall Imaging in Migraine

Background

Migraine is a primary headache disorder affecting 2 – 3 million Canadians. (National Population Health Survey, 1998/99). It ranks as the 8th most disabling medical condition globally, and accounts for 7 million lost working days annually in Canada.(Pryse-Phillips et al., 1992; Vos et al., 2010) Despite the staggering personal and societal cost of migraine, the pathomechanism of this disorder is poorly understood. A popular “vascular theory” suggested that cerebral vasospasm results in migraine aura, and dilatation and inflammation of external carotid artery (scalp) branches result in migraine pain.(Schumacher & Wolff, 1941; Wolff, Tunis, & Goodell, 1953) Parts of this theory have been disproven.(Amin et al., 2013; Iversen, Nielsen, Olesen, & Tfelt-Hansen, 1990; Olesen, Larsen, & Lauritzen, 1981) yet there remains evidence that scalp arteries are
implicated in the pathogenesis of migraine pain:

- Patients with migraine headache are more likely to have tender points along the extra-cranial arteries than those without migraine headaches. (Cianchetti, 2012)
- Prolonged compression of the superficial temporal artery is effective at interrupting an early migraine headache (Drummond & Lance, 1983; Hmaidan & Cianchetti, 2006; Lipton, 1986; Vijayan, 1993) and compression elsewhere on the scalp does not have this effect. (Cianchetti, Serce, Pisano, & Ledda, 2010)

Despite multiple investigations on blood flow and arterial diameter in migraine, there is a near-complete lack of experimental evidence to support or refute the existence of arterial wall inflammation during migraine. High-resolution contrast-enhanced vessel wall MRI is an emerging technique that enables direct evaluation of the arterial wall in vivo. In patients with temporal arteritis, vessel wall MRI demonstrates arterial wall thickening and enhancement. (Bley et al., 2009; Bley et al., 2007; Bley et al., 2005) There are no reports of arterial wall imaging in patients with migraine. Evidence of arterial wall thickening and/or enhancement in migraine patients would:

- Provide evidence an underlying inflammatory process in the arterial wall
- Demonstrate that thickening and/or enhancement of external carotid artery branches in patients with headache is not specific to giant cell arteritis
- Potentially provide a biomarker the pathophysiological process underlying migraine pain

Specific Aims and Hypotheses
We aim to determine whether high-resolution contrast-enhanced MRI of branches of the external carotid artery demonstrates:

1) arterial wall thickening and/or enhancement during migraine headache
2) resolution of arterial wall abnormalities during a period without a recent migraine headache

Hypotheses:

(I) In patients with an established diagnosis of migraine headache, vessel wall MRI performed 12-48 hours after the onset of migraine headache will demonstrate external carotid artery branch arterial wall thickening and enhancement ipsilateral to the side of the headache using the contralateral side as a reference standard.

(II) Follow-up vessel wall MRI (during a period without headache in the preceding 2 weeks) will demonstrate normalization of any arterial wall thickening and/or enhancement.

Methods

Design: This is a prospective observational study. IRB application has been submitted.

Subject Recruitment: Participating hospital neurologists will identify potential subjects using the following criteria:

Inclusion criteria:

- Neurologist-established diagnosis of migraine headaches
• Age > 16 years
• Capacity to provide informed consent

Exclusion criteria:

• Claustrophobia
• Pregnant or likely to become soon
• Prior gadolinium reaction or other severe allergic reaction
• Renal impairment
• Other contraindication to 3-Tesla MRI

Initial Data Collection: At the time of the consent discussion, we will collect data on subject age, sex, headache history, and medications for participating subjects. We will provide the subjects with contact information for one of the study investigators, and ask the subjects to contact us by email or phone as soon as possible after the onset of migraine headache. We will then utilize gaps in our daytime MR schedule, and regularly unbooked slots in the evenings and weekends, to schedule a MRI within 12-48 hours of headache onset.

Imaging: All imaging will be performed using a Signa HDx 3-Tesla MR scanner with 8-channel head coil (GE Healthcare, Milaukee, Wis) at the Toronto Western Hospital. At the time of MRI, we will record time since headache onset, whether aura, current severity (1-10 pain scale), sidedness, medication during current headache. The vessel wall MRI protocol will cover a volume that is 9 cm in craniocaudal span, extending from the mid C1 cervical level superiorly. This will include the superficial temporal artery and occipital artery.
(1) Localizer scan (30 seconds)

(2) 3D Time-of-flight MR angiogram (8 minutes)

(3) T1-weighted black blood vessel wall sequence in the axial plane (inversion recovery-prepared two-dimensional fast spin echo acquisition with fat sat and field of view=22x22cm, matrix 512x512; slice thickness=3 mm; TR/T1/TE = 2263/860/13 ms) (12 minutes)

(5) T1-weighted black blood vessel wall sequence repeated following intravenous administration of gadolinium at standard dose. (12 minutes)

These MR sequences are already in regular use at our site (Figure 1). We are currently implementing a three-dimensional vessel wall sequence that will likely replace the two-dimensional sequence by the time recruitment begins.

![Figure 8.3](image)

**Figure 8.3** Time-of-flight MRA of the superficial temporal artery (left) and axial high-resolution contrast-enhanced T1-weighted vessel wall image (right) in a patient with biopsy-proven temporal arteritis. The arterial wall is abnormally thick and enhancing.
Sample Size: There are no published reports of external carotid branch arterial wall imaging in patients with migraine headache. We will scan 15 patients for this preliminary study.

Data Analysis: Vessel wall MRI evaluated by consensus reading of two neuroradiologists

- Grading of arterial wall thickness and degree of enhancement for the superficial temporal arteries and occipital arteries using the methodology detailed by Bley et al.\(^\text{14}\)
- t-tests tests to determine whether arterial wall thickness is greater ipsilateral to migraine headache pain, whether arterial wall enhancement is greater ipsilateral to pain, and whether there is a difference in arterial wall thickening and degree of enhancement between the initial and follow-up imaging sessions
- Potential future analyses using the data from this study include evaluation of intracranial arterial wall and evaluation of intracranial and extracranial arterial diameter

Impact

Migraine is a prevalent disease with huge personal and social cost. The pathogenesis is unknown. We will apply an emerging imaging technique, high-resolution contrast-enhanced vessel wall MRI, to determine whether there are inflammatory changes in the scalp arteries of patients during acute migraine attack. Evidence of arterial wall inflammation would truly advance our understanding of this disease, and the study would...
open the possibility of arterial wall MRI for clinical and research purposes in patients with migraine.


