DIMINISHED CEREBROVASCULAR RESERVE IN THE CEREBRAL WHITE MATTER OF ELDERLY INDIVIDUALS WITH LEUKOARAIOSIS

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
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Abstract

The cerebral white matter contains millions of fibers that serve to connect different regions of the brain and supports sophisticated information processing. A sudden loss of these fibers in a brain region as seen in a stroke to a large blood vessel, can lead to severe loss of function especially when the affected fibers are in a strategic location. Little is known about the fine network of blood vessels supplying nutrition to the white matter that can become diseased with age, producing a very similar pattern of injury to the white matter termed “leukoaraiosis”. Considerable research has been carried out studying the effects of injury to large vessels supplying the gray matter of the brain. However, understanding of the relationship between dysfunctional white matter blood vessels and injury to the white matter itself is limited. The purpose of the present research is to study this relationship in a unique way. The present work will use MRI and manipulation of blood carbon dioxide (CO$_2$) levels for mapping the capacity of the cerebral vasculature to control blood flow, i.e. assessing cerebrovascular reactivity (CVR). CVR is a measure of the response of the arterial vasculature to respond to a vasoactive stimulus, such as CO$_2$. The response to vasodilatory stimuli can fail in diseases that affect the integrity of the vasculature leading to interruption of blood supply, resulting in varying degrees of brain injury, including slowly progressive ischemic demyelination in the white matter. Research within this thesis has demonstrated that: CVR is reduced in leukoaraiosis compared to
normal-appearing white matter (NAWM); (2) Cross-sectionally, abnormalities in diffusion and perfusion MRI metrics have been observed in the NAWM with steal physiology; (3) Reduced CVR is associated with the subsequent development of visible white matter injury when subjects completed a follow-up MRI scan separated 12-months apart from baseline and (4) The speed of the vascular response is also abnormal in leukoaraiosis compared to NAWM. This thesis provides insight into the mechanism of white matter injury and highlights a novel method for predicting injury in susceptible individuals.
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subjects for the studies.
INTRODUCTION ................................................................. 1

1.1 The anatomy of the cerebral circulation ................................................................. 1
  1.1.1 The macrovascular supply to the brain .............................................................. 1
  1.1.2 The microvascular structure ............................................................................. 6

1.2 The Neurovascular Unit ......................................................................................... 6
  1.2.1 Development of the Neurovascular Unit ......................................................... 8
  1.2.2 Components of the neurovascular unit ............................................................ 8
    1.2.2.1 Neurons and Astrocytes .............................................................................. 8
    1.2.2.2 Pericytes and myocytes ............................................................................. 10
    1.2.2.3 Endothelial cells ....................................................................................... 11
  1.2.3 Physiology of the neurovascular unit under normoxic and hypoxic conditions ......................................................................................................................... 11

1.3. Control of Blood Flow ......................................................................................... 13
  1.3.1 Rheological factors influencing cerebral blood flow ......................................... 13
  1.3.2 Cerebral Neurovascular Regulation ................................................................. 14
    1.3.2.1 Autoregulation .......................................................................................... 15
    1.3.2.2 Functional Hyperaemia and Neurovascular coupling .............................. 17
    1.3.2.2.1 Neurophysiology of neurovascular coupling and its contribution to fMRI signals ....................................................................................................................... 18

1.4 Mechanisms of Neurovascular Coupling ............................................................ 20
  1.4.1 Main molecular players mediating neurovascular coupling ............................ 20
1.4.2 Myogenic regulation of cerebral blood flow ................................................... 22
1.4.3 Metabolic feedback mechanism ................................................................... 23
1.4.4 Neurotransmitter-mediated feed-forward signaling ....................................... 24
1.4.5 Mechanisms of vasodilation ......................................................................... 24
1.4.5.1 Mechanisms of CO\textsubscript{2}-mediated vasodilation ................................. 27

1.5 Leukoaraiosis ..................................................................................................... 28

1.5.1 Risk factors of leukoaraiosis ......................................................................... 30
1.5.1.1. Hypertension ............................................................................................ 30
1.5.1.2. Age .......................................................................................................... 31
1.5.1.3. Race ...................................................................................................... 31
1.5.1.4. Sex .......................................................................................................... 31
1.5.1.5 Diabetes Mellitus ...................................................................................... 32
1.5.1.6 Dyslipoproteinaemia .................................................................................. 32
1.5.1.7 Smoking ................................................................................................... 32
1.5.1.8 Large vessel atherosclerosis ...................................................................... 32
1.5.1.9 Homocysteine and Vitamin B\textsubscript{12} ......................................................... 33
1.5.1.10 Genetic Factors influencing leukoaraiosis ................................................ 33
1.5.2 Comorbidities of leukoaraiosis ..................................................................... 34
1.5.3 Pathophysiology of leukoaraiosis .................................................................. 35
1.5.3.1 Blood supply to the white matter ............................................................... 35
1.5.3.2 Tortuous vessels ....................................................................................... 37
1.5.3.3 String Vessels .......................................................................................... 37
1.5.3.4 Age-related basement membrane thickening .......................................... 38
1.5.3.5 Hypoxia and angiogenesis in aging ......................................................... 38
1.5.3.6 Age-related capillary loss in leukoaraiosis .............................................. 39
1.5.3.7 Periventricular venous collagenosis ......................................................... 40
1.5.3.7.1 Periventricular venous collagenosis and interstitial flow ..................... 42
1.5.3.8 Jugular venous reflux ................................................................................ 44
1.5.3.9 Age-related changes to cerebral blood flow .......................................... 44
1.5.3.10 Reduced cerebral blood flow in leukoaraiosis ...................................... 45
1.5.3.11 The role of hypertension in leukoaraiosis ............................................. 46
1.5.3.12 Endothelial Dysfunction ....................................................................... 46
1.5.3.13 Cerebral autoregulation and leukoaraiosis ................................................. 47
1.5.4 Location and Progression of Leukoaraiosis .................................................... 47

1.6 Radiological imaging ........................................................................................... 48
1.6.1 Functional magnetic resonance imaging (fMRI) .......................................... 49
1.6.1.1 What is the fMRI BOLD Signal? ................................................................. 50
1.6.1.2 BOLD fMRI signal quantification ............................................................... 52
1.6.2 Quantitative T2 Changes in leukoaraiosis ..................................................... 54
1.6.3 Diffusion Tensor Imaging ............................................................................... 54
1.6.3.1 Diffusion MRI in leukoaraiosis ................................................................. 59
1.6.4 Perfusion MRI .................................................................................................. 60
1.6.4.1 Dynamic Susceptibility Contrast Perfusion .............................................. 61
1.6.4.1.1 What causes the perfusion signal to change? ......................................... 62
1.6.4.1.2 How does perfusion signal change relate to tracer concentration? ....... 63
1.6.4.1.3 How does the time-concentration tracer curve become a measure of perfusion? .................................................................................................................. 64
1.6.4.2 Perfusion MRI in Leukoaraiosis ................................................................. 65

1.7 Quantification of Leukoaraiosis ......................................................................... 66
1.7.1 Rating Scales .................................................................................................. 66
1.7.2 Machine learning approaches ....................................................................... 66
1.7.3 Intensity cut-off approaches ......................................................................... 67
1.7.4 Template-based approaches ......................................................................... 67
1.7.5 Fluid-attenuated inversion recovery (FLAIR) imaging ................................ 68
1.7.6 Lesion Explorer ............................................................................................. 68

1.8 Imaging Cerebral Blood Flow Regulation ....................................................... 70
1.8.1 Methods for Changing Cerebral Blood Flow .............................................. 70
1.8.1.1 Acetazolamide .......................................................................................... 70
1.8.1.2 Carbon Dioxide ....................................................................................... 70
1.8.1.3 The RespirAct ......................................................................................... 71

1.9 Imaging Methods Available for measuring cerebral blood flow .................... 71
1.9.1 Doppler Ultrasound, Xenon CT, and PET ................................................. 71
1.9.2 SPECT .......................................................................................................... 71
1.9.3 ASL MRI ...................................................................................................... 71
AIMS AND HYPOTHESES ..................................................................................................... 74

2.1 Study I. Vascular dysfunction in elderly subjects with leukoaraiosis. .......... 74
2.2 Study II. Impaired Cerebrovascular Reactivity is Associated with abnormal T2,
Diffusion and Perfusion MRI metrics in Normal-appearing White Matter .......... 76
2.3 Study III. Neurovascular uncoupling predicts future development of
leukoaraiosis. .................................................................................................................... 77
2.4 Study IV. Impaired Temporal Dynamics of the Cerebromicrovasculature in
elderly subjects with leukoaraiosis. ............................................................................ 78

GENERAL MATERIALS AND METHODS ......................................................................... 79

3.1 Subject Recruitment .................................................................................................. 79
3.1.1 Subject Inclusion/exclusion criteria ................................................................ 79
3.2 Imaging Sequences ..................................................................................................... 80
3.2.1 MRI Acquisition .............................................................................................. 80
3.3 Psychometrics ............................................................................................................. 82
3.4 Vasodilatory stimulus ................................................................................................ 82
3.5 General research design ............................................................................................. 85
3.6 General data analysis ................................................................................................. 85
3.7 Sample size calculation .............................................................................................. 85

Study I. Vascular dysfunction in elderly subjects with leukoaraiosis. .................... 87

4.1 Abstract ....................................................................................................................... 87
4.2 Introduction ................................................................................................................ 88
4.3 Methods ....................................................................................................................... 89
4.3.1 Subject Recruitment ......................................................................................... 89
4.3.2 MRI Acquisition .............................................................................................. 89
4.3.3 CVR measurement ........................................................................................... 90
4.3.4 Vasodilatory Stimulus ..................................................................................... 90
4.3.5 Image Reconstruction ...................................................................................... 90
Study IV. Impaired Temporal Dynamics of the Cerebromicrovasculature in elderly subjects with leukoaraiosis. ......................................................................................................................... 129

7.1 Abstract ........................................................................................................................................................................ 129

7.2 Introduction ................................................................................................................................................................. 130

7.3 Methods ......................................................................................................................................................................... 131

7.3.1 Subject Recruitment and Assessment ......................................................................................................................... 131

7.3.2 Image Acquisition .......................................................................................................................................................... 132

7.3.4 CVR measurement ......................................................................................................................................................... 132

7.3.4.1 Vasodilatory Stimulus (Gas Manipulation, End-tidal pCO2 and pO2 Manipulation) ......................................................... 132

7.3.4.2 Image Reconstruction .................................................................................................................................................. 133

7.3.5 Generating CVR maps .................................................................................................................................................. 133

7.3.6 Generating steady-state CVR and \( \tau \) maps .................................................................................................................. 134

7.3.7 Generating ROIs of WMH and NAWM ....................................................................................................................... 1355

7.3.8 Statistical Analyses ...................................................................................................................................................... 137

7.4 Results .............................................................................................................................................................................. 137

7.5 Discussion ................................................................................................................................................................. 141

GENERAL DISCUSSION ....................................................................................................................................................... 144

8.1 Summary and Novel Aspects of Findings ....................................................................................................................... 144

8.2 Competition for blood flow in the cerebral white matter ............................................................................................. 146

8.3 Haemodynamic Implications of the findings ................................................................................................................. 148

8.4 What causes CVR to be lower in elderly individuals with leukoaraiosis? ................................................................ 149

8.5 Thesis strengths and limitations .................................................................................................................................. 150

8.6 Future Studies ............................................................................................................................................................... 152

8.7 Conclusions ................................................................................................................................................................. 155

REFERENCES ........................................................................................................................................................................ 156

APPENDICES ......................................................................................................................................................................... 184

Appendix Figures ............................................................................................................................................................. 195

Appendix Tables ............................................................................................................................................................. 196

COPYRIGHT ACKNOWLEDGEMENTS ............................................................................................................................. 207
List of Tables

Table 4-1. Measurements of CVR, FA, mD, T2, and perfusion metrics in white matter hyperintensities and NAWM. ................................................................. 95
Table 5-1. Comparison of average values for MRI metrics in NAWM with positive and negative CVR. ................................................................................................. 109
Table 6-1. Comparison of average values for MRI metrics in regions of grey matter, normal-appearing white matter, and future. ................................................................. 123
Table 6-2. Comparison of average values for MRI metrics in future WMHs and contralateral homologous regions. ................................................................. 124
Table 7-1. Comparison of CVR MRI metrics in regions of grey matter, normal-appearing white matter, and future leukoaraiosis. ................................................................. 138
Table 7-2. Comparison of average values for CVR MRI metrics in pre-existing lesions and contralateral homologous regions. ................................................................. 139
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>The circle of Willis</td>
<td>3</td>
</tr>
<tr>
<td>1-2</td>
<td>Schematic drawing of the capillary basement membrane</td>
<td>5</td>
</tr>
<tr>
<td>1-3</td>
<td>The neurovascular unit</td>
<td>7</td>
</tr>
<tr>
<td>1-4</td>
<td>A schematic representation of the possible relationships between mean arterial pressure and cerebral blood flow</td>
<td>16</td>
</tr>
<tr>
<td>1-5</td>
<td>Neurovascular coupling pathways</td>
<td>26</td>
</tr>
<tr>
<td>1-6</td>
<td>Schematic drawing of blood supply to the cerebral white matter seen in coronal view</td>
<td>36</td>
</tr>
<tr>
<td>1-7</td>
<td>FLAIR images demonstrating the spectrum of leukoaraiosis</td>
<td>48</td>
</tr>
<tr>
<td>1-8</td>
<td>Schematic representation of white matter fibres and diffusion ellipsoid</td>
<td>57</td>
</tr>
<tr>
<td>1-9</td>
<td>Generation of the concentration-time series</td>
<td>63</td>
</tr>
<tr>
<td>3-1</td>
<td>Set up of the prospective targeting method</td>
<td>84</td>
</tr>
<tr>
<td>4-1</td>
<td>Identification of NAWM</td>
<td>92</td>
</tr>
<tr>
<td>4-2</td>
<td>Calculation of ROIs used to account for differences in spatial location of MRI metrics</td>
<td>95</td>
</tr>
<tr>
<td>4-3</td>
<td>Comparison of MRI metrics between regions of WMH and NAWM</td>
<td>95</td>
</tr>
<tr>
<td>5-1</td>
<td>Identification of NAWM</td>
<td>105</td>
</tr>
<tr>
<td>5-2</td>
<td>Identification of NAWM with steal physiology</td>
<td>106</td>
</tr>
<tr>
<td>5-3</td>
<td>ROIs used to assess the influence of spatial location on MRI metrics</td>
<td>107</td>
</tr>
<tr>
<td>5-4</td>
<td>Comparison of MRI metrics between regions of NAWM with positive CVR and steal physiology</td>
<td>110</td>
</tr>
<tr>
<td>6-1</td>
<td>MRI metrics used in the assessment of future leukoaraiosis</td>
<td>118</td>
</tr>
<tr>
<td>6-2</td>
<td>ROIs used in the assessment of MRI metrics while considering the confounding effect of spatial location</td>
<td>120</td>
</tr>
<tr>
<td>6-3</td>
<td>Comparison of MRI metrics in regions of NAWM destined to be WMH and the contralateral NAWM</td>
<td>125</td>
</tr>
<tr>
<td>7-1</td>
<td>CVR metrics used to characterize future leukoaraiosis</td>
<td>134</td>
</tr>
<tr>
<td>7-2</td>
<td>ROIs used in the comparison of NAWM and hyperintensities considering the confounding spatial factor</td>
<td>137</td>
</tr>
<tr>
<td>7-3</td>
<td>Comparison of CVR metrics in regions of WMH compared to NAWM</td>
<td>140</td>
</tr>
</tbody>
</table>
## List of Appendices

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix 1</td>
<td>Recruitment Letter</td>
<td>184</td>
</tr>
<tr>
<td>Appendix 2</td>
<td>Consent Form</td>
<td>185</td>
</tr>
<tr>
<td>Appendix Figure 1</td>
<td>MRI metrics assessed in elderly subjects with age-related leukoaraiosis.</td>
<td>195</td>
</tr>
<tr>
<td>Appendix Table 1</td>
<td>Toronto Western Hospital - Patient Characteristics</td>
<td>196</td>
</tr>
<tr>
<td>Appendix Table 2</td>
<td>Sunnybrook Health Sciences Centre - Patient Characteristics</td>
<td>200</td>
</tr>
<tr>
<td>Appendix Table 3</td>
<td>Toronto Western Hospital - Patient Characteristics for Study III and IV.</td>
<td>202</td>
</tr>
<tr>
<td>Appendix Table 4</td>
<td>Sunnybrook Health Sciences Centre - Patient Characteristics for Study III and IV.</td>
<td>205</td>
</tr>
</tbody>
</table>
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-HETE</td>
<td>20-hydroxyeicosatetraenoic acid</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid-β</td>
</tr>
<tr>
<td>ACA</td>
<td>Anterior Cerebral Artery</td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer's Disease</td>
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<tr>
<td>ADC</td>
<td>Average Diffusion Coefficient</td>
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<td>ASL</td>
<td>Arterial Spin Labeling</td>
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<td>ADP</td>
<td>Adenosine Diphosphate</td>
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<td>ARWMC</td>
<td>Age-Related White Matter Changes</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>BK</td>
<td>Bradykinin</td>
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<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent</td>
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<tr>
<td>Cav</td>
<td>Voltage-dependent Calcium Channel</td>
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<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
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<td>Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy</td>
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<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
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<td>Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral Blood Flow</td>
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<tr>
<td>CBV</td>
<td>Cerebral Blood Volume</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic Guanosine Monophosphate</td>
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<td>Carbon Dioxide</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<td>Computed Tomography</td>
</tr>
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<td>CVR</td>
<td>Cerebrovascular Reactivity</td>
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<tr>
<td>DAG</td>
<td>Diacyl-glycerol</td>
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<tr>
<td>dHb</td>
<td>Deoxyhemoglobin</td>
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<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted Imaging</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>DTPA</td>
<td>Diethylene Triamine Pentaacetic Acid</td>
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<tr>
<td>EDHF</td>
<td>Endothelium-derived Hyperpolarizing Factor</td>
</tr>
<tr>
<td>EET</td>
<td>Epoxyeicosatrienoic acid</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
</tr>
</tbody>
</table>
GABA  γ-aminobutyric acid
GERD  Gastroesophageal Reflux Disease
GTP  Guanosine Triphosphate
HSPG  Heparan Sulfate Proteoglycan
ICA  Internal Carotid Artery
IKCa  Intermediate-conductance Calcium-dependent Potassium Channel
IP₃  Inositol Triphosphate
Kᵢᵢᵣ  Inwardly Rectifying Potassium Channel
mD  Mean Diffusivity
MCA  Middle Cerebral Artery
MEGJ  Myoendothelial Gap Junction
MI  Myocardial Infarction
MNI  Montreal Neurological Institute
MoCA  Montreal Cognitive Assessment
MRI  Magnetic Resonance Imaging
MTT  Mean Transit Time
N₂  Nitrogen
NAWM  Normal-appearing White Matter
NGF  Nerve Growth Factor
O₂  Oxygen
OSA  Obstructive Sleep Apnea
PaCO₂  Partial Pressure of Arterial Carbon Dioxide
PCA  Posterior Cerebral Artery
PD  Proton Density
PET  Positron Emission Tomography
Pₐₑ₃CO₂  Partial Pressure of End-tidal Carbon Dioxide
Pₐₑ₃O₂  Partial Pressure of End-tidal Oxygen
PG  Prostaglandin
PG₁₂  Prostaglandin I₂
PLA₂  Phospholipase A₂
PLC  Phospholipase C
RF  Radiofrequency
SABRE  Semi-Automated Brain Region Extraction
SPECT  Single Photon Emission Computed Tomography
SPM  Statistical Parametric Mapping
SHSC  Sunnybrook Health Sciences Centre
SKCa  Small-conductance Calcium-dependent Potassium Channel

XVI
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<thead>
<tr>
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<th>Full Form</th>
</tr>
</thead>
<tbody>
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<td>SVD</td>
<td>Small Vessel Disease</td>
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<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
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<tr>
<td>Tmax</td>
<td>Time-to-maximum</td>
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<tr>
<td>TRP</td>
<td>Transient Receptor Potential</td>
</tr>
<tr>
<td>TTP</td>
<td>Time-to-peak</td>
</tr>
<tr>
<td>TWH</td>
<td>Toronto Western Hospital</td>
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<tr>
<td>UTP</td>
<td>Uridine Triphosphate</td>
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<td>VaD</td>
<td>Vascular Dementia</td>
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<td>VBM</td>
<td>Voxel-based Morphology</td>
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<td>VCI</td>
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<td>VIP</td>
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<td>WMC</td>
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<td>WMH</td>
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CHAPTER 1

INTRODUCTION

My thesis focuses on the structural and functional integrity of the cerebral white matter. Its integrity depends on the fine-tuned balance between substrate delivery through cerebral blood flow and energy demands during neural activity (Iadecola, 2004). Pathophysiology of the cerebral white matter arises when this balance is disturbed. Therefore, complex cerebrovascular control mechanisms exist to ensure neurogliovascular coupling, i.e. that active brain areas receive an adequate blood supply. This thesis will examine white matter vascular control integrity in elderly individuals with small vessel disease. It is hypothesized that dysfunction in neurogliovascular coupling plays an important role in white matter injury. In this thesis, mapping of cerebrovascular reactivity (CVR), defined as the change in blood flow per unit change in stimulus, will be used to better understand the relationship between blood flow dysregulation and white matter injury. To understand white matter pathophysiology, it is important to have a clear view of the anatomy of the cerebral circulation. An understanding of the physiology of the small vessels relies on knowledge of the arborization of vessels into networks, intrinsic mechanism of local blood flow control, and the mechanism of neurogliovascular coupling. The following sections will describe cerebral vascular anatomy, the different cell types (neurons, astrocytes, and vascular cells) that form a functional unit for controlling the brain’s microenvironment i.e. “the neurovascular unit,” and the mechanisms involved in neurovascular regulation. This description will provide the foundation for understanding ischemic white matter disease, forming the basis of research performed in this thesis.

1.1 The anatomy of the cerebral circulation

1.1.1 The macrovascular supply to the brain

The brain receives its arterial blood supply via two major routes; namely, the internal carotid arteries and the vertebral arteries. The right and left vertebral arteries unite to form the basilar artery at the junction of the medulla and the pons. The carotid system is mainly responsible for the anterior circulation of the brain while the basilar artery provides flow to the posterior cerebral circulation. These anterior and posterior circuits are interconnected by communicating arteries that form the circle of Willis at the base of the brain, providing potential
shortcuts and collateral flow between antero-posterior cerebral circulation. However, by infusing vital dyes in each artery, animal studies of the vertebral (posterior) and carotid (anterior) systems have demonstrated that they supply distinct brain regions and do not mix (Baldwin & Bell, 1963; McDonald & Potter, 1951). Nevertheless, if the pressure gradient in the circle of Willis changes due to insufficient flow, in the case of steno-occlusive disease, blood originating from a different circuit can redistribute via the collateral intercommunication in the circle of Willis. This degree of compensation depends on the individual variation of vessel diameter and the symmetry of the circle of Willis (Dickey, Kailasnath, Bloomgarden, Goodrich, & Chaloupka, 1996).

Arteries stemming from the basilar artery predominantly supply the brainstem and cerebellum. The cerebral hemispheres are vascularized by vessels stemming from both the internal carotid artery and basilar artery. The two largest pairs of vessels originating from the internal carotid arteries are the anterior cerebral arteries (ACA) and middle cerebral arteries (MCA). The MCA supply approximately 80% of the blood reaching the cerebral hemispheres. MCA vessels supply the temporal lobe, parietal lobe, basal ganglia, and choroid plexus in the lateral ventricles. The ACA sends vessels to supply the frontal lobe, the globus pallidus, and the amygdala. As the basilar artery ascends, it eventual splits to become the posterior cerebral artery and supplies blood to the occipital lobes and thalami (Figure 1-1).
Figure 1-1. The circle of Willis. Blood is supplied to the brain through two major routes, the internal carotid arteries and the vertebral arteries. The two vertebral arteries join to form the basilar artery, which then ascends and branches into the two posterior cerebral arteries, supplying the thalami and occipital lobes. The anterior circulation is supplied by the internal carotid arteries, which branch into the anterior cerebral arteries and middle cerebral arteries. Note that there are left and right versions of each artery except for the basilar artery and anterior communicating artery. The posterior communicating artery provides collateral flow preferentially between the anterior and posterior circulations whereas the anterior communicating artery preferentially provides collateral flow between the left-right hemispheres. ACA, anterior cerebral artery; Acom, anterior communicating artery; BA, basilar artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; Pcom, posterior communicating artery; VA, vertebral artery.
The major arteries eventually advance dorsally and spread on the surface of the cerebral hemispheres in the subarachnoid space, becoming the pial arteries (also known as leptomeningeal arteries). These vessels lie within the pia-arachnoid (also known as the leptomeninges) or glia limitans (the outermost layer of the cortex, comprised of astrocytic end-feet) (E. G. Jones, 1970). Pial vessels are surrounded by cerebrospinal fluid (CSF) and give rise to smaller arteries that eventually penetrate the brain parenchyma perpendicular to the cortical surface without establishing anastomoses with each other. Penetrating arterioles lie within the Virchow-Robin (VR) space, a continuation of the subarachnoid space, and branch off the pial arteries. The penetrating arteries become parenchymal arterioles once they leave the VR space and penetrate the brain parenchyma and also become completely surrounded by astrocytic end-feet (Z. Cohen, Bonvento, Lacombe, & Hamel, 1996).

VR spaces gradually disappear as the arterioles penetrate deeper in the brain tissue, with only the leptomeningeal cell layer remaining to form the outer layer of the arteriolar wall the tunica adventitia (also known as the tunica externa). The second and the thickest layer of the vessel wall, the tunica media, consists of one or two layers of smooth muscle cells, which are separated from the tunica adventitia by elastin and collagen fibres, known as the external elastic lamina. However, cerebral arteries do not possess an external elastic lamina unlike systemic arteries (R. M. K. W. Lee, 1995). The smooth muscle cells in the tunica media can regulate blood pressure and flow in the vessel by changing vessel diameter through contraction or relaxation. Lastly, the luminal layer of the artery, called the tunica intima, is composed of endothelial cells. The capillaries do not possess tunica media or externa and only contain tunica intima.

The endothelial cells comprising the tunica intima are surrounded by a 30-40 nm thick basement membrane. The extracellular matrix components of the basement membrane are collagen type IV, heparan sulfate proteoglycan, (HSPG) and laminin. These constituents are arranged into a trilaminar structure composed of an endothelial layer (lamina rara interna), an astrocytic layer (lamina rara externa) and a transitory, fused layer in between the two layers (lamina densa). Collagen type IV is the major structural element of the basement membrane and is preferentially found in the lamina densa while the proteins laminin and HSPG are associated with the other two laminae, which promote cell adhesion and attachment (Figure 1-2) (Perlmutter & Chui, 1990).
Figure 1-2. Schematic drawing of the capillary basement membrane. The capillary basement membrane is a trilaminar structure comprised of the lamina rara externa, lamina densa, and lamina rara interna. All three components contain collagen IV. The lamina rara externa and interna both contain heparin sulphate proteoglycan and laminin. These components are all expressed by the endothelium.
1.1.2. The microvascular structure

The function of the fine network of parenchymal vessels and capillaries inherently differs from that of arteries. Arteries regulate blood pressure while brain capillaries maintain the blood-brain barrier (BBB) and sustain continuous nutrient, electrolyte, and waste product trafficking between the blood and neural tissue (Farkas & Luiten, 2001). In general, capillary density in the grey matter is found to be three times as much as that of white matter but these differences may be due to the local blood flow and utilization in a given brain area (Klein, Kuschinsky, Schrock, & Vetterlein, 1986). The phenomenon that metabolically active brain regions are more heavily vascularized than less active areas is supported by the observation that capillary density appears to be most pronounced in areas that are rich in synapses, cell body populations, and neural fibre bundles. Microvascular density also appears to coincide with the task of a given region as sensory and association centres are usually more vascularized than motor centres. The laminar structure of the cortex also displays a typical layer-dependent density pattern where lamina IV and I are the most vascularized (Hudetz, 1997).

1.2 The Neurovascular Unit

Neurogliovascular coupling is a phenomenon produced by a group of cells called the neurovascular unit. The neurovascular unit is composed of cells of both vascular and neural origin, which include neurons, astrocytes, endothelial cells, myocytes, pericytes, and extracellular matrix components (Harder, Zhang, & Gebremedhin, 2002). Cells of the neurovascular unit have close anatomical and chemical relationships with one another in order to trigger necessary vascular responses to provide tissue nutrient supply during metabolic demands (Muoio, Persson, & Sendeski, 2014). In other words, the neurovascular unit establishes an anatomical and functional whole, which results in a highly efficient system for the regulation of cerebral blood flow (Figure 1-3) (Abbott & Friedman, 2012; Armstead & Raghupathi, 2011). Interplay between components of the neurovascular unit is made possible by gap junctions as well as adhesion molecules, such as cadherins and integrins (del Zoppo, 2010; Figley & Strom, 2011). The gap junctions, adhesion molecules, and ion channels involved in the neurovascular unit facilitate the influx and efflux of ions such as Ca$^{2+}$, K$^+$, Na$^+$, as well as the action of neuromodulators such as adenosine triphosphate (ATP) (Filosa, 2010; Gordon, Mulligan, & MacVicar, 2007).
Figure 1-3. **The neurovascular unit.** Neurons (blue) establish synapses with interneurons (purple) and astrocytes (green). Neurotransmitters (pink) are used as a form of communication to transmit information concerning metabolic requirements and indirectly command vascular contraction or relaxation via the astrocytes. Astrocytes communicate to vessels through calcium waves through the astrocytic endfeet, which can be in direct contact with endothelial cells, pericytes (yellow) or myocytes (orange).
1.2.1 Development of the Neurovascular Unit

The intimate relationship between the multiple cell types of the neurovascular unit is not by chance. The architecture of the neurovascular unit is the result of a cascade of events that are genetically programmed, which result in the anatomical and functional coupling of neuronal with vascular components during early embryogenesis (Kraemer & Hempstead, 2003; Shima & Mailhos, 2000; Ward & Lamanna, 2004). During embryogenesis, neuronal progenitor cells (derived from the neural tube) and vascular progenitor cells (derived from the neural crest) become juxtaposed. Once anatomically apposed, both neural and vascular progenitor cells are exposed to growth factors (Bagnard et al., 2001), such as VEGF and nerve growth factor (NGF) (Sanchez et al., 2013; Soker, Miao, Nomi, Takashima, & Klagsbrun, 2002). After development, both neural and vascular components remain in close anatomical and functional coupling. However, the precise role of each component during development has not yet been fully established (Kowianski, Lietzau, Steliga, Waskow, & Morys, 2013).

1.2.2 Components of the neurovascular unit

1.2.2.1 Neurons and Astrocytes

The neuron acts in a similar fashion to a pacemaker in the control of the highly sophisticated homeostatic mechanisms involved in the neurovascular unit (Banerjee & Bhat, 2007). Neurons are able to detect small variations in the supply of nutrients and oxygen. These variations are transformed into an electrical and chemical signals to adjacent interneurons or astrocytes, consequently making the necessary adjustment to mediate the metabolic demand (Figley & Stroman, 2011). When necessary, a neuron can communicate directly with an astrocyte or indirectly through an interneuron, influencing the vascular tone and blood supply in surrounding regions. Although pathways of neural-glial communication still remain to be largely explored, it is known that astrocytes are able to detect neuronal levels of glutamate and γ-aminobutyric acid (GABA) and translate this into a vasomotor command (Duchemin, Boily, Sadekova, & Girouard, 2012; Pelligrino, Vetri, & Xu, 2011).

Astrocytes were first functionally described as a support for neurons and considered to play a passive secondary role in the neurovascular unit. This role was questioned after astrocytes demonstrated a great capacity for propagating calcium waves and can form extensive and specialized networks of intercommunication (Cornell-Bell, Finkbeiner, Cooper, & Smith, 1990). Harder et al. (2002) raised the hypothesis that astrocytes are important for maintaining vascular tone. Researchers proposed that a buildup of glutamate during local neuronal activity...
is sensed by metabotropic glutamate receptors found on astrocytes. This leads to increased astrocytic calcium concentrations and the generation of arachidonic acid derivatives such as prostaglandins, which are capable of causing vasodilation through direction action on perivascular smooth muscle cells (Kowianski et al., 2013; Spector, 2009).

There is some debate on the role of astrocytes in neurovascular coupling. A recent study using in vivo two-photon imaging suggested that measurable increases in astrocytic calcium occur after the onset of arteriolar dilation (Nizar et al., 2013). Also, mice lacking astrocytic inositol triphosphate type-2 receptors still exhibited normal stimulus-evoked functional hyperemia (Takata et al., 2013). These receptors responsible for the primary mechanism of astrocytic calcium increase and are necessary for generating intracellular calcium increases (that activates a inositol triphosphate-dependent pathway), which in turn would trigger the release of vasoactive arachidonic acid derivatives. Therefore, these results challenge the intermediary role of astrocytic calcium surges in stimulus-evoked functional hyperaemia. In addition, astrocytes do not directly contact pial arteries external to the cortical surface and therefore the role of astrocytes in pial artery vasodilation is unlikely (B. R. Chen, Bouchard, McCaslin, Burgess, & Hillman, 2011). However, astrocyte involvement in neurovascular coupling has become widely accepted following a study that demonstrated in vivo uncaging of calcium in astrocytic end-feet that causes dilation of adjacent penetrating arterioles (McCaslin, Chen, Radosevich, Cauli, & Hillman, 2011).

Astrocytes appear to be the most versatile cells of the neurovascular unit as they communicate simultaneously with both neurons and blood vessels (Lopez-Bayghen & Ortega, 2011; Santello, Cali, & Bezzi, 2012). The interplay between neurons and vascular components such as the capillaries and pericytes occur both physically and chemically through the release of gliotransmitters, including glutamate and ATP (Kowianski et al., 2013; Petzold & Murthy, 2011). Similar to neurons, astrocytes can be organized into syncytial structures of up to 100 units that are anatomically connected by gap junctions and functionally through calcium waves (Giaume, Koulakoff, Roux, Holcman, & Rouach, 2010). This organization enables the propagation of electrical messages through large distances and transmission to the smooth muscle cells and pericytes. This enables astrocytic regulation of vascular tone not only locally, but also at longer distances, even recruiting other astrocytic syncytia (Haydon & Carmignoto, 2006). Neural activity may alter astrocytic regulation of vessels indirectly causing vasoconstriction or vasodilation. Astrocytes produce and release numerous substances such as
prostaglandins, ATP, and nitric oxide (NO) to directly trigger vasodilation and vasoconstriction (Gordon et al., 2007).

Anatomically, gliovascular communication takes place through the astrocyte endfoot. The endfoot consists of a highly specialized astrocyte extension that is in contact with the surface of the pericytes and smooth muscle cells (Kacem, Lacombe, Seylaz, & Bonvento, 1998; Kann, 2011). The endfoot wraps around these cells and provides a broad surface that enables fast and efficient transmission and modulation of vasoactive signals to the pericytes and smooth muscle cells (Kowianski et al., 2013).

### 1.2.2.2 Pericytes and myocytes

Pericytes were described more than 100 years ago as perivascular cells that wrap around blood capillaries. Because of their contractile fibres, pericytes are also known as vascular smooth muscle cells (Hirschi & D'Amore, 1996). Pericytes possess a cell body with a prominent nucleus and a small content of cytoplasm with several long processes embracing the abluminal endothelium wall. Gap junctions provide direct connections between the cytoplasm of the pericytes and endothelial cells, enabling the exchange of ions and small molecules. Adhesion plaques anchor pericytes to endothelial cells and support transmission of mechanical contractile forces between the two cell types (Rucker, Wynder, & Thomas, 2000). These junction complexes are composed of N-cadherin, cell-adhesion molecules, β-catenin-based adherent junctions, and extracellular matrix molecules such as fibronectin (Gerhardt & Betsholtz, 2003).

Historically, pericytes were thought to serve solely as scaffolding cells. Recently, pericytes have shown communication with endothelial cells by direct physical contact and paracrine signaling pathways. Through anatomical contact, pericytes participate in the development and maturation of endothelial cells, and take part in the metabolic performance of myocytes (Sa-Pereira, Brites, & Brito, 2012). Pericytes contract in response to ATP increases and secrete growth factors and adhesion molecules (H. Kawamura et al., 2003); in addition, pericytes also contain contractile proteins that are able to control the capillary diameter and probably help modulate blood flow in response to neuronal activity (Peppiatt, Howarth, Mobbs, & Attwell, 2006). There is evidence that pericytes constrict in a synchronized manner when calcium waves spread across the neurovascular unit, leading to a constriction of associated capillaries (Fernandez-Klett, Offenhauser, Dirnagl, Priller, & Lindauer, 2010). Moreover, pericytes play a fundamental role in the blood-brain barrier, phagocytosis, and angiogenesis (Armulik et al., 2010).
Myocytes play a role in the neurovascular unit of larger vessels by establishing a close relationship with pericytes, endothelial cells, and astrocytic endfeet. Myocytes are the main effectors of changes in the luminal diameter of large vessels. Their basal state of contractility, which is maintained by the balance of calcium and potassium released from glial endfeet, can be modified in accordance with astrocytic signaling (Haydon & Carmignoto, 2006).

1.2.2.3 Endothelial cells

Endothelial cells have recently been shown to produce trophic and vasoactive factors that are extremely important for the control of vascular tone, such as NO for vasodilation and vasoconstrictors like endothelin and thromboxane (Duchemin et al., 2012; Emanueli, Schratzberger, Kirchmair, & Madeddu, 2003). Nitric oxide is a signaling molecule that is synthesized by the enzyme NO synthase (NOS) from the amino acid L-arginine (Moncada, Palmer, & Higgs, 1991). Three isoforms of NOS have been described; namely endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) (Moncada et al., 1991). eNOS and nNOS are expressed primarily in the endothelial cells and neurons, respectively. The activation of eNOS and nNOS isoforms depends on an increase in the intracellular concentration of \( \text{Ca}^{2+} \). The complex interplay of substances produced by endothelial cells might theoretically be influenced by the extracellular matrix, which provides an environment conducive to the diffusion of ions, ATP, and neurotransmitters (Ballabh, Braun, & Nedergaard, 2004).

1.2.3 Physiology of the neurovascular unit under normoxic and hypoxic conditions

As the neurovascular unit is a vital structure in maintaining brain homeostasis, dysfunction of one or more of its components may have very serious consequences (Kovacs, Heinemann, & Steinhauser, 2012). This thesis will examine the consequences of age-related reductions in the white matter vasculature’s sensitivity to carbon dioxide (CO$_2$). This section will describe the regulation of the neurovascular unit under various physiological conditions. Numerous authors have reported dysfunction in neurovascular unit components in conditions such as traumatic brain injury (Wang et al., 2012), stroke (Ruhrberg & Bautch, 2013), dementia (Itoh et al., 2012), and Alzheimer’s disease (Sagar, Bell, & Zlokovic, 2012). In all of these conditions, possible pathophysiology may include a loss in the permeability and selectivity of the blood-brain barrier, degradation of the extracellular matrix and basal lamina components, and an inflammatory response (Hamilton, Attwell, & Hall, 2010).

Under normoxic conditions, myocytes and pericytes remain in a state of basal contractility. This tone is the result of the balance between vasodilation and vasoconstriction.
signals determined by neuronal input (Zonta et al., 2003). In this situation, neurons are able to communicate with astrocytes through the release of glutamate. Consequently, astrocytic calcium concentrations increase significantly, causing a chain reaction that culminates in the release of vasoactive substances, such as eicosanoids (Zonta et al., 2003). These peptides are released by astrocyte endfeet directly into myocytes or pericytes, thereby causing cell contraction. In addition, the permeability of potassium channels is changed, which may trigger an influx of potassium in the intracellular compartment of myocytes. This influx of potassium leads to hyperpolarization and subsequent vasodilation. The effective vascular tone is therefore the result of the dominant neuronal signal and dependent on which signaling cascades are activated. Once again, endothelial cells may also modulate vascular tone with vasoconstrictive agents, such as thromboxane and endothelin, as well as vasodilating substances, such as nitric oxide (Duchemin et al., 2012).

Among all the factors that may influence the neurovascular unit in the regulation of functional hyperaemia, astrocytic calcium concentrations seem to play a prominent role. Pathways involving calcium influx may lead to vasoconstriction or dilation depending on several factors, such as neuropeptides, ATP, or NO (Gordon et al., 2007). One of these mechanisms involves the role of Phospholipase A\textsubscript{2} (PLA\textsubscript{2}), which stimulates the production of arachidonic acid, a vasoconstrictor activated with increasing calcium concentrations (Devor et al., 2007).

Experiments of brain slices with induced hypoxia have demonstrated that vasoconstriction/vasodilation are directly related to oxygen levels in the neuronal environment (Gordon et al., 2007). Hypoxic conditions induce an increase in extracellular lactate and adenosine surrounding neurovascular units, which reduce the production and action of prostaglandins, promoting a vasodilatory response (T. J. Lee et al., 2011). In hyperoxic conditions, the production of lactate by neurons decreases, leading to a higher bioavailability of prostaglandins and favouring vasoconstriction (Iadecola, Li, Ebner, & Xu, 1995; Pillai, Shanbhag, Dittmar, Bogdahn, & Schlachetzki, 2013).

There is evidence that the effective vascular tone is strongly influenced by the changes in the bioavailability of NO and that NO maintains cerebral blood flow in situations of increased demand for oxygen and nutrients (Carletti, Ferraro, Rizzo, Friscia, & Sardo, 2012; Sardo et al., 2011). Furthermore, NO can be produced not only by endothelial cells, but also by interneurons and neurons (Bredt & Snyder, 1989). NO production depends on several factors, such as
hypoxia, second messengers, noradrenaline, acetylcholine, and hormones such as estrogen (T. J. Lee et al., 2011). NO may also play a role in the regulation of the neurovascular unit at the level of interneurons, which may lead to vasodilation in moderate concentrations or vasoconstriction at high concentrations (Duchemin et al., 2012).

1.3. Control of Blood Flow

1.3.1 Rheological factors influencing cerebral blood flow

The physical pattern of CBF and its pathological changes in the brain microvessels will be described with reference to fluid dynamics. The dynamics of blood flow in the cerebral vessels are characterized by flow velocity, microturbulent flow, blood viscosity, and shear stress created by the vascular resistance (de la Torre & Mussivand, 1993). These factors are dynamically interrelated and are important to consider for this thesis during discussions of age-related changes of the cerebrovasculature.

Flow velocity is not equal at all points in the vessel lumen throughout its transverse profile. A typically flow gradient can be characterized with decreasing flow velocity from the midline of a vessel towards the endothelial wall. Moreover, near the endothelium, blood flow is reduced to a near standstill where the blood has a cell-free plasma layer. This plasma layer serves a biological purpose in allowing nutrient transport between the blood and brain parenchyma (Fung, 1981)

Microturbulent flow disrupts the regular passage of blood and can develop when the vascular lumen has an irregular shape. Instances of this include local thickening of the lumen (fibrotic arteries or capillaries with local basement membrane thickening), partial obstruction (atherosclerosis), or with compression. This leads to a turbulent flow pattern, which could compromise the slow flow of the plasma layer, thereby impairing nutrient exchange through the BBB. Suboptimal support for cerebral metabolism ensues (Eckoldt, 1985).

The third factor influencing cerebral blood flow is the viscosity of blood. Blood viscosity has an inverse relationship with flow velocity and CBF. This means that higher whole blood viscosity is associated with lower CBF. Two major factors influencing viscosity (and oxygen-carrying capacity of the blood) are the hematocrit (Harrison, 1989) and the propensity for erythrocytes to form aggregates (Schmid-Schonbein, 1983). Early clinical studies have demonstrated that an increased hematocrit could contribute to lower CBF under neuropathological conditions. For example, greater infarct sizes after ischemic stroke have been found to be associated with lower CBF (due to a high hematocrit value) (Allport et al., 2005;
Moreover, a causal relationship between CBF and hematocrit has been observed in a study that demonstrated improvement in CBF in patients with occlusive vascular disease and reduction in hematocrit (Thomas et al., 1977). The impact of viscosity on CBF is dwarfed by the impact of reduced oxygen-carrying capacity of the blood; furthermore, hemodilution has not been shown to improve outcome after acute stroke (Chang & Jensen, 2014) and increased viscosity has been shown to increase capillary density (Tsai et al., 2005).

The rigidity of the erythrocyte membrane and their affinity to form aggregates can also affect CBF. The aggregation of erythrocytes has been shown to compromise microvascular perfusion in rat models. The rigidity of erythrocytes membrane affects CBF by limiting the rate of capillary perfusion. The ratio of cholesterol to phospholipids on the erythrocyte membrane determines its rigidity. Cholesterol plays a key role in erythrocyte membrane fluidity as it is the main bilayer matrix that regulates the mobility of phospholipid fatty acyl chains by condensing hydrophobic interactions, thereby leading to increased rigidity to membrane lipids. With age, the cholesterol concentration increases and erythrocyte membranes become more rigid (Marino et al., 2002). A rigid cell membrane may hinder the passage of erythrocytes through capillaries and therefore the membrane fluidity of erythrocytes indirectly interferes with CBF (G. McHedlishvili, Gobejishvili, Mamaladze, Momtselidze, & Varazashvili, 1999).

The contribution of shear stress to changes in CBF has been demonstrated to have an impact on vascular autoregulation by increasing vessel diameter (Rubanyi, Freay, Kauser, Johns, & Harder, 1990). The effects of shear stress on CBF play an important role particularly in tortuous vessels where the difference in flow velocity between the middle axis and vascular wall is highest. Increases in shear stress may present a physical stimulus to the endothelium and may hinder regeneration following endothelial damage (de la Torre & Mussivand, 1993). Shear stress can stimulate mechanoreceptors on endothelial cells, which would activate inward rectifying K⁺ channels. This would ultimately lead to the production of NO and prostaglandin I₂, which would dilate the vessel (Rubanyi et al., 1990). Changes in vascular diameter are associated with changes in vascular resistance and CBF, which are two inversely related physiological parameters. Changes in lumen radius affect the vascular resistance exponentially (Farkas & Luiten, 2001).

### 1.3.2 Cerebral Neurovascular Regulation

The brain is a very energy demanding organ as it only represents approximately 2% of body mass but consumes approximately 20% of the total energy in a resting state (Raichle &
Gusnard, 2002). Despite this, the brain has limited intracellular energy reserves and thus proper functioning is highly dependent on cerebral blood supply and the continuous delivery of oxygen and glucose. Because of these high energetic requirements, evolution has provided the brain several protective control mechanisms to safeguard against dangerous fluctuations in blood supply. One mechanism, cerebrovascular autoregulation, prevents harmful fluctuations in CBF that result from changes in arterial pressure. Another mechanism, functional hyperaemia, matches the delivery of blood flow to the activity level of localized brain regions. These control mechanisms determine changes in the vessels intraluminal diameter (T. J. Lee et al., 2011).

What happens when these protective mechanisms fail? This thesis will address this question in subsequent chapters. Before this discussion, it is essential to have a clear definition and understanding of these mechanisms. The following sections will describe pressure autoregulation and functional hyperaemia in more detail along with the role of the neurovascular unit in mediating the signal transduction cascades involved in these protective mechanisms.

Understanding these control mechanisms and the factors that drive them is necessary as functional brain imaging is based on mapping the changes in CBF caused by neural activity (Raichle & Mintun, 2006). This thesis presents work that uses functional brain imaging to examine the role of dysfunction in neurovascular coupling in the context of white matter disease. Functional brain imaging will be described later in more detail. For now, it is important to note that functional brain imaging is an excellent tool for examining hemodynamic changes underlying small vessel disease. Therefore, understanding the cellular mechanisms coupling neural activity to CBF may provide insight into the specific neural processes underlying functional brain imaging signals (Lauritzen, 2005). However, the mechanisms controlling blood flow may become compromised in the presence of vascular risk factors such as hypertension and diabetes and after an ischaemic event, promoting vulnerability of the brain to cellular dysfunction and death (Jackman & Iadecola, 2015).

1.3.2.1 Autoregulation

Arterial pressure heavily influences CBF (Faraci & Heistad, 1998). Arterial pressure fluctuates widely during the activities of daily living, which could induce potentially dangerous increases or decreases in CBF. Therefore, evolution has provided the cerebral circulation with a control mechanism, termed autoregulation, that renders CBF independent of arterial pressure within a certain range, termed the autoregulatory range (of roughly 60-150 mmHg mean arterial pressure) (Cipolla, 2007). Prior research examining the nature of cerebral autoregulation has
used linear models to characterize the interaction between blood pressure and cerebral blood flow. However, current research using a multiple regression model that does not assume linearity, demonstrates that the characteristic nature of the relationship between arterial pressure and cerebral flow fluctuations possesses a gain within the autoregulatory range that is dependent on the frequency of fluctuations in arterial pressure (Figure 1-4) (Tan, 2012; J. A. Taylor, Tan, & Hamner, 2014). In other words, faster fluctuations in arterial pressure (~12 seconds) are transmitted to the cerebral circulation almost linearly thereby demonstrating high coherence between flow and perfusion pressure changes. In this situation, the cerebral vessels are too slow to respond to the sudden changes in perfusion pressure. Conversely, slow fluctuations (~30 seconds) in perfusion pressure are effectively counter-regulated, thereby demonstrating effective dynamic autoregulation in which the vessels have time to respond to changes in perfusion pressure (Hamner, Cohen, Mukai, Lipsitz, & Taylor, 2004; Hamner, Tan, Lee, Cohen, & Taylor, 2010).

![Figure 1-4](image.png)

**Figure 1-4. A schematic representation of the possible relationships between mean arterial pressure and cerebral blood flow.** The left panel is Lassen’s classic cerebral autoregulation curve, which describes an autoregulatory region wherein slow arterial pressure fluctuations (> 20 seconds) are effectively counter-regulated and two passive regions wherein pressure fluctuations result in parallel changes in flow. The right panel demonstrates that the effectiveness of autoregulation is significantly reduced as pressure fluctuations become faster (above 0.07 Hz) as described in recent studies [figure is reproduced with permission from (Tzeng & Ainslie, 2014)].
The cellular bases of autoregulation reside in the intrinsic ability of smooth muscle cells to constrict when the intravascular pressure is increased and to relax when intravascular pressure decreases (known as the myogenic response) (M. A. Hill, Sun, Martinez-Lemus, & Meininger, 2007). Capillary vasodilation and vasoconstriction is controlled by endothelial cell action, mainly through the production of nitric oxide and its metabolites (Iadecola et al., 1995). In the larger calibre vessels, such as the carotid artery, myocytes play an important role in contraction and relaxation dynamics (Peppiatt et al., 2006). Ca\(^{2+}\)-dependent K\(^+\) channels and 20-HETEs (arachidonic acid metabolites of the cytochrome P450 \(\omega\)-hydroxylase pathway) have a key role in the myogenic response (Fleming & Busse, 2006). Increases in intravascular pressure activate stretch-sensitive Ca\(^{2+}\) channels, which increase intracellular Ca\(^{2+}\) concentrations and activate PLA\(_2\) leading to arachidonic acid production. Arachidonic acid is then metabolized to 20-HETEs, which in turn inhibit Ca\(^{2+}\)-dependent K\(^+\) channels, leading to smooth muscle-cell depolarization and vasoconstriction (M. A. Hill et al., 2007).

Autoregulatory mechanisms ensure that transient pressure increases do not result in increased flow and decreases do not result in decreased flow and ischemia. Therefore, if autoregulation is compromised or the ability to autoregulate is reduced, changes in systemic pressure could lead to greater vulnerability to neurological damage. Indeed, prior research has demonstrated that the autoregulatory capacity is compromised after acute brain injury and in subjects with steno-occlusive disease (Han et al., 2011). This results in CBF dysregulation and induces neuronal damage as seen with cortical thinning (Fierstra et al., 2010). The subsequent chapter of this thesis will examine the areas where the autoregulatory capacity is compromised in the white matter of elderly subjects.

**1.3.2.2 Functional Hyperaemia and Neurovascular coupling**

Cerebral functional hyperaemia is the mechanism by which blood flow is locally regulated, ensuring that the delivery of oxygen and nutrients meets the metabolic demands during changes in activity of specific brain regions (Filosa, 2010). Functional hyperaemia occurs in physiological situations such as moving, reading, calculating, or during any other mental exercise (Gordon et al., 2007). It can also occur in pathological situations, for example, during seizures when the oxygen demand increases in a specific brain area (Itoh et al., 2012).

Neurovascular coupling refers to the relationship between local neural activity and subsequent changes in cerebral blood flow. Understanding of the mechanisms that coordinate neurovascular coupling is fundamental to interpreting signals obtained from brain imaging.
techniques such as functional MRI. By the end of the nineteenth century, evidence arose supporting the coordination between neural activity and energy supply. In 1881, Mosso measured brain pulsations over the right prefrontal cortex in a subject with an abnormally thinned skull and reported increased pulsations when the subject performed a mathematical task (Mosso, 1881). By 1890, Sherrington used a more direct approach by demonstrating that stimulation of the sensory nerves, or the medulla oblongata, produced an increase in brain blood pressure (Roy & Sherrington, 1890). This hemodynamic response that accompanies brain activation was found to also exist in pathological situations such as ischaemia, in which Ames et al. (1968) demonstrated impaired reperfusion to localized areas of the rabbit brains after inducing ischaemia for short periods ranging from 2 to 15 minutes. This vascular response was interpreted as a compensatory mechanism that fuels the brain either during increased energy expenditure or during times of restricted metabolic substrate delivery (Ames, Wright, Kowada, Thurston, & Majno, 1968; Moreno, Jego, de la Cruz, & Canals, 2013). A majority of the cerebral metabolic demand comes from the restoration of the resting membrane potential of activated neurons (Ames, 2000).

1.3.2.2.1 Neurophysiology of neurovascular coupling and its contribution to fMRI signals

It is an important matter in neuroimaging to understand the aspects of neuronal activity that are reflected in increased cerebral blood flow (CBF). Synaptic transmission and action potentials (population spikes) represent two different aspects of neural activity, each having their own molecular basis and energy requirements. Synaptic activity as reflected in the local field potential can be considered as the input to the neuron. The local field potential refers to the electric potential in the extracellular space around neurons and demonstrates a collective measure of synaptic and active dendritic currents. Action potentials are the output signals that represent communication between neurons. Local field potentials and action potentials may be highly correlated and both will correlate with vascular responses. An example of this can be seen in bottom-up sensory processing where both efferent and afferent activity increase proportionally (Logothetis & Wandell, 2004). However, there are situations in which action potentials and subthreshold responses (not eliciting spikes) are dissociated. For example, higher-level brain areas may be subject to feedback and neuromodulatory signals that may induce changes in subthreshold membrane potentials that do not elicit spikes. Experiments simultaneously using fMRI and electrophysiological recordings in the primary visual cortex of anesthetized monkeys (allowing simultaneous acquisition of fMRI, local field potentials, and
spiking activity) demonstrated that the imaging signal evoked by visual stimulation maximally correlates with the local field potential (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). Anesthetized monkeys viewed contrast gratings while electrophysiological and fMRI data was recorded in the primary visual cortex. By correlating the simultaneously recorded local field potential and spiking activity with the fMRI signal, the authors showed that correlation of the fMRI blood-oxygen-level-dependent (BOLD) signal was only slightly higher toward local field potentials compared with spiking activity. The signals arising from local field potentials were the only predictor of the hemodynamic response when long stimulation protocols that habituate spiking activity were used (Mathiesen, Caesar, & Lauritzen, 2000; Thomsen, Offenhauser, & Lauritzen, 2004).

It has been argued that neuroimaging signals reflect the local processing of afferent neuronal activity rather than efferent activity. Recent studies demonstrating this idea use both fMRI and electrophysiological recordings to demonstrate that local synaptic plasticity modulates the amplitude of the BOLD signal (Canals, Beyerlein, Merkle, & Logothetis, 2009). Of note, the hippocampus is an ideal location to examine due to the axial organization of the cellular elements. There is a precise alignment of dendritic trees and somas, which minimizes the cancellation of current sources from the LFP generators and facilitates the neurophysiological interpretation of the electrically-evoked field potentials (such as synaptic currents reflected in the excitatory post-synaptic potential and spiking activity in the population spike). Moreno et al. (2013) used electrical microstimulation of the perforant pathway to activate the dentate gyrus in rats while simultaneously recording electrophysiological and fMRI signals. The authors demonstrated that the glutamate-evoked post-synaptic currents were a precise predictor of BOLD signal amplitude.

In summary, these studies suggest that when afferent and efferent activities are correlated, the vascular response may be considered to reflect both population spiking and local field potentials. However, when input is dissociated from output activity, the vascular response more closely reflects local field potentials (synaptic activity) and may be serve as an indicator of spiking activity in the local neuronal population. Moreno et al. (2013) found that glutamate evoked synaptic currents critically contribute to the BOLD signal. This is intuitively satisfying as the supply of energy substrates is potentially coupled to the process that consumes most of the energy used for neuronal signaling (Ames, 2000; Attwell & Laughlin, 2001).
1.4 Mechanisms of Neurovascular Coupling

There are currently two major concepts that have been put forward to mechanistically explain the coupling between neuronal activity and hyperaemia. The classical concept supports a feedback mechanism in which the by-products of energy expenditure act as signaling molecules to increase blood supply and restore energy reserves. A more recent concept of a feed-forward mechanism has been proposed in which neurotransmitter-mediated signaling plays a major role in the regulation of cerebral blood flow. Intuitively, neurotransmitter signaling is intrinsically correlated with energy consumption, thus the feed-forward model maintains that a causal link with cerebral blood flow regulation only exists with neurotransmission. A large body of recent experimental data by Atwell et al. (Atwell et al., 2010) as well as Petzold and Murthy (Petzold & Murthy, 2011) support the feed-forward model. However, both feedback and feed-forward models may coexist in the context of specific physiological states.

1.4.1 Main molecular players mediating neurovascular coupling

The role of NO as a modulator of the hemodynamic response to neuronal activation is well established. NO exerts its vasodilatory effects mostly by binding to soluble guanylyl cyclase found in smooth muscle cells (Friebe & Koesling, 2003), endothelial cells, astrocytes, and neurons (Baltrons, Pifarre, Ferrer, Carot, & Garcia, 2004). NO inhibition has been demonstrated to reduce CBF responses in rodent whisker and forepaw stimulation models. For example, rat whisker stimulation using a non-selective NO synthase inhibitor, N-nitro-L-arginine, which inhibits NO production produced a significant 50% reduction of CBF responses (Dirnagl, Lindauer, & Villringer, 1993). When using a specific inhibitor of neuronal NO synthase, 7-nitroindazole, local cortical CBF was reduced by 50% in response to forepaw stimulation. Despite these striking results, NO is not considered a primary mediator of the CBF response to neural activity, but rather acts as a permissive factor, which is required for vasodilation by other pathways (Lindauer et al., 2010).

Adenosine is a byproduct of cellular activity and is another mediator involved in neurovascular coupling (Iadecola, 2004). Adenosine can bind to four different receptors: A₁, A₂A, A₂B, and A₃. The vasodilatory effect of adenosine is mainly attributed to the A₂ receptor, which is expressed by neurons, astrocytes, as well as endothelial and smooth muscle cells (Ngai, Coyne, Meno, West, & Winn, 2001). For in vivo studies, the role of adenosine was established using either the non-specific adenosine receptor antagonist theophylline (Ko, Ngai, & Winn, 1990) or the selective A₂B receptor antagonists alloxazine and MRS-1754 (Shi et al., 2008) that...
decreased up to 40% of the CBF response to neuronal activity. In contrast to the other receptor subtypes, neither the selective A3 receptor antagonist, MRS-1191, nor the selective A\textsubscript{2A} receptor antagonist, SCH-58261, had any effect on the CBF response, suggesting no involvement in neurovascular coupling. However, ZM-241385 reduced pial artery vasodilation in response to rat sciatic nerve stimulation, suggesting an effect of A\textsubscript{2A} receptors in partially mediating an upstream vasodilatory response.

Arachidonic acid metabolites also play a role in neurovascular coupling. These metabolites are synthesized and released following the release of glutamate by neuronal activation (Stella, Tence, Glowinski, & Premont, 1994). Vasomediators derived from arachidonic acid can be produced by several enzymatic pathways, which include cyclooxygenase (COX) enzymes and cytochrome P450 epoxygenases or hydroxygenases (Bosetti, 2007; Kroetz & Xu, 2005). COX enzymes consist of COX-1, expressed in glial cells, and COX-2, expressed by cortical glutamatergic neurons. COX enzymes are responsible for the production of diverse prostaglandins, including the potent vasodilator PGE\textsubscript{2}. PGE\textsubscript{2} binds to EP1 to EP4 receptors, of which EP2 and EP4 receptors may mediate vascular effects. These receptors are expressed by neurons but their specific cellular localization across the components of the neurovascular unit has not been fully characterized (Andreasson, 2010). Both isoforms of CPX can be non-selectively inhibited with indomethacin, which decreases the cortical CBF response to whisker stimulation by 30% (Dahlgren, Rosen, & Nilsson, 1984).

Arachidonic acid products of the cytochrome P450 include 20-hydroxyeicosatetraenoic acid (20-HETE), formed by CYP4A and CYP4F \(\omega\)-hydroxylases (Kroetz & Xu, 2005). 20-HETE is a potent vasoconstrictor (Harder et al., 2002), which may be active depending on NO levels, since its blockade by HET-0016 has a significant effect on the CBF response to whisker stimulation when applied after neuronal NO synthase inhibition (X. Liu et al., 2008).

Epoxyeicosatrienoic acids (EETs) are synthesized by CYP2C and CYP2J epoxygenases (Kroetz & Xu, 2005) and exhibit vasodilatory effects. The exact mechanism of EETs still remains to be elucidated. EETs may be directly taken up by cells and incorporated into phospholipid membranes(Spector, 2009). The role of EETs in neurovascular coupling was confirmed by using specific inhibitors of EET synthesis (MS-PPOH and miconazole) or antagonist of EET receptors, in which EETs have been shown to contribute to 40-60% of the CBF response to sensory stimulation (Shi et al., 2008). EETs are currently known to be released by astrocytes, making them important intermediaries in the communication between neurons.
and microvessels (Alkayed et al., 1996). In addition to paracrine effects on the vasculature, EETs may also act in an autocrine fashion by enhancing calcium in astrocytes (V. M. Blanco, Stern, & Filosa, 2008). This may lead to the activation of several calcium-dependent pathways, such as the opening of astrocytic calcium-sensitive K\(^+\) channels (Gebremedhin et al., 2003).

It is also well established that increases in potassium concentrations in the extracellular space may induce vasodilation. This effect is likely mediated by inwardly rectifying K\(^+\) channels (Filosa & Blanco, 2007). Astrocytic endfeet contacting blood vessels possess several types of potassium channels, including inwardly rectifying potassium channels and large conductance, calcium-sensitive K\(^+\) channels. K\(^+\) channels are involved in both vasodilation and vasoconstriction depending on Ca\(^{2+}\) concentrations. This is of particular interest since it has the potential of explaining rapid modulations of microvascular tone following neuronal activation (Girouard et al., 2010).

### 1.4.2 Myogenic regulation of cerebral blood flow

Autoregulatory mechanisms act to maintain CBF independent of perfusion pressure, within a very tight autoregulatory range (Paulson, Strandgaard, & Edvinsson, 1990; Tan, 2012; J. A. Taylor et al., 2014; Wagner & Traystman, 1985). Outside of the autoregulatory range, these mechanisms become uncoupled from perfusion pressure and lose control of CBF. One of the mechanisms of dynamically maintaining CBF is achieved by altering vascular resistance through a myogenic response. The myogenic component of cerebral autoregulation is defined as the intrinsic capacity of vascular smooth muscle cells to contract in response to mechanical stress, such as an increase in transmural pressure (Ursino, 1991). Studies in isolated rat and human artery preparations demonstrated an increase in vascular tone and decrease in luminal diameter when perfusion pressure was gradually increased (Halpern & Osol, 1985; Wallis, Firth, & Dunn, 1996). These increases in transmural pressure caused little change in CBF unless the perfusion pressure drops below the lower limit of the autoregulatory range (Tan, 2012; Tzeng & Ainslie, 2014), indicating that stretch-dependent vasoconstriction maintains CBF constant when the perfusion pressure stays within the autoregulatory range. The cellular components of the myogenic response to autoregulation are located in the vascular smooth muscle, which depolarize as mechanical pressure increases (Harder, 1985). This pressure-activated contraction of smooth muscle cells is described to be dependent on the extracellular calcium concentration and is mediated by an arachidonic acid signal transduction pathway (Harder, Lange, Gebremedhin, Birks, & Roman, 1997).
1.4.3 Metabolic feedback mechanism

Several observations have examined the effect of glucose and oxygen on blood flow control via (Lindauer et al., 2010) (Powers, Hirsch, & Cryer, 1996) in vivo showing minimal effect on CBF regulation. However, other metabolic byproducts like adenosine can regulate CBF, linking the action of the Na\(^+/K^+\) ATPase pump to local vasodilation. Also, extracellular adenosine acting on adenosine A\(_{2A}\) receptors in vascular smooth muscle inhibits the arteriolar vasoconstriction mediated by the arachidonic acid metabolite, 20-HETE (Gordon, Choi, Rungta, Ellis-Davies, & MacVicar, 2008). The resulting vasodilatory effect has been demonstrated in the cortex (Ko et al., 1990) and the cerebellum (J. Li & Iadecola, 1994) following neuronal stimulation. The effects of adenosine on CBF mediated by adenosine A\(_{2B}\) receptors also involve interaction with calcium signaling in astrocytes and arachidonic acid metabolism (Shi et al., 2008). One caveat in interpreting the source of extracellular adenosine is that the ATP used as a gliotransmitter may also be hydrolyzed to adenosine by extracellular ectonucleotidases (Latini & Pedata, 2001). This means that adenosine will couple CBF to either energy consumption or neuronal signaling through glio-transmission, depending on its origin.

Lactate is another key energy metabolite in the brain, which reinforces the feedback model of neurovascular coupling. The mechanism linking lactate to vasodilation involves the inhibition of the prostaglandin transporter, increasing the extracellular concentration of PGE\(_2\) released from astrocytes and potentiating vasodilation (Gordon et al., 2008). Studies have shown that increased glycolytic activity during brain activation (Raichle & Mintun, 2006) results from the uptake of synaptically-released glutamate into astrocytes, in addition to sodium (Pellerin & Magistretti, 2004). Astrocytic glutamate is converted into glutamine and the excess sodium is released to the extracellular space, with both processes consuming ATP (Erecinska & Silver, 1994). Refilling of energy reserves appears to be critically dependent on glycolysis and it is linked to blood flow modulation through lactate (Raichle & Mintun, 2006). Several studies have shown that the CBF response to an increase in neuronal activity is partly modulated by changes in the plasma lactate/pyruvate ratio (Mintun et al., 2001; Vlassenko, Rundle, Raichle, & Mintun, 2006). Accordingly, it has also been shown that lactate may modulate the BOLD fMRI signal in the visual cortex of non-human primates (von Pfostl et al., 2012). These results support a direct metabolic effect on CBF regulation and identify astrocytes as important players in the generation of fMRI signals.
1.4.4 Neurotransmitter-mediated feed-forward signaling

The feed-forward model is mainly represented by NO and arachidonic acid metabolites released from neurons and glial cells as a consequence of glutamatergic neurotransmission. To a lesser extent, GABA and other vasoactive peptides released by interneurons (Kocharyan, Fernandes, Tong, Vaucher, & Hamel, 2008) have been shown to contribute to hyperemia in rats but the experimental evidence remains sparse.

Synaptically released glutamate acting on NMDA receptors increases post-synaptic calcium levels and activates neuronal nitric oxide synthase increasing NO release. NO has been demonstrated in slice and *in vivo* preparations to mediate cerebral vasodilation (Busija, Bari, Domoki, & Louis, 2007). However, there are regional differences in the extent of how NO contributes to blood flow regulation (Sokoloff et al., 1977). For example, unlike the cerebellum, cortical NO is required for functional hyperemia but does not directly mediate neuron-to-vessel signaling (Lindauer et al., 2010) as the astrocytes play a more important role. The role of NO in the cortex has been suggested to be the modulation of the arachidonic acid metabolic pathways in astrocytes as described by Attwell et al. (2010). Blood flow is regulated through the production and release of arachidonic acid metabolites in response to synaptic glutamate. The mechanism for this starts with a metabotropic glutamate receptor-dependent increase in the astrocytic intracellular calcium concentration that activates phospholipase A$_2$ and releases arachidonic acid from membrane phospholipids. Subsequently, arachidonic acid metabolites with vasodilatory activity (such as prostaglandins and epoxyeicosatrienoic acids) are produced and released. Interestingly, vasoconstriction may be mediated by increases in astrocytic calcium concentrations through the arachidonic acid metabolite 20-HETE (Mulligan & MacVicar, 2004). This dichotomy has been elegantly explained by demonstrating that the metabolic state of the tissue ultimately determines the direction of astrocytic control over vascular responses (Gordon et al., 2008), with decreasing oxygen concentrations favouring the production of vasodilatory responses. In effect, glial cells act as oxygen sensors in the tissue milieu.

1.4.5 Mechanisms of vasodilation

Astrocytes can sense increasing glutamate via metabotropic glutamate receptors promoting an increase in intracellular calcium that results in arachidonic acid formation from phospholipase A$_2$ (PLA$_2$). Arachidonic acid is converted by COX1 or COX3 to prostaglandins (PGs) and can also be converted by p450 epoxygenase to epoxyeicosatrienoic acid (EET). Both
PGs and EETs can relax smooth muscle cells through the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP).

The vasodilatory process starts with targets of endothelial receptors, which include acetylcholine (ACh), bradykinin (BK), adenosine diphosphate (ADP), ATP, uridine triphosphate (UTP), and adenosine. Endothelial receptor binding can activate phospholipase C (PLC) or PLA₂. These phospholipases hydrolyze membrane phospholipids and can produce a second messenger called diacyl-glycerol (DAG). The activation of DAG ultimately leads to the production of EETs and arachidonic acid derivatives including prostaglandin I₂ (PGI₂). These signaling molecules can drive smooth muscle cell relaxation via cAMP, while increased intracellular calcium can drive the production of endothelial NO. Endothelial NO can drive smooth muscle cell relaxation through conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP).

Another mechanism by which smooth muscle cells can relax stems from endothelial cell hyperpolarization. Endothelial cells can increase their intracellular calcium through transient receptor potential (TRP) receptor cation channels, which activate inositol triphosphate (IP₃)-mediated release of calcium from intracellular stores in the endoplasmic reticulum. Intracellular calcium increases and results in endothelial hyperpolarization (and therefore vascular relaxation) through the opening of calcium-dependent potassium channels. Endothelial hyperpolarization could be coupled to adjacent smooth muscle cells through myoendothelial gap junctions (MEGJs) or other endothelium-derived hyperpolarizing factors (EDHF) such as potassium efflux through endothelial small-conductance calcium-dependent potassium channels (SKCa) and intermediate-conductance calcium-dependent potassium channels (IKCa) by activating inwardly rectifying potassium channels (KIR) and/or activation the Na⁺/K⁺ ATPase. Neighbouring endothelial hypolarizations can spread rapidly to adjacent cells through gap junctions. Pericytes also possess many muscle cell-like properties and could relax in response to NO and PGI2 released from astrocytes cells (Wiencken & Casagrande, 1999), neurons, or endothelial. Pericytes could also relax in response to neuropeptides such as vasointestinal peptides (VIPs) and also to neurotransmitter like catecholamines. Therefore, endothelial cells, astrocytes, and pericytes play important roles in signaling to smooth muscle cells in response to neurovascular coupling (Figure 1-5) (Attwell et al., 2010; Feletou & Vanhoutte, 2004; Hillman, 2014).
Figure 1-5. Neuromodulatory coupling pathways. Vasodilatory mechanisms are initiated from neuronal metabolic demands (feed-forward model) in which metabotropic glutamatergic signaling from neurons to astrocytes increases intracellular calcium. This leads to a signaling cascade that eventually leads to the production of prostaglandins (PG) and epoxyeicosatrienoic acid (EET) which eventually leads to vasodilation by hyperpolarizing myocytes/smooth muscle cells. A number of endothelial receptor targets possess similar signal transduction mechanisms. See section 1.4.5 for more details. AA, arachidonic acid; ADP, adenosine diphosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanine monophosphate; GTP, guanine triphosphate; DAG, diacyl glycerol; EDHF, endothelium-derived hyperpolarizing factor; IKCa, intermediate-conductance calcium-dependent potassium channel; IP3, inositol triphosphate; MEGJ, myoendothelial gap junction; mGluR, metabotropic glutamate receptors; NO, nitric oxide; SKCa, small-conductance calcium-dependent potassium channel; TRP, transient receptor potential channel.
1.4.5.1 Mechanisms of CO$_2$-mediated vasodilation

The cerebral vasculature is profoundly affected by the partial pressure of arterial CO$_2$ (PaCO$_2$) (Brian, Faraci, & Heistad, 1996). The sensitivity of CBF to changes in PaCO$_2$ is an important homeostatic function that helps to maintain central pH (Chesler, 2003). The interaction between PaCO$_2$ and its vasoactive properties begins at the level of the neurovascular unit (Busija & Heistad, 1984). Increases in CO$_2$ result in vascular smooth muscle relaxation in all cerebral vessels, although smaller calibre vessels appear to be more responsive. However, vasoconstrictive effects of hypocapnia is not affected by vessel size (Wei, Kontos, & Patterson, 1980).

Mechanisms of CO$_2$-mediated vasodilation/vasoconstriction have not been fully elucidated. There is evidence that indicate elevations in CO$_2$ and accompanying changes in pH activate K$^+$ channels in the vascular smooth muscle cells. The cerebral endothelial cells express four classes of K$^+$ channels: inward rectifying K$^+$ channels, Ca$^{2+}$-activated K$^+$ channels, ATP-sensitive K$^+$ channels, and voltage-gated K$^+$ channels (Kinoshita & Katusic, 1997; Nelson & Quayle, 1995). Reductions in pH activate ATP-sensitive K$^+$ channels, and voltage-gated K$^+$ channels, contributing to acidosis-induced vasodilation (Berger, Vandier, Bonnet, Jackson, & Rusch, 1998; Xu et al., 2001). As channels open and close in a stochastic fashion, elevated CO$_2$ levels results in a higher open probability of K$^+$ channels, which result in K$^+$ efflux and endothelial cell hyperpolarization (Faraci & Sobey, 1996; Jackson, 2005). These hyperpolarizing currents may then spread to adjacent smooth muscle cells via myoendothelial gap junctions (Golding, Marrelli, You, & Bryan, 2002) where they may elicit vasodilation secondary to the inactivation of Cav channels, reduction in intracellular Ca$^{2+}$, and vascular relaxation through hyperpolarization (Jackson, 2005; Kitazono, Faraci, Taguchi, & Heistad, 1995){Nelson, 1995 #372. Therefore, K$^+$ channels play an important role in coordinating the vasoactive properties of CO$_2$.

Another mechanism in which CO$_2$-induced vasodilation can occur is through vasoactive factors. CO$_2$-induced CBF increases cause shear stress on the endothelial wall, which result in the release of NO and PGs (Leffler, Mirro, Pharris, & Shibata, 1994; Parfenova, Shibata, Zuckerman, & Leffler, 1994). Evidence exists for other potential vasodilators, including adrenomedullin and C-natriuretic peptide (also a EDHF). The role of vasoactive factors is
highly complex and much research is needed to elucidate the mechanisms by which CO₂ interacts with these signaling molecules.

This thesis will test the sensitivity of the cerebrovasculature to CO₂ particularly in the white matter. Subsequent chapters will demonstrate phenomena in brain areas that are operating at their vasodilatory capacity. If the vasodilatory capacity of a particular area is at its limit, vessels will no longer respond to CO₂ and therefore will have little if any further vasodilatation. When this happens, blood will redistribute to areas with robust vasodilatory capacity and steal physiology will occur in this region. The effects of steal physiology in grey matter are well known as it is spatially associated with cortical thinning (Fierstra et al., 2010) and cognitive decline (Balucani, Viticchi, Falsetti, & Silvestrini, 2012; Buratti et al., 2014). However, the effects of steal physiology in the white matter have not been elucidated. We believe that steal physiology will lead to white matter damage that will be visible on modern structural imaging techniques. These white matter changes are known as leukoaraiosis and will be described in the following section. This latter half of the thesis will examine leukoaraiosis describing its history, definition, risk factors, and pathophysiology. Once a solid understanding of leukoaraiosis has been developed, MRI techniques used to characterize and quantify leukoaraiosis will be explained. Particular emphasis will be placed on techniques that examine CO₂-dependent vasodilation.

1.5 Leukoaraiosis

Changes occur in nearly every body system with advanced age. Many older individuals express significant concern about loss of cognitive function and development of dementia with advanced age. These concerns are by no means a focus restricted to modern society. Advanced age and its association with impaired cognitive abilities was recognized by the Egyptians in 2000 BC and some records suggest that dementia was so common among the elderly that it was considered a normal aspect of the aging process by Plato and other scholars in those times (Boller, 2008).

Setting history aside, there is no question regarding the overwhelming prevalence of cognitive dysfunction and dementia today. Currently, 750,000 Canadians are living with Alzheimer’s disease and other dementias, account for approximately 15% of those over the age of 65. The prevalence is expected to double by 2030 if nothing changes in Canada. The current individual and societal costs of dementia are no less striking, with the combined direct (medical) and indirect (lost earnings) costs of dementia totaling $33 billion per year. This number will
climb to a staggering $293 billion a year by 2040 is nothing changes (Dao, Robin Hsiung, Sossi, Jacova, & Liu-Ambrose, 2013). The magnitude of these effects will continue to parallel the changing demographics throughout the coming years as the first wave of baby boomers turned 65 in 2011; furthermore, the risk of dementia doubles every five years after the age of 65 (Bachman et al., 1993).

The modern history of dementia begins in 1894 with neurologists Otto Binswanger and Alois Alzheimer, who realized the heterogeneity of dementia subtypes. Both neurologists described discrete vascular lesions in the brain that were presumed to underlie declines in cognitive functions. Otto Binswanger promoted this idea by reporting that arteriosclerosis and associated reductions in brain perfusion were responsible for mental decline in older adulthood. The neurologists at that time described four clinico-pathological variants of vascular dementia: dementia post-apoplexy (later known as post-stroke dementia), arteriosclerotic brain degeneration, vascular cortical atrophy (granular atrophy), and subcortical encephalopathy (Binswanger’s disease). One of the earliest illustrations of Binswanger’s disease was published in the 1910 edition of Emile Kraepelin’s famous Textbook of Psychiatry, in the chapter called “Das senile und präsenile Irresein” (“Senile and Presenile Dementia”). In this book, Kraepelin’s ideas and findings fell closely in line with that of Binswanger and Alzheimer. However, Kraepelin’s influence was so pervasive that following his description of vascular dementia under the name arteriosclerotic dementia or cerebral arteriosclerosis, these terms became synonymous with senile dementia for the next 70 years, from 1910 until 1974 (G. Roman, 2003; G. C. Roman, 2002). As Alzheimer’s disease became recognized as the common cause of senile dementia, the old term cerebral arteriosclerosis was replaced in 1974 with the term multi-infarct dementia by Hachinski et al. (V. C. Hachinski, Lassen, & Marshall, 1974). Hachinski et al. emphasized, “when vascular disease is responsible for dementia, it is through the occurrence of multiple small or large cerebral infarcts.” Multi-infarct dementia then became synonymous with vascular dementia.

With the advancement of modern brain imaging techniques such as the computed tomography (CT) and magnetic resonance imaging (MRI), many neurological changes associated with dementia could be identified in living patients. During this advancement in technology, Binswanger’s disease was being described in hundreds of patients based on radiological findings. This apparent epidemic was occurring at a time when hypertension, a hallmark of Binswanger’s disease, was becoming increasingly medically manageable and only
some patients who had hypodensities indicating ischemic changes on CT were hypertensive. Doctors realized that many different causes underlie the neurological changes seen on CT or MRI, particularly in the cerebral white matter. For example, a symmetrical periventricular white matter abnormality may represent the penetration of cerebrospinal fluid into shrinking brain, thereby producing pallor and the appearance of edema at autopsy. Alternatively, the asymmetric patchy and more intense changes may represent a confluence of microinfarcts from hypertension and/or cerebral amyloid angiopathy (Huang, Wu, & Luo, 1985). As the common denominator of white matter changes in the elderly is decreased density on CT or a change in MR signal, Hachinski et al. introduced the term “leuko-araiosis” (“leuko” = white and “araios” = rarefied) (V. C. Hachinski, Potter, & Merskey, 1986). Leukoaraiosis indicates that these white matter changes result in reduced x-ray absorption in the white matter. On MRI using T2-weighted fluid attenuated inversion recovery (FLAIR) images show changes in cerebral white matter and in particular, the periventricular white matter, as the cerebrospinal fluid signal is suppressed (DeCarli, 2013). These abnormal regions, seen as bright spots in the white matter of T2-weighted images, are called white matter hyperintensities. The use of the term leukoaraiosis is a purely descriptive term to describe white matter lesions seen on brain imaging and does not clearly define a particular pathological entity. The expressions “cerebral white matter disease” and “cerebral small vessel disease” are often used synonymously. The use of the term “white matter disease” is once again purely descriptive and may also include inflammatory disorders or leukodystrophies. The clinical context in these disorders is different and usually “white matter disease” implies a presumed ischaemic origin of the lesions. Cerebral SVD addresses the underlying pathology of the lesions and includes the presence of diffuse white matter changes as well as distinct lacunar infarction (Grueter & Schulz, 2012).

1.5.1 Risk factors of leukoaraiosis

The risk factors for leukoaraiosis have been extensively investigated in recent years and study findings have been conflicting, possibly due to differences in study methodology. Some risk factors are non-modifiable, some are acquired and can be medically managed, and some may reflect confounding by hypertension. Inzitari D. et al (1987) was probably the first to establish a relationship between vascular risk factors and leukoaraiosis (Inzitari et al., 1987).

1.5.1.1. Hypertension

Hypertension is strongly associated with leukoaraiosis and is the most important modifiable risk factor (Matsushita et al., 1994; Streifler et al., 1995). Studies of white matter
changes clearly indicate that hypertension plays an important role in the development and progression of leukoaraiosis, with increases in both systolic and diastolic blood pressure both being relevant (M Simoni, Rothwell, & Mehta, 2010). There is no threshold value above which white matter changes start; furthermore, abnormalities in the diurnal blood pressure variation may also contribute to the development of leukoaraiosis (Pantoni & Garcia, 1997).

1.5.1.2. Age

In addition to hypertension, age is also an important risk factor for developing leukoaraiosis (Streifler et al., 1995), hence why leukoaraiosis is also commonly referred to as “age-related white matter disease.” Although leukoaraiosis is a pathological phenomenon, it may be part of the normal aging process. However, it is uncertain at what age white matter changes begin to develop and precise data on the extent of leukoaraiosis that can be regarded as “normal” at a certain age does not exist. Studies suggest that at least some white matter lesions can be expected in 95% of individuals after the age of 50 years (Grueter & Schulz, 2012; Launer et al., 2006). Without a doubt, leukoaraiosis is a very common finding in elderly people and becomes more prevalent as well as severe with increasing age (Pantoni & Garcia, 1995).

1.5.1.3. Race

Leukoaraiosis occurs more frequently in Afro-Caribbean than Caucasian populations (Gottesman et al., 2010). However, this result may be due to a higher prevalence of hypertension in Afro-Caribbean subjects. These subjects may also have more severe hypertension and possess different genetic factors that may alter the effect that hypertension has on the development of leukoaraiosis (Meadows et al., 2011).

1.5.1.4. Sex

Results from studies demonstrating the association between sex prevalence of leukoaraiosis have been conflicting. Some studies found a trend towards a higher prevalence of leukoaraiosis in women (Longstreth et al., 1996; van Dijk et al., 2008) and others found a higher prevalence in men (K. Park et al., 2007). One possible reason for the differing results could be explained by the characteristics of the study and other confounding factors, such as geographic location, age, or whether the subjects had a prior history TIA/stroke. For example, one study found the prevalence of leukoaraiosis to be higher in men but solely examined a Japanese population (K. Park et al., 2007) whereas other studies examined solely American subjects (Longstreth et al., 1996; van Dijk et al., 2008). Moreover, there may have been differences in the prevalence of hypertension between the men and women in these studies, which would

### 1.5.1.5 Diabetes Mellitus

Like the sex studies, the association between diabetes mellitus and leukoaraiosis has been conflicting. One study suggested an association between periventricular white matter lesions and diabetes mellitus (Bokura, Yamaguchi, Iijima, Nagai, & Oguro, 2008). Elevated fasting glucose was found to be associated with leukoaraiosis (K. Park et al., 2007). Another study found higher insulin levels in diabetic patients with leukoaraiosis compared to those without leukoaraiosis (Anan et al., 2009). Together, these studies suggest that increased insulin resistance is a risk factor for leukoaraiosis. On the other hand, Streifler et al. conducted a study to assess whether high-grade carotid stenosis is associated with leukoaraiosis by examining patients enrolled in the North American Symptomatic Carotid Endarterectomy Trial (NASCET). After controlling for known stroke risk factors using an ordinal regression analysis, no association between leukoaraiosis and diabetes mellitus was found (Streifler et al., 1995).

### 1.5.1.6 Dyslipoproteinaemia

Dyslipoproteinaemia is an important risk factor for large vessel carotid disease but it is uncertain whether abnormalities in lipid metabolism are also a risk factor for small vessel disease. Several studies have demonstrated that low levels of high-density lipoprotein cholesterol and hypertriglyceridaemia may increase the risk of developing leukoaraiosis (Anan et al., 2009; K. Park et al., 2007). However, other studies such as that conducted by Streifler et al. (1995) did not find an association between dyslipidaemia and leukoaraiosis.

### 1.5.1.7 Smoking

Similar to dyslipoproteinaemia, it is unclear whether the effect of current or past history of smoking has an influence on the development of leukoaraiosis. Some studies found an association (van Dijk et al., 2008), whereas other studies failed to find a difference (Streifler et al., 1995) in the prevalence of white matter disease between smokers and non-smokers.

### 1.5.1.8 Large vessel atherosclerosis

As the pathogenesis of leukoaraiosis is thought to be disease of the small vessels, atheroma of the large vessels and leukoaraiosis may not necessarily be associated. Indeed, a study in Chinese patients with acute ischemic stroke did not show a relationship between large artery stenosis and burden of leukoaraiosis (Pu et al., 2009). The NASCET study conducted by Streifler et al. (1995) also did not find an association. Nevertheless, significant large vessel
stenosis reduces blood flow to the brain, which increases the risk of chronic ischaemia and may increase the risk of developing leukoaraiosis. Atherosclerotic disease and white matter lesions have common vascular risk factors and may therefore occur concurrently.

1.5.1.9 Homocysteine and Vitamin B12

Several studies have reported an association between hyperhomocysteinaemia and white matter disease (Censori, Partziguian, Manara, & Poloni, 2007; Feng et al., 2013; Sachdev et al., 2004). High concentrations of plasma homocysteine causes vascular injury through a plethora of mechanisms which include endothelial injury, DNA dysfunction, proliferation of smooth muscle cells, reduced activity of glutathione peroxidase, increased oxidative stress, and promoting inflammation (Abdulle, Pathan, Moussa, & Gariballa, 2010). As hyperhomocysteinaemia causes direct damage to endothelial cells, this manifests as impaired vasodilatory capacity, possibly due to reduced nitric oxide synthesis and bioavailability. Impairment of nitric oxide release may potentiate atherothrombogenesis and oxidative stress (Pushpakumar, Kundu, & Sen, 2014). However, even though there is evidence that hyperhomocysteaemia and low vitamin B12 levels are associated with leukoaraiosis (Clarke, Lewington, Sherliker, & Armitage, 2007; Quadri et al., 2004), there is currently no data demonstrating that lowering homocysteine levels or treatment with vitamin B12 improves leukoaraiosis or reduces its progression.

1.5.1.10 Genetic Factors influencing leukoaraiosis

Although vascular risk factors are important in the development of leukoaraiosis (Wardlaw, Smith, & Dichgans, 2013), the exact cause still remains to be elucidated because not all individuals with those risk factors develop small vessel disease and often, known risk factors are not present in some patients with leukoaraiosis (Lammie, Brannan, Slattery, & Warlow, 1997). The etiology of leukoaraiosis is complex in which interactions between candidate genes and environmental factors or established vascular factors play an important role in the development of clinical phenotypes (Meschia, 2006). Several single-nucleotide polymorphisms have been identified through genome-wide association studies or meta-analysis of individual candidate genes on susceptible genetic loci associated with individuals with severe leukoaraiotic burden (Fornage et al., 2011; Paternoster, Chen, & Sudlow, 2009). Several single-gene disorders causing leukoaraiosis have been discovered, including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL),
COL4A1-related cerebral SVD, autosomal dominant retinal vasculopathy with cerebral leukodystrophy (RVCL), and Fabry disease. CADASIL is one of the most common single-gene disorders of the cerebral small blood vessels, which is caused by mutations in the NOTCH3 gene on chromosome 19q12 (Joutel et al., 1996). The main clinical features include migraine, recurrent stroke, and progressive cognitive decline (J. C. Choi, 2010). Interestingly, common single-nucleotide polymorphisms that are frequently found at the NOTCH3 gene, apart from CADASIL-related mutations, also increase the risk of age-related leukoaraiosis in individuals with hypertension (H. Schmidt et al., 2011). However, such genetic factors may not be directly linked to the presence of leukoaraiosis but may contribute to an individual’s tendency to develop a risk factor for leukoaraiosis, as well as their vulnerability to develop end-organ damage as a consequence of having a particular risk factor. For instance, the predisposition for developing hypertension is, to a certain extent, determined genetically and the tendency to develop leukoaraiosis as a consequence of hypertension may also be influenced by genetic factors (J. C. Choi, 2015; Grueter & Schulz, 2012).

1.5.2 Comorbidities of leukoaraiosis

Falls represent a major problem in the elderly with cognitive impairment. Leukoaraiosis is associated with gait abnormalities and with falls (Baezner et al., 2008; Blahak et al., 2009; Ogama, Sakurai, Shimizu, & Toba, 2014). Lesions in the parietal lobe are most highly associated with falls. The loss of neural connections between the white matter and cortex produced by the oxidative damage to the tissue appears to be the major reason for these gait disturbances (Murray et al., 2010).

Atrial fibrillation is highly associated with leukoaraiosis, falls, and cognitive dysfunction (Ampadu & Morley, 2015). Elderly individuals with atrial fibrillation have less falls when they are anticoagulated (Hui, Morley, Mikołajczak, & Lee, 2015). Depression is another major problem in the elderly with leukoaraiosis (Firbank et al., 2012). Diabetes is also an increased risk factor for depression (Rosenthal, Fajardo, Gilmore, Morley, & Naliboff, 1998). People with diabetes have a higher volume of leukoaraiosis (Verdelho et al., 2010). Diabetics are more likely to be frail (Formiga et al., 2014). Persons with white matter hypertintensities are also more likely to have stroke (Enzinger, Fazekas, Ropele, & Schmidt, 2007; H. Markus & Cullinane, 2001). Frailty is now recognized as one of the major geriatric syndromes of the 21st century (Morley et al., 2013). There has been increased awareness that in many cognitive impaired individuals, there is an increase in the frailty physical phenotype (Malmstrom &
Morley, 2013). This has become known as cognitive frailty (Halil, Cemal Kizilarslanoglu, Emin Kuyumcu, Yesil, & Cruz Jentoft, 2015). Frailty is also a predictor of the development of depression. There is increasing awareness that this combined form of frailty may be due to leukoaraiosis (Collard et al., 2015).

1.5.3 Pathophysiology of leukoaraiosis

Although the high prevalence and clinical importance of leukoaraiosis have been increasingly recognized in recent years, the pathophysiology is still incompletely understood. There are many age-related brain microvascular pathologies associated with aging and leukoaraiosis. Understanding the cerebrovascular dysfunction that precedes cognitive dysfunction and neurodegeneration seen in aging may give us insight on the pathogenesis of leukoaraiosis. Pathological features comprise several patterns of alterations including pallor or swelling of myelin, clasmatodendrosis (characterized by beading and fragmentation of the astrocytic processes with cytoplasmic swelling and vacuolation of the astrocytic soma), areas of reactive astrogliosis, and loss of oligodendrocytes, axons, and myelin fibres (Simpson et al., 2007). Age-related leukoaraiosis is associated with preceding cerebral microvascular pathology that ultimately leads to cognitive dysfunction and neurodegeneration, which will be described in more detail. Knowledge of the cerebrovascular anatomy and its age-related pathology assists in providing a better understanding of what makes the cerebral white matter prone to leukoaraiosis.

1.5.3.1 Blood supply to the white matter

The surface of the cerebral cortex is covered by an arterial network known as the leptomeningeal arteries. These arteries penetrate the brain to supply the white matter becoming end arteries that terminate in a capillary bed. There are no shunts between arteries or arterioles and veins within the brain (Anstrom et al., 2002). However, this model of vascular supply is not uniform throughout the brain, thus creating regional heterogeneity in the brain’s vulnerability to chronic hypoperfusion. The deep white matter, a region that has a high incidence of leukoaraiosis, is particularly vulnerable to chronic hypoperfusion because its major blood supply is through long medullary arterioles that arise from the border zone between the anterior cerebral artery and middle cerebral artery [Figure 1-6 (2)] (Moody, Bell, & Challa, 1990).

Some portions of the deep white matter may also receive blood supply from the medial and lateral brain surfaces. Branches of the MCA known as the lenticulostriate arteries provide additional blood supply to the deep white matter [Figure 1-6 (3)]. These arteries project upwards from the MCA and around the lateral ventricles. As a result, the white matter above
the lateral ventricles is not supplied by the lenticulostriates. The corpus callosum is interestingly spared from leukoaraiosis [Figure 1-6 (4)]. Blood supply to the corpus callosum is supplied by short penetrating vessels with low vascular resistance, creating less of a susceptibility to hypoperfusion (De Reuck, 1971; Van den Bergh, 1969).

Figure 1-6. Schematic drawing of blood supply to the cerebral white matter seen in coronal view. 1) The leptomeningeal arteries supply the surface of the brain. These vessels are well connected at the surface. This area represents the subcortical U-fibres with blood supply coming from different conduits 2) Once the leptomeningeal arteries penetrate the brain, they become end-arteries and do not possess arteriolar-arteriolar or arterio-venous shunts. These vessels also display age-related increases in tortuosity, which increases their vascular resistance 3) The basal ganglia and thalamus of the brain is supplied by lenticulostriate arteries that are well vascularized 4) The corpus callosum is well vascularized and supplied mainly by branches of the anterior cerebral artery (i.e. the pericallosal arteries) and posterior cerebral artery (i.e. the posterior callosal arteries).
1.5.3.2 Tortuous vessels

Arterioles supplying the deep cerebral white matter have the longest course through the brain, possess high vascular resistance, and become tortuous with aging (Ravens, 1978; Thore et al., 2007). Tortuous vessels are relatively sparse in subjects under 60 years but are common after the age of 70 years (Hassler, 1967). One study found tortuosity to appear in individuals in their 50s and occurred in every specimen obtained from individuals above 80 years old (Akima, Nonaka, Kagesawa, & Tanaka, 1986). Tortuosity tends to occur abruptly as the arteriole penetrates the cortex into the white matter, which suggests an intrinsic vulnerability of white matter to vascular dysfunction. Brown et al. used collagen IV staining in thick celloidin sections to visualize coiled vessels in a cavity where some brain parenchyma had been lost. These were referred to as tortuosity lesions by the authors (W. R. Brown, Moody, Thore, Anstrom, & Challa, 2009; W. R. Brown & Thore, 2011). Tortuosity increases the vessel length, and with each turn and loop, causes a loss in kinetic energy such that increased blood pressure is required to maintain flow in these vessels (Moody, Santamore, & Bell, 1991). Brown et al. (2011) found a trend towards increasing tortuosity in individuals with leukoaraiosis. Therefore, tortuous vessels may be a contributing factor in cerebromicrovascular dysfunction leading to leukoaraiosis.

1.5.3.3 String Vessels

String vessels are defined as basement membrane remnants of capillaries that have lost their endothelium (Henry-Feugeas, 2008) and cannot transport blood or plasma (De Venecia, Davis, & Engerman, 1976). A study of retinal digest preparations found that ischaemia caused string vessels to begin to form at 3-5 days with the loss of endothelial cells. By day 8, some of the acellular capillary remnants were reduced to thin strands that remained at 40 days (Reinecke, Kuwabara, Cogan, & Weis, 1962). Ischaemia has been shown to cause capillary loss. Regression of capillaries occurs by apoptosis synchronously along capillary segments from one vascular junction to the next, often with macrophage engulfment of the apoptotic endothelial cells (Lang, Lustig, Francois, Sellinger, & Plesken, 1994). There have been two proposed mechanisms for the synchronous apoptosis of endothelial cells. First, ischaemia may result in the loss of available vascular endothelial growth factor (VEGF). Second, ischaemia causes the loss of fluid shear stress on the endothelial cell surface that is needed to maintain endothelial cell survival (Resnick et al., 2003).
String vessels are common in the healthy brain, spinal cord, and eyes of humans and animals. Vessel stringing increases in ischaemia, irradiation, and Alzheimer’s disease. The number of string vessels is found to be highest in pre- and post-natal subjects when vascular remodeling is prominent. Although string vessels increase in the aging brain and in pathology, Brown et al. (2011) found the density of string vessels in leukoaraiotic lesions to be decreased. The authors found capillary density to be decreased in the deep white matter. The authors suggested that leukoaraiosis may begin as early as midlife with a progressive loss in the deep white matter and increase in string vessels. However, the string vessels would decrease as they are resorbed such that by the time of death and autopsy, many of the string vessels associated with leukoaraiotic lesions may disappear.

1.5.3.4 Age-related basement membrane thickening

With age, the basement membrane becomes thicker. Basement membranes are highly cross-linked insoluble material composed of approximately 50 proteins (50% collagen, especially collagen IV). The composition of the basement membrane is unique for each tissue and the two basement membrane polymer sheets of collagen IV and laminin expose various signaling constituents, such as VEGF, to the endothelial cells (Kalluri, 2003). Basement membrane thickening occurs through the deposition of collagen fibrils in and around the membrane. This thickening is correlated with atherosclerosis in large peripheral vessels (Farkas et al., 2006).

1.5.3.5 Hypoxia and angiogenesis in aging

There appears to be an age-related decline in the capacity for cerebral angiogenesis (J. E. Black, Polinsky, & Greenough, 1989; Rivard et al., 1999). Angiogenesis may occur when VEGF is induced in response to hypoxia through the transcription factor, hypoxia-inducible factor-1 (HIF-1) (Saint-Geniez & D'Amore, 2004). One factor in capillary stability is that the endothelial cells of vascular sprouts have tips that are similar to axonal growth cones and many of the signaling pathways are shared between the nervous system and vasculature. For example, VEGFs are growth factors for both endothelial and neural cells. Additionally, semaphorins control both axon guidance and vascular patterning (Adams & Alitalo, 2007). Another factor in capillary stability is pericyte signaling through Tie-2 receptors on endothelial cells (Eklund & Olsen, 2006). Under normal conditions, angiopoietin-1 is released by pericytes and activates the Tie-2 receptors on endothelial cells, which help to maintain vascular integrity (Dore-Duffy & LaManna, 2007). Under hypoxic conditions, another Tie2 ligand, angiopoietin-2, is expressed
by endothelial tip cells and competitively binds to the Tie2 receptor, thereby preventing angiopoietin-1 activation. This causes pericytes to move away from the capillaries and destabilizes them (Dore-Duffy & LaManna, 2007).

Once again, hypoxia induces VEGF expression via the transcription factor, HIF-1, promoting angiogenesis (Saint-Geniez & D’Amore, 2004). In the presence of VEGF, angiogenesis occurs whereas the absence of VEGF causes capillaries to undergo apoptotic regression (LaManna, Chavez, & Pichiule, 2004). The capacity for cerebral angiogenesis declines with age as the responsiveness of HIF-1 to hypoxia wanes with age, decreasing VEGF expression (Chavez & LaManna, 2003). This HIF-1 decline is associated with neuronal loss (Rapino et al., 2005). Therefore, in leukoaraiosis, there may be a failure of vascular recovery from hypoxia-induced bouts of capillary loss. In addition, age-related cerebrovascular changes and failure of vascular recovery may consequently represent an anatomic and functional loss of brain vascular reserve.

1.5.3.6 Age-related capillary loss in leukoaraiosis

There are many studies demonstrating declines in vascular density in aging and leukoaraiosis. Most of these studies report declines in capillary number, capillary length, capillary density, and increases in intercapillary distance (Jucker, Battig, & Meier-Ruge, 1990; Wilkinson, Hopewell, & Reinhold, 1981). For example, an experimental animal study has examined age-related changes of the capillaries in the cerebral white matter of young and old rats using immunohistochemistry and stereological techniques and found a decrease in capillary length (19%) and volume (24%) in older rats (Shao et al., 2010). Others have reported a 39% decrease in arterioles penetrating the cortex (Hutchins, Lynch, Cooney, & Curseen, 1996). Brown et al. reported an age-related decline in the vessel density of deep white matter from years 57 to 90 in normal subjects without leukoaraiosis whereas vessel density in leukoaraiotic lesions did not decrease with age. At 80 years, the vessel densities were equivalent in the deep white matter between both those with and without leukoaraiosis. A non-significant decrease in vascular density (11%) in the deep white matter was observed in subjects with leukoaraiosis. However, a subset of subjects who died before 60 years had a decrease of 47%. Cortical vessel density was also decreased (7%) in subjects with leukoaraiosis and the subset of subjects who died before 60 years had a decrease of 38% (Moody et al., 2004). Leukoaraiotic regions had 20% less vessel density compared to the deep white matter in age-matched normal controls (W. R. Brown, Moody, Thore, Challa, & Anstrom, 2007). The white matter in leukoaraiotic lesions
appears to become hypoxic due to capillary loss, followed by spongiosis and enlargement of the ventricles as the cells in the white matter progressively die. In addition, histological specimens of leukoaraiotic regions have found apoptosis with a loss of oligodendrocytes (W. R. Brown, Moody, Challa, Thore, & Anstrom, 2002a). As vessels are lost, hypoxia stimulates angiogenesis, but failure of cerebral vascular recovery is associated with aging. In this case, vessel density may decline, which causes neuronal dysfunction and ultimately neuronal and glial cell death (W. R. Brown & Thore, 2011).

1.5.3.7 Periventricular venous collagenosis

Moody et al. (1995) examined the brains of 22 elderly patients (obtained at autopsy) with MRI and histopathologic methods using alkaline phosphatases (used to distinguish arterioles and capillaries) and trichome (used to distinguish venules) microvascular staining. The authors found non-inflammatory collagenous intramural thickening of the venous walls that resulted in severe periventricular venous stenosis in many of these patients with white matter disease; furthermore, the authors also found greater venous disease in brains with more severe leukoaraiosis. This landmark study identified a new type of cerebral vascular pathology, periventricular venous collagenosis, which is strongly associated with leukoaraiosis. The authors concluded that the observed concentric collagen deposition causing intramural thickening and luminal occlusion of the deep cerebral veins might promote the development of leukoaraiosis (W. R. Brown, Moody, Challa, Thore, & Anstrom, 2002b; W. R. Brown et al., 2009). The collagen deposition occurs preferentially around the anterior and posterior horns of the ventricles, consistent with where periventricular leukoaraiosis usually begins (Moody, Brown, Challa, & Anderson, 1995; Moody, Brown, Challa, Ghazi-Birry, & Rebourssin, 1997). Once again, Brown et al. (2002) found an increase in the thickness of the walls of veins and venules in the periventricular white matter in elderly individuals. In leukoaraiotic cases, the authors found a much greater degree of venous wall thickening in the lesions, which resulted in narrowed Lumina and occlusion in some cases. The thickened walls were stained for collagens type I and III, which corresponds to excessive collagen deposition as that of hyalinization. Collagen IV was stained as a marker of the basal lamina and of the endothelium and the glia limitans at the brain parenchyma (Moody et al., 1997). Similar findings have demonstrating perivascular fibrosis have been reported in multiple sclerosis lesions (Mohan et al., 2010) and in brain capillaries in aging rats as well as hypertensive rats (Farkas et al., 2006; Farkas & Luiten, 2001). A recent study by Zhou et al. (2015) aimed to determine whether arterial hypertension could
affect the cerebral venous system in 30 male Spague-dawley rats. The rats were divided into two groups, sham rats and hypertensive rats that underwent laparotomy for the bilateral placement of a partially occlusive silver clip of the renal arteries to induce arterial hypertension. Rats in the sham group underwent laparotomy and isolation of the bilateral renal arteries without clip placement. Brains were extracted 20 weeks after surgery and Masson trichome staining demonstrated normal thin walls of the cerebral veins in the sham group and severe venous collagenosis of the cerebral veins in the hypertensive group. Susceptibility-weighted imaging (SWI) was also performed at 12, 16, and 20 weeks after surgery. The hypertensive group showed higher prominence of the cerebral veins. The authors suggested that distension of the cerebral veins in the hypertensive group may be either secondary to increased blood flow (from imperfect cerebral autoregulatory vasoconstrictive response to hypertension) or because of actual changes in venous oxygen saturation (from the inadequate tissue oxygen supply caused by hypertensive arterial changes) (Zhou et al., 2015). Hayashi et al. (2003) noted that when the vein is chronically exposed to high blood pressure, wall thickness is increased to restore circumferential wall stress to normal. Venular mechanical stress depends on both blood pulse wave amplitude and the mechanical properties of the tissue environment. Venous collagenosis is a mechanical consequence of age-related changes in arterial pulsations and mechanical fatigue of vascular smooth muscles (Hayashi, Mori, & Miyazaki, 2003). The chronic increase in arterial pulsations in hypertension results in arteriolar myogenic “fatigue”, reduction in arteriolar myogenic tone, and abnormal penetration of the insufficiently dampened arterial pulse wave into the venules. Hypertension-related high pulsatile motion causes mechanical damage to the veins and may play a role in dilation of the venular lumen (Henry-Feugeas & Koskas, 2012). Collectively, these studies suggest that hypertension may play a role in developing periventricular venous collagenosis and leukoaraiosis. Although a strong association exists between severe periventricular venous collagenosis and leukoaraiosis (Moody et al., 1997), hypertension is not always found in leukoaraiosis (Pantoni & Garcia, 1997).

With MRI investigation of venous collagenosis, Black et al. (2009) found stenosis or occlusion of veins at the margin of confluent leukoaraiosis and suggested that leukoaraiosis may involve venous insufficiency. In an MRI investigation of leukoaraiosis in patient’s with Alzheimer’s disease and healthy controls followed over 1 year, Gao et al. (2008) demonstrated that focal periventricular hyperintensities often relate to venules. By tracing individual perivenular hyperintensities, they can increase, decrease, or remain stable in volume over this...
period. The authors proposed that venous collagenosis is associated with apparent venous dilatation on MRI; thereby making them visible even at 1.5T. The authors suggest that periventricular confluent white matter hyperintensities represent vasogenic edema from perivenular leakage, a form of venous insufficiency (Gao et al.). Occlusive venous and arterial disease may be mutual aggravating factors in periventricular leukoaraiosis (S. Black, Gao, & Bilbao, 2009).

Periventricular venous collagenosis promotes ischaemia not only by increasing vascular resistance, but also by vasogenic edema, compromising interstitial fluid circulation (leading to reduced clearance of amyloid-beta along perivascular spaces and enhanced amyloid deposition), which could contribute to the progression of leukoaraiosis (Moody et al., 1995; Moody et al., 1997). It is unknown, however, why venular collagen deposition occurs and why periventricular venous collagenosis is associated with leukoaraiosis. Three mechanisms have been suggested for the development of venous collagenosis leading to leukoaraiosis (W. R. Brown & Thore, 2011). First is a genetic predisposition to excessive collagen deposition in veins. Second is a genetic predisposition to chronic periventricular ischaemia that causes over-production of collagen in the veins. Lastly, leukoaraiosis may stem from mechanical damage to small vessels due to abnormally high pulsatile motion in periventricular leukoaraiosis, causing over-production of collagen (Henry-Feugeas & Koskas, 2012).

1.5.3.7.1 Periventricular venous collagenosis and interstitial flow

Venous stenosis leads to increased resistance to venous blood flow, reduced perfusion pressure, and disordered venous drainage (Zhou et al., 2015). Full venous occlusion can lead to chronic ischaemia, edema, infarct, or hemorrhage. The lymphatic system in the brain was recently understood to be controlled by aquaporin-4 water channels localized to astrocytic endfeet. Transport of excess fluid and waste products from interstitial spaces between cells and blood requires para-arterial influx of sub-arachnoid CSF into the brain interstitium. This is followed by the clearance of interstitial fluid along large-caliber draining veins. Both of these influx and efflux pathways depend on interstitial bulk flow that is dependent upon trans-astrocytic water movement through aquaporin-4 channels. This movement of fluid is through the “glymphatic system” is a critical contributor to the clearance of interstitial solutes, such as the Amyloid β protein (Iliff et al., 2012). Venous collagenosis may impair passage into the veins of fluids, solutes, and toxins that are destined for removal from the brain via the blood stream (W. R. Brown et al., 2002b; W. R. Brown et al., 2009). It has been demonstrated that
cerebrospinal fluid is pumped through the brain via the peri-arteriolar spaces by the pulsations of the arterioles (Rennels, Blaumanis, & Grady, 1990). Thickened venous walls in venous collagenosis may hinder the function of the perivascular route for CSF drainage.

One hypothesis on the pathophysiological mechanism underlying leukoaraiosis stems from alterations in CSF flow. Normal pressure hydrocephalus is known to be associated with leukoaraiosis and experimental hydrocephalus causes changes in the white matter that can be reversed by shunting (Murata, Handa, Mori, & Nakano, 1981). It is possible for leukoaraiosis to cause *ex vacuo* dilatation of the ventricular system. The increased accumulation of CSF in the ventricles raises interstitial pressure in the periventricular parenchyma and can further exacerbate ischaemia of the cerebral white matter (G. C. Roman, 1991).

Arteriolosclerosis might impede the pulsatile pumping that moves CSF along the arterioles. Theoretical and experimental studies suggest that the motive force for perivascular draining of extracellular fluid and solutes is derived from vascular pulsations (Arbel-Ornath et al., 2013; Schley, Carare-Nnadi, Please, Perry, & Weller, 2006). Arteries become stiffer with age, implying that the amplitude of vascular pulsations becomes attenuated (Weller, Boche, & Nicoll, 2009). This attenuation may reduce the motive force for perivascular drainage and impair drainage of substances from the cortex. Among the substances that drain through the perivascular pathway is Amyloid-β (Aβ) (Preston, Steart, Wilkinson, Nicoll, & Weller, 2003; Weller et al., 1998). The low concentrations of Aβ in the CSF of patients with Alzheimer’s disease is thought to be due to decreased cerebral clearance of Aβ into CSF (Pirttila et al., 1996). Aβ deposits in the walls of capillaries may lead to luminal stenosis and cause degeneration as well as disappearance of the capillary, leaving a deposit of Aβ that may form a neuritic plaque (Roher et al., 1993). In addition, Aβ deposition in the perivascular drainage pathway may cause cerebral amyloid angiopathy that may block the function of interstitial fluid drainage (Weller et al., 1998). In cerebral amyloid angiopathy, Aβ deposition occurs in and around the vascular walls, disrupts the basement membrane of small vessels, causes smooth muscle cell and endothelial damage, and can also lead to stenosis of the vascular lumen (Yamaguchi, Yamazaki, Lemere, Frosch, & Selkoe, 1992). Brown et al. (2011) has suggested that sluggish perivascular flow caused by cerebrovascular pathology (such as venous collagenosis, vessel tortuosity, and thickened arteriolar walls) facilitate Aβ deposition in the perivascular draining pathway and further blocks the clearance of toxins. Therefore, clearance
of Aβ may be disrupted by microvascular pathology and impairment of Aβ clearance may contribute to further microvascular pathology leading to leukoaraiosis.

1.5.3.8 Jugular venous reflux

Periventricular venous collagenosis may not be the only source of venous insufficiency leading to leukoaraiosis, the internal jugular venous reflux may also play a role. The internal jugular vein is the main venous outflow pathway for cerebral venous drainage. The internal jugular valve, which sits 0.5 cm above the union of the subclavian vein and the internal jugular vein at the lower limit of the jugular bulb, serves an important role in preventing backflow of venous blood and backward venous pressure into the cerebral venous system. This occurs during conditions of increased proximal venous pressure or intrathoracic pressure (Fisher et al., 1982). Jugular venous reflux results from an abnormal pressure gradient, such as elevated proximal venous pressure, which exceeds the functional limits of the jugular valves (Chung & Hu, 2008). Dural AV fistulas may cause jugular venous reflux and this condition may lead to elevated cerebral venous pressure (by a chronic or long-term episodic retrograde-transmitted venous pressure) and venous outflow insufficiency. Cerebral venous hypertension may cause impaired autoregulation, pathological changes in microvessels, and reduced cerebral blood flow since elevated cerebral venule pressure would lower cerebral perfusion pressure (Chung & Hu, 2008). Cerebral venous hypertension may also cause endothelial dysfunction, damage to the blood-brain barrier, and vasogenic edema leading to leukoaraiosis (Schaller & Graf, 2004).

1.5.3.9 Age-related changes to cerebral blood flow

The aging brain is typically characterized by a gradual functional decline and concomitant disintegration in morphology. In human aging studies comparing young and old groups, aged groups had significantly lower CBF (J. Kawamura et al., 1993; Reich & Rusinek, 1989). When a correlation analysis was performed between CBF and lifetime, CBF negative correlated to the age of the individuals (Schultz et al., 1999; Tachibana, Meyer, Okayasu, & Kandula, 1984). Studies using TCD measuring blood flow velocity in the MCA, ACA, and PCA also agree with the age-dependent decline in regional CBF in these vessels (Krejza et al., 1999; Vriens, Kraaier, Musbach, Wieneke, & van Huffelen, 1989).

This relationship between CBF with age is also regionally specific. The CBF of the cerebral cortex and basal forebrain was found to be consistently lower in older individuals. In contrast, CBF in the subcortical white matter was reported to be either decreased in the elderly (Tachibana et al., 1984) or not significantly different from younger subjects (Reich & Rusinek,
Interestingly, in elderly individuals with leukoaraiosis, the severity of frontal leukoaraiosis correlates with a lower CBF within frontal white matter and caudate nucleus (J. Kawamura et al., 1993), thus suggesting a possible causal relationship. These studies were followed up by experiments designed to elucidate the functional mechanisms underlying the age-related CBF changes and focused on either impaired vasodilation or enhanced vasoconstriction as mechanisms causing the age-related decrease in CBF. These possibilities were tested by infusing either adenosine (vasodilator) or high doses of serotonin to young adult and older experimental animals (Hajdu, McElmurry, Heistad, & Baumbach, 1993; Jiang, Chen, Sobin, & Giannotta, 1992). Infusing adenosine to the CSF elicited vasodilation in both age groups but the increase in vessel caliber was considerably lower in older animals (Jiang et al., 1992). The application of a high dose of intravascular serotonin led to an enhanced vasoconstrictive response in the aged animals that coincided with an additional decrease in CBF (Hajdu et al., 1993). These results demonstrate that the reduced CBF in aging probably reflects a shift in the autoregulatory capacity towards dominating vasoconstrictive responses. This may be due to decreased sensitivity to vasodilatory mechanisms.

### 1.5.3.10 Reduced cerebral blood flow in leukoaraiosis

Reduced vascular density is consistent with findings of reduced cerebral blood flow (CBF) in leukoaraiosis and Alzheimer’s disease (Schuff et al., 2009). Cerebral blood flow is influenced by many components of the neurovascular unit. For example, perivascular nerves can constrict or dilate arteries and arterioles (Drake & Iadecola, 2007), astrocytic end feet can influence arteriolar diameter (Takano et al., 2006), endothelial cells can release vasodilators such as nitric oxide and vasoconstrictors such as endothelin (Faraci & Heistad, 1998) and pericytes can cause capillary contraction and relaxation (Peppiatt et al., 2006).

Leukoaraiotic lesions show increased expression of the hypoxia-inducible factor HIF-1α (Fernando et al., 2006) and white matter lesion load correlates with the degree of hypoperfusion (O’Sullivan et al., 2002), and cognitive impairment (Au et al., 2006). O’Sullivan et al. (2002) demonstrated that reduced cerebral blood flow is not only confined to lesions but also extends into NAWM in subjects with leukoaraiosis. The authors examined 21 patients with leukoaraiosis and 16 age-matched control subjects using exogenous contrast-based quantitative perfusion MRI. The cerebral blood flow in NAWM was reduced in the periventricular regions of subjects with leukoaraiosis compared to controls. The authors suggested that hypoperfusion may be an early feature in the development of periventricular lesions in leukoaraiosis and may
play a direct pathogenic role. Indeed, a recent serial study by Conklin et al. (2014) followed 5 patients with leukoaraiosis and examined de novo lesions in the cerebral white matter that were ischaemic in origin. By using multiparametric MRI (DTI and quantitative T2), the lesions demonstrated signature features of acute ischemic stroke and with time, the characteristics of these lesions approached those of pre-existing leukoaraiosis (Conklin, Silver, Mikulis, & Mandell, 2014).

1.5.3.11 The role of hypertension in leukoaraiosis

Hypertension, elevated blood pressure, and fluctuations in blood pressure are associated with cerebrovascular pathology. Leukoaraiosis increases with age and linearly increases with higher blood pressure levels (Brickman et al., 2010). Hypertension is also associated with declines in cognitive function (Reitz, Tang, Manly, Mayeux, & Luchsinger, 2007). In midlife, hypertension can cause cerebromicrovascular changes, but with advancing age, the lower boundary of autoregulation in the brain shifts upward, promoting vulnerability to hypoperfusion (van Beek, Claassen, Rikkert, & Jansen, 2008). The resulting hypoperfusion may cause ischaemia in the white matter and leukoaraiosis. Therefore, aggressive treatment of hypertension in the elderly may cause brain hypoperfusion (Birns, Markus, & Kalra, 2005). Conversely, treatment of hypertension in older individuals has been shown to improve blood pressure levels and restore blood pressure regulation (Lipsitz et al., 2005). Consequently, blood pressure control in the elderly becomes a balancing act between risk and benefit.

1.5.3.12 Endothelial Dysfunction

Endothelial dysfunction is thought to be one of the first steps in the development of leukoaraiosis (Knottnerus, Ten Cate, Lodder, Kessels, & van Oostenbrugge, 2009). Several factors may cause endothelial damage. For example, mechanical factors such as hypertension results in damaged endothelium, which allows plasma proteins to leak into the vessel wall. The walls then swell and may subsequently develop hyaline degeneration and fibrosis. This leads to intramural thickening, narrowing of the vessel lumen, reduced blood flow, and lead to ischaemia in the downstream tissues supplied by these vessels (Topakian, Barrick, Howe, & Markus, 2010). Chronic ischaemia leading to tissue damage is one possible pathogenic mechanism for the development of leukoaraiosis.

In addition to ischaemia, endothelial dysfunction may also result in damage to the blood-brain barrier. Plasma components, which normally cannot penetrate the blood-brain barrier, are capable of entering the interstitial space and brain parenchyma, leading to neuronal and glial
Leukoaraiosis may therefore be a consequence of chronic ischaemia, breakdown of the blood-brain barrier, and leakage of potentially toxic substances into the brain parenchyma, or a combination of these mechanisms (Fernando et al., 2006; Pantoni & Garcia, 1997).

1.5.3.13 Cerebral autoregulation and leukoaraiosis

There is evidence that autoregulation is reduced in leukoaraiotic white matter. In a study of 51 subjects with chronic hypertension using postural or drug induced changes in blood pressure measured against cerebral blood flow (CBF) using an argon inhalation induced desaturation of arterovenous oxygen differences (brachial artery and jugular bulb), impaired autoregulation was significant and an independent determinant of the severity of periventricular lesions seen on CT (Matsushita et al., 1994). The relationships between cerebrovascular reactivity and white matter lesions were also investigated in 33 individuals (mean age of 76.2 years) with carotid stenosis using transcranial Doppler (TCD) of the middle cerebral arteries (MCAs) before and after intravenous administration of acetazolamide. Impaired CVR was associated with the extent of leukoaraiosis leading the authors to suggest that dysfunction of autoregulation might be an important factor in the development of leukoaraiosis (Fu et al., 2006). BOLD MRI CVR during inhalation of 5% CO₂ has demonstrated that CVR values in leukoaraiotic white matter was approximately half of that found in normal-appearing white matter (Uh, Yezhuvath, Cheng, & Lu, 2010), suggesting that the pathogenesis of leukoaraiosis may be associated with ischemic conditions due to the episodic reduction of systemic arterial blood pressure.

1.5.4 Location and Progression of Leukoaraiosis

Leukoaraiosis may be located periventricularly, in the deep white matter, or both. White matter changes do not typically occur in the tangentially travelling white matter fibres at the junction of the grey and white matter, known as the subcortical U-fibres (O'Sullivan, Jarosz, et al., 2001).

Progression of leukoaraiosis tends to follow a general pattern. Periventricular lesions initially form at the top of the horns of the lateral ventricles, known as capping. As the severity of the disease progresses, white matter changes extend around the ventricles. Deep white matter lesions generally occur in the frontal lobes first and subsequently affects the parieto-occipital lobes and, in rare cases, the brain stem and basal ganglia. Leukoaraiosis is rarely found in the temporal lobes, which is an important distinguishing feature from an autosomal dominant small vessel arteriopathy known as CADASIL, in which the anterior temporal lobes are affected (van
Den Boom et al., 2002). In individuals with mild leukoaraiosis, the lesions are distinct from each other but with increasing severity, the lesions become confluent (Figure 1-7) (Fazekas, Chawluk, Alavi, Hurtig, & Zimmerman, 1987).

![Figure 1-7. FLAIR images demonstrating the spectrum of leukoaraiosis.](image)

Fazekas grade of 0 represents no white matter hyperintensities. With Fazekas grade 1, periventricular hyperintensities can be seen and focal hyperintensities exist in the deep white matter. At Fazekas grade 2, the hyperintensities in the deep white matter become fluent and diffuse periventricular hyperintensities can be seen at the frontal and occipital horns of the lateral ventricles. Individuals with Fazekas grade 3 have severe leukoaraiosis involving a majority of the white matter where diffuse confluent hyperintensities are seen on T2-weighted FLAIR images.

1.6 Radiological imaging

A major breakthrough in structural imaging occurred with the development of X-ray CT methods in the mid-1970s that led to the development of functional imaging methods as well. Previously, radiological imaging was limited by the two-dimensional nature of standard X-ray procedures, which provided minimal detail of brain structure. The development of CT imaging was extremely important from a neuroscience perspective as it provided the first noninvasive method for imaging neuroanatomy in cross-section. Compared to previous methods, CT had incredible spatial resolution and could be obtained quickly, making it a vital and routine part of clinical practice by the 1980s. CT imaging continues to be routinely used today as it offers
certain advantages over MRI by being less expensive to implement and useful for detecting many disorders, such as tumours. However, MRI provides much higher contrast resolution (i.e. the ability to distinguish tissue types such as grey and white matter) and the ability to image different types of brain tissue without the use of X-rays or radioactive ligands.

Soon after the introduction of CT methods for structural neuroimaging, researchers combined this approach with principles of nuclear medicine to measure blood flow, glucose metabolism, and oxygen consumption (Ackerman, Subramanyam, Correia, Alpert, & Taveras, 1980; T. C. Hill, 1980). These nuclear medicine methods, including positron emission tomography (PET) and single photon emission computed tomography (SPECT), involve the introduction of a radioactive agent into the blood stream, which becomes metabolized by the brain. The rate of metabolism differs regionally and by types of brain tissue as a function of blood flow and glucose metabolism. Furthermore, it soon became evident that not only can these methods be used to show differential activity across brain areas at rest, but were also able to detect differences in the response of particular brain regions to cognitive and behavioural tasks.

Leukoaraiosis appears as low attenuation areas on CT and as areas with high signal on T2-weighted or FLAIR MRI. For the detection of white matter lesions, MRI is more sensitive than CT because of superior contrast resolution. Gradient-echo sequences or susceptibility weighted imaging on MRI shows the presence of cerebral microbleeds, which cannot be distinguished from ischaemic small vessel disease on CT. Cerebral microbleeds are a feature of hypertensive small vessel disease and of cerebral amyloid angiopathy (Pantoni & Garcia, 1997).

1.6.1 Functional magnetic resonance imaging (fMRI)

Approximately a decade after the routine use of CT imaging as a clinical radiological tool, magnetic resonance imaging (MRI) evolved to the point where it could be used clinically as an alternative for structural imaging. MRI is based on the concept that magnetic fields cause the alignment of the nuclei of hydrogen atoms in the water of body tissues into one of two quantum states either parallel or anti-parallel to the applied static field. The magnetic field also induces the protons to precess around an axis in the direction of the applied field. Normally, there is a slight excess of protons in the parallel state resulting in bulk tissue magnetization aligned with the applied static field. Radiowaves are then applied to alter the bulk tissue magnetization. The varying magnetic component of radiowaves of the appropriate (resonant or “Larmor”) frequency determined by the applied static magnetic field increases the number of protons in the higher
energy anti-parallel state to increase in effect “tipping” the bulk magnetization of the tissue. This condition is unstable and the protons “relax” back into the low energy state when the radiowaves are turned off. This is termed T1 relaxation. The applied radiowave also produces alignment of proton precession, which begins to decay after the radiowave is turned off due to heterogeneity in the local magnetic field caused by movement of the water protons themselves. This decay is termed T2 relaxation. Proton relaxation occurs along different spatial dimensions. T1 relaxation occurs along the direction of the applied static field and T2 occurs perpendicular to this in the transverse plane. Both are exponential over time with time constants “T1” and “T2.”

In an MR environment, the transverse magnetization decay is much faster than predicted due to static magnetic field inhomogeneities, this decay is represented by T2*. T2* can be considered the “effective” T2 whereas the T2 represents the “true” T2 of the tissue being examined. T1 is associated with the enthalpy of the spin system, whereas T2 and T2* are associated with its entropy. In the soft tissues of the body, T1 is around 1 second while T2 and T2* are much shorter, typically under 100 milliseconds. However, the exact value for T1, T2, and T2* can vary dramatically depending on external magnetic fields and in various types of tissue. This fact provides MRI with very high soft tissue contrast. T1, T2, and T2* differ in their sensitivity and resolution of particular types of tissue, thereby providing MRI with high spatial resolution to different types of brain tissue under various physiological conditions.

1.6.1.1 What is the fMRI BOLD Signal?

Functional MRI (fMRI) depends on the measurement of T2* relaxation, which is sensitive primarily to local concentrations of paramagnetic deoxyhemoglobin (dHb) (Ogawa, Lee, Kay, & Tank, 1990). The paramagnetic nature of dHb makes the magnetic field less uniform. Therefore the fMRI blood-oxygen-level-dependent (BOLD) signal increases with decreasing dHb maximizing when blood is 100% oxygenated. It can be used to produce localized maps of functional activity in the brain based on the relationship between blood flow and oxygen extraction of the tissue. However, despite widespread use for almost 20 years, the exact physiological mechanism underlying the fMRI BOLD signal in tissues is complex and is not yet fully elucidated (Attwell et al., 2010).

Knowledge of the underlying physiological processes in the brain that modulate blood flow gives insight in the interpretation of the fMRI BOLD signal. Without functional hyperemia, the oxygen consumption driving neuronal activity would increase deoxyhemoglobin concentrations and therefore decrease the MRI signal. However, BOLD maps show an increase
in signal intensity where the brain is most active. This occurs because the increase in blood flow evoked by neuronal activity delivers more oxygen than can be consumed by the tissue, decreasing deoxyhemoglobin levels and increasing the MRI signal. This was initially termed “neurovascular uncoupling.” There are also other factors coming into play such as cerebral blood volume, which increases with increased flow (Attwell et al., 2010).

A common misconception is that BOLD provides a direct measurement of neuronal oxygen consumption. However, classical positive BOLD signals seen in response to sensory stimuli represent a decrease in dHb and thus an overoxygenation of the responding region (Attwell & Iadecola, 2002). In other words, the increase in BOLD signal is a combination of increases in oxygen consumption, increases in cerebral blood flow, and changes in blood volume. These volume changes induce non-linear changes in tissue magnetization affecting T2* relaxation. If the geometry of the microcirculation is unknown a correlation between blood flow, blood volume, and BOLD signal cannot be made. Nevertheless, these positive BOLD responses correspond to a local increase in blood flow delivering more oxygen than the tissue consumes increasing local oxygenation levels (Raichle, 1998). Thus, the effect of functional hyperaemia dominates the contribution from metabolic demands induced by neuronal activity. This directs current research into the causes of functional hyperaemia serving as the basis of the BOLD fMRI signals.

In order to address questions of whether this neurovascular coupling simply matches supply to demand, several studies have been conducted in hyperoxic and hyperglycemic states. These studies have demonstrated that animals with these conditions continued to exhibit functional hyperaemia in response to stimulation despite an abundance of nutrients (Lindauer et al., 2010; Wolf, Lindauer, Villringer, & Dirnagl, 1997). Hypoglycemic human subjects also exhibit functional hyperemia (Powers et al., 1996). These studies demonstrate that blood flow increases are not triggered by local sensing of depleted oxygen/glucose supply. The fact that CBF response to activated regions of the brain increases substantially more than the oxygen consumption suggests an indirect relationship between oxygen supply and demand. One recent study has suggested that high oxygen gradients are required to supply all active cells (Devor et al., 2011). It has also been inferred that the role of the CBF response is to provide higher levels of glucose rather than oxygen (Paulson, Hasselbalch, Rostrup, Knudsen, & Pelligrino, 2010). Yabonskiy et al. (2000) has suggested roles for functional hyperaemia in waste removal and temperature regulation. Hyperemia may play a role in heat removal as cellular respiration and
the process of oxygen release from hemoglobin produces heat (Ackers, Doyle, Myers, & Daugherty, 1992; Yablonskiy, Ackerman, & Raichle, 2000).

The BOLD response to a short stimuli lasting less than 1 second typically begins within 500 milliseconds and peaks 3-5 seconds after onset of the stimulus (Martindale et al., 2005). This relative delay in the peak of increased blood flow further confirms that neurons do not rely on hyperemia to meet their initial demands for increased oxygen and glucose since neuronal firing may have ended prior to measurable changes in blood flow. One explanation is that neurons have sufficient stores of glucose to support initial neuronal responses, which may be necessary if the speed of the vascular response is physically limited. This later blood flow peak may serve to replenish nutrients, or in the case of prolonged stimulation, could be required to sustain neuronal responses once initial stores are depleted (A. M. Brown & Ransom, 2007).

The astrocyte-neuron lactate shuttle is a model that is in line with this view of sufficient nutrient stores in the neuron (Pellerin et al., 2007). This model postulates that astrocytes undergo anaerobic glycolysis in response to elevated glutamate levels, thereby preserving local oxygen supplies for neuronal use. Mechanism for lactate shuttle begins with synaptic activity, in which glutamate is released into the synaptic cleft. A glutamate transporter system on astrocytes enhances removal of glutamate from the synapse. The entry of sodium co-transported with glutamate activates the sodium/potassium ATPase. Activation of the sodium/potassium ATPase is coupled with an increased glycolytic flux, resulting in the stimulation of glucose uptake from the capillaries. Anaerobic glycolysis occurs and lactate, the byproduct, is released by astrocytes and taken up by neurons where it can enter the tricarboxylic acid (TCA) cycle and provide 18 ATP per molecule (Pellerin & Magistretti, 2012). The astrocytes are well positioned for this role as they are located between neurons and blood vessels; however, the lactate shuttle model remains controversial (Hertz, 2004; Kasischke, Vishwasrao, Fisher, Zipfel, & Webb, 2004).

The details underlying the full purpose of functional hyperemia remain to be resolved. Current literature points toward functional hyperemia being driven by an active process that is initiated soon after stimulus onset, but which is not causally dependent on local metabolic demands (Attwell & Iadecola, 2002).

1.6.1.2 BOLD fMRI signal quantification

The goal of traditional MRI is to acquire high-resolution anatomical images to identify structural abnormalities, whereas the primary goal of functional MRI is to detect variations in
MRI signal that occur over time to infer neural demands for oxygen-rich blood. BOLD fMRI is based on the magnetic properties of hemoglobin as an endogenous contrast agent to follow task-associated changes in oxygen. This method relies on the observation that neural activity reliably leads to the delivery of excess oxyhemoglobin takes advantage of the fact that deoxyhemoglobin paramagnetic while oxyhemoglobin is not.

BOLD MRI involves the use of rapid whole brain multi-slice MRI acquisitions using echoplanar T2* sequences sensitive to paramagnetic distortions caused by deoxyhemoglobin. Repetition of the acquisition on the average of every two seconds provides a time series where both spatial and temporal information is obtained. When this is coupled with precise experimental challenges that enable the time series signal to be altered by different cognitive conditions, it is possible to obtain data that reflects changes in brain function associated with specific processes. The use of fMRI is to infer brain activity that is temporally associated with a particular paradigm by tracking hemodynamic functions. Relative cerebrovascular changes in oxygenation are quantified in response to neural activity elicited by task-related challenges. Therefore, fMRI relies on quantification of cerebrovascular responses that are spatially and temporally coupled with the underlying neural activity (R. A. Cohen & Sweet, 2010).

In general, the acquisition of fMRI data typically involves a stimulus presentation and some type of behavioural response. During these events, the whole brain is typically sampled using 30 contiguous 2.5 mm thick axial slices with 2.5 mm² in-plane resolution (2.5mm² voxels) acquired every 2 seconds. The resulting raw data then undergoes several preprocessing steps to yield a motion-correlated time-dependent signal course for each image voxel acquired.

The raw BOLD signal obtained from each voxel of an individual is typically converted to a standardized metric to allow comparison across subjects or time. For example, in a finger tapping experiment, a simple method of subtracting an individual’s mean baseline signal (during rest) from the mean signal during finger tapping is done for each brain voxel. Since BOLD signal changes are 2-8% above baseline signal that contains 1-2% noise, stimulus/rest cycles are repeated to extract the signal from the noise. The mean difference scores are calculated for each cycle and may be compared to a hypothetical mean of zero (the null hypothesis) using a Student’s t-test for each voxel.

Other methods, such as cross-correlation and multiple regression, can be used to determine how closely each voxel’s time course is synchronized with the time course of the stimulus. In multiple regression analyses, individual scores are assigned a value for each
condition (i.e. predictor). Each value accounts for a component of the variance in that voxel’s BOLD signal (i.e. criterion) that is associated with each condition. These values are often called parameter estimates and are typically assigned a statistical value based on sampling rates and multiple comparisons to determine significance thresholds. The term activation is used to denote clusters of voxels in which the association of the BOLD signal to the task condition exceeds a significance threshold. Several dependent variables may be calculated for each individual to generate activation maps, these include percent signal change as well as z and t statistics (R. A. Cohen & Sweet, 2010).

1.6.2 Quantitative T2 Changes in leukoaraiosis

T2 proton relaxation in tissues is primarily a function of free water. Most white matter pathologies disrupt the integrity of myelin and axons leading to an increase in free water content. The ensuing increase in T2 relaxation becomes visible in the abnormal white matter as areas of increased signal compared to unaffected normal-appearing white matter (NAWM). Measurement of T2 relaxation therefore can provide a quantitative index of the degree of white matter injury. In fact, measurable changes in T2 relaxation within epileptogenic tissue can occur before they are visible on T2-weighted images (Laule et al., 2007; Okujava, Schulz, Ebner, & Woermann, 2002). Despite the sensitivity of T2 relaxometry, surprisingly little work has been done in the assessment of leukoaraiosis.

1.6.3 Diffusion Tensor Imaging

By the late 1980s, MRI was widely adopted for detecting neurological disorders. The introduction of diffusion-tensor imaging (DTI) in the mid-1990s (Basser, 1995) provided a new in vivo MRI tool for gaining insight into the structure of white matter and its functional correlates. DTI provides information on the structural coherence and topography of biological tissue based on measurement of the rate and direction of water diffusion. This MRI method is particularly useful in directionally organized tissue, such as cerebral white matter or muscle, where the linear arrangement of cellular structures constrain water to diffuse faster along the fibres than in other directions.

Diffusion describes the essential physical process in which particles mix under thermodynamic equilibrium without requiring bulk motion. Robert Brown first reported the random motion of pollen particles under a microscope (which later came to be known as Brownian motion), providing a framework for describing the process of diffusion (R. Brown, 1828). It is now known that the random motion of particles in water is caused by the constant
random thermal movement of water molecules. The squared displacement $<x^2>$ of water molecules in three dimensions over a period of time $t$ can be described by Einstein’s equation, $<x^2> = 6Dt$. The constant $D$ is the diffusion coefficient, which varies according to temperature, the properties of the diffusing molecules, and the microstructural features of the medium. The sensitivity of $D$ to the microstructural environment of the medium makes it a useful quantity for examining the structure of biological tissues (Alexander, Lee, Lazar, & Field, 2007).

The diffusion of water molecules is constrained in biological tissues as it contains cell membranes, organelles, and other macromolecular structures. In free water, diffusion is isotropic, describing the fact that the probabilistic displacement of water molecule over time is equal in all directions. In biological structures such as the ventricles, water diffusion is mainly unrestricted and is therefore highly isotropic. Interestingly, diffusion in the cerebral grey matter is also largely isotropic despite being restricted by cell bodies and organelles. The cerebral white matter contains axons and their corresponding microtubules and myelin, which are organized in an orientationally specific manner. Thus, diffusion of water in white matter is anisotropic with diffusion occurring faster along the direction of the axons (R. A. Cohen & Sweet, 2010).

The extent to which water diffusion is isotropic or anisotropic and its directionality can be measured using MRI. The MRI method used for these measurements is based on a spin echo acquisition, in which a pair of brief but strong magnetic field gradient pulses (called diffusion-encoding gradients) are applied during a spin echo sequence (Nagy, Weiskopf, Alexander, & Deichmann, 2007). The first gradient pulse causes dephasing of proton ($^1$H) spins and the second pulse fully rephases the spins if they do not move in the direction of the applied gradient. Protons that are bound to macromolecules, such as proteins anchored in cell membranes, are essentially stationary.

Protons bound to water molecules are not stationary. After application of the first gradient pulse, these protons will diffuse in the applied gradient field to a new location incurring a precessional phase shift. When the second gradient pulse is applied, these protons will experience a different gradient because they have shifted location. Therefore, unlikely stationary protons, these protons in water molecules will not regain their initial phase. As a result, there will be signal loss compared to the stationary protons (R. A. Cohen & Sweet, 2010). In other words, voxels with proton displacement due to diffusion will show attenuated signal ($S$) after the application of diffusion-encoding gradients when compared with non-diffusion-weighted signals.
(S₀) acquired at the same voxels. Voxels will have greater signal attenuation if there is greater diffusion. The degree of signal attenuation can be expressed with the following equation:

\[
\frac{S}{S_0} = e^{-\gamma^2 G^2 \delta^2 (\Delta - (\delta/3)) D} = e^{-b D},
\]

\[
b = \gamma^2 G^2 \delta^2 \left( \Delta - \frac{\delta}{3} \right).
\]

The diffusion weighting factor, b, may also be referred to the b-value and depends on the strength of the diffusion-encoding gradient (G), its duration (δ), the time interval between the pair of diffusion-encoding gradient pulses (Δ), and the gyromagnetic ratio (γ). S and S₀ are measured and the acquisition parameters are known, thus the diffusion coefficient D can be calculated.

This calculation of the diffusion coefficient has been used clinically in the form of diffusion-weighted imaging (DWI). In general, diffusion-encoding gradients are applied in three orthogonal directions, which produce three corresponding diffusion-weighted images. A diffusion coefficient can then be calculated separately for each image to reflect the diffusion magnitude along that particular axis in each voxel. This value is represented by the apparent diffusion coefficient (ADC), as it is dependent on the gradient duration and time interval between the diffusion gradients. DWI has been used clinically to detect acute ischemia, which causes restricted water diffusion due to cytotoxic edema (T. Q. Li, Chen, & Hindmarsh, 1998). This reduction in water diffusion results in less signal attenuation on the diffusion-weighted images and a lower ADC, therefore the ischemic regions appear hyperintense on the diffusion-weighted image and hypointense on the ADC image (Warach, Gaa, Siewert, Wielopolski, & Edelman, 1995). This MRI method provides an advantage in detecting acute ischemia over conventional T2-weighted and T1-weighted images, which may appear normal in this situation.

Tissues may be characterized by a single D value that is invariant regardless of applied gradient direction or tissue anisotropy. However, in tissues where diffusion is directionally specific, a scalar index of diffusion is inadequate for describing preferred water diffusion directions within a three-dimensional voxel. Therefore, when characterizing anisotropic tissues like the white matter, DWI is of limited utility.

Diffusion tensor imaging (DTI) addresses this limitation by using a tensor model to fully describe water diffusion in a three-dimensional space. A tensor is a mathematical construct
used to represent multidirectional forces, such as diffusion (H. J. Park, 2005). The diffusion tensor $D$ is a $3 \times 3$ symmetric matrix,

$$D = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{bmatrix}$$

The diffusion tensor is usually estimated using a linear regression model based on log-transformed diffusion signals (Basser, 1995). The diffusion tensor for each voxel can be decomposed into three orthogonal principal eigenvectors $\mathbf{e}_1$, $\mathbf{e}_2$, and $\mathbf{e}_3$, which are ordered by the magnitudes of their corresponding eigenvalues, ($\lambda_1 > \lambda_2 > \lambda_3$). Thus, $\mathbf{e}_1$ represents the dominant fibre direction (and the one with the largest diffusion magnitude) in each voxel.

A diffusion tensor may be visualized by an ellipsoid where the axes are defined by the three eigenvectors. When diffusion is perfectly isotropic, the magnitude of diffusion is equal in all directions with $\lambda_1 = \lambda_2 = \lambda_3$. In this case, the diffusion ellipsoid may be represented by a sphere. The shape of the anisotropic ellipsoids vary from voxel to voxel depending on the relative magnitudes of the three eigenvalues derived from tissue organization (Figure 1-8).

![Figure 1-8. Schematic representation of white matter fibres and diffusion ellipsoid. A) The diffusion of water molecules along axon bundles is limited by the cell membrane on the axon. Thus, water preferentially diffuses along one direction. B) A schematic representation of](image)
a diffusion ellipsoid demonstrating the probabilistic diffusion of a water molecule in an axonal environment. The direction of greatest diffusion is assumed to be oriented parallel to the axonal fibres.

A limitation of DTI is that data is typically acquired on the scale of millimeter-sized voxels. However, axon diameter is on the micron scale. Therefore each imaging voxel will contain a large number of axons. Since the tensor is calculated using the diffusion signal from each voxel, the data represents the bulk diffusion characteristics of the whole voxel. Therefore, in brain regions containing multiple populations of crossing, merging, or diverging fibres (such as the internal capsule), DTI may not accurately characterize the diffusion. Other diffusion methods such as diffusion spectrum imaging (DSI) using multiple diffusion strengths and a greater number of diffusion gradient directions can be used to resolve these complicated geometries.

Scalar metrics can be derived from the eigenvalues of the diffusion tensor. These metrics may broadly reflect two properties of diffusion: the degree of diffusivity and anisotropy within each voxel. Mean diffusivity (mD) is a widely reported diffusivity metric and is an average of the three eigenvalues, hence representing an average magnitude of the water diffusion in a particular voxel \[ mD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \] (Le Bihan et al., 2001). Other diffusivity metrics include axial diffusivity and radial diffusivity. Axial diffusivity corresponds to the direction with the highest magnitude of diffusion (\(\lambda_1\)) and is considered to be a marker of axon integrity. Radial diffusivity is corresponds to the average diffusion along the two minor axes \(\frac{\lambda_2 + \lambda_3}{2}\) and is considered to be a marker of myelin integrity.

The most widely used scalar metric for characterizing anisotropic diffusion is fractional anisotropy (FA), which describes the variance of the three eigenvalues normalized for the overall magnitude of diffusion in each voxel (Le Bihan et al., 2001). FA values range from 0 representing fully isotropic diffusion to 1 for perfect anisotropy. FA and mD are derived using the following equation:
59

\[
FA = \frac{3}{2} \sqrt{ \frac{(\lambda_1 - \overline{\lambda})^2 + (\lambda_2 - \overline{\lambda})^2 + (\lambda_3 - \overline{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2} }
\]

\[
\overline{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} = MD.
\]

Understanding the biology of white matter diffusion anisotropy is important for the interpretation of DTI studies. Axonal membranes provide the primary contribution to diffusion anisotropy. Myelin makes a secondary contribution, whereas microtubules, and neurofilaments make limited contributions to diffusion anisotropy (Beaulieu, 2002). Collectively, diffusivity measures (mean diffusivity, axial diffusivity, and radial diffusivity) provide additional information about the underlying physiology than fractional anisotropy alone. In many pathological conditions, a decrease in the white matter structural integrity is typically characterized by decreased anisotropy and increased diffusivity. However, this is not always the case as there are increases and decreases in FA in cerebral edema. In this case, a decrease in FA may represent either decreased diffusion along the primary axis (along the axons) probably representing axon loss or increased diffusion along the secondary or tertiary axis (perpendicular to the axons) probably representing demyelination (Beaulieu, 2002).

1.6.3.1 Diffusion MRI in leukoaraiosis

Diffusion-weighted imaging has the ability to detect acute and subacute infarcts with great accuracy (R. A. Cohen & Sweet, 2010), even in asymptomatic individuals. This makes diffusion MRI useful in the longitudinal assessment of patients with leukoaraiosis to monitor the development of new ischemic lesions. In one study involving subjects with leukoaraiosis, more than a third of patients with small vessel disease had high signal abnormalities on diffusion-weighted images, suggesting a recent ischemic origin (S. H. Choi et al., 2000). In addition, 20% of asymptomatic individuals had evidence of covert ischemia in the white matter and multiple small lesions, suggesting either a proximal source of embolism or global hypoperfusion (S. H. Choi et al., 2000). The severity of leukoaraiosis also seems to correlate with higher white matter ADC values (Helenius, Soinne, Salonen, Kaste, & Tatlisumak, 2002). A characteristic DTI pattern in leukoaraiotic regions is a moderate elevation in mean diffusivity with a marked loss of FA, consistent with the pathological correlate of axonal loss and gliosis (D. K. Jones et
al., 1999). In studies examining normal-appearing white matter, similar findings of increased mean diffusivity and decreased FA were also found (Conklin et al., 2011). Previous work in multiple sclerosis (MS) has shown the value of assessing NAWM using DTI. In fact, increased water diffusivity has been observed in NAWM of MS patients that subsequently evolved into new visible plaques (Werring et al., 2000). The concept that diffusion abnormalities in NAWM in patients with leukoaraiosis are detectable using DTI was confirmed by O’Sullivan (O’Sullivan, Summers, et al., 2001). In this study, significant elevations in mean diffusivity (mD) and decreases in FA were observed in NAWM in subjects with leukoaraiosis but not in age-matched controls. Furthermore, it has been shown that FA demonstrates the highest correlation between cognitive function and any other imaging parameter including lesion load and brain volume (T2 relaxometry not assessed in this study). This correlation explained 82% of the variance in cognitive function (Nitkunan, Barrick, Charlton, Clark, & Markus, 2008). The ability of DTI MRI to predict the evolution of leukoaraiosis in NAWM with pre-existing abnormal diffusion parameters has yet to be tested.

A more recently study by Conklin et al. (2014) followed 5 subjects with leukoaraiosis in the absence of significant vascular disease for 16 consecutive weeks. By using DTI, the authors found that tiny lesions arising de novo in the white matter had the hallmarks of acute ischemic stroke (abrupt decreases in mean diffusivity and fractional anisotropy during lesion detection), suggesting acute infarcts as being a possible cause of leukoaraiosis (Conklin et al., 2014).

### 1.6.4 Perfusion MRI

Although the term fMRI has become nearly synonymous with BOLD, there are many other MRI methods to image functional or physiological signal variations. A variety of methods exist to image tissue perfusion and other aspects of vascular function in the brain. Perfusion is defined as the rate at which blood is delivered to tissue and is expressed in units of millilitres of blood delivered per 100 grams of brain tissue per minute. Perfusion is a useful marker for brain activity and integrity due to its essential role in supporting neuronal and glial function. The tissue perfusion rate can indicate many physiological conditions, such as increased energy demand during neuronal activity or deficits related to pathological conditions, such as ischaemia.

Perfusion MRI techniques can be categorized into two groups based on the physical process used to generate image contrasts: one method is based on the injection of a blood-borne contrast agent and the other method is arterial spin labeling (ASL). ASL involves the
application of radio frequency (RF) pulses to achieve image contrast related to blood flow through selectively tagging blood water protons in the major arteries supplying the brain. MRI methods based on contrast agents are often referred to as susceptibility contrast techniques because the injected contrast agent changes the magnetic susceptibility of the blood. Changing the susceptibility of blood leads to changes in the $T_2^*$ of tissues. Each has advantages and disadvantages. Contrast based methods are faster with higher signal to noise ratios but because the relationship between susceptibility and blood concentration of MR contrast agents is non-linear, accurate calculation of true CBF is problematic. On the other hand, techniques that use selective RF tagging of major arteries sample tissue before and after the tag is applied with the difference representing true blood flow once T1 relaxation effects of the tagged protons are factored in. The advantage of ASL is that it can accurately quantitate CBF once the T1 relaxation of blood is known, but because it is a subtraction method, the images are noisier and the technique requires longer imaging times. For the purposes of this thesis, further details will be only be explained for dynamic susceptibility contrast methods (Swanson, 2005).

1.6.4.1 Dynamic Susceptibility Contrast Perfusion

Dynamic susceptibility contrast (DSC) perfusion methods are based on rapid, intravenous bolus injections of a paramagnetic rare earth compound, such as gadolinium. This method is routinely used for making a single measurement of resting brain perfusion in patients who suffer from acute and focal hemodynamic impairment. The rapid passage of contrast agent through a voxel causes the MR signal to decrease due to a decrease in $T_2^*$. Due to the moderately toxic nature of gadolinium, it is administered with a chelating agent, usually diethylene triamine pentaacetic acid (DTPA). This chelating agent helps reduce biological interaction and to accelerate clearance of gadolinium from the body through the kidneys. The typical elimination half-life for the gadolinium-DTPA contrast agent in humans is approximately 90 minutes (Swanson, 2005).

The pulse sequences used to measure MRI signal changes during the passage of gadolinium-DTPA are typically single-shot echo-planar imaging (EPI) acquisitions with either gradient or spin-echo readouts. This procedure assumes that the contrast agent does not leak out of the vessels into the tissue and does not cause significant T1 enhancement. Spin-echo sequences are believed to emphasize capillary microvascular effects, representing parenchymal perfusion whereas gradient-echo sequences are believed to be biased toward signals from larger blood vessels such as venues and small veins (Boxerman et al., 1995; Weisskoff, Zuo,
Boxerman, & Rosen, 1994). Typically, the EPI pulse sequence samples tissue every 2 seconds per slice for roughly 20 slice locations through the brain for 40-50 images per slice location just prior to and during the bolus passage of gadolinium-DTPA through the tissues. The final image sequence will depict abrupt signal reductions that are mathematically converted to gadolinium concentration time curves. These curves can then be modeled to extract several hemodynamic parameters including relative cerebral blood flow (CBF), regional cerebral blood volume (CBV), time-to-peak (TTP), time-to-maximum or the residue function (Tmax), and mean transit time (MTT). The TTP reflects the time at which the peak signal change occurred. TTP is inversely related to CBF, in which a reduction of blood flow results in an increase in the time that is needed for the contrast to reach its peak concentration. The physiological meaning of Tmax is not well understood but it is believed to reflect a combination of primarily dispersion, secondarily delay, and to a lesser extent, mean transit time (Calamante et al., 2010). MTT refers to the average length of time a certain volume of blood spends in the cerebral capillary circulation, equivalent to CBV/CBF. CBV may be computed as the integral of the time-concentration curve. It is important to note that all of these parameters represent the average value over the period of contrast passage. For functional studies, in which transient variations in blood flow must be continually monitored, DSC perfusion would not be practical because it requires analysis of the entire time-concentration curve in order to obtain a single estimate of blood flow (Swanson, 2005).

1.6.4.1.1 What causes the perfusion signal to change?

The perfusion MR signal intensity decreases with gadolinium because of its paramagnetic nature, disrupting the magnetic fields in a small region surrounding the capillaries as it passes through the tissue. This alteration in the magnetic field decreases T2* (or increases R2*, as R2* = 1/ T2*) of water protons in the tissue near the contrast agent. Because the echo of the spins is recorded at a time TE following the pulse (in which T2* has decreased), the perfusion signal intensity will have decreased. Without considering contrast agents, the signal intensity arising from water protons at time TE can be calculated by the following equation:

\[ S_{TE} \] = \[ S_0 e^{-R_2 TE} \]

where R2 is the transverse relaxation rate of the tissue (R2 = 1/T2). However, the addition of contrast agent requires the consideration of additional relaxation due to the susceptibility-induced dispersion created from gadolinium. The new relaxation rate can be calculated as:
where $R_2^*$ is the effective transverse relaxation rate in the presence of contrast agent and $r_2^*$ is the relaxivity (defined as the relaxation rate per unit concentration of gadolinium) and $C_{Gd}(t)$ is the concentration of gadolinium at a particular time. These equations use rate constants (instead of time constants such as $T_2$ because the rates are additive and simply the math. Taking gadolinium into consideration, the signal intensity at time $TE$ can now be defined as:

$$S_{TE}^{Gd}(t) = S_0 e^{-R_2^*(t)TE} = S_0 e^{-R_2TE} e^{-r_2^*C_{Gd}(t)TE}$$

1.6.4.1.2 How does perfusion signal change relate to tracer concentration?

Tracer concentration could be mathematically derived from perfusion signal changes (Figure 1-9) by taking the logarithm of the ratio of the signal with and without gadolinium using the following equations:

$$\frac{S_{TE}^{Gd}(t)}{S_{TE}} = \frac{S_0 e^{-R_2TE} e^{-r_2^*C_{Gd}(t)TE}}{S_0 e^{-R_2TE}} = e^{-r_2^*C_{Gd}(t)TE}$$

$$C_{Gd}(t) \propto r_2^* C_{Gd}(t) = -\frac{\ln\left(\frac{S_{TE}^{Gd}(t)}{S_{TE}}\right)}{TE}$$

**Figure 1-9. Generation of the concentration-time series.** A) A schematic illustration of a representative curve of the signal intensity in a voxel as gadolinium passes through the volume. The passage of the contrast agent causes a decrease in MR signal due to a decrease in $T_2^*$ (or
increase in $R^*_2$). The signal is transformed into a time-concentration curve (B) by using equation 4) and 5). An arterial input function is derived from empirical measurement of signal intensity using an ROI near a major large feeding vessel.

1.6.4.1.3 How does the time-concentration tracer curve become a measure of perfusion?

After a time-concentration curve for each voxel or region of interest has been calculated, deconvolution of the data with an arterial input function (AIF) will yield hemodynamic parameters. The indicator-dilution theory of perfusion imaging for non-diffusible tracers states that the profile of the time-concentration curve is equal to product of the CBF, convolution of the AIF, and the tissue residue function (Meier & Zierler, 1954; Zierler, 1965). This can be summarized with the equation:

$$C_{Gd}(t) = CBF \int_{u=0}^{t} AIF(u)R(t-u)du$$

6)

The arterial input function in equation 6) simply represents the concentration of gadolinium in the arteries as a function of time. In a perfect scenario, the shape of the AIF would be a delta function to reflect an instantaneous spike of the contrast agent. This scenario would simplify the mathematics and make quantitative perfusion trivial. In reality, this ideal delta function could never be attained in practice because of the injection and dispersion of the contrast agent into the blood. In practice, the AIF takes on the shape of a broad, Gaussian-like function that is determined by the rate at which gadolinium is injected and by the dispersion of the contrast that occurs as the blood flows from the injection site to the brain.

The residue function in equation 6) represents the manner in which gadolinium is retained by the tissue. Since there are many pathways that the gadolinium molecules can flow through the capillary bed on its way out of the tissue, the residue function represents a statistical probability of time dependent gadolinium retention. The residue function is mathematically determined by deconvolving the measured time-concentration function (which was obtained from the perfusion signal) with the AIF (R. A. Cohen & Sweet, 2010).

Deconvolution is a process that estimates the residue function using the AIF (Ostergaard, Chesler, Weisskoff, Sorensen, & Rosen, 1999). Based on equation 6) one can calculate a quantitative estimate of CBF, which can be summarized with the following equation:
Since the time-concentration curve and AIF can both be assessed, the CBV may also be derived using the following equation:

\[ CBF = \frac{C_{Gd}(t)}{\int_{u=0}^{t} AIF(u)R(t-u)du} \]

where \( C_{Gd}(t) \) is the concentration of Gd at time \( t \), \( AIF(u) \) is the arterial input function, \( R(t-u) \) is a delay function, and the integral represents the area under the curve from \( u=0 \) to \( t \).

Also, based on the central volume theorem (Meier & Zierler, 1954), MTT can also be calculated with:

\[ CBV = K \frac{\int C_{Gd}(t)dt}{\int AIF(t)dt} \]

where \( K \) is a scaling factor that depends on hematocrit and vessel geometry.

1.6.4.2 Perfusion MRI in Leukoaraiosis

Resting brain perfusion is believed to decline gradually with age (Parkes, Rashid, Chard, & Tofts, 2004) and concomitantly with age-related changes in the cerebral microvasculature to meet energy demands (Moody et al., 1997). Perfusion MRI has been applied to examine CBF and CBV in white matter lesions. Markus et al. (2000) found that the mean white matter CBF in patients with ischaemic leukoaraiosis was reduced by 40% when compared to age-matched healthy controls. The authors found that grey matter CBV was significantly increased in subjects with leukoaraiosis. The relevance of this finding is not clear but the authors have proposed cortical dysfunction or injury triggered by subcortical insults (H. S. Markus, Lythgoe, Ostegaard, O'Sullivan, & Williams, 2000). Another study examining perfusion in the normal-appearing white matter of subjects with leukoaraiosis demonstrated that CBF is significantly reduced in the periventricular white matter compared to age-matched healthy controls. The CBF in the centrum semiovale was not significantly different from those of healthy controls (O'Sullivan et al., 2002). These findings suggest chronic hypoperfusion and ischemic damage as the underlying pathophysiological mechanism in leukoaraiosis.
1.7 Quantification of Leukoaraiosis

Progress in the study of leukoaraiosis has been impeded by inconsistencies in neuroimaging methodology to quantify leukoaraiosis and terminology to describe the associated vascular changes. There is currently no single methodological approach that has been applied uniformly throughout many clinical study population and neuroimaging research centers, which may explain some of the conflicting results in the literature of leukoaraiosis. There are several qualitative rating scales and methods to quantify leukoaraiosis. These classification and quantification methods fall into 3 major categories: 1) machine learning 2) intensity cut-off points and 3) template based.

1.7.1 Rating Scales

There are various visual rating scales to quickly estimate the disease burden in subjects with leukoaraiosis. These scales require a trained user to evaluate the disease burden along a severity scale (Bocti et al., 2005; Fazekas et al., 1987; Wahlund et al., 2001). The Fazekas scale is used to quantify deep white matter hyperintensities and periventricular hyperintensities on a 4 point scale where a score of 0 indicates no lesions, 1 indicates focal lesions, 2 indicates the beginning of lesion confluence, and 3 indicates diffuse lesion involvement in entire regions (Fazekas et al., 1987). Another scale, the Age-Related White Matter Changes (ARWMC) scale, is a widely used rating scale that can be applied to both CT and MRI images (Wahlund et al., 2001). This scale categorizes disease burden on a 4-point scale in 5 different brain regions consisting of the frontal, parieto-occipital, temporal, infratentorial, and basal ganglia. Interestingly, this scale categorizes changes in the basal ganglia as white matter lesions even through it is a subcortical grey matter structure. Although these visual rating scales provide rapid impression of disease burden, they are subjective qualitative measures that are not suitable for quantitatively studying the progression of leukoaraiosis. MRI intensity based lesion segmentation methods provide a more accurate estimate of disease burden.

1.7.2 Machine learning approaches

A common machine learning approach uses the k-nearest neighbour (kNN) algorithm. This approach is a supervised pattern recognition technique that performs segmentation by comparing new data to a collection of labeled examples (truth data) in a training set (Steenwijk et al., 2013). When classifying new voxels, the kNN algorithm computes the probability of the voxel being leukoaraiosis by determining the fraction of k nearest neighbours that were labeled as a lesion in the feature space of the training set (Anbeek, Vincken, van Osch, Bisschops, &
van der Grond, 2004). Another commonly machine learning approach is the fuzzy C-means clustering algorithm that assesses hyperintensities on FLAIR images. This approach involves an iterative process where each voxel is assigned “fuzzy” membership to a predefined set of tissue classes after a supervised training period. The goal of this technique is to develop a reasonable level of classification that mimics those of expert reviewers. Machine learning approaches to lesion quantification offer the promise of a fully automated segmentation of leukoaraiosis, however manual validation for false-positives and expert training of each algorithm is still required (Admiraal-Behloul et al., 2005; Gibson, Gao, Black, & Lobaugh, 2010).

1.7.3 Intensity cut-off approaches

Intensity cut-off approaches to the quantification of leukoaraiosis involves the use of intensity histograms obtained from the imaging voxels. The classification of voxels are derived from histograms fitted to Gaussian curves where >3 standard deviations on a Gaussian curve can be used to label voxels containing leukoaraiosis (DeCarli, Fletcher, Ramey, Harvey, & Jagust, 2005). Another approach is to use modal intensity cut-off points applied on a slice-by-slice basis, in which 1/3 of the height of the mode value can be used as the cut-off value for the identification of leukoaraiosis (Jack et al., 2001). These approaches also require manual correction arising from flow-related artifacts in the posterior limb of the internal capsule and higher signal intensity in the deep grey nuclei and limbic cortex.

1.7.4 Template-based approaches

Template-based approaches to lesion segmentation are based on normalization, which is a computational process that co-registers each individual scan to a template. This can be done with software packages such as Statistical Parametric Mapping (SPM) and Voxel-Based Morphometry (Ashburner & Friston, 1999). Templates are obtained using averaged and scaled scans from a study sample or may be downloaded from a standardized averaged template, such as that provided by the Montreal Neurological Institute (MNI).

The process of normalization involves the matching of two images based on intensity differences between their corresponding regions by using linear and non-linear transformations. Linear transformation matches an individual image to the template by using sheers, zooms, shifts, and rotations to roughly align both images. Non-linear transformation (also known as warping) can further optimize the alignment between the two images. This process compresses certain regions of the source image while expanding others and tends to be more sensitive to local differences than linear transformations. However, non-linear transformations can also
eliminate a particular region of leukoaraiosis by compressing it, causing significant distortion of the brain because lesions usually have very different signal intensities than the corresponding area in a template. This can be resolved by masking the lesion during the process of non-linear transformations (Brett, Leff, Rorden, & Ashburner, 2001).

The template-based approach to segmentation is limited by several factors. The normalization process is not optimal in patient populations with large variations in ventricle size, such as Alzheimer’s disease. Also, this approach is limited to group-wise comparisons, which do not allow for individual volumetric analysis; therefore, this would not be ideal in longitudinal studies looking at individual profiles (Ramirez et al., 2011).

1.7.5 Fluid-attenuated inversion recovery (FLAIR) imaging

The use of FLAIR imaging for identifying and quantifying leukoaraiosis has recently increased in popularity. A FLAIR image is similar to a T2 image but with the CSF signal attenuated or nulled, thereby enabling discrimination of leukoaraiosis lesions from CSF in the ventricular system (Jack et al., 2001). The attenuation of the CSF signal results in the distinctiveness of white matter pathology as its signal is hyperintense relative to other tissues as well. Therefore, intensity histograms based on FLAIR images have an easily identifiable intensity peak for voxels containing white matter lesions, which enables the use of reproducible and fully-automated segmentation approaches (Admiraal-Behloul et al., 2005; Gibson et al., 2010; Jack et al., 2001).

1.7.6 Lesion Explorer

Lesion explorer is a semi-automated MRI-based tissue volumetric processing pipeline that addresses many of the aforementioned issues associated with the identification of leukoaraiosis. In general, this pipeline uses all three categories of the methodological approaches described (machine learning, intensity cut-off points, and templates) to give a volumetric profile, which includes regionalized measures for intracranial tissue (grey matter, white matter, and CSF) as well as leukoaraiosis (periventricular, deep white, and lacunes) (Ramirez et al., 2011). In contrast to previous MRI segmentation methods, the Lesion Explorer pipeline addressing many lesion identification issues by using an innovative tri-feature approach, which is based on intensity information from co-registered T1, T2, and proton density (PD) images. For example, to correct for voxel misclassification arising from leukoaraiosis, T1-based tissue segmentation is corrected with additional PD- and T2-based lesion segmentation. The overall processing pipeline consists of three main components: an automated T1-based
tissue segmentation protocol, Brain-Sizer (Kovacevic et al., 2002), a parcellation procedure called Semi-Automated Brain Region Extraction (SABRE) (Dade et al., 2004), and Lesion Explorer (Ramirez et al., 2011), all of which were developed at Sunnybrook Health Sciences Centre (SHSC).

To briefly explain the image processing protocol, PD- and T2-weighted images are used to remove the skull and other non-brain tissues, and to identify white matter hyperintensities (WMH) for co-registration with T1-weighted volumetric anatomical images (Woods, Grafton, Holmes, Cherry, & Mazziotta, 1998; Woods, Grafton, Watson, Sicotte, & Mazziotta, 1998). The T1-weighted image is segmented into gray matter, white matter, sulcal and ventricular cerebrospinal fluid compartments (Kovacevic et al., 2002). Based on a small number of manually traced landmarks on the surface-rendered T1 image rotated into Talairach space, a mask defining 26 bilateral brain regions is generated automatically and then rotated back down into acquisition space (Dade et al., 2004). Typically, WMH will segment as gray matter or CSF on T1 and as hyperintensities on T2-weighted images. To overcome this limitation of T1 segmentation, WMH are identified separately using a semiautomatic tool based on the PD/T2-weighted images, called Lesion Explorer, which enables automated WMH followed by manual selection of valid WMH yielding the number, size, and volume of the hyperintensities (Levy-Cooperman, Ramirez, Lobaugh, & Black, 2008). The WMH mask and T1-lesion ROIs are combined with the T1 segmentation to clearly separate normal-appearing tissues from abnormal ones. This co-registration allows any lacunes within hyperintense regions, representing cystic necrosis, to be separately documented. Volumetric measures are expressed as a ratio of a tissue volume to the total supratentorial intracranial volume with infra-tentorial structures removed in order to control for head size (Andersen, Zhang, Zhang, Gash, & Avison, 1999; Arndt, Cohen, Alliger, Swayze, & Andreasen, 1991). The WMH lesion load is also calculated regionally, including the number of lesions > 3 mm in diameter (commonly accepted as potentially clinically significant) as well as lacunes. Location within the Talairach and Tournoux proportional grid representing the cortical cholinergic projections, the anteromedial thalamus, head of caudate and putamen, are also separately coded (Selden, Gitelman, Salamon-Murayama, Parrish, & Mesulam, 1998). In summary, Lesion Explorer outputs number, size, and location (periventricular, deep white, basal ganglia, and thalamus) separately for hyperintense only and cystic lesions (black holes on T1). SABRE can then parcellate into 26 lobular regions.
1.8 Imaging Cerebral Blood Flow Regulation

The autoregulatory capacity of the brain blood vessels to control blood flow can be assessed with cerebrovascular reactivity (CVR). CVR is a measure of the sensitivity of the cerebrovasculature to CO2 and is defined as the change in cerebral blood flow induced by a vasoactive stimulus. This definition is important to remember when considering the tools available for inducing changes in blood flow and measuring blood flow since all methods have important limitations. Mapping CVR has two components: 1) application of a vasodilatory stimulus, and 2) measuring the ensuing changes in cerebral blood flow. The commonly used vasodilatory stimuli include carbon dioxide (CO2) and acetazolamide. Blood pressure reduction has been used on occasion. Methods available for measurement of changes in blood flow include Doppler ultrasound (usually of the MCA), inhaled xenon CT, CT perfusion, MR perfusion, single photon emission computed tomography (SPECT), positron emission tomography (PET), Blood-oxygen-level-dependent (BOLD) MRI, and arterial spin labeling (ASL) MRI.

1.8.1 Methods for Changing Cerebral Blood Flow

1.8.1.1 Acetazolamide

Acetazolamide is a drug that produces maximal vasodilatation for 20 minutes beginning 12-20 minutes after injection (Kuroda et al., 2001). The problems with this method include stability of the blood flow response, reproducibility, and quantitation. These factors are impacted by the relatively short 20 minute time window where the blood flow response is maximal representing the optimal time for acquiring the flow data. According to the definition of CVR, the change in blood flow would be measured against an administered dose of acetazolamide with accuracy dependent on the assumption that the flow response to the drug is linear, and that the concentration of the drug is not changing during the time frame of the flow measurement.

1.8.1.2 Carbon Dioxide

CO2 directly modulates vascular tone with hypocapnea resulting in vasoconstriction and hypercapnea producing vasodilatation. However, it is difficult to induce stable reproducible changes in end-tidal CO2 using conventional methods, such as inhalation of 5% carbogen or breath hold. These methods do not control the extent of the rise or fall of blood CO2 levels, which are a function not only of the administered concentration of CO2, but of the patient’s ventilatory response to CO2 which varies from person to person.
1.8.1.3 The RespirAct

The RespirAct is a device now being manufactured by Thornhill Research Inc. that can rapidly and precisely change end-tidal CO\textsubscript{2} concentrations independent of the subject’s minute ventilation. These changes can be induced within three breaths and are accurate to within +/- 1 mmHg (Slessarev et al., 2007). The device can also carefully control end-tidal oxygen levels since blood oxygen can shorten T1 relaxation therefore affecting the BOLD signal (Prisman et al., 2008). End-tidal CO\textsubscript{2} measurements obtained from this device have been validated by using arterial lines to accurately reflect arterial partial pressure of CO\textsubscript{2} (Ito et al., 2008). This device is therefore the method of choice for enabling quantitative analysis of CVR since the changes in the flow stimulus, end-tidal CO\textsubscript{2}, are precisely known. The ability to rapidly control end-tidal CO\textsubscript{2} is essential for efficiently acquiring MRI data and reducing overall scan time thus allowing translation of the method to the clinical setting.

1.9 Imaging Methods Available for measuring cerebral blood flow

1.9.1 Doppler Ultrasound, Xenon CT, and PET

Doppler ultrasound is a simple method for measuring CVR since it can measure flow changes in large feeding vessels. It cannot do this at the level of the microcirculation and is therefore incapable of measuring CVR in the white matter. Xenon CT is capable of measuring changes in blood flow in standard units (ccs/100mg/min) but the technology is not widely available. PET is capable of measuring CBF but requires a cyclotron since oxygen 15 is required.

1.9.2 SPECT

SPECT imaging does not measure CBF since it assesses the first pass deposition of lipophilic tracer into the tissue and is therefore a measure of tracer distribution. Despite the non-linear deposition of the tracer with CBF, it is possible to quantitate CBF, but arterial blood samples are needed to measure tracer concentrations. Both quantitative and non-quantitative methods require two separate imaging sessions, one to measure resting CBF, and the second after washout of the tracer usually 24 hours later, to measure CBF during a flow augmentation stimulus.

1.9.3 ASL MRI

ASL MRI is an excellent method since it is arterial input function independent and measures CBF in standard units. There are several implementations of the tool with the most desirable not currently supported by MR vendors. A version with high temporal resolution and
less sensitive to proton labeling delay is called FAIR ASL but it is limited to 3-5 slices and therefore will not provide the needed coverage of mapping the entire white matter.

1.9.4 DSC-perfusion MRI

DSC MR perfusion is a technique in which the first pass of a bolus of gadolinium-based contrast agent through brain tissue is monitored by a series of T2- or T2*-weighted MR images. The susceptibility effect of the paramagnetic contrast agent leads to a signal loss in the signal intensity-time series. Using the principles of the indicator dilution theory, the signal information can then be converted into a contrast concentration–time series. From this data, parametric maps of cerebral blood flow may be derived after deconvolution of the concentration-time series with an arterial input function. However, there are a number of issues that can affect the accuracy of these measurements such as inaccuracy of the arterial input function due to gadolinium bolus delay and dispersion as well as partial voluming effects of the arterial input function (Ostergaard, 2005). These confounds increase as the extent and complexity of vascular disease increases. DSC-MR perfusion methods are capable of measuring CBF but require injections of contrast and are dependent on selection of arterial input function for deconvolution of signal changes in the microcirculation during transit of the contrast. Delay time correction must be used if significant vascular stenoses are present. Reliability of flow quantification is unknown in the setting of bilateral carotid or multi-vessel stenoses (Ostergaard, 2005).

1.9.5 BOLD functional MRI

Functional MRI, based on the blood oxygen level-dependent (BOLD) signal, is the principal neuroimaging technique for basic and clinical research in humans. It is based on the paramagnetic nature of deoxygenated hemoglobin (Pauling & Coryell, 1936) and the excess blood supply in response to brain activation that produces a net increase in oxygenated hemoglobin (Fox & Raichle, 1986). These changes are accompanied by an enhancement of the MRI signal (Ogawa & Lee, 1990; Ogawa et al., 1990).

BOLD MRI is arterial input function independent but does not measure CBF directly. It measures the washout of deoxyhemoglobin (dHb) in the setting of constant metabolic activity. In this setting, the change in dHb concentration is proportional to the change in CBF following a flow stimulus. The images are noisy and the baseline signal has a low frequency variation similar to that with the EPI sequence used for DSC studies. These issues are minimized when multiple baseline and flow augmented conditions are obtained for comparison. This method however requires rapid manipulation of CO₂ levels to keep imaging times acceptable.
1.9.5.1 Advantages of BOLD MRI CVR

BOLD MRI CVR can be performed in a single setting during an 8-9 minute acquisition without tracers or injections providing whole brain coverage. Although BOLD CVR measures blood flow change indirectly, it has been validated against blood flow measurements obtained with FAIR ASL MRI (Mandell, Han, Poub lanc, Crawley, Stainsby, et al., 2008). For the range of CO\textsubscript{2} manipulation required for CVR analysis, the response is linearly proportional to end-tidal CO\textsubscript{2} (P\textsubscript{ET}CO\textsubscript{2}) (Tancredi & Hoge, 2013). To date, we have performed over 900 CVR studies without complication in patients with severe cerebrovascular disease (Spano et al., 2013). Approximately 5% of subjects were unable to tolerate hypercapnea and were not able to complete the examination.

1.9.5.2 BOLD CVR MRI Reproducibility

CVR reproducibility was studied on a 1.5T MRI system in 10 healthy male volunteers with ages ranging between 25-42 years. Each was imaged 4 times with 2 sequential runs on day 1 and 2 sequential runs on a separate day 1-2 weeks later. Results for the day-to-day variation of runs show excellent reproducibility with an interclass correlation coefficient (ICC) of 0.9 (95% CI: 0.7-0.97). Within a session, there is a 95% chance that repeated measures for entire brain values on a subject would lie within $\pm$ 0.017 % BOLD signal change/mmHg P\textsubscript{ET}CO\textsubscript{2} (Kassner et al., 2002).
CHAPTER 2

AIMS AND HYPOTHESES

General Aim

The general aim of this thesis is to determine whether patients with leukoaraiosis have signs of dysfunction in neurogliovascular coupling as assessed with CVR, particularly in the cerebral white matter. By manipulating PaCO$_2$ for CVR assessment, the sensitivity of the vasculature to respond to CO$_2$ will be tested. Areas of the white matter with reduced vasodilatory capacity are particularly vulnerable to neurogliovascular uncoupling and ischemic injury. If this is true, the ensuing white matter injury will be corroborated by other MRI metrics. Four MRI-based studies have been conducted to assess the role of CVR in white matter physiology. The rationale, specific aims, and hypotheses for each of these studies are presented below.

2.1 Study I. Vascular dysfunction in elderly subjects with leukoaraiosis.

Background and Rationale

Leukoaraiosis is defined as diffuse white matter lesions that are visible on CT or MRI. Previous studies report that leukoaraiosis can be associated with various pathological conditions including Alzheimer’s disease (Gootjes et al., 2004), vascular dementia (C. K. Liu et al., 1992), hypertension (Verdelho et al., 2010), small vessel disease (Hainsworth & Markus, 2008), atherosclerosis (Bots et al., 1993), cerebral hemorrhage (E. E. Smith et al., 2004), and aging (R. Schmidt et al., 2002). Unlike the white matter lesions arising from a stroke or brain tumour, the pathogenesis of normal age-related leukoaraiosis is not well defined (Uh et al., 2010). Almost all elderly individuals have some degree of leukoaraiosis, particularly in the periventricular regions (Piguet et al., 2003; Soderlund, Nyberg, Adolfsson, Nilsson, & Launer, 2003). A number of studies have suggested a strong contribution of vascular dysfunction in leukoaraiosis associated with ischemic diseases (Lammie, 2000; Wardlaw et al., 2013). Mandell et al. (2008) has demonstrated impaired vascular reactivity in the white matter of young healthy individuals in areas that spatially correlate with the distribution of leukoaraiotic lesions. Cerebrovascular autoregulation is a protective homeostatic mechanism that maintains cerebral blood flow (CBF) despite changes in cerebral perfusion pressure. There is a failure to maintain normal CBF in
response to a challenge, such as a reduction in perfusion pressure, neural activation, or chemical stimulation (hypoxia or hypercapnea), when the autoregulatory capacity is exhausted. Evidence has shown that the severity of leukoaraiosis and the impaired hemodynamics is also is predictive of cognitive function (Balucani et al., 2012; Breteler et al., 1994; Buratti et al., 2014; Gunning-Dixon & Raz, 2000). Therefore, it is important to characterize the physiological mechanisms underlying age-related leukoaraiosis.

**Specific Aims:**

1. To determine if there is a relationship between impaired vascular reserve in the cerebral white matter and the presence of leukoaraiosis.
2. To characterize white matter pathophysiology by using MRI metrics such as diffusion tensor imaging (DTI), quantitative T2, and dynamic susceptibility contrast (DSC)-perfusion in leukoaraiosis compared to normal-appearing white matter (NAWM).

**Specific Hypotheses**

1. Vascular reserve will be reduced in leukoaraiosis compared to NAWM, indicating impaired sensitivity of the vasculature in meeting blood flow demand.
2. DTI metrics will show decreased FA and increased mD in leukoaraiosis compared to NAWM, indicating degeneration of the white matter fibres.
3. T2 will be higher in leukoaraiosis DTI metrics in leukoaraiosis compared to NAWM, indicating a higher water content and loss in structural integrity.
4. DSC-perfusion metrics will show lower cerebral blood flow (CBF), higher time-to-maximum (Tmax), higher time-to-peak (TTP), and mean transit time (MTT) in leukoaraiosis compared to NAWM, indicating dysfunction in the local vascular network of leukoaraiosis.
2.2 Study II. Impaired Cerebrovascular Reactivity is Associated with abnormal T2, Diffusion and Perfusion MRI metrics in Normal-appearing White Matter.

Background and Rationale

The human cerebrovasculature changes with age, but the rate of change varies regionally (Raz & Rodrigue, 2006). For example, the small connecting fibres of the anterior corpus callosum have been demonstrated by several studies to be particularly vulnerable to age-related changes (Aboitiz, Rodriguez, Olivares, & Zaidel, 1996; Sullivan & Pfefferbaum, 2003). Relatively new approaches to studying age-related changes in the white matter include DTI and DSC-perfusion, providing insight into the microstructural changes in the white matter. Advanced age is associated with an increase in mean diffusivity of the whole brain, frontal white matter but not in the posterior limb of the internal capsule and corpus callosum (Abe et al., 2002; Rovaris et al., 2003). Another diffusion MRI metric that demonstrates age-related changes is FA, in which decreases in anisotropy tend to be greater in the white matter of the centrum semiovale, parietal pericallosal regions, genu of the corpus callosum, and lentiform nucleus (Abe et al., 2002; Pfefferbaum et al., 2000). The results from Study I demonstrate that impaired CVR is associated with the extent of leukoaraiosis, suggesting that impaired blood flow regulation may be an important factor for the progression of white matter disease. The sequence of events in the evolution of leukoaraiosis is a key question that has important implications for understanding lesion pathogenesis.

Specific Aims

To determine if MRI metrics within NAWM with steal physiology will show abnormalities compared to areas of NAWM with normal CVR.

Specific Hypotheses

1. FA will be lower and mD values will be higher in NAWM with steal physiology compared to areas with normal CVR.
2. T2 will be slightly higher in NAWM with steal physiology compared to areas with normal CVR.
3. CBF and CBV will be slightly reduced while MTT, Tmax, and TTP will be slightly higher in NAWM with steal physiology compared to areas with normal CVR.
2.3 Study III. Diminished cerebrovascular reserve predicts future development of leukoaraiosis.

Background and Rationale.

Leukoaraiosis describes cerebral white matter changes that are frequently observed on MRI scans of elderly individuals and is associated with cognitive decline (Verdelho et al., 2010), balance disturbances (Whitman, Tang, Lin, & Baloh, 2001), depression (Collard et al., 2015), as well as increased stroke risk (Pu et al., 2009). Previous research by Mandell et al. (2008) has shown reduced cerebrovascular reactivity (CVR) in the white matter of young healthy individuals, particularly in locations that spatially correspond with regions where the elderly develop leukoaraiosis. This raises the possibility that vascular reactivity may play a significant role in the pathogenesis of white matter disease. The relationship between neurovascular coupling and future development of leukoaraiosis remains unresolved. As such, it is unclear if NAWM with reduced CVR is more likely to progress to leukoaraiosis that NAWM with normal CVR.

Specific Aims

To determine if CVR deficits in normal-appearing white matter are associated with the subsequent evolution of leukoaraiosis.

Specific Hypotheses

1. Regions of NAWM with reduced CVR will be more likely to develop leukoaraiosis than regions of NAWM with normal CVR over a one-year period.

2. Subjects will be more likely to develop visible T2-signal hyperintensities in regions of normal-appearing white matter with lower FA and higher mD over a one-year period.

3. Subjects will be more likely to develop visible T2-signal hyperintensities in regions of normal-appearing white matter with higher T2 values over a one-year period.
2.4 Study IV. Impaired Temporal Dynamics of the Cerebromicrovasculature in elderly subjects with leukoaraiosis.

Background and Rationale

CVR is defined as the change in flow in response to a vasoactive stimulus. CVR may be characterized into static and dynamic components when an abrupt and vigorous vasodilatory stimulus is administered. With this vasodilatory paradigm, it is possible to assess the rate of CBF increase in response to vasodilation (thereby measuring the dynamic aspect of CVR) by convolving the measured $P_{ET}CO_2$ with a set of first-order exponential functions whose time constant ($\tau$) is increased in 2-second intervals between 2 and 100 seconds. The $\tau$ that corresponds to the best fit between the BOLD signal and the convolved $P_{ET}CO_2$ is used as the rate of response for that particular voxel. Previous assessment of CVR in Study I assessed steady-state CVR by taking the slope of the regression between the BOLD signal and convolved $P_{ET}CO_2$. The Study I has demonstrated that areas of leukoaraiosis have reduced CVR compared to NAWM. Whether a difference in dynamic CVR exists between NAWM and leukoaraiosis remains to be elucidated.

Specific Aim

The aim of this study is to determine whether a difference in dynamic CVR exists between NAWM and leukoaraiosis.

Specific Hypothesis

Areas of leukoaraiosis will demonstrate a depressed dynamic response to a vasodilatory stimulus ($CO_2$) compared to NAWM.
CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Subject Recruitment

For all studies, both male and female elderly adults (age range: 50-91; 40 males) were recruited for participation and provided informed consent to procedures approved by both the University Health Network (UHN) and Sunnybrook research ethics boards (See Appendix Tables 1 and 2). Subject recruitment was achieved through both the memory and stroke prevention clinic at Toronto Western Hospital (TWH) and memory clinic at Sunnybrook Health Sciences Centre (SHSC).

Magnetic resonance angiogram (MRA) or computed tomography angiography (CTA) and T2-weighted fluid-attenuated inversion recovery (FLAIR) images of all patients were screened by an experienced neuroradiologist and subjects were included in the study based on the following inclusion criteria: (1) previous neurological event involving white matter > 3 months from presentation; (2) over the age of 50; (3) MRI white matter disease burden > Fazekas Grade 2 indicating a moderate number of FLAIR hyperintensities in the white matter; (4) no evidence of bilateral ICA stenosis greater than 70%; (5) no evidence of significant vertebral or basilar stenosis greater than 70%; (7) no evidence of dissection (8) no evidence of pulmonary or cardio-embolic disease. Subjects with motion artifacts on BOLD images were excluded. Forty-three patients from Toronto Western Hospital (age range, 50 to 87 years; 23 males and 20 females) and thirty-two patients from Sunnybrook Health Sciences Centre (age range, 51 to 91 years; 17 males and 15 females) with moderate-severe leukoaraiosis met the inclusion criteria and were considered in subsequent analysis (See Appendix Tables 1 and 2).

3.1.1 Subject Inclusion/exclusion criteria

Inclusion:

1. Previous ischemic neurological event involving white matter or TIA greater than three months from presentation
2. Over the age of 50
3. MRI white matter disease burden > Fazekas grade 2.
4. Recent (6 months) cervico-cerebral MRA, CTA, or DSA

Exclusion:
1. Unsafe for 3T MRI
2. Cortical or sub-cortical infarcts > 2 cm
3. Cardio-embolic disease
4. Dissection
5. Medications known to alter CVR including nitrates, calcium channel blockers.
6. Pulmonary disease
7. Carotid stenosis > 70% determined on pre-existing MRA or CTA studies.

3.2 Imaging Sequences

Studies were performed at the TWH and SHSC. CVR data were obtained on identical Generation III RespirAct gas blenders used at both sites for control of end-tidal CO₂. The following MRI protocol with whole brain coverage will be applied at both subject time points:

1. Sagittal Localizer 0:42 minute
2. FAST-SPGR 3D sequence 7:36 minutes
   for co-registration and segmentation
3. Axial single shot EPI-GRE sequence 8:30 minutes
   for BOLD CVR data acquisition
4. DTI sequence (23 directions) 6:18 minutes
   for mD and FA measurements
5. 2D FLAIR 5:48 minutes
6. Multi-echo T2 sequence 10:36 minutes
   for quantitative T2 measurements
7. PD/T2 sequence 5:12 minutes
   for tissue segmentation
8. DSC-perfusion 2:00 minutes

Total Scan Time: 55:42 minutes

3.2.1 MRI Acquisition

For all studies at TWH, subjects underwent MRI scans on a 3-Tesla GE system (Signa HDx platform, GE Healthcare, Milwaukee, Wis) and fitted with an eight-channel phased array head coil. Subjects were asked to refrain from heavy exercise and drinking on the day of each scan. Scans included a T₁-weighted anatomical scan, one or two runs of BOLD fMRI with the
CO₂ stimulus administered by the RespirAct, a FLAIR scan, diffusion tensor imaging, proton density/T₂-weighted imaging, multiecho T₂ scan, and a DSC-perfusion scan. The imaging acquisition parameters were as follows:

T₁-weighted scan using a 3D spoiled gradient echo sequence [slice thickness = 1.5 mm; no interslice gap; matrix size = 256 x 256; field of view = 22 x 22 cm; flip angle = 20°; TE = 3 ms; TR = 7.8 ms; TI = 300 ms; 146 slices per volume];

BOLD fMRI assessing cerebrovascular reactivity using a T₂*-weighted echoplanar imaging gradient echo sequence [slice thickness = 5.0 mm; field of view = 24 x 24 cm; matrix size = 64 x 64; flip angle = 85°; TE = 30 ms; TR = 2000 ms; 255 frames];

Conventional FLAIR images [slice thickness = 3 mm; 36 slices per volume; matrix size = 256 x 224; field of view = 22 x 22 cm; flip angle = 90°; TE = 165 ms; TR = 9145 ms; TI = 2200 ms];

Diffusion tensor imaging with echoplanar imaging spin-echo sequence [slice thickness = 3 mm; matrix size = 128 x 128; field of view = 22 x 22 cm; 47 axial slices per volume; b = 1000 s/mm²; 23 diffusion-encoding gradients; 2 non-diffusion-weighted B0 images; TE = 80 ms; TR = 14500 ms];

Proton density/T₂-weighted images using fast spin echo-XL [slice thickness = 3 mm; matrix size = 128 x 128; field of view = 22 x 22 cm; flip angle = 90°; TE = 11.1/90 ms; TR = 7200 ms; 48 images per volume each];

Multiecho T₂ mapping using a fast spin echo-XL [slice thickness = 3 mm; no interslice gap; matrix size = 256 x 192; field of view = 22 x 22 cm; 22 axial slices per volume; TE = 7, 21, 35, 49, 63, 77, 91, 105 ms; TR = 6000 ms];

DSC perfusion scan using gradient-echo echoplanar imaging sequence [slice thickness = 5 mm; matrix size = 128 x 128; field of view = 27 x 27 cm; flip angle = 90°; TE = 31.5 ms; TR = 1725 ms; 50 slices per location] during which a single dose of 0.1 mmol/kg of gadolinium contrast agent was injected at a rate of 5 mL/s (See Appendix Figure 1 for all parametric maps).

For studies at SHSC, subjects underwent MRI scans on a 3-Tesla Philips Achieva system (Philips Medical Systems, Best, Netherlands) and fitted with an eight-channel phased array head coil. Subjects were asked to refrain from heavy exercise and drinking on the day of each scan. Scans included a T₁-weighted anatomical scan, one or two runs of BOLD fMRI with the CO₂ stimulus administered by the RespirAct, a FLAIR scan, diffusion tensor imaging,
proton density/T2-weighted imaging, and a multiecho T2 scan. The imaging acquisition parameters were as follows:

T1-weighted scan using a 3D turbo field echo sequence [slice thickness = 1.2 mm; no interslice gap; matrix size = 256 x 164; field of view = 240 x 192 mm; flip angle = 8°; TE = 2.3 ms; TR = 9.5 ms; TI = 822 ms; 144 slices per volume];

BOLD fMRI assessing cerebrovascular reactivity using a T2*-weighted echoplanar imaging gradient echo sequence [slice thickness = 3.0 mm; field of view = 230 x 187 mm; matrix size = 64 x 64; flip angle = 90°; TE = 30 ms; TR = 2000 ms; 255 frames];

Conventional FLAIR images [slice thickness = 3 mm; 52 slices per volume; matrix size = 240 x 217; field of view = 240 x 240 mm; flip angle = 90°; TE = 125 ms; TR = 9000 ms; TI = 2800 ms];

Diffusion tensor imaging with echoplanar imaging spin-echo sequence [slice thickness = 3 mm; matrix size = 76 x 62; field of view = 224 x 224 mm; 52 axial slices per volume; b = 1000 s/mm²; 23 diffusion-encoding gradients; 2 non-diffusion-weighted B0 images; TE = 55 ms; TR = 9150 ms];

Proton density/T2-weighted images using turbo spin echo imaging [slice thickness = 3 mm; matrix size = 256 x 209; field of view = 230 x 183 mm; flip angle = 90°; TE = 11/102 ms; TR = 2500 ms; 48 images per volume each];

Multiecho T2 mapping using a turbo spin echo imaging [slice thickness = 3 mm; no interslice gap; matrix size = 232 x 183; field of view = 230 x 184 mm; 16 axial slices per volume; TE = 13, 26, 39, 52, 65, 78, 91, 104, 117, 130, 143, 156 ms; TR = 5301 ms].

3.3 Psychometrics

Before each scan, all subjects completed the Montreal Cognitive Assessment (MoCA) as this cognitive test only takes 10 minutes to administer (Nasreddine et al., 2005). The MoCA is a cognitive test used to screen for mild cognitive impairment (MCI) based on 8 cognitive domains, which include memory, orientation, visuospatial perception, language, conceptual ability, attention and concentration, executive function, and calculation. A score of 26 out of 30 or above indicates that a patient is not cognitively impaired (Nasreddine et al., 2005). See Appendix 3.

3.4 Vasodilatory stimulus

Control of end-tidal CO₂ (P_{ET}CO₂) and O₂ (P_{ET}O₂) was achieved using the RespirActTM. This is a gas blender uses a prospective targeting method in which P_{ET}CO₂ and P_{ET}O₂ is
controlled independently from each other and from minute ventilation (Slessarev et al., 2007). The RespirAct™ uses a sequential gas delivery circuit to achieve rapid changes in end-tidal gas concentrations within 2-3 breaths that can be sustained for long durations to within ± 2 mmHg of the target. Ito et al. (Ito et al., 2008) has shown that when breathing using the RespirAct™, the $P_{ET}CO_2$ achieved provides an accurate measurement of arterial PCO$_2$.

In brief, the function of the sequential delivery circuit starts with an open-ended gas reservoir that is added to the expiratory end, referred to as the G2 bag, as well as an inspiratory reservoir, referred to as the G1 bag, that are connected through a series of one-way valves (Hi-Ox, VIASYS Health Care, Yorba Linda, CA, USA) (See Figure 3-1). The flow of gases emitted from the inspiratory and expiratory reservoir, referred to as G1 and G2 respectively, are provided to the subject upon inspiration through the circuit. The composition of G1 is predetermined based on 4 potential source gases (A: Medical Air B: 100% O$_2$ C: 10% O$_2$, 90% N$_2$ and D: 10% O$_2$, 20% CO$_2$, 70% N$_2$) and is continuously fed into the inspiratory reservoir. To precisely control $P_{ET}CO_2$, G1 is provided to the subject at a flow that is either equivalent to or a bit less than the subject’s minute ventilation. If the subject’s minute ventilation is higher than the G1 flow, the G1 reservoir is depleted and the subject will begin inhaling flow form the G2 reservoir. All of G1 gas is always inhaled before any of the G2 gas. This is made possible through a series of 3 one-way valves called the expiratory, inspiratory, and cross-over valves. The opening pressure of the cross-over valve is higher than the other two valves, assuring that the G1 reservoir is empty before any G2 is inhaled. This set up also prevents contamination of the G1 reservoir with gases in the exhaled breath as only the expiratory valve can open during exhalation. The composition of G2 is determined by the gas exhaled from the previous breath and is held in the G2 reservoir. G2 gas can be referred to as “neutral” gas because G2 gas is already equilibrated with pulmonary capillary blood and there is no CO$_2$ partial pressure gradient between the lungs with the blood. In normal situations, an increase in minute ventilation would increase alveolar ventilation, thereby reducing $P_{ET}CO_2$ and increase $P_{ET}O_2$. By using this sequential gas delivery circuit, an increase in ventilation will supply G1 for alveolar ventilation and the balance of gas will also consist of neutral gas from the exhaled gas reservoir. Therefore, this circuit is unaffected by minute ventilation and the blend of gases to achieve the next prospective target can be calculated. The RespirAct™ provides blends of G1 to a sequential gas delivery circuit according to the model outlined by Slessarev et al. (2007) for prospective targeting of $P_{ET}CO_2$ and $P_{ET}O_2$. 
Figure 3-1. Set up of the prospective targeting method.

Prior to the start of the breathing sequence used in for the BOLD CVR studies, the O$_2$ and CO$_2$ sensors are calibrated. The CO$_2$ sensor within the RespirAct™ (Ir3107, Servomex Group Ltd, Sugar Land, TX, USA) is accurate to within ± 0.1% of the CO$_2$ within the range of 0-10% CO$_2$. The O$_2$ sensor (UFO 130, Teledyne Analytical Instruments, City of Industry, CA, USA) is accurate to within 1%. The gas blends required to achieve the described breath-by-breath control of P$_{ET}$CO$_2$ and P$_{ET}$O$_2$ are calculated using customized software. These calculations require subject parameters, such as age, height, weight, gender. Other parameters are also required, which include functional residual capacity, metabolic uptake of O$_2$, and production of CO$_2$, which can be referenced from tables of normal values or calculated using the RespirAct™. The respiratory protocol used in this study consisted of P$_{ET}$CO$_2$ 40 mmHg baseline for 60 s, a hypercapnic step change to a P$_{ET}$CO$_2$ of 50 mmHg for 90 s, a return to baseline for 90 s, and a second hypercapnic step for 120 s with a final return to baseline. All steps were implemented while maintaining normoxia (P$_{ET}$CO$_2$ ~110 mmHg). Previous work describes the P$_{ET}$CO$_2$ and
P_{ET}CO_2 sequences used during the analysis of BOLD MRI CVR in more detail (Fierstra et al., 2013; Vesely et al., 2001). During the test, end-tidal gases were continuously sampled at 20 Hz, digitized, and recorded to a file where it could be further analyzed (Labview, National Instruments Corporation, Austin, TX).

3.5 General research design

The aforementioned tools were applied for assessment of the possible link between CVR deficits and future development of leukoaraiosis in older individuals who have leukoaraiotic white matter lesions and are at a risk of developing new lesions. Subjects were recruited from the stroke prevention or memory clinic at TWH and SHSC who were being followed clinically for non-disabling ischemic events and had MR or CT cervico-cerebral angiography for work-up. These subjects were studied with identical MRI protocols 12 months apart. The protocol consisted of high-resolution 3D anatomical images, FLAIR images, multi-echo T2 images for generating quantitative T2 maps, diffusion tensor acquisitions for measurement of mean diffusivity (mD) and fractional anisotropy (FA), and BOLD CVR acquisitions. The multi-metric data from the two time points were co-registered for each subject making available a temporal comparison of each of the parameters (CVR, T2, FA, mD). The use of the Sunnybrook Lesion explorer pipeline (Ramirez et al., 2014) was applied to identify leukoaraiotic lesion load on the initial and follow-up scans in order to identify normal white matter that converts to abnormal white matter.

3.6 General data analysis

Data from the first CVR study will be processed to yield a whole brain CVR map. Grey/white matter/CSF segmentation and masking will provide a white matter map of CVR consisting of red voxels (increased BOLD signal in response to increased CO_2) and blue voxels (decreased BOLD signal in response to increased CO_2). The multi-echo T2 series will be used to generate mono-exponential T2 relaxation maps. DTI data will be processed to provide FA, and mean diffusivity maps using AFNI (Cox, 1996) and FSL (S. M. Smith et al., 2004). Lesion Explorer will be applied to identify normal vs. abnormal white matter for both the initial and follow-up MRI study. CVR, the independent variable, will be compared against the dependent variables T2, FA, and mD in both leukoaraiosis and normal-appearing white matter.

3.7 Sample size calculation

In their longitudinal studies, Taylor et al. found a WMH volume of 4.91 (± 7.01) ccs per subject at baseline (mean age 69.1 years) and 6.42 (± 9.53) ccs at 2 year follow-up (W. D.
Taylor et al., 2003). Whitman et al. found 3.1 (± 2.5) ccs per subject at baseline (mean age 79 years) and 4.2 (± 3.5) ccs at 4 year follow-up (Whitman et al., 2001). These represent increases in lesion load of 0.76 cc/year and 0.28 cc/year. These studies represent the lower end of the spectrum. At the high end is Sachdev’s work that showed a yearly increase in the volume of abnormal white matter in healthy elderly subjects with a mean age of 71 years of 2.17 ccs (Sachdev, Wen, Chen, & Brodaty, 2007). For the purposes of sample size calculation, it was assumed that the recruited subjects, who were pre-selected for entry with Fazekas score 2 or higher, will have a higher rate of progression at a rate of 3 ccs of abnormal white matter per year. However, the subjects in the presented work represent the full spectrum of leukoaraiosis severity (Fazekas grade 1 to 3). Normal white matter volume in healthy young adults is approximately 450 ccs (Shuter, Yeh, Graham, Au, & Wang, 2008) but can decrease to 350 ccs with age. Three ccs of new WMH voxels developing over 12 months per patient corresponds to about 83 CVR size voxels (3.0 x 3.0 x 4.0 mm).

Previously acquired CVR data indicates that CVR measurements in WM are quite noisy, due to the fact that CVR is 2-3 times lower in WM than GM, and due to signal artifacts from patient motion arising near the ventricles. It was estimated that 30 subjects would be required to establish a correlation between CVR and newly developing WMH, using a fixed effects analysis that is insensitive to between subject differences in WMH load. Others have shown that global CVR is negatively correlated to hypertension.

We investigated whether quantitative tissue parameters (T2, mD, and FA) demonstrate changes over 12 months in areas that do not yet show up on FLAIR images. We found these areas to be restricted to WM close to pre-existing WMH and within the known high-probability areas for the development of WMH (Sachdev et al., 2007). Each subject will yield only a relatively small number of voxels. We anticipate a relatively small effect size related to the temporal changes in tissue parameters. Therefore, it was proposed to image 50 patients at each site. See Appendix Tables 1 and 2 for a full description of subject demographics.
CHAPTER 4

Study I. Vascular dysfunction in elderly subjects with leukoaraiosis.

4.1 Abstract

The purpose of this study is to evaluate the vascular hallmarks of age-related leukoaraiosis in elderly subjects with particular emphasis on cerebrovascular reactivity (CVR) and its relation to neurovascular coupling.

Methods: In this prospective observational study, 75 subjects with leukoaraiosis (age range, 50 to 91 years; 40 males) were studied. CVR was calculated as the change in blood oxygen level dependent (BOLD) MRI signal (as a surrogate of cerebral blood flow), in response to a consistently applied step change in the arterial partial pressure of carbon dioxide (PaCO$_2$) in normal-appearing white matter (NAWM) and white matter hyperintensities (WMH). The presence of structural and vascular pathophysiology in leukoaraiosis was corroborated by diffusion, quantitative T2, and DSC-perfusion MRI metrics.

Results: CVR values were significantly lower by 35.7 ± 19.1% in WMH compared to NAWM (P<0.01). FA values were significantly lower by 44.9 ± 6.9%, CBF values were significantly lower by 10.9 ± 11.9%, CBV values were significantly lower by 10.2 ± 15.0% in WMH compared to the contralateral NAWM (P<0.01). T2 values were significantly higher by 61.7 ± 13.5%, mD values were significantly higher by 59.0 ± 11.7%, Tmax values were significantly higher by 44.4 ± 30.4%, TTP values were significantly higher by 6.8 ± 5.8% in WMH compared to NAWM (P<0.01). MTT values were not significantly different between WMH and NAWM (12.4 ± 45.6% increase from NAWM values).

Conclusions: These findings support vascular pathophysiology in leukoaraiosis. The results confirm that hemodynamic impairment occurs in leukoaraiosis as demonstrated by a reduced CBF and CBV. The reduced CVR in leukoaraiosis suggests dysfunction in neurogliovascular coupling as we have demonstrated that blood flow to leukoaraiosis does not meet demand. These findings support the role of chronic hypoperfusion to white matter changes in the aging brain.
4.2 Introduction

Age-related changes in the cerebral white matter become apparent on computed tomography (CT) by a decrease of X-ray attenuation, which causes pronounced hypodensity. These areas may be characterized by myelin pallor, reactive astrogliosis, as well as loss of oligodendrocytes, axons, and myelin fibres (Simpson et al., 2007). This rarefaction of the white matter tissue led to the creation of the term leukoaraiosis derived from the Greek words “leuko-“ for white and “araios” for rarefied (V. C. Hachinski et al., 1986). In contrast to magnetic resonance imaging (MRI), these white matter changes appear bright on T2-weighted images and are called white matter hyperintensities (WMHs) if they are of presumed vascular origin. As much as 95% of individuals over the age of 50 have some degree of white matter changes, particularly in the periventricular and deep white matter (de Leeuw et al., 2001; Launer et al., 2006). WMHs were once thought to be benign age-related changes but recent studies have shown that they associated with cognitive impairment (Verdelho et al., 2010) and disability (Malmstrom & Morley, 2013; Ogama et al., 2014; Whitman et al., 2001).

Several studies have demonstrated vascular changes with aging that may lead to WMHs, including increased vessel tortuosity (Moody et al., 1991) and increased stringed vessels, and vessel basement membrane thickening (Farkas et al., 2006). Histopathological analysis of abnormal white matter has demonstrated venular intramural collagen deposition leading to wall thickening stenosis (Moody et al., 1995). Areas with excellent collateral blood supply, such as the subcortical U-fibers, are spared of age-related WMH, which suggests a vascular etiology (Henry-Feugeas, 2008).

The purpose of this study is to characterize the vascular physiology of WMHs further with cerebrovascular reactivity (CVR). CVR is defined as the change in cerebral blood flow induced by a vasoactive stimulus. Previous studies examining age-related white matter changes have not been able to provide specific CVR measurements solely in WMHs due to their inability to precisely control their vasoactive stimulus. In this study, we are able to examine CVR specifically in WMH and compare them to normal-appearing white matter (NAWM) by controlling the end-tidal partial pressure of carbon dioxide (P$_{ET}$CO$_2$) and oxygen (P$_{ET}$O$_2$) independently of each other by using an automated gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada). With the high spatial resolution of MRI, we combined these two technologies to quantitate CVR in WMHs. Blood-oxygen-level-dependent (BOLD) CVR is
a measure the sensitivity of the cerebral microvasculature to demands in blood flow to carbon 
dioxide (CO₂) (Spano et al., 2013), enabling the examination of dysfunction in neurovascular 
coupling in WMH compared to NAWM. Other MRI metrics obtained from diffusion tensor 
imaging (DTI) and dynamic susceptibility contrast (DSC) perfusion MRI were used to 
corroborate CVR findings. We hypothesize that CVR and other MRI metrics will be abnormal 
in leukoaraiosis compared to NAWM.

4.3 Methods

4.3.1 Subject Recruitment

Elderly adults with age-related leukoaraiosis (age range: 50-91; 40 males) were recruited for 
participation and provided informed consent to procedures approved by both the University 
Health Network (UHN) and Sunnybrook research ethics boards. Subject recruitment was 
achieved through both the memory and stroke prevention clinic at Toronto Western Hospital 
(TWH) and memory clinic at Sunnybrook Health Sciences Centre (SHSC). Magnetic resonance 
angiogram (MRA) or computed tomography angiography (CTA) and T2-weighted fluid- 
attenuated inversion recovery (FLAIR) images of all patients were screened by an experienced 
neuroradiologist and subjects were included in the study based on the following inclusion 
criteria: (1) previous neurological event involving white matter > 3 months from presentation; 
(2) over the age of 50; (3) MRI white matter disease burden > Fazekas Grade 2, moderate 
number of FLAIR hyperintensities in the white matter; (4) no evidence of bilateral ICA stenosis 
greater than 70%; (5) no evidence of significant vertebral or basilar stenosis greater than 70%; 
(7) no evidence of dissection (8) no evidence of pulmonary or cardio-embolic disease. Subjects 
with motion artifacts on BOLD images were excluded. Forty-three patients from Toronto 
Western Hospital (age range, 50 to 87 years; 23 males and 20 females) and thirty-two patients 
from Sunnybrook Health Sciences Centre (age range, 51 to 91 years; 17 males and 15 females) 
with moderate-severe leukoaraiosis met the inclusion criteria and were considered in subsequent 
analysis (See Appendix Tables 1 and 2).

4.3.2 MRI Acquisition

Subjects scanned at TWH underwent MRI scans on a 3-Tesla GE system (Signa HDx 
platform, GE Healthcare, Milwaukee, Wis) and fitted with an eight-channel phased array head 
coil. Subjects were asked to refrain from heavy exercise and drinking on the day of each scan. 
Please refer to section 3.2.1 for the imaging acquisition parameters at Toronto Western Hospital 
and Sunnybrook Health Sciences Centre.
4.3.3 CVR measurement

CVR was assessed by measuring the change in Blood Oxygen Level Dependent Magnetic Resonance Imaging (BOLD MRI) in response to changes in end-tidal (i.e., end expiratory) partial pressure of carbon dioxide (\(\text{P}_{\text{ET}}\text{CO}_2\)) as the vasoactive stimulus (Han et al., 2011). CVR was calculated as \(\%\) change BOLD / \(\Delta\) \(\text{P}_{\text{ET}}\text{CO}_2\). A colour scale ranging from blue to red was used to identify the magnitude of CVR. Negative CVR values representative of steal physiology were represented as shades of blue. Positive CVR values were defined as “normal-appearing” and are represented as shades of yellow, orange, and red.

4.3.4 Vasodilatory Stimulus

\(\text{P}_{\text{ET}}\text{CO}_2\) and end-tidal partial pressure of oxygen (\(\text{P}_{\text{ET}}\text{O}_2\)) were targeted independently of each other, independent of the subjects’ minute ventilation, and independent of the subject’s breathing pattern by using an automated gas blender and sequential gas delivery breathing circuit (RespirAct\textsuperscript{TM}, Thornhill Research Inc., Toronto, Canada). Target \(\text{P}_{\text{ET}}\text{CO}_2\) and \(\text{P}_{\text{ET}}\text{O}_2\) are achieved by administering blends of gas according to algorithms as previously described (Slessarev et al., 2007). The respiratory protocol used in this study was, inducing a baseline PETCO2 of 40 mmHg for 60 seconds (normocapnea), a hypercapnic step change to PETCO2 of 50 mmHg for 90 s, a return to baseline for 90 s, and a second hypercapnic step for 120 s with a final return to baseline (Vesely et al., 2001). All steps were implemented while maintaining normoxia (\(\text{P}_{\text{ET}}\text{O}_2\sim110\) mmHg).

4.3.5 Image Reconstruction

The BOLD time series at each voxel was orthogonalized to 6 motion directions and co-registered to the initial frame of the BOLD dataset to minimize any influence from hypercapnea-related head motion. The acquired BOLD MRI and \(\text{P}_{\text{ET}}\text{CO}_2\) data were imported to AFNI (Cox, 1996) software, a freeware program used for analysis. BOLD images were slice time-corrected, volume-registered, and aligned to axial anatomical T1-weighted images. T1- and T2-weighted images were reviewed to identify regions of parenchymal infarction, or old hemorrhages. Using the draw plugin in AFNI, we then created a mask of each parenchymal lesion for exclusion of this tissue on CVR maps. CVR maps were also generated using AFNI. T1-weighted anatomical images were segmented into cerebrospinal fluid, grey matter, and white matter using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK). The final CVR maps used in our calculations only contained healthy appearing brain parenchyma without CSF.
To confirm tissue dysfunction in the cerebral white matter, maps of the transverse relaxation time (T2) were calculated. T2 reflects white matter water content and myelination. Quantitative T2 maps were obtained using a multiecho fast spin-echo sequence (voxel size = 0.86 x 0.86 x 3 mm for TWH and 1.0 x 1.0 x 3 mm for SHSC). T2 maps were calculated in AFNI using methods previously described (Miller & Joseph, 1993). To calculate FA and mD maps, diffusion-weighted images were imported into FSL 4.1.8 (http://www.fmrib.ox.ac.uk/fsl) for quality control (S. M. Smith et al., 2004). Pre-processing included eddy current and motion artifact correction using FMRIB's Diffusion Toolbox (FDT) (Jenkinson, Bannister, Brady, & Smith, 2002). Then, individual brain masks were created using BET (S. M. Smith, 2002). The preprocessed images were fit with a diffusion tensor model using DTIFIT in FDT (S. M. Smith et al., 2004).

The time-signal attenuation curve obtained from perfusion-weighted T2* images were converted to time-concentration curves using PerfTool (Kosior & Frayne, 2007). PerfTool uses delay-insensitive reformulated singular value decomposition approach to deconvolution of the time-concentration curves (M. R. Smith, Lu, Trochet, & Frayne, 2004). The arterial input function was selected from an ROI placed on the middle cerebral artery. The pre-processed perfusion-weighted images were used to generate maps of cerebral blood flow (CBF), cerebral blood volume (rCBV), mean transit time (MTT), time-to-maximum (Tmax), and time-to-peak (TTP) using PerfTool (Kosior & Frayne, 2007).

4.3.6 Generating CVR maps

$P_{ETCO2}$ data were first synchronized with the whole brain average BOLD signal using MATLAB software (Mathworks, Natick, Massachusetts, USA). The synchronization compensated for delays in breath sample analysis and delay of blood flow from pulmonary to cerebral circulation. A voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $P_{ETCO2}$ was performed and the slope of the line of best fit was taken as the CVR. CVR values are expressed as percent MR signal change per mmHg of $PETCO2$ (mean ± SD).

4.3.7 Generating ROIs of WMH and NAWM

Segmentation of WMH was performed using the Lesion Explorer processing pipeline (Ramirez et al., 2011; Ramirez et al., 2014). To account for spatial location of MRI metrics, T1-weighted images were transformed into Montreal Neurological Institute space using SPM8. The transformation matrix was applied to other MRI metrics, thereby transforming these maps to a standard space but retaining the native structure. AFNI was used to identify NAWM.
contralateral to WMH (Figure 4-1). In brief, a diamond shaped structuring element was used to erode the white matter in five iterations at the resolution of the T1-weighted image to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a NAWM mask that is contralateral to WMHs. MRI metrics in WMH were compared to the corresponding NAWM. MRI metrics were also compared between WMH and the entire NAWM using either Wilcoxon matched-pairs signed ranks test (for CVR and MTT measurements) or two-tailed paired t-tests where appropriate.

![Figure 4-1. Identification of NAWM. A, Example of a T1-weighted image from a subject with diffuse hyperintensities in the deep white matter. B, FLAIR image highlights the WMHs seen in this subject. C, The white matter was segmented from other subdural structures such as cerebrospinal fluid and grey matter. D, NAWM mask; a diamond shaped structuring element was used to erode the white matter (image C) in five iterations at the resolution of the T1-weighted image (image A) to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a final NAWM mask.](image)

4.4 Results

Seventy-five subjects (age range: 50-91 years, 40 males) with confluent white matter changes participated in this study. Subjects were referred to either the memory or stroke prevention neurology clinic for a variety of reasons including chronic imbalance, gait disturbances, transient episodes of paresthesia, syncopal episodes, headaches, cognitive decline, or memory impairment.

At SHSC, subjects had an average WMH volume of $36.7 \pm 26.3$ ccs per subject (mean age: 77.0 years). At TWH, subjects had an average WMH volume of $19.3 \pm 18.4$ ccs per subject (mean age: 72.5 years).

The average $\pm$ SD for each CVR metric in each group is summarized in Table 4-1. Spatial factors can strongly influence differences in these results and were accounted for with ROIs using NAWM contralateral to WMH (Figure 4-2). Statistical significance remained the same after using these ROIs and results are summarized in Figure 4-3. Comparisons involving
CVR and MTT were made using Wilcoxon matched-pairs signed ranks test and all other comparisons were made using two-way paired t-tests. Compared to NAWM, WMH CVR values were significantly lower by 35.7 ± 19.1% (P<0.01), FA values were significantly lower by 44.9 ± 6.9% (P<0.01), mD values were significantly higher by 59.0 ± 11.7%, T2 values were significantly higher by 61.7 ± 13.5%, CBF values were significantly lower by 10.9 ± 11.9% (P<0.01), CBV values were significantly lower by 10.2 ± 15.0% (P<0.01), Tmax values were significantly higher by 44.4 ± 30.4% (P<0.01), and TTP values were significantly higher by 6.8 ± 5.8% (P<0.01). MTT values were not significantly different between WMH and NAWM (12.4 ± 45.6% increase from NAWM values).
Figure 4-2. Calculation of ROIs used to account for differences in spatial location of MRI metrics. A, An example FLAIR map of a patient with periventricular and deep white matter hyperintensities. B, WMHs are highlighted in yellow and overlaid on the FLAIR map. C, WMHs (yellow) with the underlying FLAIR map removed. D, The WMHs are left-right flipped about the y-axis to give rise to the contralateral NAWM (pink). This transformation is in MNI coordinates but retains the native structure (no warping of the brain). E, Final contralateral NAWM mask used in measurements of MRI metrics; WMH (image C) is subtracted from contralateral NAWM (pink). F, Final WMH mask, the contralateral NAWM (image F) is left-right flipped; thus, only WMH with contralateral NAWM will be used in the analysis of MRI metrics in WMH. G, Final masks (image E and F) overlaid on a FLAIR map.

Figure 4-3. Comparison of MRI metrics between regions of WMH and NAWM. The values in MRI metrics are derived from paired comparisons and are given as % change from NAWM. The ROIs used for these measurements are taken from Figure 4-2(G), thereby accounting for differences in spatial location that may give rise to differences in MRI metrics. CVR, FA, CBF, and rCBV values are significantly lower in WMH compared to NAWM while mD, T2, MTT, Tmax, and TTP were significantly higher in WMH compared to NAWM (* P < 0.01, compared to NAWM; • P < 0.05, compared to NAWM). Bars indicate minimum and maximum, boxes indicate the interquartile range (25th to 75th percentile) and the line within each box indicates the median.

Table 4-1. Measurements of CVR, FA, mD, T2, and perfusion metrics in white matter hyperintensities and NAWM.

<table>
<thead>
<tr>
<th></th>
<th>White Matter Hyperintensities (WMH)</th>
<th>Normal-appearing White Matter (NAWM)</th>
<th>WMH (Contralateral to NAWM)</th>
<th>NAWM (Contralateral to White Matter Hyperintensities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR (at TWH) [%BOLD/mmHg]</td>
<td>0.04 ± 0.03</td>
<td>0.10 ± 0.04</td>
<td>0.05 ± 0.07</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>CVR (at SHSC)</td>
<td>0.05 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>[%BOLD/mmHg]</td>
<td>*</td>
<td>◆</td>
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<tr>
<td>FA [unitless]</td>
<td>0.24 ± 0.03 0.46 ± 0.04 *</td>
<td>0.23 ± 0.03 0.44 ± 0.02 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA [unitless]</td>
<td>0.36 ± 0.03 0.51 ± 0.04 *</td>
<td>0.35 ± 0.04 0.61 ± 0.1 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mD [mm²]</td>
<td>1.35 ± 0.11 0.84 ± 0.04 *</td>
<td>1.36 ± 0.10 0.85 ± 0.04 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mD [mm²]</td>
<td>1.34 ± 0.12 0.82 ± 0.04 *</td>
<td>1.37 ± 0.11 0.84 ± 0.03 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>138.3 ± 15.5 85.7 ± 5.6 *</td>
<td>142.8 ± 13.3 87.1 ± 3.6 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>131.1 ± 7.7 82.8 ± 2.4 *</td>
<td>133.1 ± 16.7 83.4 ± 6.1 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF [mL/100g/min]</td>
<td>20.1 ± 4.5 23.2 ± 5.8 *</td>
<td>18.2 ± 3.5 21.0 ± 4.4 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rCBV [AI]</td>
<td>105.2 ± 9.4 114.1 ± 7.7 * (P &lt; 0.05)</td>
<td>103.9 ± 10.1 117.5 ± 9.3 (P &lt; 0.05)</td>
<td></td>
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</tr>
<tr>
<td>MTT [s]</td>
<td>4.3 ± 1.5 3.9 ± 1.1</td>
<td>4.6 ± 0.5 4.1 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax [s]</td>
<td>3.5 ± 1.2 2.4 ± 0.7 *</td>
<td>3.5 ± 0.3 2.4 ± 0.2 ◆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTP [s]</td>
<td>22.3 ± 3.8 20.6 ± 2.9 *</td>
<td>22.3 ± 1.3 20.8 ± 1.0 ◆</td>
<td></td>
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</tr>
</tbody>
</table>

Grey and white boxes are measurements from TWH and SHSC, respectively. * P < 0.01 compared to WMH, unless indicated otherwise; ◆ P<0.01 compared against WMH with contralateral NAWM (WMH with contralateral hyperintensities are excluded from this analysis). Statistical analyses were performed using two-tailed paired t-tests (other than CVR and MTT, which used Wilcoxon matched-pairs signed ranks test).

4.5 Discussion

We have demonstrated vascular dysfunction in regions of leukoaraiosis as seen on multiple MRI metrics. The CVR of regions of leukoaraiosis was significantly reduced compared to the NAWM, which demonstrates the sensitivity of the local microvasculature to demands in blood flow is impaired in leukoaraiosis. In our study, subjects started off at an average baseline $P_{ETCO_2}$ of 40 mmHg and were given an abrupt (within 1 to 2 breaths) square-wave stimulus to 50 mmHg on two occasions. The 40-50 mmHg change in $P_{ETCO_2}$ represents a vigorous vasodilatory stimulus that was sustained for at least two minutes, therefore any CVR measurements could be considered a measure of vascular reserve as well (Sobczyk et al., 2014).
In our study, the reduced CVR values in leukoaraiosis also implies vascular reserve in regions of leukoaraiosis is reduced. From DTI findings, mean diffusivity was higher and FA was reduced in leukoaraiosis, indicating the white matter structural integrity has been compromised as water is able to diffuse more freely in axons in regions of leukoaraiosis representing areas of demyelination as well as axonal degeneration (Feldman, Yeatman, Lee, Barde, & Gaman-Bean, 2010). These findings are consistent with previous findings of increased mean diffusivity, axial diffusivity, and radial diffusivity in regions of leukoaraiosis (Bastin et al., 2009). Quantitative T2 values are higher in leukoaraiosis, which is to be expected as this indicates increased water content due to loss in tissue structure. CBF was found to be reduced in leukoaraiosis, indicating that the blood supply to the abnormal tissue in leukoaraiosis is reduced. CBV was also found to be reduced in leukoaraiosis, suggesting reduced density the local vasculature. Time-dependent MRI metrics such as MTT (although not significant), Tmax, and TTP were prolonged in leukoaraiosis which demonstrate delays in blood supply to areas of leukoaraiosis. Collectively, these MRI metrics all point towards evidence of vascular dysfunction in leukoaraiosis.

Our results confirm findings from a previous study by Uh et al. (2010) that report a lower CVR values in leukoaraiosis (Uh et al., 2010). Although this finding has been replicated in our study, we believe our approach to assess CVR is more accurate as we have the ability to control $P_{ET}CO_2$ and $P_{ETO_2}$ precisely and independent of each other. Our ability to control the rise in $P_{ET}CO_2$ of subjects to 50 mmHg and maintain it for an extended period provides confidence in the accuracy of our assessment of vascular reserve. The inability to precisely control $P_{ET}CO_2$ and $P_{ETO_2}$ significantly limits the interpretation of CVR measurements. The method of inhaling 5% CO$_2$ is not robust as it produces a small change in $P_{ET}CO_2$ subject to change depending on the subject’s minute ventilation or breathing pattern, which could inflate CVR values (Sobczyk et al., 2014). Like Uh et al. (2010), we found reduced CVR in leukoaraiosis, suggesting a role in vascular endothelium dysfunction leading to development of leukoaraiosis, that is consistent with the findings of Hassan et al. (2003) who demonstrated upregulated markers of endothelial activation and damage in leukoaraiosis.

Our results are also consistent with other studies in finding CBF reductions in leukoaraiosis compared to NAWM (Brickman et al., 2009; Marstrand et al., 2002; O'Sullivan et al., 2002; Uh et al., 2010). We expected the CBV to be lower in leukoaraiosis due to a potential loss in vascular density; however, these results are inconsistent with previous findings of no
difference in CBV between leukoaraiosis and NAWM (Marstrand et al., 2002). The observation of reduced CBF in leukoaraiosis does not necessarily imply hypoperfusion and may simply reflect the lower the metabolic demand of white matter (Villringer & Dirnagl, 1995). Nevertheless, the reduction in CVR demonstrates that during a vasodilatory challenge from CO$_2$, leukoaraiotic regions may be unable to meet demands in blood supply. The observed reduced perfusion metrics and CVR in leukoaraiosis are also in agreement with predilection maps demonstrating that regions of white matter with lower perfusion have a higher occurrence frequency of leukoaraiosis (Brickman et al., 2009; Holland et al., 2008).

We also found prolonged time-dependent perfusion measures in leukoaraiosis. MTT was longer in leukoaraiosis compared to NAWM in almost all subjects although not significant. These findings are consistent with report of TTP and Tmax values obtained with CT in the infarct core of acute stroke patients (Campbell et al., 2011). The prolonged TTP and Tmax values found in leukoaraiosis mimic signs of acute ischemic infarcts and is in agreement with a recent serial MRI study in elderly subjects with age-related leukoaraiosis who examined DTI characteristics of de novo WMHs and found characteristics that were also similar to those of acute ischemic infarcts (Conklin et al., 2014).

Our study is limited by several factors. Our measure of CVR is based on percent BOLD change per mmHg P$_{ET}$CO$_2$. The BOLD signal does not measure blood flow directly but represents an interaction of arterial PO$_2$, cerebral blood flow, cerebral blood volume, hematocrit, and cerebral metabolic rate of oxygen (Ogawa, Lee, & Barrere, 1993). However, we have shown a highly correlated BOLD MRI signal response to hypercapnea against CBF measurements obtained with arterial spin labeling in patients with steno-occlusive disease (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008). Also, perfusion measurements were only performed on a subset of subjects from TWH and not SHSC (43 out of 75 subjects. To our knowledge, this is the first report of time-dependent perfusion MRI parameters in leukoaraiosis.

In conclusion, CVR and several other MRI metrics are abnormal in WHH indicating a diminished vascular reserve capacity and increased vulnerability to transient ischemia compared to NAWM. Whether the impaired reactivity in white matter precedes white matter tissue injury or vice versa remains unclear. However, this will be addressed with one-year follow-up studies. The results suggest that dysfunction in neurovascular coupling is an important association in white matter disease and may contribute to its progression.
CHAPTER 5

Study II. Impaired Cerebrovascular Reactivity is Associated with abnormal Diffusion and Perfusion MRI metrics in Normal-appearing White Matter.

5.1 Abstract

Purpose: Impaired cerebrovascular reactivity (CVR) is associated with the extent of leukoaraiosis, suggesting that impaired autoregulation may be an important factor for the progression of white matter disease. The sequence of events in the evolution of leukoaraiosis is a key question that has important implications for understanding lesion pathogenesis and for targeting therapeutic interventions. The aim of this study to characterize the extent of tissue injury before presumed development of leukoaraiosis by examining T2, diffusion, and perfusion values in normal-appearing white matter (NAWM) with impaired cerebrovascular reactivity (CVR).

Method: This is a multi-centre study in which 75 subjects from Toronto Western Hospital and Sunnybrook Research Institute with moderate-severe leukoaraiosis (Fazekas score >2) underwent BOLD CVR MRI at 3T (Signa HDx, GE Healthcare, Milwaukee; Philips Achieva, Best, the Netherlands, respectively). Precise carbon dioxide manipulation was performed during BOLD imaging (RespirActTM, Thornhill Research Inc., Toronto, ON) to measure flow related wash-out of deoxyhemoglobin during hypercapnea, thus enabling quantitative analysis of CVR. Multi-echo T2 sequences were used to generate quantitative T2 maps. Diffusion-tensor MRI sequences were used to calculate maps of the fractional anisotropy (FA) and mean diffusivity (mD). Twenty-five of the 43 subjects from Toronto Western Hospital underwent DSC-MRI perfusion imaging to calculate parametric maps of cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), time-to-peak (TTP), and time-to-maximum (Tmax). PD/T2 images were used to segment the white matter hyperintensities using Lesion Explorer (Sunnybrook Research Institute, Toronto, ON). Anatomical images were used to segment the white matter and the lesions were then subtracted to generate masks of NAWM. In all subjects, T2, perfusion, and diffusion parameters were compared between areas of NAWM with positive and negative CVR. Statistical analyses between voxels with negative and positive CVR were performed using one-way ANOVA with Bonferroni correction for multiple comparisons.
Results: Areas of normal-appearing white matter with negative CVR show a significant reduction in FA by 3.7 ± 2.4%, CBF by 22.1 ± 8.2%, rCBV by 22.2 ± 7.0% and significant increase in mD by 3.9 ± 3.1% as well as Tmax by 10.9 ± 13.2% (P<0.01; one-way ANOVA) compared to areas with positive CVR.

Conclusion: We have shown that areas with impaired cerebrovascular reactivity are associated with subtle changes in the tissue integrity of NAWM as seen on conventional images. This provides new information about the pathogenesis of white matter lesions and NAWM changes. The results suggest that impaired autoregulation is an important association in white matter disease and may contribute to its progression. Whether the impaired reactivity in white matter precedes white matter tissue injury and whether the evolution of lesions will occur in areas of impaired CVR remains unclear.

5.2 Introduction

The occurrence of diffuse changes in periventricular and subcortical white matter observed on both CT and MRI have been identified as a significant finding in elderly individuals (Moody et al., 1997). These changes in the cerebral white matter appear hyperintense on T2-weighted images on MRI and have been referred to as white matter hyperintensities (WMHs) if they are of presumed vascular origin. The clinical importance of WMHs is significant as they are associated with increased risk of dementia (Verdelho et al., 2010), stroke (M. Simoni et al., 2012), and disability (Ogama et al., 2014; Whitman et al., 2001). Changes in the white matter are significantly correlated with age; as much as 95% of individuals over the age of 50 years have some degree of WMHs (de Leeuw et al., 2001; Launer et al., 2006). Therefore, preventing or slowing down the progression of WMH may have the potential to lower disease burden. Several modifiable risk factors have been associated with WMH progression, such as smoking and high blood pressure(Soderlund et al., 2003; van Dijk et al., 2008). However, the pathogenesis of WMHs is still largely unknown. Previous studies have suggested chronic low grade ischemic injury in the white matter (Conklin et al., 2010; Conklin et al., 2011).

This study aims to characterize vascular pathophysiology in normal-appearing white matter (NAWM) that may be prone to developing into WMH by assessing cerebrovascular reactivity (CVR). CVR can be defined as the change in cerebral blood flow in response to a vasodilatory stimulus. Negative CVR values are representative of steal physiology, in which a redistribution of blood flow away from regions of exhausted cerebrovascular reserve to areas
with preserved vasodilatory capacity occurs during a demand in blood flow (Sobczyk et al., 2014). We believe that regions that demonstrate dysfunction in neurovascular uncoupling as assessed with CVR, will be prone to developing into WMH. Tissue dysfunction will be verified with diffusion tensor imaging (DTI) and dynamic susceptibility contrast (DSC)-perfusion MRI.

DTI and DSC-perfusion MRI allows in vivo study of white matter tissue microstructure. DTI provides imaging metrics such as fractional anisotropy (FA) and mean diffusivity (mD) that can detect changes in the white matter that are not distinguished on typical T1- or T2-weighted images. Steal physiology has been shown to be spatially correlated with higher diffusion in normal-appearing white matter (Conklin et al., 2010; Conklin et al., 2011). A recent study by Mandell et al. (2015) demonstrated restricted diffusion in de novo WMHs in a serial longitudinal MRI study, indicative of acute ischemic infarcts (Conklin et al., 2014). These lesions later developed increased diffusion characteristic of chronic WMH. DSC-perfusion provides imaging metrics such as cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), time-to-maximum (Tmax), and time-to-peak (TTP). Markus et al. (2000) found CBF to be lower in WMH than NAWM (H. S. Markus et al., 2000). These studies collectively demonstrate that performing DTI and perfusion MR imaging enables investigation of NAWM microstructure before development of WMH.

In this study, 75 elderly subjects with age-related WMHs, were assessed for the utility of using CVR as a biomarker of vascular dysfunction in NAWM. Metrics used to assess the effects of reduced CVR included comparisons of water diffusion and blood flow perfusion measures between NAWM with steal physiology and NAWM with robust CVR. We hypothesize that NAWM with steal physiology will demonstrate abnormal MRI metrics that will be similar to pre-existing WMH when compared to NAWM with robust CVR.

5.3 Subjects and Methods

5.3.1 Subject Recruitment and Assessment

Two MRI datasets were included in this study, one from Toronto Western Hospital and the other from Sunnybrook Health Sciences Centre. Subjects were participants in a study of age-related white matter changes and written informed consent was obtained from all participants. This study was approved by both the University Health Network and Sunnybrook research ethics boards. Patients were recruited from either the Memory or Stroke Prevention Clinic at Toronto Western Hospital and the memory clinic at Sunnybrook Hospital. Magnetic resonance angiogram (MRA) or computed tomography angiography (CTA) and T2-weighted fluid-
attenuated inversion recovery (FLAIR) images of all patients were screened by an experienced neuroradiologist and subjects were included in the study based on the following inclusion criteria: (1) previous neurological event involving white matter > 3 months from presentation; (2) over the age of 50; (3) MRI white matter disease burden > Fazekas Grade 2, moderate number of FLAIR hyperintensities in the white matter; (4) no evidence of bilateral ICA stenosis greater than 70%; (5) no evidence of significant vertebral or basilar stenosis greater than 70%; (7) no evidence of dissection (8) no evidence of pulmonary or cardio-embolic disease. Patients with motion artifacts on BOLD images were excluded. Forty-three patients from Toronto Western Hospital (age range, 50 to 87 years; 23 males and 20 females) and thirty-two patients from Sunnybrook Health Sciences Centre (age range, 51 to 91 years; 17 males and 15 females) with moderate-severe leukoaraiosis met the inclusion criteria and were considered in subsequent analysis (Appendix Tables 1 and 2).

5.3.2 MRI Acquisition

Please refer to section 3.2.1 for the imaging acquisition parameters at Toronto Western Hospital and Sunnybrook Health Sciences Centre.

5.3.3 CVR measurement

CVR was assessed by measuring the change in Blood Oxygen Level Dependent Magnetic Resonance Imaging (BOLD MRI) in response to changes in end-tidal (i.e., end expiratory) partial pressure of carbon dioxide (P_{ET}CO_2) as the vasoactive stimulus (Han et al., 2011). CVR was calculated as % change BOLD / ∆ P_{ET}CO_2. A colour scale ranging from blue to red was used to identify the magnitude of CVR. Negative CVR values representative of steal physiology were represented as shades of blue. Positive CVR values were defined as “normal-appearing” and are represented as shades of yellow, orange, and red.

5.3.3.1 Vasodilatory Stimulus (Gas Manipulation, End-tidal pCO2 and pO2 Manipulation)

To precisely manipulate carbon dioxide, we used an automated gas blender that adjusts the gas composition and flow to a sequential gas delivery mask and breathing circuit (RespirActTM, Thornhill Research Inc., Toronto, Canada) according to previously described methods (Ito et al., 2008; Kisilevsky, Hudson, Mardimae, Wong, & Fisher, 2008). The RespirAct enables independent manipulation of the participants end-tidal partial pressure of carbon dioxide (P_{ET}CO_2) and end-tidal partial pressure of oxygen (P_{ET}O_2). Measurements of gases using the RespirAct are also independent of minute ventilation and breathing pattern of
the subject. Previous work describes the $P_{\text{ETCO}_2}$ and $P_{\text{ETO}_2}$ sequences used during the analysis of BOLD MRI CVR in more detail (Slessarev et al., 2007; Vesely et al., 2001). The respiratory protocol used in this study was, inducing a baseline PETCO2 of 40 mmHg for 60 seconds (normocapnea), a hypercapnic step change to PETCO2 of 50 mmHg for 90 s, a return to baseline for 90 s, and a second hypercapnic step for 120 s with a final return to baseline (Vesely et al., 2001). All steps were implemented while maintaining normoxia ($P_{\text{ETO}_2}$ $\sim$110 mmHg).

**Image Reconstruction**

The acquired BOLD MRI and $P_{\text{ETCO}_2}$ data were imported to AFNI software (Cox, 1996) for analysis. BOLD images were slice time-corrected, volume-registered, and aligned to axial anatomical T1-weighted images. The CVR maps were then constructed by first time-shifting the acquired $P_{\text{ETCO}_2}$ data to the point of maximum correlation with the whole brain average BOLD signal using MATLAB software. This compensates for the temporal discrepancy from pulmonary to cerebral circulation. Afterwards, a voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $P_{\text{ETCO}_2}$ data was performed. The BOLD time series at each voxel was orthogonalized to the 6 motion parameters estimated by the volume registration to minimize the effect of hypercapnea-correlated head motion.

To confirm tissue dysfunction in the cerebral white matter, maps of the transverse relaxation time (T2) were calculated. T2 reflects white matter water content and myelination. Quantitative T2 maps were obtained using a multiecho fast spin-echo sequence (voxel size = 0.86 x 0.86 x 3 mm for TWH and 1.0 x 1.0 x 3 mm for SHSC). T2 maps were calculated in AFNI using methods previously described (Miller & Joseph, 1993). To calculate FA and mD maps, diffusion-weighted images were imported into FSL 4.1.8 (http://www.fmrib.ox.ac.uk/fsl) for quality control (S. M. Smith et al., 2004). Pre-processing included eddy current and motion artifact correction using FMRIB's Diffusion Toolbox (FDT) (Jenkinson et al., 2002). Then, individual brain masks were created using BET (S. M. Smith, 2002). The preprocessed images were fit with a diffusion tensor model using DTIFIT in FDT (S. M. Smith et al., 2004).

The time-signal attenuation curve obtained from perfusion-weighted T2* images were converted to time-concentration curves using PerfTool (Kosior & Frayne, 2007). PerfTool uses delay-insensitive reformulated singular value decomposition approach to deconvolution of the time-concentration curves (M. R. Smith et al., 2004). The arterial input function was selected from an ROI placed on the middle cerebral artery. The pre-processed perfusion-weighted images were
used to generate maps of cerebral blood flow (CBF), cerebral blood volume (rCBV), mean transit time (MTT), time-to-maximum (Tmax), and time-to-peak (TTP) using PerfTool (Kosior & Frayne, 2007).

5.3.4 Generating CVR maps

$P_{ET}CO_2$ data were first synchronized with the whole brain average BOLD signal using MATLAB software (Mathworks, Natick, Massachusetts, USA). The synchronization compensated for delays in breath sample analysis and delay of blood flow from pulmonary to cerebral circulation. A voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $P_{ET}CO_2$ was performed and the slope of the line of best fit was taken as the CVR. CVR values are expressed as percent MR signal change per mmHg of PETCO2 (mean ± SD).

5.3.5 Generating ROIs of WMH and NAWM

Segmentation of WMH was performed using the Lesion Explorer processing pipeline (Ramirez et al., 2011; Ramirez et al., 2014). T1-weighted anatomical images were segmented into cerebrospinal fluid, grey matter, and white matter using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK). A diamond shaped structuring element was used to erode the white matter segmentation in five iterations at the resolution of the T1-weighted image to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a NAWM mask (Figure 5-1). To identify regions of steal physiology in NAWM, CVR maps were overlaid on the NAWM using AFNI (Figure 5-2). To account for spatial location of MRI metrics, T1-weighted images were transformed into Montreal Neurological Institute space using SPM8. The transformation matrix was applied to other MRI metrics, thereby transforming these maps to a standard space but retaining the native structure. AFNI was used to identify NAWM contralateral to WMH (Figure 5-3). MRI metrics in WMH were compared to the corresponding NAWM. MRI metrics were also compared between WMH and the entire NAWM using either Friedman test with Dunn’s post-hoc correction for multiple comparisons for non-parametric MTT data. All other MRI metric was parametric and statistical analyses involved repeated measures one-way analysis of variance using the metrics as dependent variables and ROIs as the matched-pairs independent variable. Mauchly’s test was used to assess deviations from sphericity and degrees of freedom was corrected using the Greenhouse–Geisser method. Results were considered significant and accounted for multiple comparisons if the per-comparison P value was less than 0.05/(3 comparisons) = 0.0167, that is, Bonferroni corrected.
Figure 5-1. Identification of NAWM.  

A, Example of a T1-weighted image from a subject with diffuse periventricular and deep WMHs. 

B, FLAIR images highlight the hyperintensities seen in this subject. 

C, The white matter was segmented from other subdural structures such as cerebrospinal fluid and grey matter (green). 

D, Regions of hyperintensities are identified in yellow. 

E, NAWM mask; a diamond shaped structuring element was used to erode the white matter (image C) in five iterations at the resolution of the T1-weighted image (image A) to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a final NAWM mask (pink).
Figure 5-2. Identification of NAWM with steal physiology.  A, Example of a mD map from a subject with periventricular and deep WMHs.  B, The NAWM (green) is overlaid on the mD map.  C, Regions of positive CVR (red) and negative CVR (steal physiology) in blue are overlaid on a mD map.  D, NAWM mask with only regions of positive CVR.  E, NAWM mask with only regions of negative CVR.
Figure 5-3. ROIs used to assess the influence of spatial location on MRI metrics. A, NAWM mask with only regions of positive CVR. B, NAWM mask with only regions of steal physiology (negative CVR). C, Image B is left-right flipped about the y-axis in MNI coordinates while retaining native structure. D, Image C is overlaid on Image A and overlapping voxels are retained, giving rise to a contralateral NAWM mask that has only positive CVR. E, Image D is left-right flipped about the y-axis, giving rise to the original NAWM with steal physiology that also has contralateral positive CVR. F, The two final masks, NAWM with steal physiology (image E) and the corresponding contralateral homologous NAWM with positive CVR (image D), are overlaid on a mD map.
5.4 Results

Seventy-five subjects (age range: 50-91 years, 40 males) with moderate-severe leukoaraiosis participated in this study. Subjects were referred to neurology clinics due to transient episodes of paresthesia, chronic imbalance, gait disturbances, syncopal episodes, headaches, cognitive decline, or memory impairment.

At SHSC, subjects had an average WMH volume of 36.7 ± 26.3 ccs per (mean age: 77.0 years). At TWH, subjects had an average WMH volume of 19.3 ± 18.4 ccs per subject (mean age: 72.5 years).

The average ± SD for each CVR metric in each group is summarized in Table 5-1. The focus of this study is to examine whether a difference in MRI metrics occurs in NAWM regions with steal physiology as opposed to regions with robust CVR. Therefore, attention will be paid to ROIs derived from Figure 5-3 and results with the confounding spatial factor are presented in Figure 5-4. When comparing NAWM with steal physiology to NAWM with robust CVR, FA significantly decreased by 3.7 ± 2.4 %, mD significantly increased by 3.9 ± 3.1%, CBF significantly decreased by 22.1 ± 8.2%, rCBV significantly decreased by 22.2 ± 7.0% and Tmax significantly decreased by 10.9 ± 13.2% (P < 0.01; repeated measures one-way ANOVA). MTT values did not significantly differ between NAWM with steal physiology and CVR (3.9 ± 0.2 vs. 4.0 ± 0.2 seconds; Friedman test). TTP values were initially significant between NAWM with steal and CVR; however, after considering the influence of spatial location, differences were no longer significant (20.8 ± 0.7 vs. 20.6 ± 0.7 seconds; repeated measures one-way ANOVA).
Table 5-1. Comparison of average values for MRI metrics in NAWM with positive and negative CVR.

<table>
<thead>
<tr>
<th></th>
<th>White Matter Hyperintensities</th>
<th>Normal-appearing White Matter (NAWM)</th>
<th>NAWM With positive CVR</th>
<th>NAWM With negative CVR</th>
<th>Contralateral NAWM (with positive CVR)</th>
<th>Ipsilateral NAWM (with negative CVR)</th>
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<tbody>
<tr>
<td>FA (TWH) [unitless]</td>
<td>0.24 ± 0.03</td>
<td>0.46 ± 0.04</td>
<td>0.47 ± 0.04</td>
<td>0.42 ± 0.05</td>
<td>0.438 ± 0.028</td>
<td>0.420 ± 0.028</td>
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<tr>
<td>FA (SRI) [unitless]</td>
<td>0.36 ± 0.03</td>
<td>0.51 ± 0.04</td>
<td>0.51 ± 0.05</td>
<td>0.50 ± 0.04</td>
<td>0.53 ± 0.02</td>
<td>0.51 ± 0.03</td>
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<td>mD [mm(^2)]</td>
<td>1.35 ± 0.11</td>
<td>0.84 ± 0.04</td>
<td>0.83 ± 0.04</td>
<td>0.86 ± 0.04</td>
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<tr>
<td>mD [mm(^2)]</td>
<td>1.34 ± 0.12</td>
<td>0.82 ± 0.04</td>
<td>0.81 ± 0.04</td>
<td>0.84 ± 0.04</td>
<td>0.87 ± 0.03</td>
<td>0.90 ± 0.05</td>
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<td>T2 [ms]</td>
<td>138.3 ± 15.5</td>
<td>85.7 ± 5.6</td>
<td>85.4 ± 5.8</td>
<td>87.1 ± 6.1</td>
<td>87.5 ± 1.0</td>
<td>87.8 ± 1.0</td>
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<tr>
<td>T2 [ms]</td>
<td>131.1 ± 7.7</td>
<td>82.8 ± 2.4</td>
<td>82.8 ± 2.6</td>
<td>82.7 ± 2.3</td>
<td>82.0 ± 2.0</td>
<td>82.5 ± 2.2</td>
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<td>CBF [mL/100g/m in]</td>
<td>20.1 ± 4.5</td>
<td>23.2 ± 5.8</td>
<td>23.8 ± 6.5</td>
<td>22.0 ± 4.9</td>
<td>26.4 ± 1.0</td>
<td>20.5 ± 0.9</td>
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<td>CBV [AI]</td>
<td>105.2 ± 9.4</td>
<td>114.1 ± 7.7</td>
<td>116.4 ± 8.4</td>
<td>110.1 ± 7.8</td>
<td>125.0 ± 4.9</td>
<td>98.7 ± 4.4</td>
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<td>MTT [s]</td>
<td>4.3 ± 1.5</td>
<td>3.9 ± 1.1</td>
<td>3.9 ± 1.1</td>
<td>4.0 ± 1.1</td>
<td>4.0 ± 0.2</td>
<td>3.9 ± 0.2</td>
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<tr>
<td>Tmax [s]</td>
<td>3.5 ± 1.2</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>2.7 ± 0.8</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.1</td>
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</table>
Grey and white boxes are measurements from TWH and SHSC, respectively. WMHs were compared with three different groups of normal-appearing white matter 1) a mask containing the entire NAWM 2) the entire NAWM segmented based on CVR 3) NAWM with contralateral homologous regions defined in Figure 5-3. NAWM with positive CVR was also compared with negative CVR. * denotes significance against WMHs (P<0.01) and † denotes significance against NAWM with positive CVR (P<0.01) using repeated measures ANOVA with a Bonferroni correction for all MRI metrics other than MTT. Comparisons involving MTT used Friedman test with Dunn’s correction for multiple comparisons. Measurements for each metric are given in average ± SD.

![Figure 5-4. Comparison of MRI metrics between regions of NAWM with positive CVR and steal physiology.](image)

**5.5 Discussion**

This study demonstrates that NAWM may not be as normal as defined with current conventional MRI sequences. In fact, areas in in NAWM can show steal physiology. Areas with positive CVR demonstrate a robust sensitivity for blood supply to meet demands whereas areas with steal physiology display a dysfunction in neurovascular coupling. Our results of abnormal
MRI metrics in areas of neurovascular uncoupling is consistent with animal models of chronic hypoperfusion, in which the white matter has been associated with demyelination, axonal loss, and gliosis (Hainsworth & Markus, 2008; Kurumatani, Kudo, Ikura, & Takeda, 1998).

NAWM with steal physiology can show subtle increase in mean diffusivity and lower fractional anisotropy compared to areas with robust CVR. This implies that steal physiology is capable of defining areas in the NAWM with subtle pathological features of structural damage to white matter that results in increased water diffusivity. This is in agreement with a previous study that found an association between steal physiology and elevated diffusivity in the white matter of patients with Moyamoya disease (Conklin et al., 2010). To our knowledge, this study is the first to report the association between steal physiology and decreases in anisotropy in NAWM. Another study also reported increased diffusivity in white matter ipsilateral to extracranial internal carotid artery stenosis compared to the contralateral unaffected white matter (Conklin et al., 2011). However, our subjects are free of significant stenosis in both intracranial and extracranial vessels and differ from populations with Moyamoya and steno-occlusive disease. Patients with vascular disease have development of parenchymal and leptomeningeal collateral vessels to supply the hemodynamically compromised brain parenchyma and represent a different pathophysiology; therefore, these results cannot be generalized to our study population.

We have found no significant difference in T2 values between NAWM with steal physiology and positive CVR. This finding is not surprising as the definition of NAWM was made on T2-weighted images so any significant increase in T2 would have been found to be WMH. Longer T2 values were found in populations with multiple sclerosis and phenylketonuria compared to healthy controls (Laule et al., 2007). Prolonged T2 in the NAWM may indicate signs of diffuse demyelination (Haughton et al., 1992), astrogliosis, and vasogenic edema (Armspach, Gounot, Rumbach, & Chambron, 1991). Results from TWH demonstrate T2 values were initially higher in areas with steal physiology but after taking the regionally variability of T2 into account, the difference was no longer significant.

Perfusion MRI metrics in NAWM were found to be abnormal in regions with steal physiology. A previous study that found a reduction in the CBF of NAWM in subjects with known leukoaraiosis compared to controls (17.9 vs. 21.6 mL/100g/min, respectively). Our CBF
values are higher as the average CBF in NAWM with physiological was 20.5 ± 0.9 mL/100g/min compared to 26.4 ± 1.0 mL/100g/min in the contralateral NAWM with positive CVR (O'Sullivan et al., 2002). One study found the CBF of WMHs to be 40% reduced compared to healthy controls although white matter CBV did not differ (H. S. Markus et al., 2000). To our knowledge, this is also the first report demonstrating the association of steal physiology with perfusion metrics in the NAWM. CBV was reduced and Tmax was higher in NAWM with pathologic steal. We interpret these findings as a potential loss in tissue vascularity that may signal the eventual development of WMHs. These perfusion results support the theory that chronic hypoperfusion and ischemic damage may be the underlying pathophysiological mechanism underlying age-related leukoaraiosis.

These findings raise the question of whether all tissue with steal physiology is prone to pathological changes. Steal physiology has been shown to be correlated with cortical thinning (Fierstra et al., 2010), enhanced risk of stroke (H. Markus & Cullinane, 2001; Silvestrini et al., 2000), and cognitive decline (Balucani et al., 2012; Buratti et al., 2014). A reduction in CVR and steal physiology has been observed in the white matter of young healthy individuals (Mandell, Han, Poublanc, Crawley, Kassner, et al., 2008). Conklin et al. (2010) has suggested that the answer lies in the regional distribution of steal. Similar to ischemic preconditioning, areas that are chronically exposed to physiological steal may be able to tolerate a sudden loss in blood flow during a vasodilatory challenge whereas areas that normally possess robust positive CVR may be more prone to pathological changes when exposed to steal physiology. Mandell et al. (2008) has suggested that large areas of steal physiology in healthy young adults are anatomically similar to where the elderly develop leukoaraiosis. We have demonstrated subtle pathological changes in NAWM towards values of WMHs that are associated with areas of steal physiology; however, whether or not these regions will develop into WMHs will need to be explained by longitudinal studies.

The strength of this study lies on our ability to provide accurate assessment steal physiology in NAWM. A submaximal vasodilatory stimulus will yield higher CVR values than a supramaximal stimulus and details explaining the physiology have been previously described (Sobczyk et al., 2014). In our study, subjects had a 10 mmHg square wave increase in PETCO2 that was sustained for at least two minutes from an average baseline of 40 mmHg. This gives us confidence that we have reached the autoregulatory capacity of the local microvasculature and
can accurately define regions of pathologic steal, which demonstrate redistribution of blood flow to areas with robust positive CVR. Another strength in our study is the multicentre aspect as we have merged datasets from both sites and represent a wide range of leukoaraiosis severity (Fazekas grade 2 to 3). Our study is limited by several factors. A limitation of this study is that only the TWH subjects had perfusion-weighted imaging (43 out of 75 subjects). Another limitation is that we currently use the BOLD as a surrogate for blood flow, which represents an interaction of arterial PO\textsubscript{2}, cerebral blood flow, cerebral blood volume, hematocrit, and cerebral metabolic rate of oxygen (Ogawa et al., 1993). However, we have shown a highly correlated BOLD MRI signal response to hypercapnea against CBF measurements obtained with arterial spin labeling in patients with steno-occlusive disease (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008).

In conclusion, we have shown that areas with steal physiology are associated with subtle changes in the tissue integrity of NAWM. This provides new information about the changes to NAWM and pathogenesis of white matter lesions. The results suggest that impaired neurovascular coupling is an important association in white matter disease and may contribute to its progression. Whether the impaired reactivity in white matter precedes white matter tissue injury and whether the evolution of lesions will occur in areas of impaired CVR remains unclear. However, this will be addressed with longitudinal studies in these subjects.
Study III. Diminished cerebrovascular reserve predicts future development of leukoaraiosis.

6.1 Abstract

Background: Leukoaraiosis describes cerebral white matter changes that are frequently observed on MRI scans of elderly individuals and is associated with cognitive decline, balance disturbances, depression, as well as increased stroke risk. Previous research has shown reduced cerebrovascular reactivity (CVR) in the white matter of young healthy individuals, particularly in locations that spatially correspond with regions where the elderly develop leukoaraiosis. This raises the possibility that vascular reactivity may play a significant role in the pathogenesis of white matter disease. We hypothesized that elderly subjects will be more likely to develop lesions in regions of impaired cerebrovascular reactivity over a 1 year period.

Methods: In this prospective observational study, 45 subjects (age range, 50 to 91 years; 25 males) with moderate-severe leukoaraiosis (Fazekas score >2) were enrolled following IRB approval. CVR was quantitated as BOLD MRI signal (as a surrogate of cerebral blood flow), in response to a consistently applied step change in the arterial partial pressure of carbon dioxide (RespirActTM, Thornhill Research Inc., Toronto, ON). Diffusion-tensor MRI was used to calculate parametric maps of the fractional anisotropy (FA) and mean diffusivity (mD). Parametric quantitative T2 maps were generated from a multi-echo T2 sequence. All parametric maps generated from baseline and follow-up sessions were registered to the baseline anatomical scan. Newly identified lesions on the follow-up scan were identified using the Lesion Explorer pipeline (Sunnybrook Research Institute, Toronto, ON). CVR, T2, and diffusion parameters were determined in areas that developed leukoaraiosis. Statistical analysis was performed using one-way ANOVA with Bonferroni correction for multiple comparisons.

Results: CVR and FA values in regions that will eventually develop future leukoaraiosis are significantly lower by 26.5 ± 23.2% and 11.0 ± 7.5%, respectively, compared to stable NAWM (P<0.001). T2 and mD values in regions that will develop future leukoaraiosis are significantly higher by 8.7 ± 7.9% and 17.0 ± 8.5%, respectively, compared to stable NAWM (P<0.001).
Conclusions: The sequence of events in the evolution of leukoaraiosis is a key question that has important implications for understanding lesion pathogenesis and for targeting therapeutic interventions. We have shown that areas with reduced cerebrovascular reactivity are associated with future white matter injury, assessed with DTI and quantitative T2 measures. This study gives new insight by suggesting a strong vascular component in the pathogenesis of white matter disease. We have shown that impairment in the brain’s ability to autoregulate leads to future development of white matter disease. The results suggest that impaired autoregulation is an important association in white matter disease and may contribute to its progression.

6.2 Introduction

In 1986, Hachinski et al. introduced the term leukoaraiosis (“leuko” = white and “araios” = rarefied) to describe the reduced x-ray absorption or high signal intensity on T2-weighted MRI images seen in the cerebral white matter (V. C. Hachinski et al., 1986). The use of the term leukoaraiosis is a purely descriptive term to describe white matter changes seen on brain imaging and does not clearly define a particular pathological entity. The term white matter hyperintensities (WMHs) is used to describe high signal intensities on MRI and if the etiology is suspected to be of vascular origin. WMHs were once thought to be benign age-related changes in the white matter but are now shown to be strongly associated with clinical symptoms, such as cognitive impairment (Verdelho et al., 2010), progression to dementia (Gunning-Dixon & Raz, 2000; R. Schmidt et al., 2002), and disability (Malmstrom & Morley, 2013; Whitman et al., 2001). WMHs are also strongly associated with age, as some studies reported that as much as 95% of individuals over the age of 50 have some degree of WMHs (de Leeuw et al., 2001; Launer et al., 2006). As the pathophysiology of WMH still remains poorly understood, it is important to understand the progression of WMH in hopes of decreasing disease burden.

This study will examine the use of cerebrovascular reactivity (CVR) in assessing the progression of WMHs. CVR is defined as the change in flow in response to a vasoactive stimulus. CVR may be measured with the use of blood-oxygen-level-dependent (BOLD) MRI as a surrogate for blood flow and an automated gas blender (RespirAct™, Thornhill Research Inc, Toronto, Canada) to control a vasoactive stimulus, end-tidal partial pressure of carbon dioxide ($P_{ET\text{CO}_2}$) and oxygen ($P_{ET\text{O}_2}$). Therefore, CVR is a measure of the sensitivity of the cerebral microvasculature to demands in blood flow through CO$_2$, thereby demonstrating neurovascular coupling. Negative CVR values are representative of steal physiology, in which a redistribution of flow occurs during a blood flow demand from areas of exhausted
cerebrovascular reserve to areas with robust vasodilatory reserve (Sobczyk et al., 2014). Therefore, areas of negative CVR or even abnormally reduced CVR indicate dysfunction in neurovascular coupling.

An advanced MRI technique, diffusion tensor imaging (DTI), has been shown to be sensitive to white matter changes in apparently normal-appearing white matter (Conklin et al., 2010; Conklin et al., 2011). Conklin et al. (2010) has recently demonstrated the use of CVR in identifying diffusion changes in normal-appearing white matter. The authors demonstrate that areas with steal physiology (negative CVR) have abnormally high diffusion values in the normal-appearing white matter (Conklin et al., 2010). A recent serial DTI and quantitative T2 MRI study followed elderly subjects with age-related WMHs over 16 weeks and found abnormally low FA, high mD, and high T2 values in de novo WMHs, indicative of an etiology similar to that of an acute ischemic infarct (Conklin et al., 2014).

Whether a dysfunction in neurovascular coupling may be involved in the progression of WMHs remains to be explored. In order to assess this relationship, we examined 45 elderly subjects with age-related leukoaraiosis acquiring two multiparametric MRI scans set one-year apart. Our approach to assessing CVR quantitatively is well established (Spano et al., 2013) enabling us to compare tissues over time since repeating the same vasodilatory stimulus is achievable with the RespirAct. These potential abnormalities in CVR (indicative of impaired neurovascular coupling) may be potentially useful surrogate disease markers that can used to assess therapeutic approaches. We hypothesize that normal-appearing white matter (NAWM) that is destined to becoming WMH will demonstrate abnormal CVR and other MRI metrics when compared to NAWM that remains stable over time.

6.3 Methods

6.3.1 Subject Recruitment and Assessment

This longitudinal prospective observational study involved MRI datasets from Toronto Western Hospital and Sunnybrook Health Sciences Centre. Subjects were participants in a study of age-related white matter changes, which involved image acquisition at two timepoints set one-year apart. Forty-five elderly adults (age range: 51-90; 25 males) were recruited for participation and provided informed consent to procedures approved by both the University Health Network (UHN) and Sunnybrook research ethics boards. Subject recruitment was achieved through both the memory and stroke prevention clinic at Toronto Western Hospital (TWH) and memory clinic at Sunnybrook Health Sciences Centre (SHSC). Magnetic resonance
angiogram (MRA) or computed tomography angiography (CTA) and T2-weighted fluid-attenuated inversion recovery (FLAIR) images of all patients were screened by an experienced neuroradiologist and subjects were included in the study based on the following inclusion criteria: (1) previous neurological event involving white matter > 3 months from presentation; (2) over the age of 50; (3) MRI white matter disease burden > Fazekas Grade 2, moderate number of FLAIR hyperintensities in the white matter; (4) no evidence of bilateral ICA stenosis greater than 70%; (5) no evidence of significant vertebral or basilar stenosis greater than 70%; (7) no evidence of dissection (8) no evidence of pulmonary or cardio-embolic disease. Subjects with motion artifacts on BOLD images were excluded. Twenty-four subjects from Toronto Western Hospital (age range, 50 to 83 years; 13 males and 11 females) and twenty-one subjects from Sunnybrook Health Sciences Centre (age range, 51 to 90 years; 12 males and 9 females) with moderate-severe leukoaraiosis met the inclusion criteria and were considered in subsequent analysis (Appendix Tables 3 and 4).

6.3.2 Image Acquisition
Please refer to section 3.2.1 for the imaging acquisition parameters at Toronto Western Hospital and Sunnybrook Health Sciences Centre. Figure 6-1 illustrates the metrics used in this study.

Figure 6-1. MRI metrics used in the assessment of future leukoaraiosis. A, Example of T1-weighted images from a subject with diffuse periventricular and deep WMHs. B, T2 mapping demonstrates that WMHs indeed have higher water content than NAWM. C, FLAIR images highlight the WMHs seen in this subject on the baseline scan. D, FLAIR images on the one-
year follow-up scan of the same subject. E, WMHs from the baseline scan are highlighted in yellow. F, WMHs from baseline are highlighted in yellow whereas new hyperintensities are highlighted in red. G, FA map of this subject demonstrates reduced anisotropy in areas of WMHs. H, mD map of this subject demonstrates higher diffusivity in areas of WMHs. I, The CVR map of this subject demonstrates reduced reactivity, if not steal physiology (negative CVR), in WMHs.

6.3.4 CVR measurement

CVR was assessed by measuring the change in Blood Oxygen Level Dependent Magnetic Resonance Imaging (BOLD MRI) in response to changes in end-tidal (i.e., end expiratory) partial pressure of carbon dioxide (\(P_{ETCO_2}\)) as the vasoactive stimulus (Han et al., 2011). CVR was calculated as \(% \text{ change BOLD} / \Delta P_{ETCO_2}\). A colour scale ranging from blue to red was used to identify the magnitude of CVR. Negative CVR values representative of steal physiology were represented as shades of blue. Positive CVR values were defined as “normal-appearing” and are represented as shades of yellow, orange, and red.

5.3.4.1 Vasodilatory Stimulus (Gas Manipulation, End-tidal pCO2 and pO2 Manipulation)

Manipulation of end-tidal partial pressure of carbon dioxide (\(P_{ETCO_2}\)) and end-tidal partial pressure of oxygen (\(P_{ETO_2}\)) was achieved using an automated gas blender that adjusts the gas composition and flow to a sequential gas delivery mask and breathing circuit (RespirActTM, Thornhill Research Inc., Toronto, Canada) according to previously described methods (Ito et al., 2008; Kisilevsky et al., 2008). The RespirAct can independently manipulate \(P_{ETCO_2}\) and \(P_{ETO_2}\) that is also independent of the subject’s minute ventilation and breathing pattern. Previous work describes the \(P_{ETCO_2}\) and \(P_{ETO_2}\) sequences used during the analysis of BOLD MRI CVR in more detail (Slessarev et al., 2007; Vesely et al., 2001). The vasodilatory stimulus used in this study mimicked a boxcar protocol in which a baseline \(P_{ETCO_2}\) of 40 mmHg for 60 seconds (normocapnea), followed by an abrupt hypercapnic step change to \(P_{ETCO_2}\) of 50 mmHg for 90 s, then a return to baseline for 90 s, followed by second abrupt hypercapnic step change for 120 s with a final return to baseline (Vesely et al., 2001). Normoxia (\(P_{ETO_2} \sim 110 \text{ mmHg}\)) was maintained throughout all step changes in \(P_{ETCO_2}\).

6.3.4.2 Image Reconstruction

The acquired BOLD MRI and \(P_{ETCO_2}\) data were imported to AFNI software (Cox, 1996) for analysis. BOLD images were slice time-corrected, volume-registered, and aligned to axial anatomical T1-weighted images. The CVR maps were then constructed by first time-shifting the acquired \(P_{ETCO_2}\) data to the point of maximum correlation with the whole brain average BOLD signal using MATLAB software. This compensates for the temporal discrepancy from
pulmonary to cerebral circulation. Afterwards, a voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $P_{ET}CO_2$ data was performed. The BOLD time series at each voxel was orthogonalized to the 6 motion parameters estimated by the volume registration to minimize the effect of hypercapnea-correlated head motion.

To confirm tissue dysfunction in the cerebral white matter, maps of the transverse relaxation time (T2) were calculated. T2 reflects white matter water content and myelination. Quantitative T2 maps were obtained using a multiecho fast spin-echo sequence (voxel size = 0.86 x 0.86 x 3 mm for TWH and 1.0 x 1.0 x 3 mm for SHSC). T2 maps were calculated in AFNI using methods previously described (Miller & Joseph, 1993). To calculate FA and mD maps, diffusion-weighted images were imported into FSL 4.1.8 (http://www.fmrib.ox.ac.uk/fsl) for quality control (S. M. Smith et al., 2004). Pre-processing included eddy current and motion artifact correction using FMRIB's Diffusion Toolbox (FDT) (Jenkinson et al., 2002). Then, individual brain masks were created using BET (S. M. Smith, 2002). The preprocessed images were fit with a diffusion tensor model using DTIFIT in FDT (S. M. Smith et al., 2004).

6.3.5 Generating CVR maps

$P_{ET}CO_2$ data were first synchronized with the whole brain average BOLD signal using MATLAB software (Mathworks, Natick, Massachusetts, USA). The synchronization compensated for delays in breath sample analysis and delay of blood flow from pulmonary to cerebral circulation. A voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $P_{ET}CO_2$ was performed and the slope of the line of best fit was taken as the CVR. CVR values are expressed as percent MR signal change per mmHg of PETCO2 (mean ± SD).

6.3.6 Generating ROIs of WMH and NAWM

Segmentation of WMH was performed using the Lesion Explorer processing pipeline (Ramirez et al., 2011; Ramirez et al., 2014). Follow-up FLAIR, PD, and T2-weighted images were reviewed to identify newly appearing lesions. An ROI of newly developed lesions were manually traced using AFNI (Cox, 1996). T1-weighted anatomical images were segmented into cerebrospinal fluid, grey matter, and white matter using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK). A diamond shaped structuring element was used to erode the white matter segmentation in five iterations at the resolution of the T1-weighted image to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a NAWM mask. To account for spatial location of MRI metrics, T1-weighted images were transformed into Montreal Neurological Institute space using
SPM8. The transformation matrix was applied to other MRI metrics, thereby transforming these maps to a standard space but retaining the native structure. AFNI was used to identify NAWM contralateral to WMH (Figure 6-2).

![Figure 6-2. ROIs used in the assessment of MRI metrics while considering the confounding effect of spatial location. A, Example of FLAIR images from a subject with diffuse periventricular and deep WMHs. B, WMHs are highlighted in yellow. C, WMHs are displayed without the underlying FLAIR map. D, Image C is left-right flipped about the y-axis in MNI coordinates while maintaining native structure. E, Image D is subtracted from C, giving rise to NAWM that is contralateral to lesions. F, Image E is left-right flipped about the y-axis to give rise to the original WMH mask but only voxels with contralateral NAWM remain. G, The final two masks, NAWM contralateral to WMH (image E) and WMH containing contralateral NAWM (image F) are overlaid on FLAIR images.](image)

### 6.3.7 Statistical Analyses

MRI metrics were compared in the following five groups: 1) grey matter 2) pre-existing WMH 3) the entire NAWM 4) a subset of NAWM that will develop into WMH on the follow-up scan (with measurements made on the initial scan) and 5) WMH that appear only on the follow-up scan. Statistical significance in CVR between groups was tested using Friedman test with Dunn’s correction for multiple comparison. For DTI and quantitative T2 data, significance was tested using a repeated measures one-way analysis of variance with FA, mD, and T2 as dependent variables and ROIs as the matched-pairs independent variable. Mauchly’s test was used to detect significant departures from sphericity, and degrees of freedom were corrected
using Greenhouse–Geisser method. Results were considered significant and accounted for multiple comparisons with the Bonferroni post-hoc test if the per-comparison P value was less than 0.05/(10 comparisons) = 0.005. When accounting for spatial location, significance was tested with either Wilcoxon matched-pairs signed ranks test or two-tailed paired t-tests where appropriate.

6.4 Results

Forty-five subjects (age range: 50-90 years, 25 males) with moderate-severe leukoaraiosis participated in this study. Subjects were referred by their family physicians to either the memory or stroke prevention clinics due to syncopal episodes, transient paresthesia, gait disturbances, headaches, memory impairment, or cognitive decline.

At SHSC, subjects had an average WMH volume of 36.7 ± 26.3 ccs per subject at baseline (mean age: 74.0 years), average WMH volume of 38.4 ± 26.6 ccs per subject on one-year follow-up, and WMH progression rate of 0.86 ± 0.68 ccs/year. At TWH, subjects had an average WMH volume of 19.3 ± 18.4 ccs per subject at baseline (mean age: 72.2 years), average WMH volume of 20.7 ± 20.0 ccs per subject on follow-up one year later, and WMH progression rate of 0.44 ± 0.41 ccs/year. Interestingly at TWH, two subjects had lesions resolve on the follow-up MRI scans with one subject losing 0.28 ccs and the other losing 0.13 ccs of WMH volume.

The average ± SD for each CVR metric in each group is summarized in Table 6-1. In summary, when comparing stable NAWM with NAWM destined to become a WMH on follow-up, the Friedman test demonstrated a significant decrease in CVR (0.049 ± 0.023 vs. 0.118 ± 0.052 %BOLD/mmHg for TWH values), repeated measures one-way ANOVA demonstrated a significance decrease in FA (0.315 ± 0.064 vs. 0.444 ± 0.024 for TWH values), increase in mD (1.013 ± 0.120 vs. 0.849 ± 0.004 mm²/s at for TWH values) and increase in quantitative T2 measurements (91.6 ± 9.6 vs. 85.4 ± 5.2 ms for TWH values). Statistical significance was achieved at P < 0.001 when accounted for multiple comparisons with either Dunn’s or Bonferroni’s post-hoc test where appropriate.

Spatial location could influence these results. However, statistical significance remained when comparing NAWM destined to be WMH compared to the contralateral homologous NAWM using ROIs derived from Table 6-2. In particular, Wilcoxon matched-pairs signed ranks test demonstrated a significant decrease in CVR by 26.5 ± 23.2 % between both sites (P<0.001) and two-way paired t-tests demonstrated a significant decrease in FA by 11.0 ± 7.5%
between both sites, increase in mD by 17.0 ± 8.5% between both sites, and increase in T2 by 8.7 ± 7.9% between both sites (P<0.001) (Figure 6-3).

When examining ROIs of NAWM that is destined to becoming WMH, after it became a WMH, there were trends of decreases in CVR, decreases in FA, and increases in T2 as NAWM became WMH but they were not statistically significant. However, there were significant increases in mD (1.013 ± 0.120 vs. 1.086 ± 0.129 mm²/s, repeated measures one-way ANOVA with Bonferroni post-hoc correction). There was also an significant increase in T2 found only at TWH (94.7 ± 9.6 vs. 113.2 ± 12.0 ms, repeated measures one-way ANOVA with Bonferroni post-hoc correction) (Table 6-1).
Table 6-1. Comparison of average values for MRI metrics in regions of grey matter, normal-appearing white matter, and future.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Grey Matter</th>
<th>Pre-existing WMH</th>
<th>Normal-appearing White Matter</th>
<th>NAWM destined to be WMH (measurements on baseline scan)</th>
<th>WMH only on follow-up (measurements on follow-up scan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR (TWH) [%BOLD/mmHg]</td>
<td>0.241 ± 0.074</td>
<td>0.051 ± 0.062</td>
<td>0.118 ± 0.052</td>
<td>0.049 ± 0.023</td>
<td>0.036 ± 0.044</td>
</tr>
<tr>
<td>CVR (SRI) [%BOLD/mmHg]</td>
<td>0.179 ± 0.049</td>
<td>0.050 ± 0.030</td>
<td>0.110 ± 0.035</td>
<td>0.051 ± 0.063</td>
<td>0.061 ± 0.054</td>
</tr>
<tr>
<td>FA [unitless]</td>
<td>0.171 ± 0.011</td>
<td>0.228 ± 0.062</td>
<td>0.444 ± 0.024</td>
<td>0.315 ± 0.064</td>
<td>0.294 ± 0.053</td>
</tr>
<tr>
<td>FA [unitless]</td>
<td>0.269 ± 0.022</td>
<td>0.383 ± 0.054</td>
<td>0.524 ± 0.045</td>
<td>0.308 ± 0.075</td>
<td>0.309 ± 0.076</td>
</tr>
<tr>
<td>mD [x10⁻³mm²/s]</td>
<td>1.092 ± 0.005</td>
<td>1.360 ± 0.109</td>
<td>0.846 ± 0.004</td>
<td>1.013 ± 0.120</td>
<td>1.086 ± 0.129</td>
</tr>
<tr>
<td>mD [x10⁻³mm²/s]</td>
<td>1.091 ± 0.006</td>
<td>1.369 ± 0.116</td>
<td>0.844 ± 0.004</td>
<td>0.991 ± 0.103</td>
<td>1.07 ± 0.103</td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>103.7 ± 6.6</td>
<td>135.4 ± 15.3</td>
<td>84.0 ± 5.4</td>
<td>91.6 ± 9.6</td>
<td>113.2 ± 12.0</td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>108.7 ± 4.7</td>
<td>129.7 ± 8.7</td>
<td>85.5 ± 3.2</td>
<td>94.7 ± 9.6</td>
<td>105.6 ± 12.5</td>
</tr>
</tbody>
</table>

Grey and white boxes are measurements made at TWH and SHSC, respectively. All comparisons with FA, mD, and T2 were made using repeated measures one-way ANOVA with a Bonferroni correction for multiple comparisons. CVR comparisons used Friedman test with Dunn’s post-hoc correction. * P<0.01 for comparisons against grey matter (column A); • P<0.01
for comparisons against NAWM (column C); +P<0.01 between timepoints of future leukoaraiosis; WMH=white matter hyperintensities, NAWM= normal-appearing white matter

Table 6-2. Comparison of average values for MRI metrics in future WMHs and contralateral homologous regions.

<table>
<thead>
<tr>
<th>Metric</th>
<th>NAWM destined to be WMH (assessed on baseline scan)</th>
<th>Contralateral NAWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR (TWH) [%BOLD/mmHg]</td>
<td>0.036 ± 0.044</td>
<td>0.075 ± 0.048*</td>
</tr>
<tr>
<td>CVR (SRI) [%BOLD/mmHg]</td>
<td>0.061 ± 0.054</td>
<td>0.076 ± 0.068*</td>
</tr>
<tr>
<td>FA [unitless]</td>
<td>0.294 ± 0.053</td>
<td>0.317 ± 0.024*</td>
</tr>
<tr>
<td>FA [unitless]</td>
<td>0.309 ± 0.076</td>
<td>0.341 ± 0.066*</td>
</tr>
<tr>
<td>mD [mm²/s]</td>
<td>1.136 ± 0.115</td>
<td>1.086 ± 0.129*</td>
</tr>
<tr>
<td>mD [mm²/s]</td>
<td>1.168 ± 0.131</td>
<td>1.07 ± 0.103*</td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>113.2 ± 12.0</td>
<td>103.049 ± 8.140*</td>
</tr>
<tr>
<td>T2 [ms]</td>
<td>105.6 ± 12.5</td>
<td>98.698 ± 11.033*</td>
</tr>
</tbody>
</table>

* denotes significance against future leukoaraiosis (P<0.01) using a two-tailed paired Student’s t-test or Wilcoxon matched-pairs signed ranks test where appropriate.
Figure 6-3. Comparison of MRI metrics in regions of NAWM destined to be WMH and the contralateral NAWM. The values in MRI metrics are derived from paired comparisons between regions of NAWM that developed WMHs on the follow-up scan and their corresponding contralateral homologous regions of NAWM. Values are given as % change from contralateral NAWM. All values are assessed on baseline MRI scans. CVR and FA values are significantly lower on baseline NAWM that subsequently developed leukoaraiosis compared to the contralateral stable NAWM. Results from mD and T2 metrics show significant increases in regions of NAWM that will develop leukoaraiosis compared to the contralateral NAWM. * denotes significance compared to the contralateral homologous NAWM (P < 0.01). Bars indicate minimum and maximum, boxes indicate the interquartile range (25th to 75th percentile) and the line within each box indicates the median.
6.5 Discussion

The findings of our study demonstrate that NAWM that will evolve into a WMH have reduced vascular reactivity compared to NAWM that will remain stable. This is the first study to empirically demonstrate the relationship of impaired neurovascular coupling with the progression of leukoaraiosis. In our study, CVR is defined as the sensitivity of the local vasculature to blood flow demand through CO$_2$. With a near maximal vasodilatory stimulus (i.e. 10 mmHg change in P$_{ET}$CO$_2$), we can also confidently interpret our measure of CVR as vascular reserve as well. Therefore, the CVR reduction in NAWM that is destined to becoming WMH will have a decreased sensitivity to vasodilatory demands as well as a lower autoregulatory capacity.

NAWM that was destined to be WMH also had signs of pathology based on DTI metrics. An increase in mD and decrease in FA was found in areas of NAWM destined to be WMH compared to stable NAWM, suggesting signs of a loss in tissue integrity before visible changes on T1- or T2-weighted imaging. These changes are found to be consistent with previous reports demonstrating abnormal diffusivity in areas of pathologic steal phenomenon in the NAWM in patients with Moyamoya disease (Conklin et al., 2010) and steno-occlusive disease (Conklin et al., 2011). These authors examined diffusivity in NAWM with steal physiology compared to NAWM with robust positive CVR responses and found abnormalities on DTI that correlated only with steal physiology. They also questioned whether or not these subtle pathological changes would eventually manifest as WMHs. We have shown that areas with reduced CVR, although not necessarily demonstrating steal physiology (i.e. negative CVR), will be prone to developing WMH. This is probably due to chronic competition for blood flow due to the regional variability of vascular reserve. Sobczyk et al. (2014) has recently reported a conceptual model for CO2-induced redistribution of cerebral blood flow, in which the authors suggest that CVR produces a differential effect of CO$_2$ on vascular territories. CVR may be absent if the vasculature responds uniformly throughout the white matter and would not show areas of compromised vascular reserve if the vascular network was not co-dependent upon each other during limitations in blood supply (Sobczyk et al., 2014). Therefore, our findings support the notion that chronic dysfunction in neurogliovascular coupling leads to the development of leukoaraiosis.

We have found prolonged T2 values in NAWM that is prone to developing WMH. These values were approaching those of pre-existing WMH although not visible on T2-weighted
or PD images on baseline scans. This indicates pathological changes in NAWM that occur before becoming visible and are consistent with previous studies. Prolonged T2 values have been reported in the NAWM of subjects with multiple sclerosis and phenylketonuria compared to healthy controls (Laule et al., 2007). Prolonged T2 in the NAWM indicates signs of diffuse demyelination (Haughton et al., 1992), astrogliosis, and vasogenic edema (Armspach et al., 1991).

We report a WMH progression rate of $0.86 \pm 0.68$ ccs/year at SHSC and $0.44 \pm 0.41$ ccs/year at TWH, and represent values that are in the same range of lower progression rates found in previous studies. Taylor et al. (2003) and Whitman et al. (2001) examined changes in the volume of WMHs in a cohort of community-dwelling elderly subjects with no identifiable neurological disease and found a progression rate of $0.76$ cc/year and $0.28$ cc/year, respectively (W. D. Taylor et al., 2003; Whitman et al., 2001). Sachdev et al. (2007) examined elderly individuals with age-related leukoaraiosis and found a much higher progression rate of $2.17$ ccs/year. Interestingly, these authors found a decrease in WMH volume in 8 out of 51 subjects whereas we found this to occur only in 2 out of 45 subjects. One explanation for this decrease in WMH volume may be due to registration error between baseline and follow-up scans. However, we relied on multiple sequences, such as FLAIR, multiecho T2, PD, and conventional T2-weighted images to confirm for any differences of new WMH development or loss on follow-up scans.

In a recent meta-analysis of WMH volume progression by Kloppenborg et al. (2014) it was demonstrated that the prevalence of WMHs is significantly associated with declines in cognitive function. Moreover, progression of WMHs was found to be significantly associated with even greater cognitive decline over time. These effects were most pronounced for general intelligence, attention, and executive functioning (Kloppenborg, Nederkoorn, Geerlings, & van den Berg, 2014).

In terms of limitations, our study is limited by the use of the BOLD signal as a surrogate for blood flow. However, arterial spin labeling quantitated CBF has been demonstrated to be a linear function in the $P_{ET}CO_2$ range of 40 to 50 mmHg used in our study (Tancredi & Hoge, 2013). The BOLD signal also represents an interaction of arterial $PO_2$, cerebral blood flow, cerebral blood volume, hematocrit, and cerebral metabolic rate of oxygen (Ogawa et al., 1993). However, we have shown a highly correlated BOLD MRI signal response to hypercapnea
against CBF measurements obtained with arterial spin labeling in patients with steno-
occlusive disease (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008).

A strength of our study is our ability to accurately control $P_{ETCO_2}$ and $P_{ETCO_2}$
independently of each other and also of the subject’s breathing pattern and minute ventilation. Our vasodilatory stimulus is known at all times and is accurately controlled. Therefore, all subjects underwent the same $P_{ETCO_2}$ changes and experienced the same gas changes on the follow-up scan, which is important to control in a longitudinal assessment of CVR. If the stimulus was submaximal, a maximal vasodilatory state of the vessels may not have been reached; therefore CVR values may be inflated in vessels where the sensitivity to CO$_2$ is still robust. This occurs because the autoregulatory capacity has not been exhausted, therefore neurovascular coupling remains normal. However, as $P_{ETCO_2}$ continues to rise, a redistribution of blood flow from regions with exhausted vascular reserve to regions with a greater reserve occurs and impairs neurovascular coupling in regions with lower CVR (Sobczyk et al., 2014).

In summary, the sequence of events in the evolution of leukoaraiosis is a key question that has important implications for understanding lesion pathogenesis and for targeting therapeutic interventions and even prevention. Our findings show that areas in NAWM with reduced CVR are associated with future injury to the white matter, as assessed with DTI and quantitative T2 measures. This study gives new insight by suggesting a strong vascular component in the pathogenesis of white matter disease. We have shown that impairment of the vasculature’s response to CO$_2$ and inability to further augment blood flow leads to future development of white matter disease. The results suggest that dysfunction in neurogliovascular coupling is an important association in white matter disease and may contribute to its progression.
Study IV. Impaired Temporal Dynamics of the Cerebromicrovasculature in elderly subjects with leukoaraiosis.

7.1 Abstract

With the ability to manipulate the arterial partial pressure of CO$_2$ (PaCO$_2$), we can parse CVR into dynamic and steady state measurements. The purpose of this study is to evaluate the dynamic and static components of cerebrovascular reactivity in age-related leukoaraiosis of elderly subjects.

Methods: In this prospective observational study, 75 subjects with leukoaraiosis (age range, 50 to 91 years; 40 males) were studied at two time-points set one-year apart. CVR was calculated as the change in blood oxygen level dependent (BOLD) MRI signal (as a surrogate of cerebral blood flow), in response to a consistently applied step change in the end-tidal partial pressure of carbon dioxide (P$_{ET}CO_2$). The P$_{ET}CO_2$ was convolved to the BOLD signal with a set of first order exponentials with a time constant $\tau$ for each. The $\tau$ that corresponded to the best fit between the convolved P$_{ET}CO_2$ and BOLD signal was scored as the speed of the response for that particular voxel. The slope of the regression between the convolved P$_{ET}CO_2$ and BOLD signal was measured as the steady-state CVR (ssCVR) for that particular voxel. CVR, ssCVR, and $\tau$ were assessed in the normal-appearing white matter (NAWM) and white matter hyperintensities (WMH) in elderly subjects.

Results: CVR and ssCVR values in WMH were significantly lower by 35.7 ± 19.1% and 30.9 ± 18.0%, respectively, compared to NAWM (P<0.01). $\tau$ values in WMH were significantly higher by 9.4 ± 11.5% compared to NAWM (P<0.01).

Conclusions: These findings confirm that the vascular physiology in regions of leukoaraiosis is abnormal. This is the first study that demonstrates a reduced speed of vascular response to demands in blood flow in regions of leukoaraiosis. These findings support the role of chronic hypoperfusion in white matter changes in the aging brain.
7.2 Introduction

Cerebral white matter changes are frequently observed on magnetic resonance imaging (MRI) and computed tomography (CT) in elderly individuals and appear as hyperintensities on T2-weighted MRI images, hence the term white matter hyperintensities (WMHs). On CT, white matter changes appear to have low density and have been termed “leukoaraiosis” (leuko = white, araiosis = rarefied) (V. C. Hachinski et al., 1986). WMHs are believed to be of vascular origin and are found to occur predominantly in the periventricular white matter, particularly around the horns of the lateral ventricles, and in the centrum semiovale. According to several population-based studies, the prevalence of WMHs on MRI in elderly people is in the range of 62%-95% (Breteler et al., 1994; Launer et al., 2006; Liao et al., 1997). Given the high prevalence of WMHs in normal functioning elderly individuals, their significance has previously been thought to be a benign. We know now that WMHs are associated with disability (Blahak et al., 2009; Whitman et al., 2001), cognitive decline (Verdelho et al., 2010), and progression to dementia (Gunning-Dixon & Raz, 2000; R. Schmidt et al., 2002). However, the pathophysiology of WMH progression remains poorly understood.

The mechanisms leading to WMHs are complex. Age-related stenosis and hypoperfusion of medullary arterioles may cause low grade ischemic injury to the deep white matter. Also, a reduction of autoregulation may contribute to the progression of WMHs in areas that are not well capillarized, which predisposes the white matter to low perfusion damage. A study by Uh et al. (2010) demonstrated that cerebrovascular reactivity (CVR) and cerebral blood flow (CBF) are reduced in leukoaraiosis compared to normal-appearing white matter. Leukoaraiosis was also characterized by significant blood-brain barrier leakage that also correlated to with increased diffusion seen on diffusion tensor imaging.

CVR is defined as a change in blood flow in response to a vasoactive stimulus. We have established a quantitative approach to measuring CVR by using blood-oxygen-level-dependent (BOLD) MRI as a surrogate for blood flow and control of end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) and oxygen ($P_{ET}O_2$) as our vasoactive stimulus (Spano et al., 2013). By applying a square wave change in $P_{ET}CO_2$ during BOLD imaging, we are able to parse CVR into three different metrics: 1) an ordinary CVR derived using conventional methods by taking the slope of the line of the regression between the BOLD signal and $P_{ET}CO_2$, 2) a static component of CVR referred to as steady-state CVR, representing a measure of the steady-state reactivity of the vasculature and 3) a dynamic component of CVR referred to as $\tau$, representing the speed of
the cerebrovascular response to our vasoactive stimulus. Previous CVR measurements reported in literature have not been able to provide dynamic measurements due to limitations in the control of their stimulus. Details explaining the unique nature of our stimulus in providing these different CVR metrics have been previously published (Poublanc et al., 2015). In brief, our approach uses a sequential gas delivery breathing circuit through an automated gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) that enables an abrupt (within 1 to 2 breaths) square-wave increase in P_{ETCO_2}; furthermore, the rebreathing aspect of our circuit has been validated to be equivalent to the arterial partial pressure of CO₂ so an accurate assessment of our stimulus is known throughout the entire experiment (Ito et al., 2008).

To date, no one has examined the speed of the vascular response in areas of leukoaraiosis. The CBF vasodilatory response times have been found to be longer in patients with mild dementia and Alzheimer’s disease compared to healthy age-matched subjects (Cantin et al., 2011; E. R. Cohen, Ugurbil, & Kim, 2002; Vazquez et al., 2006). Poublanc et al. (2015) has recently shown, in patients with unilateral steno-occlusive disease, prolongations of τ, reductions in ssCVR, and reductions in CVR ipsilateral to the stenosis. Whether this same pattern in CVR occurs in WMHs and influences the development of WMH remains to be elucidated. We hypothesize that reductions in ssCVR and prolongations of τ would occur in WMHs as well as normal-appearing white matter (NAWM) prone to becoming WMH when compared to stable NAWM.

7.3 Methods

7.3.1 Subject Recruitment and Assessment

This longitudinal prospective observational study examined subjects with moderate-severe leukoaraiosis with two MRI scans set one year apart. Subjects were participants in a study of age-related white matter changes and written informed consent was obtained from all participants for each MRI scan. This study was approved by both the University Health Network and Sunnybrook research ethics boards. Subject recruitment was achieved through both the memory and stroke prevention clinic at Toronto Western Hospital (TWH) and memory clinic at Sunnybrook Health Sciences Centre (SHSC). Magnetic resonance angiogram (MRA) or computed tomography angiography (CTA) and T2-weighted fluid-attenuated inversion recovery (FLAIR) images of all patients were screened by an experienced neuroradiologist and subjects were included in the study based on the following inclusion criteria: (1) previous neurological event involving white matter > 3 months from presentation; (2) over the age of 50; (3) MRI
white matter disease burden > Fazekas Grade 2, moderate number of FLAIR hyperintensities in the white matter; (4) no evidence of bilateral ICA stenosis greater than 70%; (5) no evidence of significant vertebral or basilar stenosis greater than 70%; (7) no evidence of dissection (8) no evidence of pulmonary or cardio-embolic disease. Subjects with motion artifacts on BOLD images were excluded. Twenty-four subjects from Toronto Western Hospital (age range, 50 to 83 years; 13 males and 11 females) and twenty-one subjects from Sunnybrook Health Sciences Centre (age range, 51 to 90 years; 12 males and 9 females) with moderate-severe leukoaraiosis met the inclusion criteria and were considered in subsequent analysis (See Appendix Tables 3 and 4).

7.3.2 Image Acquisition

Please refer to section 3.2.1 for the imaging acquisition parameters at Toronto Western Hospital and Sunnybrook Health Sciences Centre.

7.3.4 CVR measurement

CVR is defined as the change in flow in response to a vasoactive stimulus. In this study, Blood Oxygen Level Dependent Magnetic Resonance Imaging (BOLD MRI) was used as a surrogate of blood flow as is the case in numerous previous publications (Poublanc et al., 2015; Sam et al., 2015; Sobczyk et al., 2015). End-tidal partial pressure of carbon dioxide ($P_{ETCO_2}$) was used as the vasoactive stimulus (Han et al., 2011). Thus, CVR was calculated as % change $BOLD / \Delta P_{ETCO_2}$. A colour scale ranging from blue to red was used to identify the magnitude of CVR. Negative CVR values representative of steal physiology were represented as shades of blue. Positive CVR values were defined as “normal-appearing” and are represented as shades of yellow, orange, and red.

7.3.4.1 Vasodilatory Stimulus (Gas Manipulation, End-tidal $pCO_2$ and $pO_2$ Manipulation)

Control of end-tidal partial pressure of carbon dioxide ($P_{ETCO_2}$) and end-tidal partial pressure of oxygen ($P_{ETO_2}$) was achieved using an automated gas blender that adjusts the gas composition and flow to a sequential gas delivery mask and breathing circuit (RespirActTM, Thornhill Research Inc., Toronto, Canada) according to previously described methods (Ito et al., 2008; Kisilevsky et al., 2008). The RespirAct is capable of manipulating $P_{ETCO_2}$ and $P_{ETO_2}$ independently of each other and also independent of the subject’s minute ventilation and breathing pattern. The $P_{ETCO_2}$ paradigm used during BOLD imaging started subjects at approximately 40 mmHg for 60 seconds (normocapnea), followed by an abrupt hypercapnic step change to $P_{ETCO_2}$ of 50 mmHg for 90 s, a return to baseline for 90 s, a second hypercapnic
step change for 120 s with a final return to baseline (Vesely et al., 2001). Normoxia ($\text{PETO}_2 \sim 110 \text{mmHg}$) was maintained throughout BOLD imaging. Previous work describes the $\text{PETCO}_2$ and $\text{PETO}_2$ sequences used during the analysis of BOLD MRI CVR in more detail (Slessarev et al., 2007; Vesely et al., 2001).

7.3.4.2 Image Reconstruction

The acquired BOLD MRI and $\text{PETCO}_2$ data were imported to AFNI software (Cox, 1996) for analysis. BOLD images were slice time-corrected, volume-registered, and aligned to axial anatomical T1-weighted images. The CVR maps were then constructed by first time-shifting the acquired $\text{PETCO}_2$ data to the point of maximum correlation with the whole brain average BOLD signal using MATLAB software. This compensates for the temporal discrepancy from pulmonary to cerebral circulation. Afterwards, a voxel-by-voxel linear least-squares fit of the BOLD signal time series to the $\text{PETCO}_2$ data was performed. The BOLD time series at each voxel was orthogonalized to the 6 motion parameters estimated by the volume registration to minimize the effect of hypercapnea-correlated head motion.

7.3.5 Generating CVR maps

Acquired $\text{PETCO}_2$ data were first synchronized with the whole brain average BOLD signal using MATLAB software (Mathworks, Natick, Massachusetts, USA). The synchronization compensated for delays in breath sample analysis and delay of blood flow from pulmonary to cerebral circulation. A voxel-by-voxel linear least-squares fit of the BOLD signal time series to the acquired $\text{PETCO}_2$ was performed and the slope of the line of best fit was taken as the CVR (Figure 7-1).
Figure 7-1. CVR metrics used to characterize future leukoaraiosis. A, T1-weighted images from a subject with diffuse periventricular and deep WMHs. B, Steady-state CVR (ssCVR) map in the same subject showing reduced CVR in areas of WMHs. C, CVR map in the same subject. D, Tau map demonstrating slower vascular responses to carbon dioxide in regions of WMHs. E, FLAIR images showing WMHs on the baseline scan. Two colour scales are used to represent regions with positive ssCVR (red to green) and negative ssCVR (light blue to purple). F, FLAIR images showing WMHs on the follow-up scan. G, WMHs identified on the baseline scan are highlighted in yellow. H, WMHs identified on baseline are highlighted in yellow whereas new hyperintensities identified on follow-up are highlighted in red. CVR values are in units of % BOLD/mmHg.

7.3.6 Generating steady-state CVR and τ maps

CVR was parsed into steady-state (ssCVR) and dynamic (τ) components by using a convolved $P_{ET}CO_2$ instead of using the acquired $P_{ET}CO_2$. The details of the principles behind measuring these parameters are described by Poublanc et al. (2015). In brief, the BOLD response to a stimulus was modeled in which the $P_{ET}CO_2$ was convolved with a an exponential decay function $[\exp(-t/\tau)]$ that represents a vessel hemodynamic response function, where $t$ is time and $\tau$ is the time constant of the vessel response. $\tau$ was allowed to vary from 2 to 100 seconds in 2 second increments, giving rise to 50 convolved signals. A Pearson correlation coefficient was then calculated between the BOLD signal and all 50 convolved $P_{ET}CO_2$ signals. The $\tau$ that corresponded with the maximal correlation coefficient was taken as the speed of the response for that particular voxel and was used for ROI comparison. Steady-state CVR was calculated by taking the slope of the regression between the convolved $P_{ET}CO_2$ and the BOLD response (Poublanc et al., 2015). Steady-state CVR differs from CVR as it is corrected for the speed of
the response ($\tau$). Both CVR and ssCVR values are expressed as percent MR signal change per mmHg of $P_{ET\text{CO}_2}$ whereas $\tau$ is measured in seconds (mean±SD).

7.3.7 Generating ROIs of WMH and NAWM

Segmentation of WMH was performed using the Lesion Explorer processing pipeline (Ramirez et al., 2011; Ramirez et al., 2014). Follow-up FLAIR, PD, and T2-weighted images were reviewed to identify newly appearing lesions. An ROI of newly developed lesions were manually traced using AFNI (Cox, 1996). T1-weighted anatomical images were segmented into cerebrospinal fluid, grey matter, and white matter using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College, London, UK). A diamond shaped structuring element was used to erode the white matter segmentation in five iterations at the resolution of the T1-weighted image to prevent partial voluming effects. The WMHs were subtracted after erosion to give rise to a NAWM mask. One important confound in our study is spatial factor as differences in each metric could be due to spatial location i.e. periventricular CVR could be lower than white matter near the cortical surface. To account for spatial location of MRI metrics, T1-weighted images were transformed into Montreal Neurological Institute space using SPM8. The transformation matrix was applied to other MRI metrics, thereby transforming these maps to a standard space but retaining the native structure. AFNI was used to identify NAWM contralateral to WMH (Figure 7-2).
Figure 7-2. ROIs used in the comparison of NAWM and hyperintensities considering the confounding spatial factor. A, FLAIR images from a subject with diffuse periventricular and deep WMHs. B, WMHs are highlighted in yellow and overlaid on a FLAIR map. C, WMHs without the underlying FLAIR map. D, Image C is left-right flipped about the y-axis in MNI coordinates while retaining native structure. E, Image D is subtracted from Image C, giving rise to NAWM that is contralateral to hyperintensities. F, Image E is left-right flipped about the y-axis to give rise to the original WMH mask; however, only voxels with contralateral NAWM remain. G, The final NAWM mask (image E) and WMH mask (image F) used in the characterization of CVR metrics are overlaid on FLAIR images.

7.3.8 Statistical Analyses

MRI metrics were compared in the following four groups: 1) grey matter 2) pre-existing WMH 3) the entire NAWM 4) a subset of NAWM that will develop into WMH on the follow-up scan (but measurements assessed on baseline scan). Statistical significance in CVR and ssCVR between groups was tested using Friedman test with Dunn’s correction for multiple comparison. Statistical analysis of $\tau$ between groups used repeated measured one-way ANOVA, with $\tau$ as the dependent variable and ROIs as the matched-pairs independent variable. Mauchly’s test was used to detect significant departures from sphericity and degrees of freedom were corrected with the Greenhouse–Geisser method. Results were accounted for multiple comparisons using the Bonferroni post-hoc test. Statistical significance was tested with Wilcoxon matched-pairs signed ranks test or two-tailed paired t-tests where appropriate.

7.4 Results

Forty-five subjects (age range: 50-90 years, 25 males) with moderate-severe leukoaraiosis participated in this study. Reasons for referral to either the memory or stroke prevention clinics included chronic imbalance and headaches, memory impairment, cognitive decline, transient episodes of paresthesia, or syncopal episodes.

At SHSC, subjects had an average WMH volume of 36.7 ± 26.3 ccs per subject at baseline (mean age: 74.0 years), average WMH volume of 38.4 ± 26.6 ccs per subject on one-year follow-up, and WMH progression rate of 0.86 ± 0.68 ccs/year. At TWH, subjects had an average WMH volume of 19.3 ± 18.4 ccs per subject at baseline (mean age: 72.2 years), average WMH volume of 20.7 ± 20.0 ccs per subject on follow-up one year later, and WMH progression rate of 0.44 ± 0.41 ccs/year. Interestingly at TWH, two subjects had lesions resolve on the follow-up MRI scan with one losing subject 0.28 ccs and the other losing 0.13 ccs of WMH volume.
The average ± SD for each CVR metric in each group is summarized in Table 7-1. In summary, Friedman test demonstrated a significant difference of all CVR metrics (CVR, ssCVR, and τ) in grey matter compared to other groups, namely NAWM, pre-existing WMH, and NAWM destined to be WMH (P < 0.001). All CVR metrics were significantly different when comparing the entire NAWM to all other groups. Interestingly, when comparing 1) NAWM that is destined to be WMH on follow-up to 2) stable NAWM, Friedman test demonstrated that the former had significantly lower CVR (0.04 ± 0.04 vs. 0.12 ± 0.04 %BOLD/mmHg, P < 0.01) and ssCVR (0.086 ± 0.076 vs. 0.172 ± 0.056 %BOLD/mmHg, P < 0.01) as well as significantly longer τ values (46.02 ± 15.86 vs. 35.62 ± 9.30 seconds; P < 0.01 using repeated measures one-way ANOVA).

The focus of this study, τ, was found to be longer in pre-existing WMH compared to stable NAWM (50.25 ± 11.52 vs. 35.62 ± 9.30 seconds; P < 0.01 using repeated measures one-way ANOVA). However, when using ROIs derived considering the influence of spatial location on these metrics, these differences are reduced but significance is still maintained when comparing pre-existing WMH to contralateral NAWM (36.95 ± 7.59 vs. 34.72 ± 8.01 seconds; P < 0.001, two-tailed paired t-test) (Table 7-2). Differences in CVR metrics between pre-existing WMH and stable NAWM are illustrated in Figure 7-3 and Figure 7-4.

**Table 7-1. Comparison of CVR MRI metrics in regions of grey matter, normal-appearing white matter, and future leukoaraiosis.**

<table>
<thead>
<tr>
<th></th>
<th>Grey Matter</th>
<th>Normal-appearing White Matter</th>
<th>Pre-existing WMH</th>
<th>NAWM destined to be WMH (measurements on baseline scan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR [%BOLD/mmHg]</td>
<td>0.26 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.05 ± 0.03</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>ssCVR [%BOLD/mmHg]</td>
<td>0.32 ± 0.07</td>
<td>0.17 ± 0.06</td>
<td>0.11 ± 0.06</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td>Tau [s]</td>
<td>23.50 ± 7.47</td>
<td>35.62 ± 9.30</td>
<td>50.25 ± 11.52</td>
<td>46.02 ± 15.86</td>
</tr>
</tbody>
</table>
CVR and ssCVR comparisons were made using Friedman test with Dunn’s correction for multiple comparison while comparisons involving Tau were made using repeated measures one-way ANOVA with Bonferroni correction. * denotes significance against grey matter (P<0.01); + denotes significance with pre-existing and future leukoaaraiosis (P<0.01). No significant differences were observed between pre-existing WMH and NAWM destined to be WMHs.

Table 7-2. Comparison of average values for CVR MRI metrics in pre-existing lesions and contralateral homologous regions.

<table>
<thead>
<tr>
<th></th>
<th>Pre-existing Lesions</th>
<th>Contralateral NAWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR [%) BOLD/mmHg</td>
<td>0.05± 0.03</td>
<td>0.10 ± 0.04 *</td>
</tr>
<tr>
<td>ssCVR [%) BOLD/mmHg</td>
<td>0.11 ± 0.06</td>
<td>0.18 ± 0.06 *</td>
</tr>
<tr>
<td>(\tau) [s]</td>
<td>36.95 ± 7.59</td>
<td>34.72 ± 8.01 *</td>
</tr>
</tbody>
</table>

* denotes significance against pre-existing lesions (P<0.001) using Wilcoxon matched-pairs signed ranks test for CVR and ssCVR and two-tailed paired t-test for \(\tau\).
Figure 7-3. Comparison of CVR metrics in regions of WMH compared to NAWM. Differences in CVR metrics between WMH and NAWM may be due to spatial location. Results with the confounding spatial factor removed are also presented (grey boxes) and ROIs are derived from Figure 7-2(G). Values are given as % change from contralateral NAWM. All values are assessed on baseline MRI scans. CVR and ssCVR are significantly lower and tau is significantly higher in WMH compared to NAWM. * denotes significance compared to NAWM (P<0.01). Bars indicate minimum and maximum, boxes indicate the interquartile range (25th to 75th percentile) and the line within each box indicates the median.

Figure 7-4. Comparison of CVR metrics in regions of pre-existing and future leukoaraiosis. All Values are given as % change from contralateral NAWM and assessed on baseline MRI scans. Values for future leukoaraiosis are made in the NAWM on the baseline scan (before the progression into overt WMH). CVR and ssCVR are significantly lower and tau is significantly higher in pre-existing and future leukoaraiosis compared to NAWM. * denotes significance compared to NAWM (P<0.01). Bars indicate minimum and maximum, boxes
indicate the interquartile range (25\textsuperscript{th} to 75\textsuperscript{th} percentile) and the line within each box indicates the median.

7.5 Discussion

Our results demonstrate abnormal vascular responses in leukoaraiosis. We have demonstrated reduced steady-state CVR and CVR, the latter consistent with one other report by Uh et al. (2010) who examined CVR by using the BOLD signal during inhalation of 5\% CO\textsubscript{2} and found that values in WMHs were approximately half of values in NAWM. The CVR measurements assessed by these authors differ from ssCVR that represents the time-independent magnitude of change of the BOLD response to a square-wave change in our vasodilatory stimulus. The strength of this study is the ability to accurately control our vasodilatory stimulus, P\textsubscript{ET}CO\textsubscript{2}, throughout the entire experiment. All subjects had a 10 mmHg change in P\textsubscript{ET}CO\textsubscript{2} from an average baseline of 40 mmHg, demonstrating that our method is consistent between subjects and reproducible between baseline and follow-up scans. Without knowing the precise change in P\textsubscript{ET}CO\textsubscript{2}, CVR measurements may be overinflated (Sobczyk et al., 2014).

Our findings are consistent with chronic hypoperfusion as a potential mechanism driving the progression of WMHs. Unlike the richly collateralized cerebral cortex, the white matter is particularly vulnerable to leukoaraiosis because it is perfused by long penetrating medullary endarteries with poor collateralization. This vascular architecture in the white matter provides little protection from the ischemic effects during competition of blood flow from vasodilatory demands (G. C. Roman, 1987). Our findings of reduced vascular reactivity may be used to explain some of the conflicting evidence for hypoperfusion as a mechanism for the pathogenesis of WMHs. Several studies have demonstrated reduced CBF in leukoaraiosis (Fazekas et al., 1988; Ishii, Nishihara, & Imamura, 1986) but it is unclear whether such hypoperfusion is causative of WMHs or occurs as a secondary response to the reduced metabolic activity of leukoaraiotic tissue. Therefore, our study demonstrated CVR reductions in leukoaraiotic regions by using hypercapnea as a vascular-specific vasodilatory stimulus. The CVR in the NAWM in individuals with leukoaraiosis was shown to be reduced compared to controls without leukoaraiosis (Uh et al., 2010). We have also shown reduced CVR in NAWM that is prone to developing WMH compared to NAWM that remains stable over time. This suggests
that our finding of a reduced steady-state CVR in leukoaraiosis is a factor that the cascade of events that eventually leads to WMH development.

We have demonstrated that the speed of the vascular response in leukoaraiosis and in NAWM that is prone to developing into WMH is slower than in NAWM that will remain stable over time. This physiological finding is consistent with observations in the penumbra of large infarcts, which represent a state of hypoperfusion (Englund, 2002). The hypoperfusion seen in the penumbra of large infarcts also demonstrate time-dependent metrics, such as mean transit time, time-to-peak of the tissue-concentration curve, and time-to-peak of the deconvolved tissue residue function (Campbell et al., 2012; Srinivasan, Goyal, Al Azri, & Lum, 2006). Reports of prolonged $\tau$ have also been made in patients with unilateral steno-occlusive disease (Poublanc et al., 2015). These authors found that $\tau$ was longer in the hemisphere ipsilateral to the stenosis/occlusion and normal in the contralateral hemodynamically unaffected hemisphere. This implies that hemodynamically impaired areas demonstrate a longer vascular response to a vasodilatory challenge. Our results are consistent as we have shown prolonged $\tau$ in regions with reduced CVR; namely the in pre-existing WMHs and in NAWM that is destined to becoming WMHs. This is the first report demonstrating that speed of the vascular response is slower in white matter that is susceptible to developing injury. These results are consistent with the notion that chronic low-grade ischemia has a role in leukoaraiosis progression.

It is important to note that our time course reflects the speed of the vascular response to reach maximal vasodilation and represents longer values than published reports on the time course to induce vasodilation. Previous studies demonstrated that the time courses of the cerebral pial vessels to respond to changes in pH are relative rapid, with changes in diameter occurring within approximately 10 seconds. Early studies in humans (Shapiro, Wasserman, & Patterson, 1965) following CO2 inhalation demonstrated that the CBF response started within 30 seconds and that 2 minutes were required to reach peak values (Ellingsen, Hauge, Nicolaysen, Thoresen, & Walloe, 1987). A study of TCD and end-tidal gas manipulations demonstrated that the CBF response to step changes in CO2 in human was much faster, with a delay of 6 seconds (Poulin, Liang, & Robbins, 1996, 1998).

Previous studies have suggested that differences between leukoaraiosis and NAWM may exist due to regional variability in metabolic demand. One study used 18 F fluoromethane positron emission tomography to demonstrate hypoperfusion in leukoaraiosis (Herholz et al., 1990). Blood flow reductions in leukoaraiosis were the result from the lower metabolic demands
of the cortex rendered electrophysiologically isolated by subjacent zones of degenerated white matter tissue. According to this notion, hypoperfusion may not be involved in the pathophysiology of leukoaraiosis. Also, hemodynamically significant extracranial carotid stenosis has been shown to not correlate with the presence of ipsilateral leukoaraiosis (Fazekas et al., 1988) although WMH progression has been shown to correlate with significant carotid stenosis that advanced to complete occlusion (Ylikoski et al., 1993). To circumvent issues due to spatial location, we compared values using ROIs examining the contralateral homologous region (Figure 7-2). We have shown a reduction in CVR, steady-state CVR, and prolonged tau in WMHs compared to the contralateral homologous NAWM, suggesting that abnormal vascular responses are an etiologic mechanism of leukoaraiosis progression.

Several considerations must be made with our use of the BOLD signal as a surrogate for CBF in the convolution of the actual $P_{ET}CO_2$. First, we have used the BOLD signal as a surrogate measure of CBF. During the abrupt change in $P_{ET}CO_2$, the temporal dynamics between changes in CBF and BOLD responses differ due to the slow recovery of blood volume changes (J. J. Chen & Pike, 2009). However, CBF measured with arterial spin labeling has been demonstrated to be a linear function with BOLD in the $P_{ET}CO_2$ range of 40 to 50 mmHg (Tancredi & Hoge, 2013). We have also shown a highly correlated BOLD MRI signal response to hypercapnea against CBF measurements obtained with arterial spin labeling in patients with steno-occlusive disease (Mandell, Han, Poublanc, Crawley, Stainsby, et al., 2008). Second, changes in the arterial partial pressure of $CO_2$ may influence CMRO$_2$. However, increases in CBF through vasodilation have been shown to produce very little or no increases in CMRO$_2$ (Sokoloff, 1981).

In summary, we have shown that the speed of the vascular response is abnormal in white matter that is prone to developing leukoaraiosis. These areas also demonstrate a decrease in the magnitude of the vasodilatory response. This is the first report to associate dysfunction in neurogliovascular coupling with the progression of leukoaraiosis.
8.1 Summary and Novel Aspects of Findings

This is the first comprehensive study to demonstrate that impaired vasodilatory reserve is associated with white matter pathology and is the first to demonstrate an association between the role of vasodilatory reserve with future evolution of leukoaraiosis. The present work also demonstrates the ability of BOLD MRI to assess cerebrovascular reactivity (CVR) in order to determine areas of the normal-appearing white matter (NAWM) that are susceptible to structural alterations. The main findings of this thesis are:

1) Areas of leukoaraiosis demonstrate reduced responsivity to \( \text{CO}_2 \) and reduced vascular reserve compared to white matter that is normal. These areas will also demonstrate lower cerebral blood flow (CBF) and prolonged time-dependent perfusion metrics such as time-to-maximum and time-to-peak. Areas of leukoaraiosis also demonstrate reduced fractional anisotropy (FA) and increased mean diffusivity (mD) compared to areas of NAWM.

2) Areas with steal physiology in the NAWM will demonstrate the aforementioned abnormalities in DTI and DSC-perfusion metrics compared to areas with a robust vasodilatory reserve.

3) The steady-state change in the magnitude of blood flow in response to a near maximal vasodilatory stimulus is significantly reduced in leukoaraiosis compared to NAWM. Areas of leukoaraiosis also demonstrate prolonged \( \tau \) (speed of response) compared to NAWM, indicating that the speed of the vasculature to respond to vasoactive stimuli is much slower in these territories.

4) Areas of NAWM that are destined to develop leukoaraiosis will have reduced vascular reserve. This demonstrates that the long-term consequence of neurovascular uncoupling is the development of significant white matter pathology.

Taken together, these results demonstrate the pathophysiology that occurs in areas that lose vasodilatory capacity. If the vasodilatory capacity is limited, autoregulatory mechanisms would also be impaired. Normally, cerebral pressure autoregulation acts to maintain CBF relatively constant despite variations in arterial blood pressure. It has an important protective
role against ischemia at low perfusion pressures and the risk of brain edema at higher arterial pressures (Paulson et al., 1990; van Beek et al., 2008; Wagner & Traystman, 1985). The results of this thesis demonstrate that white matter operating at its vasodilatory limits will experience disease.

CVR may be defined as a change in cerebral blood flow (CBF) in response to a vasoactive stimulus, such as the arterial partial pressure of carbon dioxide (PaCO₂). Negative CVR values represent paradoxical reductions in CBF in response to an increase in PaCO₂; this is termed steal physiology. This phenomenon has been shown to be associated with vascular pathology (Conklin et al., 2010; Conklin et al., 2011; Fierstra et al., 2010), increased risk of stroke (H. Markus & Cullinane, 2001), and dementia (Silvestrini et al., 2011). The CVR measurements made in Study I-IV advance our understanding of how the cerebral vasculature in leukoaraiosis fails to control the distribution of blood flow and also characterizes cerebrovascular pathophysiology in leukoaraiosis. The results from Study II add to the consequences of this phenomenon by demonstrating that steal physiology in the white matter is associated with compromised structural integrity and perfusion.

This thesis fills a major gap in the literature concerning the effects of chronic vascular insufficiency on cerebral white matter integrity. Study I and II demonstrated reduced CBF in leukoaraiosis compared to NAWM and also in NAWM with steal physiology compared to areas with robust vasodilatory reserve. This confirms a previous finding from a large study with 628 elderly individuals (mean age = 68.8 years) that found an association between lower CBF and leukoaraiosis as measured by transcranial doppler. It was also found that the progressive reductions in CBF were associated with the extent of leukoaraiosis (Tzourio et al., 2001).

A key question is whether any reduction in perfusion is primary or occurs secondary to white matter pathology. Neurogliovascular coupling is the coupling of the vascular system to the metabolic demands of increasingly active tissue for more blood flow through signaling. Neurogliovascular uncoupling occurs when the flow increase is insufficient to match the metabolic demand of the tissue resulting in tissue damage. One way of addressing this assumption was to measure CBF in NAWM as seen in Study II resting CBF measurements were found to be lower in areas with steal physiology. O’Sullivan et al. (2002) also found that patients with symptomatic lacunar stroke and confluent leukoaraiosis had lower CBF in periventricular NAWM. CBF was reduced to an intermediate level between leukoaraiosis and the white matter of control subjects; however, the CBF in the deep white matter (centrum
semiovale) or gray matter did not differ between patients and controls. Unfortunately a majority of the subjects who participated in this thesis did not consent to having perfusion performed on the follow-up scan and only DSC-perfusion baseline measurements are presented in Study I and II. A longitudinal study examining the association between CBF and the progression of leukoaraiosis is a substudy of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial of statin therapy that investigated 390 individuals with cardiovascular disease including myocardial infarction and stroke or with cardiovascular risk factors. This study used gradient-echo phase contrast to assess flow volume in both the carotid and vertebral arteries. No association was found between CBF and the presence of periventricular, deep, or total leukoaraiosis at baseline (ten Dam et al., 2007). After a 33-month interval, subjects were rescanned and a decline in CBF was not associated with either an increase in total or deep white matter leukoaraiosis but was with periventricular leukoaraiosis. However, this technique in assessing flow measures total CBF and is unable to separate out white matter or regional CBF. Therefore, this study is flawed methodologically in its sensitivity to detect subtle changes in white matter flow. This could explain the lack of association at baseline between CBF and leukoaraiosis. Nevertheless, the structural compromise associated with steal physiology in the NAWM suggests a chronic neurovascular uncoupling syndrome that would promote tissue injury.

8.2 Competition for blood flow in the cerebral white matter

Competition for blood flow occurs during hypercapnic square-wave challenges that are dependent on the resistance of the cerebral vasculature. For example, the MCA gives rise to lenticulostriate arteries shortly after branching from the internal carotid artery, supplying the basal ganglia and internal capsule. At more distal regions, the MCA provides perfusion to the cerebral cortex and subcortical tissues of parieto-temporal regions. As the MCA advances to the surface of the cortex, leptomeningeal arteries penetrate the brain to become long end-arteries with high vascular resistance, supplying the deep white matter. (Faraci & Heistad, 1990) As a consequence, a global hypercapnic vasodilatory PaCO\(_2\) stimulus would result in a redistribution of blood flow between co-dependent vascular territories that is based on their relative vascular resistances (Mandell, Han, Poublanc, Crawley, Kassner, et al., 2008). Therefore, the vascular response to a vasoactive challenge in a particular voxel and competition of blood flow is dependent on the temporal pattern of when each supplying vessel reduces its resistance in each co-dependent vascular territory. Therefore, the white matter is particularly vulnerable to
ischemic damage (because it is supplied by arterioles with high vascular resistance and limited vasodilatory capacity) compared to areas downstream with a robust vasodilatory capacity that can lower their vascular resistance more readily.

The major arteries of the cerebral circulation generate about 30-40% of the total cerebrovascular resistance to flow (Faraci & Heistad, 1990; G. I. McHedlishvili, Mitagvaria, & Ormotsadze, 1973). The pial surface vessels arising from the circle of Willis account for another 30% of cerebrovascular resistance at 120 mmHg intraluminal pressure and are exclusively responsible for autoregulation between 120 to 160 mmHg intraluminal pressure. Therefore, the net perfusion pressure to any vascular territory is a function of the system perfusion pressure minus the reduction in pressure from the vascular resistance of the large cerebral feeding vessels and circle of Willis. If perfusion pressure drops, the resistances of vascular territories can respond to by changing their diameter but this is limited. Therefore, each vascular territory has an autoregulatory reserve. In the face of a global vasodilatory stimulus, it is possible for the CBF capacity to exceed blood supply due to the high vascular resistance of the major feeding vessels and robust vasodilatory mechanisms. Because of this, co-dependent vascular territories will compete for a limited flow of blood. During large hypercapnic challenges (such as the square-wave 10 mmHg changes in $P_{ET\text{CO}_2}$ used for Study I-IV), flow will redistribute to vascular territories with greater vasodilatory reserve from areas with less reserve. This forms the physiological basis for steal physiology (Sobczyk et al., 2014).

During a global vasodilatory challenge, the distribution of blood flow depends on three interacting factors (Sobczyk et al., 2014): 1) the resistance of the feeding vessel 2) the relative regional autoregulatory reserve and 3) the magnitude of the stimulus. Some vessels with reduced CVR may be capable of reducing their resistance sufficiently to increase blood flow at small increases in $PaCO_2$; however, as $PaCO_2$ increases, blood flow is redistributed to vascular beds with more robust vasodilation. The inter-dependence of the reduction in flow (in territories with reduced vascular reserve) on the increase in flow in territories with robust vascular reserve is supported by their simultaneous occurrence, despite having delayed circulation and arrival of $PaCO_2$ changes to the territory with reduced reserve (Poublanc et al., 2013; Sobczyk et al., 2014). The fact that steal physiology exists in elderly subjects with age-related leukoaraiosis demonstrates the absence of global vascular pathology. Absence of CVR would occur if blood vessels throughout the brain respond uniformly, even if the response is abnormal. Also, steal physiology requires perfusion of vascular beds to be dependent on each other, i.e. co-dependent
with other vascular territories for a limited supply for blood flow.

8.3 Haemodynamic Implications of the findings

This work is consistent with the notion that leukoaraiosis is induced primarily by a chronic low-grade ischaemia caused by mechanisms of cerebral hypoperfusion and impaired vascular reactivity. These mechanisms may stem from age-related structural changes of the parenchymal small vessel walls such as tunica media thickening and hyaline degeneration (Pantoni, 2010). Both of these mechanisms are particularly harmful, especially in the white matter as blood is supplied by end-arteries with limited collateral support. Study II demonstrated that steal physiology in the NAWM was associated with subtle impairments in the structural integrity of the cerebral white matter. This is consistent with several studies using PET and MRI to show hypoperfusion not only in leukoaraiosis, but also in the NAWM of elderly individuals (O'Sullivan et al., 2002). To our knowledge, this is the first report demonstrating an association between steal physiology and structural changes in NAWM in elderly individuals.

The results of this thesis have implications in functional hyperaemia and neurovascular coupling. Functional hyperaemia describes the increase in blood flow and oxygen consumption that accompanies neural/glial activation. Study III suggests that when disease of the small parenchymal vessels is present, this normal neurovascular relationship can become uncoupled in areas with reduced vasodilatory reserve. When this happens, diminished blood flow augmentation occurs in response to neural activation, leading to leukoaraiosis. Study III demonstrates that areas of NAWM that will develop leukoaraiosis have neurovascular uncoupling. Chronic neurovascular uncoupling syndrome is a term used to describe a condition in which areas of the brain are exposed to long-term haemodynamic insufficiency during neural activation (Mikulis, 2013). Unlike the infarct core of acute ischaemia, damage to brain areas may occur over a longer time-scale secondary to repeated stress from insufficient or lack of blood flow increases in response to increased metabolic activity.

Cerebral ischemia is thought to be an important mechanism in cerebral small vessel disease and leukoaraiosis. The results of this thesis conform with studies demonstrating that leukoaraiosis may be a result of ischemic changes in the territory of the perforating artery (Conklin et al., 2014). Study III demonstrated that areas in the white matter that are particularly vulnerable to developing leukoaraiosis would have reduced vascular reserve. These areas are prone to dysfunction in neurovascular coupling and leads to future development of
leukoaraiosis, suggesting that chronic low-grade ischemia occurring in the distal territories of perforating arteries results in changes to the cerebral white matter. Our results are consistent with the idea that regions with the highest vascular resistance experience hypoperfusion during competition for blood supply. The spatial pattern of leukoaraiosis demonstrates changes first in brain regions furthest away from the origin of the major feeding vessels. These vessels supplying the deep white matter experience the highest vascular resistance and drawing on vascular reserve baseline. Any demands for blood flow would therefore subject these territories to hypoperfusion as redistribution of flow moves towards vascular territories with robust vasodilatory reserves.

CVR assessed in Studies I, II, and III represents an estimate of the magnitude of the cerebrovascular response and do not indicate the time course of the response. The haemodynamics that go into CVR measurements are complex and may reflect the dynamic interaction of the vasodilatory CO$_2$ stimulus with mechanisms of pressure autoregulation as well as the dynamic redistribution of blood flow between various areas of the microvasculature (Blockley, Driver, Francis, Fisher, & Gowland, 2011; Regan, Duffin, & Fisher, 2013). The vessels supplying the white matter are complicated and involve groups of long medullary end-arteries that dynamically interact with well-collateralized vascular networks, such as the pial vessels supplying the cortical grey matter. Vessels also differ in their response to PaCO$_2$ with respect to magnitude and speed of response. Our results from Study I demonstrating the difference in MRI metrics in WMH compared to NAWM are consistent with previous MRI and PET studies showing that the CVR of white matter is delayed and about one-third of the amplitude of grey matter (Prisman et al., 2008; Rostrup et al., 2000).

8.4 What causes CVR to be lower in elderly individuals with leukoaraiosis?

The results demonstrated in this thesis are consistent with the hypothesis that white matter damage is induced primarily by chronic hypoperfusion in areas with reduced flow response to CO$_2$ in which there is a redistribution of blood flow to areas with healthy vasodilatory reserve. What causes the reduction in sensitivity to CO$_2$? One potential factor that could be driving these changes is endothelial dysfunction by mechanisms of steno-occlusive disease of the small parenchymal vessels, blood-brain barrier leakage, and apoptosis of oligodendrocytes.

Cerebral arteriolosclerosis may develop with age (Xiong & Mok, 2011). Elements of arteriolosclerosis include microatheroma, lipohyalinosis, and fibrinoid necrosis. Microatheroma
describes the formation of atherosclerotic plaque in the parent artery from which the penetrating artery emanates and is characterized by subintimal proliferation of fibroblasts, lipid-laden macrophages, and cholesterol. These changes could induce microturbulent flow (See section 1.3.1) and impair nutrient exchange through the blood-brain-barrier, leading to structural alterations in the white matter. Lipohyalinosis involves the deposition of atheromatous lipids and hyalinization of the vessel wall. These changes may reduce the elasticity of the vessel and impair vascular reactivity. If vascular reactivity is impaired, neurovascular uncoupling would occur, which would lead to structural changes and leukoaraiosis. Fibrinoid necrosis involves a combination of necrotic smooth muscle cells and extravasated proteins in the tunica media of intraparenchymal vessels. This would impair functional hyperaemia, pressure autoregulation, and mechanisms of vasoconstriction and/or vasodilation with uncoupling of the neurovascular unit. With vasodilatation exposure of the venules and veins to higher pressures may occur leading to venous collagenosis. Collectively, all of these changes would theoretically act synergistically to develop leukoaraiosis.

Impaired neurovascular coupling and reduced vasodilatory reserves could be a result of endothelial dysfunction (Knottnerus et al., 2009). Mechanical factors such as hypertension results in damaged endothelium, which allows plasma proteins to leak into the vessel wall. Also, high arterial pulsations stemming from hypertension causes mechanical damage to the veins and periventricular venous collagenosis. This leads to intramural thickening, narrowing of the venous lumen, reduced blood flow, exacerbating ischemic conditions (Topakian et al., 2010). Chronic ischaemia and impaired vasodilatory reserve may be aggravating factors leading to white matter tissue damage and development of leukoaraiosis.

8.5 Thesis strengths and limitations

It is important to note that our vasodilatory stimulus is standardized and reproducible. All subjects experienced a change in PaCO$_2$ from an average baseline of 40 mmHg to 50 mmHg. Small changes in PaCO$_2$ result in much larger CVR values compared to a greater change in PaCO$_2$. Greater changes in PaCO$_2$ are more sensitive in identifying reductions of CVR. Without a large supramaximal stimulus, the global CBF demand may not exceed the supply capacity; therefore, redistribution of blood flow in vascular territories may not be apparent. Therefore, our use of a computerized gas-blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) enables us to have a reproducible stimulus that we can use for longitudinal assessment of changes in CVR. This is not possible with other methods of controlling
vasoactive stimuli.

One limitation of this study was that a full range of PaCO$_2$ was not assessed. Sobczyk et al. (2014) have demonstrated that with smaller changes in PaCO$_2$, there is a wider range of CVR values. With a greater CO$_2$ stimulus, the number of voxels that can retain a robust positive or negative response decreases. This can be attributed to the peculiar method of calculating CVR while considering the change in PaCO$_2$ as the denominator. The relationship between PaCO$_2$ and CBF is sigmoidal, with the midpoint of the sigmoid falling close the resting PaCO$_2$ (generally 40 mmHg) and approaches a plateau near 50 mmHg; therefore, CVR values become progressive smaller as the calculation of CVR incorporates non-linear values from the plateau of the sigmoid (PaCO$_2$ of 50 mmHg). To avoid this confound, a ramp stimulus can be employed to segment portions of the PaCO$_2$ stimulus providing a better understanding of the underlying physiology. For example, it is possible to compare the change in flow for a change in PaCO$_2$ stimulus of 30-40 mmHg compared to a change in PaCO$_2$ of 45-55 mmHg. Therefore, it is possible to categorize the response of vessels that is dependent on the full range of PaCO$_2$ (e.g. 25-60 mmHg).

With a ramp stimulus, the flow for a full range of PaCO$_2$ is known. If mean arterial pressure is also known and intracranial pressure or backpressure is not limiting, a resistance map can be calculated for the brain. Knowing the resistance of each brain area enables better understanding of the underlying physiology. The relationship between resistance and PaCO$_2$ can be characterized as sigmoidal. Therefore, as PaCO$_2$ increases, vessels will vasodilate and lower their vascular resistance. However, the midpoint of this sigmoidal relationship differs between co-dependent vascular territories. For example, if we consider two territories – one with leukoaraiosis and the other with NAWM – we can divide the PaCO$_2$ range into 20-40 mmHg and 40-60 mmHg and see how each territory competes with one another for flow. For a PaCO$_2$ ranging from 20-40 mmHg, the territory with NAWM may be first to lower its vascular resistance and increase flow, causing a redistribution of flow to this area from areas of leukoaraiosis. For PaCO$_2$ ranging from 40-60 mmHg, areas of leukoaraiosis may display a reduction of vascular resistance at 50 mmHg in which there are further reductions in flow as these areas have used up their vasodilatory capacity. Therefore, applying a ramp stimulus that captures flow information from a full range of PaCO$_2$ will yield more information about vessel physiology.

Another limitation of this thesis is the inability to corroborate our findings with healthy
age-matched control subjects without significant leukoaraiosis. With a normal reference atlas of healthy age-matched controls, it is possible to develop an objective scoring assessment of abnormality within the normal-appearing white matter. The creation of a Z-map of CVR would enhance our understanding of the extent and distribution of pathophysiology than looking at individual CVR maps alone. We have also not corroborated our findings with neuropsychology. The only cognitive test performed was the Montreal Cognitive Assessment (MoCA), providing a very weak cognitive profile of each subject. Therefore, it was not possible to examine the association between MRI metrics and cognition.

A limitation in the longitudinal studies was that no distinction was made as to whether lesions identified on the follow-up scan were either de novo or part of baseline lesion growth. New lesions were identified visually and not with the use of the semi-automated Lesion Explorer pipeline due to technical limitations.

8.6 Future Studies

In Study IV, $\tau$ was assessed in each voxel to provide an estimate of the time course of the cerebrovascular response to P$_{ETCO_2}$. Another analysis to assess response time courses is to perform a transfer function analysis (TFA) of the BOLD response to P$_{ETCO_2}$, which measures the magnitude of the response (gain), speed of the response (phase), and fidelity with which the response follows the stimulus (coherence) (Duffin et al., 2015). In TFA analysis, the P$_{ETCO_2}$ and BOLD signals are time aligned during the pre-processing stage so that phase differences primarily reflect the speed of the vascular response. Subsequently, the stimulus is convolved with a mono-exponential “dispersion” function and the time constant altered to provide the best fit with the BOLD response. Performing a TFA analysis would corroborate our CVR and $\tau$ results, thereby confirming cerebrovascular pathophysiology and provide insight into the dynamics of cerebral blood flow control.

Cognitive impairment is associated with age-related leukoaraiosis (E. E. Smith et al., 2004; Soderlund et al., 2003; van Dijk et al., 2008; Verdelho et al., 2010). Future work should include a full neuropsychological assessment as few longitudinal studies of cognition and leukoaraiosis have been conducted. Cross sectional studies have shown that the cognitive profile of subjects with small vessel disease is characterized by impairments of processing speed and executive function with relative sparing of episodic memory (Lawrence et al., 2013; Nitkunan et al., 2008). These neuropsychological deficits are associated with poor functional outcome, such as a reduction in instrumental activities of daily living (Mok et al., 2004).
Longitudinal reports of how the cognitive profile of subjects with leukoaraiosis changes over time are sparse. This information is important for providing prognosis to individuals with moderate-severe leukoaraiosis, monitoring progression of small vessel disease, and is important for planning treatment. Little is known about the rate of cognitive changes in this population and identifying neuropsychological tasks, which provide the most sensitive and relative measures of cognitive change. It is difficult to study the cognitive profiles of elderly individuals with leukoaraiosis as many who present to memory clinics may also have coexisting pathology, such as Alzheimer’s disease.

Diffusion tensor imaging (DTI) is a MRI technique that measures the mobility of water in biological tissues at the molecular level. Quantitative parameters offered by this technique include mD, which indicates the diffusivity of water molecules in tissues as well as FA, which quantifies the degree of anisotropy in a particular region. However, DTI also allows for reconstruction of cerebral nerve fibre tracts through a method called fibre tractography. A small study assessed the white matter tracts crossing NAWM and leukoaraiosis and found greater discontinuity in fibres crossing the latter (MohdTaib, Abdullah, Shuaib, Magosso, & Mat). Future work could assess the degree of discontinuity of white matter fibres crossing leukoaraiotic regions and find a possible association with neuropsychological measures.

Neurodegenerative and vascular changes are the two most prevalent types of dysfunction in the elderly brain. Outstanding questions that still need answers include whether these two types of pathology interact synergistically, whether they are independent or co-occurring processes, whether one pathology triggers causality in the other. Previous studies also report age-related changes of global morphometric properties including decline in total brain weight and cortical thinning that is particularly accelerated in the sixth and seventh decades (Raz et al., 1997; Salat et al., 2004). Future work could also assess the degree of discontinuity of leukoaraiotic fibres with cortical thinning. However, cortical thickness measurements were obtained in the 75 subjects from TWH and SHSC (Appendix Table 1) in the left and right hemisphere and did not correlate with the severity of leukoaraiosis in each hemisphere (data not shown). This finding confirms the fact that leukoaraiosis is not secondary to neurodegeneration.

A number of animal studies should be performed that could elucidate the mechanisms of reduced sensitivity to CO$_2$ by examining endothelial dysfunction. This may be a consequence of oxidative stress or inflammatory mechanisms that promote endothelial dysfunction (M. Blanco, Rodriguez-Yanez, Sobrino, Leira, & Castillo, 2005; Szmitko et al., 2003). Oxidized low-density
lipoproteins, hypertension, smoking, diabetes, and hyperhomocysteinemia have pro-
oxidative effects and increase the production of reactivity oxygen species (M. Blanco et al.,
2005). As a consequence of these oxidative stresses, various types of adhesion molecules are
released by the endothelium, recruiting leukocytes. When this happens, platelets will activate
various inflammatory molecules, such as interleukin-1β, thromboxane A2, and CD40L, which
promote leukocyte adhesion. Secretion of matrix metalloproteases, such as MMP-9, occurs
during the inflammatory process, which causes deterioration of the extracellular matrix and
permit the migration of leukocytes through the endothelium (M. Blanco et al., 2005; Szmitko et
al., 2003). If BOLD MRI CVR is performed in animals, staining for these oxidative and
inflammatory markers in animals would confirm endothelial dysfunction in the reduced
sensitivity to CO₂. It is also possible to perform in-vivo imaging of the leptomeningeal arteries
of animals and examine their CBF responses to CO₂. Vessels demonstrating less reactivity could
then be isolated and stained for hyaline degeneration or oxidative markers to confirm
endothelial dysfunction.

Once a biological basis for leukoaraiosis is established, there is also the need for
therapeutic interventions. Increased arterial pulsations due to hypertension may result in
periventricular venous collagenosis, which could further exacerbate hypoperfusion to the
cerebral white matter. Treatment of hypertension in older individuals has been shown to
improve blood pressure levels and restore blood pressure regulation (Lipsitz et al., 2005).
Future therapeutic studies should examine cerebrovascular reactivity before and after treatment
with anti-hypertensives. In the recent SPRINT study, 9361 patients with a systolic blood
pressure of 130 mmHg or higher and increased cardiovascular risk (but without diabetes) were
randomly assigned to a target systolic blood pressure of less than 120 mmHg (intensive
treatment) or less than 140 mmHg (standard treatment). There was a quarter reduction in
vascular events and in overall mortality in the group with intensive treatment compared to the
standard treated group ("A Randomized Trial of Intensive versus Standard Blood-Pressure
Control," 2015). Interestingly, although there were more side effects with intensive treatment,
orthostatic hypotension occurred less frequently in the intensively treated group. This result
demonstrates an effective dynamic autoregulation in which pressure fluctuations are counter-
regulated. Whether restoration of cerebrovascular reactivity occurs, remains to be elucidated.
Arterial stiffness is an independent predictor of cardiovascular morbidity and mortality in
patients with hypertension (Koumaras et al., 2012). Cerebral arterial stiffness occurs with age
due to hyaline sclerosis, whether anti-hypertensives have a destiffening effect on cerebral pulsatile haemodynamics also remains a promising field of research.

8.7 Conclusions

This thesis is the first to associate reductions in vasodilatory reserve with subsequent structural changes in the cerebral white matter. The inability to augment blood flow because of reduced vascular reserve during neuro/glial activation leads to neurovascular uncoupling and impaired pressure autoregulation. The results suggest that impaired vasodilatory reserve is an important factor in white matter disease and contributing to its progression. The result of this thesis validate the use of BOLD MRI CVR in detecting white matter alterations, which may lead to a vascular form of cognitive impairment. It is important to recognize, treat, and prevent vascular cognitive impairment as subclinical “silent” strokes occur five times more frequently than clinically “overt” strokes (V. Hachinski, 2008). These white matter changes are associated with increased risk of subsequent overt stroke, dementia, and cognitive decline. Therefore, this thesis adds to our understanding of the long-term consequences of chronic neurovascular uncoupling and the use of BOLD MRI CVR as a biomarker in identifying areas that are prone to structural changes. Future work should study the effects of therapeutic interventions on stabilizing or even reversing the progression of white matter disease.
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Appendix 1. Recruitment Letter

2008-11-10

Dr. David Mikulis,
Department of Radiology,
University Health Network.

Dear David,

I am supportive of the grant application by Mikulis et al. entitled “Impairment of Vascular Autoregulation in the Cerebral White Matter Predissus Future Leukkeniosis” and agree to make the Respiract™ system available to him and his research collaborators to study their subjects.

The installation of the Respiract™ system in our neurological research facility at Sunnybrook Health Sciences Centre should be complete by end of January 2009.

We ask that the investigators reimburse us for costs that we estimate at $100.00 per patient.

Leodante da Costa and I are looking forward to our collaboration on this and other projects using the Respiract™ system.

Best regards,

Michael Schwartz
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title
Impairment of Vascular Autoregulation in the Cerebral White Matter Predicts Future Leukoaraiosis

Investigator
Dr. David J. Mikulis
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Dr. Leo Da Costa
Dr. Joe Fisher
Dr. Adrian Crawley
Dr. Andrea Kassner

Funded by
GE Healthcare and Canadian Stroke Network

Study Coordinator:
Yang Sun MSc, CCRP
Introduction

You are being asked to take part in a research study. Please read this explanation about the study and its risks and benefits before you decide if you would like to take part. You should take as much time as you need to make your decision. You should ask the study doctor or study staff to explain anything that you do not understand and make sure that all of your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish. Participation in this study is voluntary.

Background and Purpose

You are invited to take part in this study because you have had an ischemic event (stroke or transient ischemic attack) involving white matter three or more months ago and you are over the age of 50.

The cerebral white matter contains millions of fibers that connect different areas of the brain with each other. It represents the essential networking architecture ("wiring") required to support complex information processing. Blood vessels supplying nutrition to the white matter can become diseased because of smoking, high blood pressure, and high cholesterol and that can lead to serious narrowing. Blood flow to the white matter then drops and the fibers become injured.

The overall result over time is a loss in the brain’s ability to carry out complex information processing resulting in a loss in mental ability.

This research will study the blood flow control mechanism to the white matter. The control mechanism, which can be thought of as valves in the smallest blood vessels, can adapt to narrowing of the larger feeding blood vessels by opening up. It can return blood flow to normal. However, this adaption mechanism has limits, and if the feeding vessels narrow even more, the valves can no longer adapt because they are already wide open. This leads to poor blood flow and injury to the white matter fibers. That we can see on MRI.

Proposed imaging is not standard of care. Research imaging is additional. The recruited patients have no change in their standard of care, which consists of follow-up in the stroke clinic according to the neurologist’s clinical practice guidelines

The purpose of this study is to identify that part of the brain’s white matter that is being supplied by vessels with valves that are already wide open and to see the effect on white matter fibers a year later.
The method for studying how wide open the “valves” are uses carbon dioxide. This is the gas that you normally breathe out at the end of each breath. It turns out that small excess amounts of this gas cause the valves to open up if they are not already fully open. Using Magnetic Resonance Imaging (MRI), we can map the increase in blood flow caused by the opening valves, and can compare it with the lack of increased blood flow in areas where valves are already fully open.

Study Design, Study Visits and Procedures

In total, 100 participants from two hospitals in Canada will participate in this study. About 50 will come from the Toronto Western Hospital/University Health Network.

If you agree to be in the study, you will have two study visits. Each visit will be approximately 60 to 90 minutes in duration.

Visit 1: We will check your health and medical history to see whether you can be in the study and receive MRI. If you can, then you will have MRI and complete a test of ‘Montreal Cognitive Assessment (MoCA)’, and tests of ‘Trail-making test A and B’.

MRI
You will be lying on your back in the MRI machine. During the MRI scan you will receive increased amounts of carbon dioxide by re-breathing some of the carbon dioxide you exhale during each breath. You will have a plastic clip blocking your nose and you will breathe through a mouthpiece connected to tubing with a bag on the end. The breathing device (an investigational device, approved by Health Canada for research purpose) uses a higher amount of oxygen than you normally breath (30% versus 21%), and adjusts the amount of carbon dioxide you breath in. The changes in carbon dioxide blood levels during this test will be the same as those you experience during every day living.

Oxygen, sometimes with a small amount of carbon dioxide mixed in, will flow into the bag. The flow into the bag will increase for two minutes and then decrease for 2 minutes. This will go on for 9 minutes. You will be asked to always breathe hard enough to keep the bag empty. During this 9 minutes there will be loud clicking noises indicating that the MRI machine is taking pictures of your brain. Your head will be held motionless by a soft pillow, which will decrease the noise from the MRI machine to comfortable levels.

In addition, you will be asked to have a gadolinium injection to measure brain blood flow. This injection is the same as an injection you may already have had previously for assessment of the blood vessels in your neck.
Montreal Cognitive Assessment (MoCA).
The Montreal Cognitive Assessment (MoCA) will ask you questions to score the level of complex information processing that you are able to achieve. It will take approximately 10 minutes. Please inform us of your first language (specify: ___________________________), the MoCA questionnaire will then be provided in your first language.

Trail-Making Test A and Trail-Making Test B

Trail-Making Test A and B are tests of visual attention and task switching. You will be instructed to connect a set of 25 dots as fast as possible while still maintaining accuracy. These tests will provide information about your visual search speed, scanning, speed of processing, mental flexibility, as well as executive functioning. Both tests will take about 10 minutes to complete.

Visit 2: Twelve (12) months after your first study visit you will have a follow up MRI and answer another MoCA, and Trail-making tests A and B.

You will be in this study for approximately 12 months.

Reminders

It is important to remember the following things during this study:

- Ask your study team about anything that worries you
- Tell study staff anything about your health that has changed
- Tell your study team if you change your mind about being in this study
- Please call the study doctor if you have any side effects even if you do not think it has anything to do with this study

Risks Related to Being in the Study

This study has risks. Some of these risks we know about. There is also a possibility of risks that we do not know about and have not been seen in study subjects to date. Some can be managed.
The MRI scanning is identical to that used for normal diagnostic tests. There is no radiation as in X-rays or CT scans. Except for the noise, it is painless and safe. Some people may feel a little “closed-in” the MRI machine but you will be able to speak with someone at all times and can stop the test at any time.

Gadolinium is a contrast agent used in MRI that helps to increase the visibility of abnormal tissues. It has been in routine use for over 20 years, and has one of the highest safety profiles of any administered contrast agent or drug as long as your kidney function is not severely impaired. If you have or have had a problem with your kidneys, please inform us of this problem before proceeding any further.

The changes in breathing have been chosen to be in the range of that expected in most people in the course of normal living. If you have not noticed any brain-related symptoms related to breathing as part of your normal life, it is unlikely that you will have any during the test.

During parts of the test you will be asked to breathe harder than you would normally. You may feel slightly light-headed. Therefore, before the test we will do a “dry run” to find a comfortable breathing level for you. Nevertheless, if during the test you feel uncomfortable in any way, you can press a button alerting the MRI technologist that you wish to end the test.

Very rapid breathing can cause seizures in patients with epilepsy. If you have epilepsy, we will not include you in this study.

Benefits to Being in the Study

This research may not benefit you personally, but could eventually lead to improved methods for diagnosing and treating patients with diseases of brain blood vessels.

Voluntary Participation

Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now and then change your mind later. You may leave the study at any time
without affecting your care. You may refuse to answer any question you do not want to answer, or not answer an interview question by saying “pass”.

We will give you new information that is learned during the study that might affect your decision to stay in the study.

**Confidentiality**

**Personal Health Information**

If you agree to join this study, the study doctor and his/her study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify you and includes your:

- name
- address
- date of birth
- new or existing medical records, that includes types, dates and results of medical tests or procedures.

Only the study team or the people or groups listed below will be allowed to look at your records. Your participation in this study also may be recorded in your medical record at this hospital.

The following people may come to the hospital to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines:

- The study sponsor or its representatives/partner companies.
- Representatives of the University Health Network Research Ethics Board.
- Other regulatory bodies (such as the Canadian Stroke Network).

**Study Information that Does Not Identify You**

Any information about you that is sent out of the hospital will have a unique study code and will not show your name or address, or any information that directly identifies you.

All information collected during this study, including your personal health information and will only be released if required by law. De-identified information, meaning information identified with a study number only without any names, will be shared with the study sponsor, GE Healthcare.
You will not be named in any reports, publications, or presentations that may come from this study.

If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

In Case You Are Harmed in the Study

If you become ill, injured or harmed as a result of taking part in this study, you will receive care. The reasonable costs of such care will be covered for any injury, illness or harm that is directly a result of being in this study. In no way does signing this consent form waive your legal rights nor does it relieve the investigators, sponsors or involved institutions from their legal and professional responsibilities. You do not give up any of your legal rights by signing this consent form.

Expenses Associated with Participating in the Study

You will not have to pay for any of the tests, examinations or medical care required as part of this study.

You will not be paid for the study participation; however, you will be reimbursed for the parking, meal, and travel expenses for all of your completed hospital visits related to the study @ $50.00 per visit.

You must inform the researchers of any metal objects in your body before testing:

Before the MRI exam, the MRI technologist will ask you if there is metal, or a metallic device in your body, in order to be sure that it is safe to put you into the MRI scanner.

Questions About the Study

If you have any questions, concerns or would like to speak to the study team for any reason, please call Yang Sun at 416-603-5008 ext. 6586.

If you have any questions about your rights as a research participant or have concerns about this study, call the Chair of the University Health Network Research Ethics Board (REB) or the Research Ethics office number at 416-581-7849. The REB is a group of people
who oversee the ethical conduct of research studies. These people are not part of the study team. Everything that you discuss will be kept confidential.

Consent

This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to take part in this study.

_________________________  ___________________  ________
Print Study Participant’s Name  Signature  Date

(You will be given a signed copy of this consent form)

My signature means that I have explained the study to the participant named above. I have answered all questions.

_________________________  ___________________  ________
Print Name of Person Obtaining Consent  Signature  Date

Was the participant assisted during the consent process?  □ YES  □ NO
If YES, please check the relevant box and complete the signature space below:

□ The person signing below acted as a translator for the participant during the consent process and attests that the study as set out in this form was accurately translated and has had any questions answered.

_________________________  ___________________  ________
The consent form was read to the participant. The person signing below attests that the study as set out in this form was accurately explained to, and has had any questions answered.

Relationship to Participant

Language

Print Name of Witness

Signature

Date

Relationship to Participant
Appendix 3. Montreal Cognitive Assessment

Montreal Cognitive Assessment (MoCA)
Version 7.1 Original Version

Visual-Spatial/Executive

- Copy cube
- Draw clock: Ten past eleven (2 points)

Naming

- Contour: [ ]
- Numbers: [ ]
- Hands: [ ]

Memory

- Read list of words; subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.
- 1st trial: [ ]
- 2nd trial: [ ]

Attention

- Read list of digits (1 digit/sec.)
- Subject has to repeat them in the forward order: [ ] 2 1 8 5 4
- Subject has to repeat them in the backward order: [ ] 7 4 2

Language

- Read list of letters, the subject must tap with his hand at each letter. A no point if ≥ 2 errors.
- Serial 7 subtraction starting at 100: [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

Abstraction

- Similarity between e.g. banana - orange - fruit: [ ]
- Train - bicycle: [ ]
- Watch - ruler: [ ]

Delayed Recall

- Has to recall words with no cue: [ ]
- Category cue: [ ]
- Multiple choice cue: [ ]

Orientation

- Date: [ ]
- Month: [ ]
- Year: [ ]
- Day: [ ]
- Place: [ ]
- City: [ ]

Total: [ ]/30

Points for uncued recall only

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Normal ≥ 26 / 30

Add 1 point if ≤ 12 yr. edu
Appendix Figures.

Appendix Figure 1. MRI metrics assessed in elderly subjects with age-related leukoaraiosis. A, FA map (unitless). B, mD map highlighting greater diffusivity in WMH (mm²). C, CVR map demonstrating reduced reactivity in the white matter compared to the robust CVR in grey matter (% BOLD/mmHg). D, CBF map demonstrating lower flow in WMH (mL/100g/min). E, CBV map demonstrating lower CBV in WMH (arbitrary units). F, MTT map showing a general trend towards longer circulatory transit times to WMH. G, Tmax map showing more dispersion of contrast to WMH. H, TTP map demonstrating delays in peak tracer concentration in WMH.
### Appendix Table 1. Toronto Western Hospital - Patient Characteristics

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M indicates male; F, female; AD, Alzheimer’s disease; Afib, atrial fibrillation; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; Dx, diagnosis; GERD, gastroesophageal reflux disease; MCI, mild cognitive impairment; MoCA, Montreal cognitive assessment; OSA, obstructive sleep apnea; SVD, small vessel disease; TIA, transient ischemic attack; VaD, vascular dementia; WMC, white matter changes
### Appendix Table 2. Sunnybrook Health Sciences Centre - Patient Characteristics

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M indicates male; F, female; AD, Alzheimer’s disease; Afib, atrial fibrillation; CAD, coronary artery disease; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CVD, cerebrovascular disease; DM, diabetes mellitus; Dx, diagnosis; GERD, gastroesophageal reflux disease; ICA, internal carotid artery; MCI, mild cognitive impairment; MoCA, montreal cognitive assessment; OSA, obstructive sleep apnea; TIA, transient ischemic attack; VCI, vascular cognitive impairment; WMC, white matter changes
Appendix Table 3. Toronto Western Hospital - Patient Characteristics for Study III and IV.

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M indicates male; F, female; AD, Alzheimer’s disease; Afib, atrial fibrillation; CAD, coronary artery disease; DM, diabetes mellitus; Dx, diagnosis; GERD, gastroesophageal reflux disease; MCI, mild cognitive impairment; MoCA, Montreal cognitive assessment; OSA, obstructive sleep apnea; SVD, small vessel disease; TIA, transient ischemic attack; VaD, vascular dementia; WMC, white matter changes
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<td>WMC</td>
<td>Age</td>
<td>Gender</td>
<td>BP</td>
<td>Lumbar</td>
<td>Carotid</td>
<td>Other</td>
<td>OSA</td>
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</table>

M indicates male; F, female; AD, Alzheimer’s disease; Afib, atrial fibrillation; CAD, coronary artery disease; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CVD, cerebrovascular disease; DM, diabetes mellitus; Dx, diagnosis; ICA, internal carotid artery; MCI, mild cognitive impairment; MoCA, montreal cognitive assessment; OSA, obstructive sleep apnea; TIA, transient ischemic attack; VCI, vascular cognitive impairment; WMC, white matter changes
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