Identifying Associations between Vascular Risk Factors and measures of Brain Health Using Multimodal Magnetic Resonance Imaging

by

Ekaterina Tchistiakova

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

© Copyright by Ekaterina Tchistiakova 2016
Identifying associations between vascular risk factors and measures of brain health using multimodal magnetic resonance imaging

Ekaterina Tchistiakova
Doctor of Philosophy
Department of Medical Biophysics
University of Toronto
2016

Abstract
Recent epidemiological evidence indicates that vascular risk factors (VRFs) play a significant role in cognitive decline that can contribute to the onset of dementia among older adults. The link between VRFs and dementia is supported by common neurological findings of structural deficits, cerebrovascular dysfunction and amyloid accumulation in both conditions. Despite the growing literature on VRFs and the brain, there are still many unanswered questions about their roles in pathoetiology and best imaging methods for detecting neurological effects of VRFs.

The main goal of this thesis was to characterize the effect of increasing number of VRFs on brain health. Measures of structural brain integrity and cerebrovascular health were acquired using non-invasive magnetic resonance imaging (MRI). The first study of this thesis identified regions of impaired vascular reactivity and thinning of the cortical
mantle in older adults with type 2 diabetes and hypertension compared to hypertension only. The next two studies focused on the associations between VRFs and brain health in two common neurodegenerative conditions: older adults with small vessel disease and older adults with mild cognitive impairment. In adults with white matter hyperintensities, a hallmark of small vessel disease, higher VRF number was associated with lower vascular reactivity in cognitively relevant regions. In older adults with mild cognitive impairment, increasing VRF number was correlated with cortical thinning in regions implicated in Alzheimer’s Disease. Together, findings of this thesis highlight an association between increasing VRF number and exacerbated brain health in otherwise healthy older adults and individuals with comorbid neurodegenerative disorders. To conclude, the implications of these findings for clinical practices are discussed with focus on proper VRF management as well as the need to properly account for VRFs in neuroimaging studies.
Acknowledgments

There are many people that I would like to thank that helped make this thesis a reality. First, and foremost, I would like to thank my supervisor Dr. Brad MacIntosh for his patience, motivation and immense knowledge. Thank you for encouraging my research and allowing me the freedom to work independently and grow as a research scientist. Your advice on both research as well as on my career have been priceless.

To my committee, Dr. Simon Graham, Dr. Chuck Cunningham and Dr. Carol Greenwood, I am extremely grateful for your insightful comments and encouragement, but also for the hard questions, which incented me to widen my research from various perspectives.

I would also like to thank Dr. Sandra Black for providing guidance and insight on the clinical data. Dr. Black’s dedication to her patients and research is truly inspiring.

My sincere thanks goes to Dr. Nicole Anderson, who provided an invaluable expertise in the field of cognitive psychology and most insightful comments on the co-authored papers.

I would also like to thank all the “Bmac” lab members that have passed through the lab at various points during my graduate work: Ilia Makedonov, Saeed Rajab, Farhang Jalilian, Dr. Andrew Robertson, Dr. Arron Metcalfe, Zahra Shirzadi, Sarah Atwi, Anna Mersov, Gena Matta and Joanna Huang. A special thank you goes to David Crane who was a great source of knowledge in many aspects including technical development, data analysis as well as stimulated great scientific discussions that helped generate ideas that contributed to this thesis.

I’m very grateful to Dr. Walter Swardfager for his help in preparation of this thesis as well as his expertise and advice on the statistical analyses throughout the years.

I’m also thankful to the members of Greenwood and Anderson labs from the Rotman Research Institute who helped with the recruitment and cognitive testing of study participants.
A special thank you goes to Chris Scott and Gregory Szilagyi for technical support and advice on image analysis throughout the years.

I thank Canadian Partnership for Stroke Recovery and the department of Medical biophysics for the student funding that supported this research. Special thank you to Merle Casci and Donna-Marie Pow who always go out of their way to help the students.

Last but not least, I would like to thank my family, especially my mom, who have supported me and gave me encouragement throughout the years. I thank my friends who stuck by my side even during weeks and sometimes months of lost communication during deadline season. Finally, I’d like to thank my beloved dog, Sam, who has been with me through my entire graduate career and helped me get through he hardest days with his quiet and unconditional love and the ability to always make me smile.
# Table of Contents

Chapter 1 ........................................................................................................................................... 1

1 Introduction ........................................................................................................................................ 1

1.1 Clinical Motivation ......................................................................................................................... 1

1.2 Healthy aging ................................................................................................................................. 1

1.3 Vascular risk factors ....................................................................................................................... 4

1.3.1 Type 2 Diabetes Mellitus .......................................................................................................... 4

1.3.2 Hypertension ............................................................................................................................ 8

1.3.3 Hypercholesterolemia .............................................................................................................. 11

1.3.4 Smoking .................................................................................................................................. 14

1.4 VRFs and cerebral small vessel disease ......................................................................................... 16

1.5 VRFs relation to dementia ............................................................................................................ 19

1.6 Magnetic Resonance Imaging ....................................................................................................... 23

1.6.1 MRI pulse sequences and contrasts (ρ, T1, T2, T2*) ............................................................... 27

1.6.2 Physiology of BOLD signal ..................................................................................................... 31

1.7 Cerebrovascular reactivity ........................................................................................................... 33

1.7.1 Cerebral autoregulation ............................................................................................................ 33

1.7.2 Clinical relevance ...................................................................................................................... 34

1.7.3 Measuring cerebrovascular reactivity ...................................................................................... 34

1.7.4 Vasodilatory challenges .......................................................................................................... 35

1.7.5 fMRI analysis .......................................................................................................................... 37

1.8 Resting state functional connectivity .............................................................................................. 39

1.8.1 Clinical relevance ...................................................................................................................... 40

1.8.2 The Default Mode Network .................................................................................................. 40

1.8.3 Resting-state fMRI analysis .................................................................................................... 41

1.9 Structural brain imaging ................................................................................................................. 44

1.9.1 Brain structure .......................................................................................................................... 44

1.9.2 Imaging biomarkers ............................................................................................................... 45

1.9.3 Cortical thickness analysis ...................................................................................................... 46

1.10 Statistical analysis ....................................................................................................................... 49
1.11 VRFs – Remaining questions.......................................................... 50

1.12 Thesis hypothesis ........................................................................... 51

1.13 Thesis Outline ................................................................................ 52

1.14 Contribution .................................................................................. 52

Chapter 2 ......................................................................................... 53

2 Associations between combined effects of type 2 diabetes and hypertension and measures of cortical thickness and cerebrovascular reactivity relative to hypertension alone in older adults.................................................. 53

2.1 Introduction .................................................................................... 53

2.2 Methods .......................................................................................... 54

2.2.1 Participants .................................................................................. 54

2.2.2 MRI ............................................................................................ 55

2.2.3 Image Analysis ........................................................................... 56

2.2.4 Correlation with cognitive scores ................................................ 57

2.2.5 Statistical Analysis ..................................................................... 58

2.3 Results ............................................................................................ 58

2.3.1 Demographics ............................................................................ 58

2.3.2 Group differences in cerebrovascular reactivity and cortical thickness .......... 59

2.3.3 Correlation with clinical measures ............................................. 61

2.3.4 Correlation with cognitive scores .............................................. 61

2.4 Discussion ....................................................................................... 62

2.5 Conclusion ....................................................................................... 64

Chapter 3 ......................................................................................... 65

3 Vascular risk factor index correlates with cerebrovascular reactivity but not resting state co-activation in the default mode network in older adults with WMH ................. 65

3.1 Introduction ....................................................................................... 65

3.2 Methods .......................................................................................... 66

3.2.1 Participants .................................................................................. 66

3.2.2 MRI ............................................................................................ 67

3.2.3 Hypercapnia challenge ................................................................ 67

3.2.4 Image Analysis .......................................................................... 68
3.3 Results ................................................................................................................. 71
  3.3.1 Demographics ................................................................................................. 71
  3.3.2 VRF index sub-group comparison ................................................................. 72
  3.3.3 RS co-activation vs. CVR ............................................................................... 74
  3.3.4 Influence of WMH and head motion ............................................................... 75
3.4 Discussion ........................................................................................................... 76
3.5 Conclusion ............................................................................................................ 78
Chapter 4 .................................................................................................................... 79
4 Associations between summative vascular risk factor effects and cortical thinning
  in mild cognitive impairment .................................................................................. 79
  4.1 Introduction ........................................................................................................ 79
  4.2 Methods ............................................................................................................ 80
  4.2.1 Participants .................................................................................................... 80
  4.2.2 Image pre-processing ..................................................................................... 81
  4.2.3 Identifying associations between summative VRF index and CThk
      with PLS ............................................................................................................ 82
  4.2.4 Cognitive group x VRF index interaction ...................................................... 82
  4.2.5 Inter-regional CThk correlations .................................................................... 83
  4.2.6 Analysis of one-year follow-up MRI ............................................................. 83
  4.2.7 Statistical analysis ........................................................................................ 83
  4.3 Results .............................................................................................................. 83
  4.3.1 Participants .................................................................................................... 83
  4.3.2 Associations between summative VRF index and CThk ................................ 84
  4.3.3 Cognitive group x VRF index interaction ...................................................... 86
  4.3.4 Inter-regional CThk correlations .................................................................... 87
  4.3.5 Analysis of one-year follow-up MRI ............................................................. 87
  4.4 Discussion ........................................................................................................... 89
  4.5 Conclusion ........................................................................................................... 91
Chapter 5 .................................................................................................................... 92
5 Thesis Discussion .................................................................................................... 92
  5.1 Summary and Conclusions ................................................................................ 92
5.2 Clinical implications ................................................................. 93
5.3 Limitations and future directions ................................................ 96
  5.3.1 Data quality considerations ..................................................... 97
  5.3.2 Characterization of VRF impact on brain perfusion with ASL ........ 102
  5.3.3 Multimodal imaging ............................................................... 102
5.4 Conclusions ................................................................................ 103
Summary of thesis dissemination ....................................................... 104
Appendices ....................................................................................... 106
References ......................................................................................... 110
Studies included in systematic review on ADNI .................................. 140
List of Tables

Table 1.1. T2DM diagnostic criteria................................................................. 6
Table 1.2. Diagnostic criteria for total and LDL cholesterol................................. 12
Table 1.3. MR properties of brain tissues at 3T..................................................... 28
Table 2.1. Participant demographics...................................................................... 59
Table 3.1. Participant demographics...................................................................... 72
Table 4.1. Participant demographics...................................................................... 85
Table 4.2. Inter-regional correlation coefficients for brain regions where CThk was found
to correlate with the VRF index by PLS............................................................ 87
List of Figures

Figure 1.1. Schematic summary of topics covered in Sections 1.3, 1.4 and 1.5 .............. 4
Figure 1.2. Natural history of Type 2 Diabetes ................................................................. 5
Figure 1.3. Cerebral autoregulation curves: normal (black) and shifted towards higher BP due to hypertension (red) ................................................................. 10
Figure 1.4. A hazard ratio for AD diagnosis calculated by comparison to a group with no VRFs and plotted as a function of the VRF number ........................................... 20
Figure 1.5. Gradient echo EPI timing diagram and schematic of interleaved sampling of k-space ............................................................................................................................ 29
Figure 1.6. MPRAGE sequence timing diagram ................................................................. 31
Figure 1.7. Schematic illustration of data decomposition performed by spatial ICA ......... 43
Figure 1.8. A schematic illustration of PLS analysis ......................................................... 48
Figure 1.9. The breakdown of VRFs and their combinations, based on a large sample of adults (age >65 years) recruited in Kaiser Permanente Medical Care program, based on 50
Figure 2.1. A CVR map and mean BOLD time course for a representative HTN participant ................................................................................................................................. 57
Figure 2.2. Regions of decreased CVR and CThk in HTN+T2DM group compared to HTN group ............................................................................................................................. 60
Figure 2.3. Regions of significant correlation between higher CThk and better executive function ............................................................................................................................... 61
Figure 3.1. Flow chart for data-driven analysis approach on RS-fMRI and CVR-BOLD data ................................................................................................................................. 70
Figure 3.2. Three networks of interest used in the VRF index sub-group comparison .... 73
Figure 3.3. Mean and SD for RS co-activation and DR-based CVR for 3 VRF index sub-groups in 3 NOIs ................................................................................................................. 74
Figure 3.4. Correlations between RS co-activation and DR-based CVR in 3 NOIs ....... 75
Figure 3.5. Probability of each voxel being classified as WMH ....................................... 75
Figure 4.1. Flow-chart of participants included in CThk analysis .................................... 84
Figure 4.2. Significant latent variable pattern and regions exhibiting the LV pattern for PLS on CThk vs. summative VRF index in MCI cohort ..................................................... 86
Figure 4.3. Main effects of VRF index and cognitive group x VRF index interaction .... 86
Figure 4.4. CThk region -by-region correlation coefficient maps shown for each of the VRF index sub-groups and pairwise VRF index sub-group comparisons...................... 88
Figure 5.1. Impact of diabetic drugs (insulin and metformin) on the relationship between brain insulin resistance and AD through the IRS- 1 - AKT pathway (pink) in MCI and AD. ......................................................................................................................... 95
Figure 5.2. CVR correlation map and R -value distribution curves for GM, WM and full brain for CO₂ inhalation vs. BH challenge ................................................................. 99
Figure 5.3. Example of FreeSurfer segmentation fail in the temporal region. ............ 101
Figure 5.4. Distribution of L. Entorhinal CThk in NC and MCI groups by collection site. ........................................................................................................................................ 101
List of Appendices

Appendix A. Alzheimer’s Disease Neuroimaging Initiative.................................106
Appendix B. Systematic review of ADNI publications for VRF inclusion...............108
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D MPRAGE</td>
<td>Three-dimensional magnetization prepared rapid gradient echo</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADNI</td>
<td>Alzheimer’s Disease Neuroimaging Initiative</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>ASL</td>
<td>Arterial spin labeling</td>
</tr>
<tr>
<td>BH</td>
<td>Breath hold</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygenation level dependent imaging</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CThk</td>
<td>Cortical thickness</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CBV</td>
<td>Cerebral blood volume</td>
</tr>
<tr>
<td>CMRO₂</td>
<td>Cerebrovascular metabolic rate of oxygen consumption</td>
</tr>
<tr>
<td>CVR</td>
<td>Cerebrovascular reactivity</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DMN</td>
<td>Default mode network</td>
</tr>
<tr>
<td>DR</td>
<td>Dual-regression</td>
</tr>
<tr>
<td>EPI</td>
<td>Echo planar imaging</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>FEAT</td>
<td>FMRI expert analysis tool</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid attenuated inversion recovery</td>
</tr>
<tr>
<td>FMRI</td>
<td>Functional MRI</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width half max</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>GM</td>
<td>Grey matter</td>
</tr>
<tr>
<td>HBA1C</td>
<td>Hemoglobin A1C</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
</tbody>
</table>
HRF  Hemodynamic response function
HTN  Hypertension
IC   Independent component
ICA  Independent component analysis
LDL  Low-density lipoprotein
LV   Latent variable
MCI  Mild cognitive impairment
MELODIC  Multivariate exploratory linear optimized decomposition into independent components
MRI  Magnetic resonance imaging
NC   Normal controls
NOI  Network of interest
PaCO₂  Partial pressure of CO₂
P_{ET}CO₂  Partial end-tidal CO₂
PET  Positron emission tomography
PLS  Partial least squares
RF   Radiofrequency pulse
RS-fMRI  Resting-state fMRI
RSN  Resting state network
ROI  Region of interest
SBP  Systolic blood pressure
SVD  Cerebral small vessel disease
T2DM Type 2 diabetes mellitus
TE   Echo time
TI   Inversion time
TR   Repetition time
VaD  Vascular dementia
VLDL Very low density lipoprotein
VRF  Vascular risk factor
WM   White matter
WMH  White matter hyperintensities
Chapter 1

1 Introduction

This thesis examines the effects of vascular risk factors (VRFs) on brain health using magnetic resonance imaging (MRI) techniques to identify their associations with brain structure, vasculature and neuronal function. This introductory section provides background information on the relevant topics and rationale for the current work.

1.1 Clinical Motivation

Risk factor is defined by World Health Organization as any attribute, characteristic or exposure that increases the likelihood of developing a disease. Cardiovascular risk factors such as type 2 diabetes (T2DM), hypertension (HTN) and hypercholesterolemia are rapidly increasing in prevalence, which is attributed largely to environment and lifestyle choices, namely poor diet, sedentary lifestyle and lack of physical exercise. The prevalence of HTN, for example, more than doubled from 1995 to 2005 (Tu et al., 2008) and the number of people living with diabetes is projected to reach 592 million by 2035 (from 382 million in 2013) (Guariguata et al., 2014). Progress in the health care and disease management allows individuals with these conditions to live for many years, however, long-term exposure to VRFs can lead to deleterious effects on cerebrovascular health and cognition. The co-occurrence of VRFs is common. HTN is present in up to 75% of individuals with T2DM (Colosia et al., 2013), and may further exacerbate the negative impact of T2DM on brain health. Higher number of VRFs, for example, is associated with increased incidences of Alzheimer’s disease (AD) (Luchsinger et al., 2005). The mechanisms behind the multiple VRF impact on the brain are, however, still unclear and will be explored in this thesis using MR imaging.

1.2 Healthy aging

Before discussing the VRFs, it is important to consider the effect of healthy aging on brain health.

Brain structure

Numerous studies observed age-related reduction in grey matter (GM) and white matter (WM)
volumes (Good et al., 2001; Lemaître et al., 2005; Tisserand et al., 2004). Beginning at the age of 20 years, the GM demonstrated a constant linear decline across the life span (20-86 years). WM, on the other hand, showed a quadratic pattern of changes with WM increase until the age of 40 years followed by a decline thereafter (Ge et al., 2002). Regionally, age-related GM volume decline was observed in the frontal lobe, anterior cingulate cortex, Heschl gyrus, insula, sensorimotor cortex and cerebellum (Good et al., 2001; Lemaître et al., 2005; Tisserand et al., 2004). The results of examining the hippocampus, a critical brain structure involved in memory, are less consistent. Several studies detected significant degeneration (Lemaître et al., 2005), while others observed a sparing effect (Good et al., 2001). A closer examination of the population demographics in these studies revealed a possible nonlinear correlation of hippocampal volumes with age. Unlike the frontal lobe atrophy that follows a linear trend of age-related decline, hippocampal volumes do not show a significant change in volume until the age of 60 years. In the later years, however, hippocampal volumes decline rapidly (Kalpouzos et al., 2009). Cortical thinning was also observed in the prefrontal cortex in normal aging, while the temporal and parahippocampal cortices appear to be spared (Salat et al., 2004).

**Brain vasculature**

Vascular and metabolic alterations are also evident across the adult lifespan. Lu et al. demonstrated an increase in cerebral metabolic rate of oxygen consumption (CMRO$_2$) of 2.6µmol/100g/min per decade and a decrease in cerebral blood flow (CBF) of 0.8 ml/100g/min per decade, which amounts to approximately 1.6% increase and 1.4% decrease per decade, respectively. The increase in CMRO$_2$ in the older adults may be compensatory in response to the decrease in neuronal computational efficiency (Lu et al., 2011), while CBF decrease can be attributed to either underlying structural changes, decreased activity of individual neurons, or both (Martin et al., 1991). Regionally, impacted areas were similar to those showing structural changes, i.e. prefrontal cortex, insular cortex and caudate (Lu et al., 2011). In a direct comparison of CBF decrease and cortical thinning, however, only a modest overlap was observed (J. J. Chen et al., 2011). CBF decrease was most prominent in frontal and insular cortices, fronto-temporal region, precuneus, anterior cingulate cortex, left lateral occipital and supramarginal regions, superior parietal, caudate and thalamus. Cortical thinning was also detected in the frontal, temporal and precuneus areas, along with many regions that did not show a concomitant decrease in CBF (J. J. Chen et al., 2011).
Aging was found to also affect the vasoregulatory capacity of the brain. A decrease in cerebrovascular reactivity (CVR), the ability to maintain sufficient blood supply to brain tissue through vasodilation or vasoconstriction in response to changes in blood CO$_2$ levels, was observed in the middle cerebral artery using transcranial doppler ultrasound in the middle-aged and older adults compared to young persons (Peisker et al., 2010). Blood oxygenation level dependent (BOLD) MRI, often used for functional and CVR measurements and discussed in more detail in Sections 1.6.2 and 1.7 is highly sensitive to changes in cerebral vasculature. Age differences were seen during the breath hold (BH) challenge, where mean activation volume was reduced in the older group compared to younger adults by 38% (Kannurpatti et al., 2010).

Studying the effects of aging on brain health is complicated by the discrepancy between what is defined as “typical” and “healthy” aging (Meusel et al., 2014). The high prevalence of VRFs in the aging population warrants the need for proper screening procedures in aging studies. Failure to account for the effects of VRFs on brain structure, vasculature and cognition may misconstrue our understanding of brain aging. A systematic review by Meusel et al. demonstrated that 58-78% of the studies related to aging had no clear indication whether VRFs, such as HTN and T2DM, were part of the exclusion criteria (Meusel et al., 2014). Presence of multiple VRFs is highly prevalent in the older adults, however, most studies to date have focused on identifying individual effects of each VRF on brain health. The primary objective of this thesis is to augment the earlier findings on the individual VRFs by examining the associations between multiple VRFs and brain health measures. To start of, however, sections 1.3.1, 1.3.2, 1.3.3 and 1.3.4 will review individual effects of T2DM, HTN, hypercholesterolemia and smoking on brain structure, vasculature and cognition, see Figure 1.1, while sections 1.4 and 1.5, will examine the contribution of VRFs to two neurodegenerative disorders common in older adults, cerebral small vessel disease (SVD) and dementia. Figure 1.1 provides a schematic summary of the sections to come.
1.3 Vascular risk factors

1.3.1 Type 2 Diabetes Mellitus

Diabetes Mellitus is a group of metabolic disorders characterized by reduced production or developed insensitivity to the hormone insulin. Insulin is a peptide hormone that serves as a regulator for glucose metabolism by facilitating glucose uptake in tissues (Biessels, 2009). T2DM, previously called non-insulin dependent diabetes, usually occurs later in life and is the most common of the diabetic disorders, accounting for up to 90% of all cases. Peripheral insulin resistance in T2DM occurs when the response of body cells to insulin stimulation is reduced. In the early stages of the disease, pancreatic β cells respond by increasing production of insulin, which results in peripheral hyperinsulinemia as a means to maintain glucose levels within the normal range. As the disease progresses the β cells can no longer offset the insulin resistance and the blood glucose levels begin to rise leading to the diagnosis of T2DM. The natural history of T2DM progression is depicted in Figure 1.2, while Table 1.1 summarizes the diagnostic criteria for T2DM based on three most commonly used tests: fasting plasma glucose, hemoglobin A1C and oral glucose tolerance test. Fasting plasma glucose test is the most commonly used diabetes test, which measures blood glucose levels following an overnight fast (at least 8 hours of no caloric intake). Hemoglobin A1c indicates an average blood sugar levels over the past 2-3 months and measures percentage of hemoglobin molecules attached to glucose. Finally, the oral
glucose tolerance test measures the glucose levels before and 2 hours following the ingestion of a sugary drink (75g of glucose), measuring the body’s ability to utilize glucose or clear it from the blood stream. In case of inconclusive results multiple tests may be required for definitive diagnosis of T2DM.

![Graph showing glucose tolerance test results](image)

**Figure 1.2. Natural history of Type 2 Diabetes.** Adapted from International Diabetes Centre, Minneapolis, Minnesota. Top: Blood glucose levels (both fasting and postmeal) increase with T2DM progression. Bottom: Insulin resistance and β-cell function increase in the years preceding T2DM onset. The insulin resistance persists throughout the T2DM progression, while β-cell function will eventually begin to decline, causing the blood glucose levels to rise (Top), signifying T2DM onset (dashed red line).

Although most individuals with T2DM are able to manage their blood glucose levels with strict diet and oral medications, as the disease progresses, some will require insulin injections due to a failure of pancreatic β cells.

Risk of T2DM increases with obesity, inactive life style, family history of diabetes, >45 years of age as well as additional co-morbidities such as high blood pressure (BP) and low HDL cholesterol (Biessels, 2009). African American, Latino, Native American and Asian American are racial/ethnic groups that are at a higher risk of developing T2DM (Biessels, 2009).
<table>
<thead>
<tr>
<th></th>
<th>FPG</th>
<th>HbA1c</th>
<th>OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>≤ 6.0 mmol/L</td>
<td>≤ 6.0%</td>
<td>≤ 7.8 mmol/L</td>
</tr>
<tr>
<td>Pre-diabetic</td>
<td>6.1-6.9 mmol/L</td>
<td>6.0% to 6.4%</td>
<td>7.8-11.0 mmol/L</td>
</tr>
<tr>
<td>Diabetic</td>
<td>≥ 7.0 mmol/L</td>
<td>≥ 6.5 %</td>
<td>≥ 11.1 mmol/L</td>
</tr>
</tbody>
</table>

Table 1.1. T2DM diagnostic criteria. Adapted from http://guidelines.diabetes.ca/Browse/Chapter3

FPG – Fasting plasma glucose
HbA1c – hemoglobin A1C
OGTT – oral glucose tolerance test

T2DM and brain structure

T2DM is associated with brain atrophy that is in excess of that seen in normal aging. Diabetic individuals show an overall decrease in GM and WM volumes and increase in cerebrospinal fluid volume relative to age-matched healthy controls (Bresser et al., 2010; Last et al., 2007). Brain atrophy in T2DM is detected in cortical and subcortical regions and is thought to contribute to accelerated cognitive decline. Cerebellar infarcts are also significantly more common in diabetic individuals and can lead to impairment in motor function (Saczynski et al., 2009). Regional analysis revealed that certain regions in the brain are more susceptible to diabetes-induced damage than others. Affected regions include those tightly correlated with memory function, such as the hippocampus. Early studies of T2DM effects on cortical thickness (CThk) are consistent with volumetric findings, with preferential regional thinning in the middle temporal gyrus, posterior cingulate cortex, precuneus, lateral occipital gyrus and entorhinal cortex (Brundel et al., 2010; Z. Chen et al., 2015). Cortical thickness was also negatively associated with blood glucose levels in the left anterior cingulate cortex and bilateral occipital regions (Leritz et al., 2011). The exact nature of diabetes induced structural atrophy is still debated. Possible contributors include chronic exposure to hyperglycemia, vascular disease, as well as the direct effect of insulin dysregulation on the brain. With respect to the latter, peripheral hyperinsulinaemia, seen in pre-diabetic and diabetic individuals, down-regulates insulin transport across the blood-brain barrier. Although insulin is not directly involved in the transport of the glucose to the brain, as evidenced by positron emission tomography (PET) studies (Hasselbalch et al., 1999), it does play an important role in the glucose metabolism in certain brain regions. Regional effects of insulin are likely due to selective distribution of insulin-sensitive GLUT4 and GLUT8 glucose transporters, which are expressed in the hippocampus, hypothalamus, sensorimotor cortex, pituitary and cerebellum. The overlapping
regions of increased brain atrophy in T2DM individuals and distribution of insulin, insulin receptors and insulin-sensitive glucose transporters provide one possible explanation for the regional effects of T2DM on the brain (Craft & Watson, 2004). Other mechanisms, including inflammation and oxidative stress, have also been implicated in T2DM effects on the brain and are discussed in more detail in the next section.

**T2DM and cerebrovascular function**

Increased glucose levels in the blood stream, i.e. hyperglycemia, plays a major role in the vascular effects of T2DM as it preferentially impacts the endothelial cells, which are less efficient in regulating glucose uptake into the cell. High glucose levels in the blood cause elevated intracellular glucose, which increases oxidative stress by inducing overproduction of reactive oxygen species within the mitochondria (Kaiser et al., 1993). This activates a cascade of biochemical events including overproduction of advanced glycation end products that can cause intracellular damage, resulting in macro- and microvascular complications associated with T2DM (Brownlee, 2005).

Hyperglycemia-induced oxidative stress also increases production of vasoconstrictor endothelin-1 while decreasing production of vasodilator nitric oxide, leading to a diminished vasodilatory capacity, an important mechanism in maintaining adequate blood flow to brain tissues. Prolonged exposure to high concentration of endothelin-1 and decreased nitric oxide can result in structural alterations of the vessel wall, thrombosis and formation of atherosclerotic plaques (Kalani, 2008). Exposure to the high levels of endothelin-1 can also cause proliferation of vascular smooth muscle cells and inflammation (Ergul, 2011). Inadequate CBF and impaired vasoregulatory capacity is detrimental as it interferes with the delivery of essential nutrients and oxygen to brain tissues. The literature suggests that the age-related decrease in CBF is exacerbated by the presence of hyperglycemia. Last et al. used arterial spin labeling (ASL) MRI to measure CBF during rest, CO₂ rebreathing of 95% air and 5% CO₂ and hyperventilation. Results showed a global decrease in both resting CBF and CO₂ reactivity in individuals with T2DM compared to those without. The decrease in the CBF was most pronounced in the frontal lobes, suggesting a high vulnerability of these regions to the disease (Last et al., 2007). Reduced CVR has also been reported in people with T2DM, using transcranial doppler ultrasound to measure the change in CBF velocity in the middle cerebral artery in response to intravenous administration of vasodilator acetazolamide (Fülesdi et al., 1999). The same study found a
significant decrease in cerebrovascular reserve capacity, defined as the maximum percent increase in CBF velocity, in individuals with long-standing T2DM (over 10 years) when compared to short-term diabetes group and healthy control group (Fülesdi et al., 1999). This finding suggests a continuing decline in vascular health with the duration of the disease.

T2DM and cognition

T2DM is associated with exacerbated cognitive deficits when compared to healthy age-matched controls, particularly when it comes to processing speed, memory, attention and executive function domains. These cognitive changes are thought to be the result of metabolic dysregulation (hyperglycemia and hyperinsulinemia), prominent in T2DM, and mediated by structural and vascular changes. Higher HbA1c levels, for example, were associated with decreased hippocampal volumes and worse memory performance (Gold et al., 2007), while lower CBF corresponded to lower brain volumes and poorer performance on tasks that require attention, executive function and information processing speed (Brundel et al., 2012). Progression of brain atrophy in T2DM was also associated with accelerated cognitive decline (Reijmer et al., 2011). Notably, the correlations between cognitive performance and poor glucose regulation were also observed in pre-diabetic and non-diabetic adults (Dahle et al., 2009; Messier et al., 2011; Yaffe et al., 2004), stressing the importance of proper glucose control even at the preclinical stages. A link between T2DM and AD and other dementias was also suggested and will be explored in more details in Section 1.5.

1.3.2 Hypertension

Hypertension can be classified as one of two forms: essential or secondary. Essential HTN is by far the most common, approximately 95% of all cases, and is defined as high BP that can not be attributed to secondary causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism or other conditions associated with secondary HTN. Although the primary causes of essential HTN are unknown, several factors are thought to play a role, including increased salt intake, obesity, insulin resistance, as well as the dysfunction of renin-angiotensin and sympathetic nervous systems (Carretero & Oparil, 2000). A contribution of several of these factors is likely to contribute to the development of HTN. Normal BP is maintained through a balance between cardiac output and peripheral vascular resistance. Peripheral vascular resistance is primarily determined by the small arterioles, the walls of which contain smooth muscle cells. In the early stages of HTN the increase in BP is thought to be caused by overactivity of the
sympathetic nervous system, which among many things increases cardiac output. The rise in the resistance is developed as a compensatory mechanism in an attempt to prevent the transfer of BP to the capillary bed (Beevers et al., 2001). Renal sympathetic stimulation is also increased in hypertensive individuals, causing salt and water reabsorption, expansion of the intravascular volume and, consequently, an increase in BP (DiBona & Kopp, 1995; Oparil et al., 2003).

**HTN and brain structure**

Chronic elevation in BP dramatically affects many of the highly vascularized organs. In the brain HTN decreases GM and WM tissue volumes and leads to the accumulation of white matter hyperintensities (WMH), which contribute to cognitive deficits in executive function, as posited by Raz et al. (Raz et al., 2003). Blood pressure levels were also associated with cortical thinning in bilateral superior temporal, supramarginal, middle and superior frontal, as well as in portions of the frontal and occipital cortices (Leritz et al., 2011). The authors also noted that the correlation was apparent even at the normal and mildly elevated BP, suggesting that the impact of HTN on brain structure is present even at the subclinical levels. The preferential impact of HTN in frontal and temporal regions may be due to the vascular supply of these brain regions since large portions of these areas are supplied by distal arteries and are therefore particularly sensitive to impaired vascular circulation, leading to hypoperfusion, decrease in oxygen supply, and, consequently, shrinkage of brain tissue (Leritz et al., 2011). Alosco et al. showed a direct association between reduced total brain perfusion and cortical thinning in frontal, temporal and parietal lobes in individuals with cardiovascular disease (Alosco et al., 2014). The rate of cortical thinning was also found to increase in hypertensive compared to normotensive individuals in the left fronto-marginal gyrus, left superior temporal, fusiform and lateral occipital cortex. Furthermore, higher midlife BP and longer duration of HTN contributed to a higher rate of cortical thinning in the right superior temporal gyrus (Gonzalez et al., 2015).

**HTN and cerebrovascular function**

Hypertension increases vascular resistance by reducing the lumen diameter and vessel number in cerebral vasculature. Animal models of hypertension demonstrate a reduction in the number of intracerebral capillaries and pial arteries (Sokolova et al., 1985). Arteriole and capillary loss can
lead to chronic hypoperfusion of brain tissues increasing the risk of ischemia. Prolonged exposure to high BP increases intraluminal pressure and raises the tangential stress on the arterial wall. Arterial wall thickens to counteract this effect, and maintain wall stress levels within physiological range, causing (Hayashi & Naiki, 2009). Hypertension-induced alterations were also seen in the smooth muscle cells, manifesting in the disruption of their normal circular arrangement around the artery wall (Arribas et al., 1999). The goal of vascular remodeling in hypertensive individuals is to normalize the blood flow in the face of increased arterial pressures by increasing vascular resistance. This leads to the shift of the autoregulatory curve to the right towards high BP as depicted in Figure 1.3. As a result, CBF is maintained at a constant level at higher BP measures (the plateau region of a red curve on Figure 1.3), however, the decrease in BP can lead to hypoperfusion and risk of ischemia (rapid CBF decline occurs at the lower BP on the red curve on Figure 1.3). The ischemic damage is thought to contribute to increased prevalence of WMH in hypertensive individuals (Raz et al., 2003). The link between HTN and WMH will be explored in more details in Section 1.4.

In older hypertensive participants, CBF was found to be reduced in several regions including occipito-temporal regions, prefrontal cortex and the hippocampus, while the longitudinal assessment revealed a negative association between CBF and the duration of HTN in several frontal regions, precentral and postcentral gyi, anterior cingulate cortex, middle temporal, parahippocampal and lingual gyri (Beason-Held et al., 2007). A study using ASL MRI also showed decreases in CBF in hypertensive subjects in right and left anterior cingulate cortices, left posterior cingulate cortex and medial precuneus, left lateral inferior and superior frontal, inferior parietal, left orbitofrontal, left superior temporal cortices, as well as several subcortical

![Cerebral autoregulation curves: normal (black) and shifted towards higher BP due to hypertension (red). Adapted from (Novak, 2012).](image)
regions including putamen, globus pallidus and left hippocampus (Dai et al., 2008).

Hypertension was also found to impair vasodilatory capacity, during neuronal activation (i.e. hyperemia) (Jennings et al., 2005) or vasodilatory challenges (i.e. CVR). With respect to the latter, Hajjar et al. demonstrated global and regional CVR decrease in hypertensive individuals, with highest impact in the frontal, temporal and parietal lobes. Lower CVR was also associated with higher systolic blood pressure (SBP) (Hajjar et al., 2010).

**HTN and Cognition**

Cognitive deficits associated with HTN include poorer performance on tests of executive function, slower processing speed, and deficits in attention and memory compared to healthy age-matched controls (Bucur & Madden, 2010; Dahle et al., 2009; Gifford et al., 2013). Decrease in cognitive performance is seen even in relatively young adults with HTN (mean age 38.2 years) (Shehab & Abdulle, 2011). Individuals with borderline hypertension had slower reaction time, while the white-coat hypertension (a transient increase in BP in the medical settings), was associated with worse performance on the memory tests compared to healthy controls (Shehab & Abdulle, 2011). Mid-life hypertension is viewed as problematic because it is linked to exacerbate cognitive decline and increased dementia risk later in life (Gottesman et al., 2014). In a 15-year longitudinal study, Reijmer et al. demonstrated an association between baseline SBP and poor information-processing speed at the 15-year follow-up (Reijmer et al., 2012). Cognitive deficits in hypertensive individuals are particularly strong in the executive function and processing speed and to a lesser extent in memory function and are likely due, in part, to cerebrovascular damage. Deficits in attention and psychomotor speed, for example, were associated with reduction in global CBF (Efimova et al., 2008). Furthermore, Mahoney et al. recently showed that low SBP (<70 mmHg) is also associated with poor performance on executive attention tests in the elderly individuals (Mahoney et al., 2010). This demonstrates there is likely a “U-shaped” effect of BP on cognitive performance and stresses the importance of normal BP levels. Similarly to T2DM, HTN has been implicated in increased risk of AD later in life. The link between the two conditions will be explored in details in Section 1.5.

### 1.3.3 Hypercholesterolemia

Cholesterol is a fat-like substance (lipid) found in the cell membranes. In the blood, cholesterol is transported as distinct particles composed of lipids and proteins (lipoproteins), which can be
subdivided into three major categories: low density proteins (LDL), very low density proteins (VLDL) and high density proteins (HDL). The majority of total serum cholesterol, 60-70%, is composed of LDL, while HDL make up 20-30%. LDL oxidation contributes to the development of atherosclerosis and increases the risk of coronary artery disease. HDL, on the other hand, has a protective effect against atherosclerosis. VLDL is a triglyceride-rich lipoprotein that makes up 10-15% of plasma cholesterol. It is a precursors of LDL produced by the liver. Generally LDL is the primary target of cholesterol-lowering medications due to its high correlation with coronary artery disease. The diagnostic criteria for total and LDL cholesterol are summarized in Table 1.2 (Grundy et al., 2002).

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol (mg/dL)</th>
<th>LDL cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>&lt;200</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Borderline high</td>
<td>200-239</td>
<td>130-159</td>
</tr>
<tr>
<td>High</td>
<td>≥240</td>
<td>160-189</td>
</tr>
</tbody>
</table>

Table 1.2. Diagnostic criteria for total and LDL cholesterol, adapted from the "Third report of the national cholesterol education program"

Low levels of HDL cholesterol (<40 mg/dL) were also seen to contribute to atherosclerotic plaque formation (atherogenesis), and can be caused by physical inactivity, smoking, obesity, high carbohydrate intake, and others (Grundy et al., 2002). Contribution of hypercholesterolemia to coronary artery disease is well established. In the brain, high cholesterol is associated with increased risk of stroke; however, less is known of its other effects on brain health.

**Cholesterol and brain structure**

Brain cholesterol and plasma cholesterol constitute two pools separated by the blood-brain barrier. Excess brain cholesterol is eliminated by converting the neuronal-specific cholesterol 24-hydroxylase to 24S-hydroxycholesterol; the latter is capable of passing the blood-brain barrier into peripheral circulation (Björkhem, 2006). The 24S-hydroxycholesterol in the plasma can, therefore, be used as a marker for cerebral cholesterol metabolism and has been implicated in several neurodegenerative disorders (V. Leoni et al., 2008; Solomon et al., 2009). In Huntington disease, for example, lower 24S-hydroxycholesterol levels were associated with caudate GM atrophy (V. Leoni et al., 2008). Associations between plasma cholesterol and brain structure, on the other hand, are less well established. Salomon et al. showed a negative association between cholesterol precursors (lanosterol and lathosterol) and total brain volume in individuals with subjective cognitive impairment, while a positive correlation was seen between total brain
volume and total cholesterol levels in AD patients. Authors attribute this finding to impairment in brain cholesterol metabolism in AD, with lower cholesterol levels associated with more advance disease (Solomon et al., 2009). Plasma HDL levels showed a positive association with GM volume in AD-related regions, such as middle temporal gyri, parahippocampal and hippocampal areas, among others (Ward et al., 2010; H. Wolf et al., 2004). Mechanisms relating cholesterol levels and AD risk will be examined in more details in Section 1.5. In contrast to these volumetric findings, a study examining cortical thickness in the population of non-demented older adults reported a positive association between cholesterol factor (combination of total cholesterol, LDL and HDL cholesterol, with high values corresponding to high total and LDL and low HDL cholesterols) and cortical thickness across a wide range of brain regions (Leritz et al., 2011).

**Cholesterol and cerebrovascular function**
High cholesterol levels also play a role in the development of extracranial and intracranial atherosclerosis contributing to vascular dysfunction in the peripheral organs and increasing the risk of stroke. Independent of atherosclerosis impact, hypercholesterolemia contributes to the impairment in endothelial and smooth muscle function (D'Uscio et al., 2001; Kitayama et al., 2007). Conflicting evidence, however, was reported on the effect of blood lipids on cerebral arteries. Bakker et al. demonstrated an association between lower HDL and higher total cholesterol/HDL ratio and decreased CVR (Bakker et al., 2000). Kerenui et al. and Rodriguez et al., on the other hand, showed no significant differences in resting CBF or CVR between individuals with hypercholesterolemia and healthy controls (Rodriguez et al., 1994; Kerenyi et al., 2000). The discrepancy in these findings may be due in part to the age groups of the participants, i.e. the former study examined older adults, mean age 66.4 years, while the adults in the latter study were younger, mean age 42.5 years. The impact of lipids may, therefore, be dependent on participants’ age and/or disease duration. Animal models support the association between vascular impairment and high cholesterol. Ayata et al. showed a reduction in resting CBF and CVR, and increase in the lower limit of autoregulation in the hyperlipidemic ApoE knockout mice (Ayata et al., 2013).

**Cholesterol and cognition**
The impact of hypercholesterolemia on cognition is multifactorial. Cholesterol is directly involved in both generation and clearance of Aβ amyloid and can therefore contribute to Aβ
accumulation, a hallmark of AD (Beeri et al., 2009). Indirectly, cholesterol contributes to atherosclerotic disease, which has also been seen to increase the risk of dementia (Kalaria, 2000). In the population-based study by van Exel et al., lower HDL levels were associated with lower mini mental state examination scores. Individuals with low HDL also had a 2.3 odds ratio for dementia compared to those with high HDL (Van Exel et al., 2002). Furthermore, lower baseline levels of cholesterol precursors (lanosterol and lathosterol) correlated with better cognitive performance at a 6-year follow-up on tests of auditory verbal learning, information processing speed and perceptual interference (Teunissen et al., 2003).

1.3.4 Smoking

Almost 2 billion people worldwide use tobacco products, most in the form of cigarettes. Smoking-related diseases affect a wide range of systems, including cardiac, pulmonary and vascular functions, and result in 4 million deaths each year (DeMarini, 2004). Deleterious effects of chronic smoking on the peripheral organs are well established (Bartal, 2001); less, however, is known of smoking impact on the brain physiology and cognition.

Smoking and brain structure

History of cigarette smoking is associated with large-scale structural brain abnormalities, with preferential impact on middle temporal and prefrontal regions, similarly to T2DM and HTN. Lower GM volumes were detected in the bilateral prefrontal and left dorsal anterior cingulate cortices in young and middle-aged smokers compared to age-matched nonsmokers (Brody et al., 2004). Decrease in GM density was also noted in the prefrontal cortex, as well as in the right cerebellum. In the former, GM density was negatively correlated with higher pack-per-year history. Furthermore, mid-life smoking was associated with decrease in total brain volume, an increase in temporal horn volume (a surrogate marker for hippocampal atrophy) and an increased risk of WMH (Debette et al., 2011), while late-life smoking was related to decreased GM density in right precuneus, left posterior cingulate cortex, right thalamus, and bilateral precentral and middle frontal gyri (Almeida et al., 2008).

Smoking and cerebrovascular function

Acute effects of smoking result in the increased CBF in frontal lobe, hippocampus, uncus, thalamus and caudate nucleus, likely due to nicotine-induced neuronal activation (Nakamura et al., 2000). Chronic smoking, on the other hand, has been linked to decreased whole brain CBF,
and particularly right and left parietal cortex and right occipital cortex (Isaka et al., 1992). The study by Rogers et al. demonstrated that the negative effects of smoking on CBF may be reversed following cessation (Rogers et al., 1985). CBF in elderly individuals that quit smoking was significantly higher at a 1-year follow-up than those who continued smoking, but still remained lower than those without history of smoking. CBF increased linearly with the duration of cessation.

**Smoking and cognition**

Chronic smoking is associated with decline in a wide range of cognitive domains across the entire lifespan. Adolescents showed a decline in working memory compared to age-matched non-smokers, with those starting at the earlier age showing greater deficits (Jacobsen et al., 2005). Smoking young adults scored lower on measures of sustained attention, impulse control, auditory memory, oral arithmetics, expressive vocabulary, information processing speed and general intelligence compared to non-smokers (Yakir et al., 2007). Impairment in audio-verbal learning (Paul et al., 2006), working memory (Ernst et al., 2001; George et al., 2002), executive function (Paul et al., 2006; Razani et al., 2004), general intelligence (Deary et al., 2003), processing speed and cognitive flexibility (Paul et al., 2006) was also seen in middle-aged and older adults. Conflicting evidence is reported on the cognitive function in the former smoker, while in some studies their cognitive performance fell in between the smokers and non-smokers (Ernst et al., 2001; Fried et al., 2006; Starr et al., 2007), others showed no difference between former smokers and non-smokers (Hill et al., 2003). Longitudinal studies showed an accelerated decline in auditory-verbal memory in middle-aged (Richards et al., 2003) and older adults smokers (Reitz et al., 2005) compared to age-matched non-smokers. A more rapid decline in smokers compared to non-smokers was also seen on indices of reasoning (Sabia et al., 2008) in middle-aged adults and global cognitive function in older adults (Fischer et al., 2006). Finally, the amount of cigarettes smoked per day and the duration of smoking were found to inversely correlate with cognitive function across a variety of cognitive domains (Fried et al., 2006; Hill et al., 2003; Stewart et al., 2006).

From the findings presented above it is evident that VRFs have a detrimental effect on brain health in healthy adults. VRFs, however, can also contribute to the onset and exacerbate the effects of other neurological disorders. SVD and AD are two neurological conditions common in older adults. Their associations with individual VRFs will be examined in more detail in the next
sections.

1.4 VRFs and cerebral small vessel disease

*Cerebral small vessel disease*

Cerebral small vessel disease is a disease affecting perforating cerebral arterioles, capillaries and venules, and results in the damage to cerebral WM and deep GM. SVD is responsible for up to a fifth of all strokes worldwide; individuals with SVD have twice the risk of future stroke and contribute to up to 45% of dementias (Wardlaw et al., 2013). The mechanisms that give rise to SVD is a topic of intense debate, however, endothelial dysfunction is thought to play a key role. Impairment of endothelial function can result in a leakage of plasma content into the vessel wall, damaging arterial smooth muscle and causing fibrin deposition. These changes to arteriolar wall result in the stiffening of the vessels and loss of autoregulation, leading to ischemia and disruption of the blood-brain barrier. The permeability of cerebrovascular endothelium increases with age and disease, i.e. AD and VRFs. The examination of the relationships between SVD and VRFs (T2DM, HTN, hypercholesterolemia and smoking) will be the topic of focus in the later sections. SVD-related damage to small blood vessels is difficult to examine directly in-vivo, however, several related features can be observed using imaging techniques, i.e. MRI. These include, acute lacunar infarcts, lacunes (fluid-filled cavities, thought to be old infarcts), WMH, visible perivascular spaces (Virchow-Robin spaces) and microbleeds (Wardlaw et al., 2013).

*White Matter Hyperintensities*

SVD white matter hyperintensities of presumed vascular origin are identified as regions of decreased attenuation on computed tomography, increased signal on T2-weighted MR images (i.e. Fluid attenuated inversion recovery - FLAIR) and decreased signal on the T1-weighted images. The prevalence of WMH is high in aging populations. It is estimated that by the fifth decade of life almost 50% of people will have some WMH (Wen et al., 2009), which tend to accumulate within periventricular and deep WM, basal ganglia and pons. WMH have been linked to 1) cognitive decline (Prins et al., 2005), 2) gait impairment (Whitman et al., 2001) and 3) increased risk of dementia and stroke (Debette & Markus, 2010; Van Straaten et al., 2008).

WMH burden is thought to play a mediating role in the age-related cognitive and functional decline (Prins et al., 2005). Reduced brain activation in dorsolateral prefrontal cortex and increased anterior cingulate cortex activation during cognitive task were seen in non-demented
individuals with high WMH compared to those with low WMH (Mayda et al., 2011). One of these regions, the anterior cingulate cortex is part of the default-mode network (DMN), a distributed group of brain regions that are typically deactivated during task execution and activated during the rest condition. Failure to deactivate the DMN during a task can hinder the performance, a finding reported in several neurological disorders, including AD (Rombouts et al., 2005; Schwindt et al., 2013; Wang et al., 2006). A study in mild cognitive impairment (MCI) patients with and without SVD reported an impaired deactivation in precuneus/posterior cingulate region of the DMN in the group with MCI and SVD during an n-back cognitive task compared to MCI only group (Papma et al., 2013). A more detailed overview of the DMN and its role in cognitive function will be provided in Section 1.8.2.

WMH are also associated with altered brain hemodynamics. CBF and CVR were found to be reduced across the brain in individuals with high WMH volumes compared to those with low (Bakker et al., 1999; Hatazawa et al., 1997; Isaka et al., 1994). Furthermore, both vascular measures were also lower in the WMH regions compared to normal appearing WM (Brickman et al., 2009; Marstrand et al., 2002).

Recent studies also suggest a link between WMH and AD, such that WMH can exacerbate the AD pathology. In a longitudinal study, baseline WMH volumes were seen to predict amyloid deposition in individuals with probable AD (Grimmer et al., 2012). Impaired perivascular drainage associated with SVD was thought to contribute to decreased amyloid clearance. In contrast, the genetic markers of AD, i.e. ApoE status and family history, were not associated with WMH differences (Birdsill et al., 2014). Despite the strong association between WMH burden and chronological age, various diseases can accelerate the rate of WMH development and significantly increase WMH burden compared to healthy aging. The next several sections will examine the association between VRFs and WMH.

**T2DM and WMH**
Results of the early studies examining the link between WMH and T2DM are highly inconsistent, which could be attributed to methodological issues and study cohort sizes (Van Harten et al., 2006). The majority of these studies used grading systems to assess WMH burden, which may lack sensitivity to detect subtle differences. Increased sensitivity of recently developed automated segmentation methods may help explain why more recent studies showed
WMH differences between T2DM individuals and controls (Manschot et al., 2006; Van Harten et al., 2007), whereas the earlier studies did not. Contribution of metabolic dysregulation in T2DM to endothelial dysfunction, described in Section 1.3.1, provides one potential mechanism for increased WMH vulnerability. High variability of WMH in T2DM individuals and common co-occurrence of other confounding factors, i.e. HTN, makes it challenging to definitively establish the relationship between them.

**HTN and WMH**

Hypertension is a major risk factor for WMH, although the relationship between high BP and WMH is complex. Several longitudinal studies demonstrated that the BP in mid-life is more closely correlated to the WMH development later in life than concurrent BP (Skoog et al., 1996; Swan et al., 1998). The damaging effects HTN on cerebral blood vessels can lead to hypoperfusion and ischemia during subsequent episodes of hypotension. Deep WM regions are particularly vulnerable to these effects as they are supplied by long penetrating end-arteries with few collateral vessels. The decrease in blood supply can lead to demyelination, contributing to reduced functional/structural cortical-subcortical connections. Notably, increased WMH correlates with both decreased and increased BP (De Leeuw et al., 1999), stressing the importance of maintaining BP within an optimal range. Higher BP was also associated with progression of WMH, which was more severe in those with uncontrolled vs. treated HTN (Verhaaren et al., 2013). A study on regional WMH distribution demonstrated that high BP or a history of HTN was associated with greater deep WMH volume in the frontal lobe, but not periventricular WMH. No differences were seen in WMH volumes in other brain regions. This suggests a preferential vulnerability of frontal regions to HTN (Raz et al., 2012). One study also suggested that HTN and SVD may have a common genetic predisposition (Kochunov et al., 2010). A proper management of HTN may, therefore, be particularly critical in these SVD vulnerable individuals.

**Hypercholesterolemia and WMH**

Studies examining the association between WMH and high cholesterol produced inconsistent results. Schilling et al. showed a positive association between triglyceride levels and WMH volumes and frequency of lacunar infarcts. Other cholesterol levels (LDL and HDL), however, were not significantly associated with either SVD biomarker (Schilling et al., 2014). A study in patients with acute ischemic stroke showed an opposite effect of hyperlipidemia (defined as
hypercholesterolemia, hypertriglyceridemia or use of lipid-lowering drugs), such that lower WMH volumes were seen in patients with hyperlipidemia compared to those without (Jimenez-Conde et al., 2010). Cholesterol plays an important role in the creation and maintenance of new synapses in the brain, which might explain the observed beneficial effect.

*Smoking and WMH*

Conflicting results were reported in the cross-sectional studies on association of smoking and WMH; while some showed an increase in WMH volume with smoking (Fukuda & Kitani, 1996; Jeerakathil et al., 2004; Liao et al., 1997), others saw no association (Murray et al., 2005). These inconsistencies may, in part, be due to the presence of confounding factors, such as other vascular conditions effecting WMH severity. In a longitudinal study increased risk of WMH progression was reported in the current but not former smokers, and was associated with the number of pack-per-year (Power et al., 2015).

1.5 VRFs relation to dementia

Prevalence of dementia in western countries is 5% to 10% in individuals >65 years of age, with AD being the most common cause. As life expectancies increase, AD is becoming a major health issue in elderly population, therefore, a large effort is made to identify potential treatment targets. MCI is considered a prodromal stage of AD, although the debate on the exact definition of this condition is still ongoing. MCI is of particular interest as it provides an opportunity for therapeutic intervention during an early disease stage, which may help delay and even reverse disease progression.

Several distinct features are often observed in MCI and AD, including accumulation of Aβ and tau proteins (Hardy & Selkoe, 2002), cognitive decline (Becker et al., 1988), regional decrease in glucose metabolism (Devanand et al., 2010), progressive structural atrophy (Fennema-Notestine et al., 2009; Misra et al., 2009), particularly in the memory related structures (i.e. hippocampus), disruption of functional connectivity (Schwindt et al., 2013), among others. Although genetic predisposition plays an important role in some AD cases, other factors also contribute to disease onset and progression. Luchnsinger et al. showed a link between VRFs (HTN, T2DM, heart disease and smoking) and the risk of AD, with higher number of VRFs being associated with increased hazard ratio (relative risk) for AD (Luchsinger et al., 2005), see Figure 1.4. Later in this section the contribution of each VRF to AD risk will be examined in more detail.
Alzheimer’s disease vs. Vascular dementia

The definition of vascular dementia (VaD) has been debated extensively in scholarly circles and as a consequence several criteria were implemented to try and distinguish AD from VaD. A recent paper on vascular contributions to dementia attempted to bring earlier reports to a consensus and provide a diagnostic guidance to health care professionals (Gorelick et al., 2011). Vascular cognitive impairment is defined as “a syndrome with evidence of clinical stroke or subclinical vascular brain injury and cognitive impairment affecting at least 1 cognitive domain”. VaD is the most severe form of vascular cognitive impairment. The difficulty in differentiating between AD and VaD stems from the common presence of both pathologies (“mixed” dementia). VRFs contribute to both AD and VaD through vascular and metabolic mechanisms which will be examined in more detail next.

T2DM and dementias

Several population studies identified T2DM as a major risk factor for increased AD and VaD (Ott et al., 1999; Peila et al., 2002). Longitudinal assessment showed a 50%-100% increased risk of AD and 100%-150% increased risk of VaD in older individuals with T2DM compared to those without. Other studies found that mid-life T2DM was associated with increased rate of AD, while late-life T2DM showed no such associations (Beeri et al., 2009; Whitmer et al., 2005; Yamada et al., 2003). This could in part be explained by a selection bias, as individuals with T2DM were shown to have a higher dropout rate from the longitudinal studies (mostly due to

Figure 1.4. A hazard ratio for AD diagnosis was calculated by comparison to a group with no VRFs and plotted as a function of the VRF number - adapted from Luchsinger et al, 2005.
death) than those without T2DM (Beeri et al., 2009). Mediating factors, such as glycemic control, presence of other comorbidities and genetic predisposition may play a role in the association between T2DM and AD. The Kungsholmen study, for example, demonstrated that relative risk of dementia (both AD and VaD) was dependent on the interaction of T2DM and HTN (Xu et al., 2004). Genetic predisposition was also examined in regards to the ApoE genotype. Individuals with T2DM and the ApoE ε4 allele were found to have double the risk of dementia compared to those with either one of this factors (Peila et al., 2002). The severity of the disease may also affect the risk of AD, such that T2DM individuals treated with insulin (more severe form of T2DM) had higher risk for dementia compared to oral hyperglycemic medications (Luchsinger et al., 2001).

Several pathophysiological mechanisms may explain the link between T2DM and dementia. Microvascular changes, described in Section 1.3.1, can lead to chronic ischemia in the brain and contribute to cognitive decline. Chronic hyperglycemia is another contributor, as it causes an increase in production of advanced glycation end products, which promote formation of neurofibrillary tangles and neuritic plaques (Korf et al., 2006). Furthermore, insulin dysregulation, a hallmark of T2DM, plays an important role in AD pathology. Insulin crosses the blood-brain barrier from the periphery and competes for insulin degrading enzyme with Aβ. Peripheral hyperinsulinemia can also inhibit production of brain insulin (Reger et al., 2006). Low brain insulin reduces the release of Aβ from intracellular compartment (Gasparini et al., 2001) and decreases production of insulin degrading enzyme (Zhao et al., 2004), both of which result in reduced clearance of Aβ contributing to the formation of amyloid plaques. Impaired insulin signaling also increases tau-phosphorylation leading to accumulation and aggregation of neurofibrillary tangles (Schubert et al., 2004).

**HTN and dementias**

Given that HTN is particularly harmful to the brain and contributes to neurodegeneration, it is not surprising that there are numerous studies that relate HTN to dementia. HTN in mid-life, in particular, has been shown to increase the risk of dementia (AD and VaD) later in life (Kivipelto et al., 2001; Whitmer et al., 2005). As is the case with late-life diabetes, causal links between late-life HTN and dementia are less common. Hypertension contributes to AD risk on its own as well as by contributing to cerebrovascular disease, i.e. stroke and WMH. Furthermore, imaging studies revealed a negative association between SBP and structural atrophy in AD relevant
regions and positive association between SBP and the amount of amyloid plaques in the hippocampus and cortex (De La Torre, 2000; Petrovitch et al., 2000). Elevated DBP (>95mHg) was also associated with increased neurofibrillary tangles in hippocampus (Sparks et al., 1995). Increased amyloid plaques and neurofibrillary tangles were also found in middle-aged hypertensive adults without dementia, suggesting the contribution of HTN to AD onset (Sparks et al., 1995).

**Cholesterol and dementias**

Similar to the relationship between T2DM, HTN and dementia, most studies found an association between mid-life cholesterol levels and late-life dementia, while the relationship with late-life cholesterol are less consistent (Kivipelto et al., 2001; Whitmer et al., 2005) and some findings report cholesterol as having a protective effect (Mielke et al., 2005). Associations between cholesterol and AD may have a genetic component, namely because the ApoE gene codes for a cholesterol protein carrier in the brain, while the ApoE ε4 variant increases the risk of AD and elevated plasma cholesterol levels (Puglielli et al., 2003). The studies examining the interaction of ApoE genotype and cholesterol levels, however, are not consistent (R. M. Evans et al., 2000; Hall et al., 2006; Solomon et al., 2007). Cholesterol metabolism in the neurons may also directly impact AD-pathology, as both generation and clearance of Aβ are reliant on the cholesterol (Papassotiropoulos et al., 2003). Indirectly, high cholesterol levels contribute to AD pathology by increasing the risk of atherosclerosis, which can result in hypoperfusion (Hofman et al., 1997). Considering the close relationship between cholesterol and AD pathology, statins have been proposed as potential intervention for cognitive decline. Although animal studies have shown some promising results (Rech et al., 2010), meta-analysis of the human studies concluded that the statins had no measurable effect on cognition (McGuinness et al., 2009).

**Smoking and dementias**

Several early case-control studies reported a potential protective effect of smoking against AD (Almeida et al., 2002; Brenner et al., 1993), which was attributed to nicotine’s ability to inhibit amyloid formation (Ono et al., 2002). The results of prospective studies, however, are more in line with known negative effects of smoking on cardiovascular system. A meta-analysis of 19 prospective studies reported a significantly higher risk of AD and VaD in smokers, with hazard ratios of 1.79 and 1.78 respectively, compared to never smokers. The risk of AD in smokers was
also higher compared to former smokers (1.70) (Anstey et al., 2007). Notably, several studies pointed out that smoking was only associated with increased AD risk in individuals without ApoE ε4 allele (Ott et al., 1998; Reitz et al., 2007). The risk of AD may also depend on the amount smoked, such that compared to light smokers (≤26.7 pack-years), medium smokers (>26.7-40.5 pack-years) had a 2.56 risk of AD at a 2-year follow-up, while the heavy smokers (>40.5-55.5 pack-years) had a 3.03 risk of AD (Juan et al., 2004). One possible mechanism relating smoking with increased risk of AD is through increased oxidative stress caused by nicotine and other components of cigarette smoke (Crowley-Weber et al., 2003).

1.6 Magnetic Resonance Imaging

Advances in the imaging technology have greatly improved our understanding of neurodegenerative disorders. MRI is a powerful, noninvasive imaging technique that has found a wide range of application in modern research and medicine. MRI techniques can provide insight on a broad spectrum of physiological parameters in the body including anatomy, vasculature, metabolism and function. Unlike other imaging techniques like computed tomography, PET and single photon emission computed tomography, MRI uses magnetic properties of biological tissues to achieve desired contrast and does not expose patients to ionizing radiation. Several MRI techniques will be used in this thesis and are discussed in the next several sections. First, however, a brief introduction to MR principles is provided.

The physical principles of MRI are based on the Nuclear Magnetic Resonance phenomenon utilizing the magnetic properties of certain atomic nuclei. Nuclei with an odd number of protons and/or neutrons possess a property of spin angular momentum. In the human body, hydrogen ($^1$H) is the most abundant nucleus with a single proton and nuclear spin quantum number $I=\frac{1}{2}$. The angular momentum of the protons produce a magnetic field referred to as a magnetic moment ($\mu$):

$$\mu = \gamma \hbar I$$  \hspace{1cm} (1)

where $\gamma$ is a gyromagnetic ratio, a constant specific to the type of a nucleus imaged (for $^1$H, $\gamma=42.58 \text{ MHz/T}$). Other nuclei with non-zero spin angular momentum include: $^{13}\text{C}$, $^{19}\text{F}$, $^{23}\text{Na}$ and $^{31}\text{P}$, which can also be used in MRI but are less common due to their lower prevalence compared to $^1$H.
MR imaging is accomplished by three types of magnetic fields: 1) the main magnetic field, \( B_0 \), ranging in field strength from 1.5-7 Tesla on human systems, 2) the radiofrequency field \( B_1 \) (often referred as “RF pulse”) and 3) linear magnetic gradient fields (Hennig, 1999).

In the absence of the external main magnetic field, the orientation of \( \mu \) is random leading to the net zero magnetization. In the presence of the external main magnetic field, \( B_0 \), protons tend to preferentially align parallel (low energy state) or antiparallel (high energy state) to the magnetic field. By convention, the direction of \( B_0 \) is the z-axis or longitudinal axis. Preference of the protons towards the low energy state results in the net magnetization, \( M_0 \), along the direction of \( B_0 \), and this effect is defined by physical constants and parameters:

\[
M_0 = \frac{N \gamma^2 \hbar^2 I_z (I_z + 1) B_0}{3kT}
\]  

(2)

where \( N \) is the total number of nuclear spins per unit volume, \( \hbar \) is a Plank’s constant, \( \kappa \) is a Boltzman’s constant and \( T \) is the absolute temperature in Kelvin.

When placed in the external magnetic field \( B_0 \) magnetic moments \( \mu \) experience a torque, which causes them to precess about \( B_0 \). The precession of the magnetic moments is described by equation:

\[
\frac{d}{dt} \mu = \mu B_0
\]  

(3)

with solution:

\[
\mu = \mu_0 e^{-i\gamma B_0 t}
\]  

(4)

where \( \mu \) is the magnetic moment rotating about \( B_0 \) with angular frequency \( \omega_0 \), referred to as the Larmor frequency and defined as:

\[
\omega_0 = \gamma B_0
\]  

(5)

The net longitudinal component of the magnetization is then composed of the magnetic moments \( \mu \) precessing at \( \omega_0 \). Since there is no phase coherence between precessing \( \mu \) the net magnetization is pointing along the direction of \( B_0 \).
MRI signal is measured by the excitation and subsequent relaxation of protons. A magnetic field \( B_1 \) applied in the transverse direction and rotating at the Larmor frequency induces a torque, causing the magnetization to tip away from the equilibrium and into the transverse plane. Following the excitation the net magnetization will precess about the longitudinal axis with the frequency proportional to the applied magnetic field and at an angle defined by:

\[
\alpha = \gamma B_1 t
\]

where \( t \) is the duration of the RF pulse, \( B_1 \). This creates a transverse component of net magnetization that can be detected using receiver RF coil.

The MR signal detection is based on Faraday’s Law of induction that states that rotating magnetization creates an electric voltage, which in this case is detected by an RF receive coil. The resulting signal is referred to as free induction decay.

The magnetization following an excitation pulse is described by the Bloch equation:

\[
\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B}_0 - \frac{M_x \vec{x} + M_y \vec{y}}{T_2} - \frac{(M_z - M_0) \vec{z}}{T_1}
\]

where \( T_2 \) is the transverse relaxation time constant that describes the decay of the transverse component of the magnetization (\( M_x \) and \( M_y \)), and \( T_1 \) is the longitudinal relaxation constant that describes the return of the longitudinal magnetization (\( M_z \)) to the equilibrium state. Different physical processes govern these two relaxation events. \( T_1 \) is called the spin-lattice time constant since relaxation occurs as the result of energy exchange between the spins and the surrounding tissue (lattice). Thermal equilibrium is reached as \( M_z \) is restored to \( M_0 \), i.e. the lowest energy state. The time-dependent relaxation of \( M_z \) is described by the following mono-exponential recovery equation:

\[
M_z = M_0 + (M_z(0) - M_0) e^{-t/T_1}
\]

The second relaxation constant, \( T_2 \), is referred to as the spin-spin relaxation and can be defined as the loss of phase coherence due to the interactions between the neighbouring spins. Interaction of the spins’ magnetic fields results in the slight differences in their precession rates leading to a
cumulative loss of phase. The relaxation of $M_{xy}$ is described by the following mono-exponential decay equation:

$$M_{xy} = M_{xy}(0)e^{-t/T_2}$$  \hspace{1cm} (9)

In practice, the transverse magnetization signal decays more rapidly than would be expected due to the $T_2$ relaxation alone. Local non-uniformities of the static magnetic field increase signal dephasing causing shortening of $T_2$ hence a second transverse relaxation term is used, defined $T_2^*$. This apparent transverse relaxation is described by:

$$M_{xy} = M_{xy}(0)e^{-t/T_2^*}$$  \hspace{1cm} (10)

where

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'}$$  \hspace{1cm} (11)

and $T_2'$ is the additional decay constant due to non-uniformities of the static magnetic field.

The signal loss due to $T_2^*$ effect can be recovered using a second 180° RF pulse that rotates magnetization causing the signal to refocus.

Spatial localization of MRI signal is achieved using three orthogonal RF coils producing gradient fields in x-, y- and z-directions. An external magnetic gradient $G$, applied in an arbitrary spatial direction, e.g. r direction, results in a spatially varying Larmor frequency:

$$\omega(r) = \omega_0 + \gamma G r$$  \hspace{1cm} (12)

One specific application of magnetic gradients is the ability to prescribe a slice of interest. Slice selection is achieved by simultaneously applying the magnetic gradient (by convention, $G_z$) and the RF excitation pulse with a specific bandwidth ($BW_{RF}$). Since the Larmor frequency can be made to vary linearly along the z-direction, following a $G_z$ gradient, selecting a specific range of frequencies for RF will limit the proton excitation to a specific plane ($\Delta z$) where $\omega_0$ matches those frequencies:
\[ \Delta z = \frac{BW_{RF}}{\gamma G_z} \] (13)

Spatial encoding in the x- and y- directions are achieved using Gx and Gy gradients and these are conventionally referred to as phase- and frequency- encoding gradients, respectively. Application of Gy gradient causes magnetic moments \( \mu \) to precess at different frequencies (i.e. increasing linearly with y) and accumulate position dependent phase relative to one another. Once the gradient is turned off the frequencies of \( \mu \) returns to the original \( \omega_0 \) but retain the accumulated y-dependent phase. Therefore, phase-encoding gradient Gy spatially encodes the y position of \( \mu \) through the y-dependent phase accumulation. Phase-encoding typically occurs prior to signal acquisition. Frequency-encoding gradient Gx, on the other hand, is applied during the acquisition, and is therefore also referred to as the readout gradient. Similarly to the phase-encoding gradient, Gx, causes the position dependent change in the precessing frequencies of \( \mu \) and phase accumulation along the x-direction.

The MR signal is recorded using the RF receiver coils. This information is stored in a data matrix referred to as k-space. The time integrals of the gradient waveform represent a position in the k-space matrix:

\[
k_x(t) = \frac{\gamma}{2\pi} \int_0^t G_x(\tau) \, d\tau
\] (14)

\[
k_y(t) = \frac{\gamma}{2\pi} \int_0^t G_y(\tau) \, d\tau
\] (15)

Once the k-space matrix information is filled, it contains all the spatial frequencies used in the spatial encoding. The inverse Fourier transform is then used to transform the data into an imaging domain producing the final MR image of the structure.

1.6.1 MRI pulses sequences and contrasts (\( \rho, T1, T2, T2^* \))

Good tissue contrast is one of the defining features of MR imaging. The contrast between different tissue types is governed by the inherent characteristics of the tissue, i.e. proton density (\( \rho \)), T1 and T2 relaxation times, and on selected pulse sequence parameters, i.e. repetition time (TR), echo time (TE) and flip angle. Preferred weighting selection often depends on the
organ/pathology of interest. Table 1.3 summarizes the MR properties of brain tissues at 3T, while the following sections provide examples of commonly used pulse sequences and imaging contrasts.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>$T_1$ (ms)</th>
<th>$T_2$ (ms)</th>
<th>$T_2^*$ (ms)</th>
<th>$\rho$-density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td>1820</td>
<td>100</td>
<td>50</td>
<td>0.69</td>
</tr>
<tr>
<td>White matter</td>
<td>1080</td>
<td>70</td>
<td>50</td>
<td>0.61</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>1932</td>
<td>275</td>
<td>46</td>
<td>0.72</td>
</tr>
<tr>
<td>CSF</td>
<td>3817</td>
<td>1442</td>
<td>n.a.</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 1.3. MR properties of brain tissues at 3T, adapted from (MacIntosh & Graham, 2013)

A pulse sequence is a series of events consisting of RF pulses, gradients and data acquisition that give experimenter the ability to manipulate magnetization and achieve a desired contrast. In the absence of any relaxation effects the variation in the MR image intensity represents the differences in proton density between different tissues, which is directly proportional to the amount of hydrogen molecules, and therefore the water content of the tissue. In the $T_1$-weighted image, the contrast between different tissue classes depends primarily on their longitudinal relaxation time $T_1$. If the pulse sequence is designed to differentiate the tissues based on their transverse relaxation, the images are said to be $T_2$-weighted. The $T_2^*$ contrast can be achieved using the gradient echo sequence, described in the next section. $T_2^*$ contrast was shown to vary with physiological status; pulse sequences utilizing this contrast have found a wide application in functional MRI (fMRI). One of the most common fMRI techniques, BOLD imaging, will be used in Chapters 2 and 3 and is discussed later in the section.

Two MRI techniques used in this thesis are: 1) Blood Oxygenation Level Dependent (BOLD) imaging used to measure CVR and resting-state fMRI, and 2) high-resolution T1-weighted three-dimensional magnetization-prepared rapid gradient-echo sequence (3D-MPRAGE) used to estimate the CThk. These two imaging techniques are discussed in more detail below. First, however, a brief introduction to gradient echo imaging is provided as it serves as the base for both BOLD and MPRAGE sequences.
**Gradient Echo**

The gradient echo (GE) sequence uses a pair of gradients with opposite polarities to first dephase and then rephase the signal. Following the initial RF excitation, a negative gradient is turned on causing the spins in the selected slice to dephase. The phase accrual continues for a certain period of time, $\tau$, after which the gradient is reversed refocusing the magnetization and forming a signal echo. At time $2\tau$, the spins regain coherence and the signal is at its maximum (Hennig, 1999). Gradient echo imaging is commonly used in fast imaging sequences since TR can be kept relatively short. Unlike the spin echo sequences where an extra 180° pulse is used to correct for field inhomogeneities, the signal in gradient echo decays with $T_2^*$. 

**BOLD imaging**

BOLD effect depends on the changes in the local $T2^*$ values and is, therefore, most pronounced on gradient echo images. The neuronally-mediated hemodynamic responses, measured by BOLD, require sufficiently high temporal resolution, thus prompting the need for fast MR imaging to detect the activation. A commonly used approach is echo planar imaging (EPI) (Mansfield, 1977; Stehling et al., 1991). During an EPI acquisition, signal readout is accomplished by sampling of k-space lines for a given slice with a rapidly oscillating readout gradient and a train of blips in phase-encoding direction, see Figure 1.5a.

![Gradient echo EPI timing diagram](image)

**Figure 1.5.a)** Gradient echo EPI timing diagram. RF - Radiofrequency pulse, GS - Slice-selective gradient, GR - Readout gradient, GP - Phase-encoding gradient, and **b)** schematic of interleaved sampling of k-space.

EPI allows for fast imaging by filling a k-space plane with a single excitation RF pulse, however, the downside to this is high susceptibility to geometric distortions that are caused by chemical
shift, magnetic susceptibility variations (i.e. tissue-air interfaces or metallic implants), magnetic field inhomogeneities and ghosting (McKinnon, 1993). Numerous techniques have been developed to correct these artifacts. For example, the effects of chemical shift, occurring due to the differences in resonance frequencies between fat and water, can be minimized by an addition of 90° pulse at the fat frequency followed by a dephasing of the fat signal using gradient pulse. This approach is referred to as fat saturation (De Kerviler et al., 1998), and is a common practice in BOLD imaging. “Ghosting” is another common EPI artifact that occurs due to the rapid gradient switching that can form eddy currents. Eddy currents can introduce a time-dependent frequency shift, which creates a phase difference between the lines of the raw data. Once the data are Fourier transformed, part of the signal appears 90° out of phase. The best approach to decrease “ghosting” artifacts is to design gradient coils to minimize the eddy current induction. Sampling a portion of a k-space at a time can also provide a substantial increase in image quality. One option of partial k-space readout is “interleaved” imaging implemented in Chapter 2. During the interleaved EPI with 2 shots, the first pass covers the entire k-space but only samples every other line. The second pass fills in the remaining portion of k-space, see Figure 1.5b. Spiral k-space trajectory can also improve image quality compared to EPI by decreasing the readout time (Glover, 2012). The implementation of the spiral methods, however, is more complex than EPI and therefore less common in fMRI. In addition to decreased geometric distortions, interleaved acquisition can also provide a substantial increase in signal-to-noise ratio in comparison to the original EPI, at the expense of temporal resolution (Butts et al., 1994). Gradient echo EPI is the most common technique for fMRI acquisition. The physiological basis of BOLD signal are discussed in Section 1.6.2.

**MPRAGE**

T1-weighted three-dimensional magnetization-prepared rapid gradient–echo sequence (3D-MPRAGE) imaging provides high-resolution T1-weighted anatomical images with excellent tissue contrast and is therefore well suited for brain structure assessment. It begins with a nonselective preparatory inversion pulse followed by a time delay (TI) to achieve T1-dependent contrast, see Figure 1.6. The readout is accomplished using the rapid gradient echo sequence. Each rapid gradient echo acquisition samples a portion of k-space. A small flip angle excitation pulse is applied to sample the image. The small flip angle is used such that the longitudinal magnetization does not vary greatly during the x-y plane sampling. Any remaining transverse
magnetization is destroyed by a crusher gradient before the next excitation pulse. Once the acquisition for the plane is complete, magnetization is allowed to recover before the next inversion pulse is introduced and the entire process is repeated for the new plane.

A complete 3D MPRAGE includes multiple cycles of the preparation-acquisition-recovery paradigm (Mugler III & Brookeman, 1990; Runge et al., 1991). 3D MPRAGE is one of the most common techniques used for structural brain imaging as it provides high tissue contrast, high spatial resolution and full brain coverage with short scan time. Several optimizations techniques for the sequence have been recently proposed including the use of parallel acquisition to further decrease the scan time (Griswold et al., 2002), and modifying the sequence to produce two images at two different TI (MP2RAGE) (Marques et al., 2010). Combining the two images improves T₁ contrast by eliminating contribution from proton density, T₂ and other field inhomogeneities. Field inhomogeneities can significantly affect image quality and render segmentation and qualitative analysis, particularly at the ultra-high fields (≥7 T). Structural imaging is discussed in more detail in Section 1.9 and is used as a biomarker in Chapters 2 and 4.

1.6.2 Physiology of BOLD signal

In 1990, Seiji Ogawa was the first to propose a type of MRI that would allow non-invasive in-vivo monitoring of blood oxygenation in the brain, from here on referred to as Blood Oxygenation Level Dependent (BOLD) imaging (Ogawa, Lee, Nayak et al., 1990). The method is based on the effect of oxygen content on the magnetic properties of hemoglobin in the blood. The normally diamagnetic oxyhemoglobin acquires paramagnetic properties as it gives up its oxygen. The shortening of blood T₂ upon deoxygenation leads to a difference in magnetic

![Figure 1.6. MPRAGE sequence timing diagram. Adapted from (Runge et al., 1991). RF - Radiofrequency pulse, GS - Slice-selective gradient, GR - Readout gradient, GP - Phase-encoding gradient.](image-url)
susceptibility between blood vessel and the surrounding tissue. This susceptibility difference produce local magnetic fields around blood vessels that result in phase dispersion on images acquired with gradient echo sequences causing the area to appear hypointense (Ogawa, Lee, Kay et al., 1990; Ogawa et al., 1990). Therefore, deoxyhemoglobin acts as an endogenous contrast agent.

The BOLD fMRI technique has been widely utilized in cognitive neuroscience and has been a tool of choice for visualization of neuronal activity during task performance and resting condition in healthy aging and neurodegenerative conditions. With almost 6000 papers published on “BOLD fMRI” in the last decade it is important to have a clear understanding of the physiological basis of BOLD fMRI signal to appreciate the strengths and limitations of the technique. Neuronal activity is characterized by the local field potentials and spiking activity, which account for synaptic and cell bodies activity respectively (S. G. Kim & Ogawa, 2012). Neuronal activation is accompanied by increased glucose consumption, resulting in heightened ATP production, and an influx of $K^+$, $Ca^{2+}$ and $Na^+$. These changes cause astrocytes and neuronal cells to send vasoactive signals to the nearby arterioles and capillaries resulting in the dilation of the upstream arteries and a focal increase in CBF (S. G. Kim & Ogawa, 2012).

Although the mechanism of neuronal activation suggests a tight coupling between glucose consumption and CBF, PET studies demonstrated that the CBF increase surpasses the increase in CMRO$_2$ (P. T. Fox & Raichle, 1986). This mismatch leads to the increased oxygenation in the capillary and venous blood.

BOLD signal is proportional to the concentration of deoxyhemoglobin in the venous blood and is therefore influenced by the factors affecting the deoxyhemoglobin levels, such as CBF, CMRO$_2$ and cerebral blood volume (CBV). Increase in the CMRO$_2$ and CBV decreases the BOLD signal while the increase in the CBF increases it. The change in the BOLD signal is approximated by Equation (16) (Davis et al., 1998):

$$\%BOLD = M \left(1 - \left(1 + \frac{\Delta CMRO_2}{CMRO_2} \right)^\alpha \left(1 + \frac{\Delta CBV}{CBV}\right)^\beta \right)$$

where M represents the maximum potential BOLD signal change that depends on a variety of factors such as baseline physiology and vasculature, the change in CBV is calculated from the Grubb’s formula: $(1+\Delta CBV/CBV) = (1+\Delta CBF/CBF)^\alpha$, and the values of $\alpha=0.38$ and $\beta = 1.5$ are assumed from previous studies (Davis et al., 1998; Grubb Jr et al., 1974). M can be
experimentally determined by measuring changes in CBF and BOLD signal during hypercapnia or hyperoxia, conditions when CMRO$_2$ is presumed to remain unchanged. It can then be used in Equation (16) to estimate the change in CMRO$_2$ during task condition based on the measured change in BOLD and CBF. Such approach is referred to as the calibrated fMRI (Davis et al., 1998). The measurement of cerebrovascular hemodynamics using hypercapnia and BOLD has also found a wide application in multiple neurodegenerative disorders and will be discussed in more details in Section 1.7.

Although BOLD fMRI continues to play a key role in the study of neural activation by contrasting task and resting conditions, new applications of the technique have emerged. Two such applications will be used in this thesis and are discussed in Sections 1.7 and 1.8. Section 1.7 will examine the use of BOLD and hypercapnia challenges to measure CVR, while Section 1.8 will introduce the concept of resting-state fMRI (RS-fMRI).

1.7 Cerebrovascular reactivity

1.7.1 Cerebral autoregulation

Although the human brain constitutes only 2% of the entire body weight it accounts for 20% of the body’s resting glucose metabolism (Kety, 1957) and receives 15% of cardiac output. The high energy demand and its low capacity to store energy emphasizes the need for an effective regulation of the brain’s blood supply. According to the Poiseuille’s law, Equation (17), blood flow is dependent on perfusion pressure and vascular resistance.

\[ F = \frac{(P_1 - P_2)\pi r^4}{8\mu L} \]  

where $F$ - flow, $P_1$ - inflow pressure, $P_2$ - outflow pressure, $r$ – radius, $\mu$ – fluid viscosity, and $L$ - length.

Regulation of blood flow during changing systemic BP is achieved by altering vascular resistance (Panerai, 1998). Fog made the first observation of cerebral autoregulation in 1930 by measuring the change in the vessel tone of pial arteries in response to the fall in BP (Fog, 1937). In 1959 Lassen established the concept of “cerebral autoregulation” as the ability to maintain blood flow constancy over a range of perfusion pressure levels (Lassen, 1959). At the limits of autoregulation control, the blood vessels are no longer able to make adjustments to alter the
vascular resistance and the CBF will decrease/increase with changing perfusion pressure, resulting in a sigmoidal relationship. Lower and upper pressure limits are approximately 50-70mmHg (Lassen, 1959) and 150-160mmHg (Symon et al., 1973), respectively. These levels may vary between individuals and are influenced by disease, for example, chronic hypertension shifts the lower end of the autoregulatory curve towards high BP, see Figure 1.3 (Strandgaard, 1976).

The mechanisms underlying cerebral autoregulation are not yet completely understood. Vascular resistance has been traditionally attributed to small arteries and arterioles, whereas the large arteries were considered to be conduit vessels. Several studies, however, demonstrated that vascular resistance of large vessels in the brain is higher than that in other organs (Faraci & Heistad, 1990).

1.7.2 Clinical relevance

Cerebral autoregulation ensures an adequate cerebral blood flow by constricting or dilating blood vessels in response to changes in BP. This ability can be compromised by atherosclerotic disease, thereby increasing the risk of ischemia and stroke. One way to indirectly evaluate the autoregulatory capacity of brain blood vessels is to measure their ability to respond to a vasoactive stimulus, referred to as CVR.

1.7.3 Measuring cerebrovascular reactivity

CVR measures the change in CBF in response to a vasodilation or vasoconstriction stimuli. Types of stimuli will be discussed in more detail in Section 1.7.4. Imaging techniques used in the earlier CVR studies included PET (Hirano et al., 1994), single photon emission computed tomography (Knop, Thie, Fuchs, Siepmann, & Zeumer, 1992), Xenon computed tomography (Yonas, Smith, Durham, Pentheny, & Johnson, 1993), transcranial doppler ultrasound (Silvestrini et al., 2000) and dynamic susceptibility contrast MRI (Soinne et al., 2003), each with their own strengths and limitations. PET, single photon emission computed tomography and xenon computed tomography use ionizing radiation, limiting the ability for repeated measurements. Transcranial Doppler ultrasound is inexpensive, non-invasive, and free of ionizing radiation, but it yields only a single measurement of the blood velocity from a major artery. Dynamic susceptibility contrast MRI has also been used for CVR measurement, but it uses an exogenous contrast and is not be suitable for patients with impaired kidney function.
An ASL MRI has also been used in CVR studies, and can provide a quantitative measure of perfusion changes during the vasodilatory stimuli. The main challenge of ASL is, however, the low signal-to-noise ratio. A common practice for improving ASL image quality involves averaging control-tag difference pairs to achieve a final perfusion map. Although this approach is valid with CO$_2$ inhalation and acetazolamide injection (discussed in more details in the next section) (Hajjar et al., 2010; Last et al., 2007), the implementation of ASL with BH remains a challenge due to the short hypercapnia period.

BOLD imaging offers a compelling alternative with no radiation, high temporal and spatial resolution and no need for contrast injection. As discussed in Section 1.6.2 BOLD signal is proportional to CBF in the absence of the change in CMRO$_2$. Vasodilatory-induced CBF increases cause the concentration of deoxyhemoglobin to decrease, leading to the increase in the BOLD signal.

1.7.4 Vasodilatory challenges

Several vasodilatory techniques have been utilized to examine CVR, including: 1) transient reduction in mean arterial pressure (MacKenzie et al., 1979), 2) intravenous injection of vasodilatory agent, e.g. acetazolamide (Vorstrup et al., 1986), and 3) increase or decrease in partial pressure of CO$_2$ (PaCO$_2$) levels (Poulin et al., 1996).

Repeatable use of hypotensive stimuli, however, is hard to implement and may increase the risk of ischemia in patients with compromised regional CBF. Furthermore, the extent of induced hypotension, either through administering vasodilators (sodium nitroprusside), abrupt thigh cuff release or lower body negative pressure, varies greatly even in the healthy subjects.

The use of intravenous acetazolamide was first introduced by Vorstrup et al. and has since been widely used in CVR studies and clinical settings. Acetazolamide acts by producing an extracellular and intracellular acidosis, lowering the pH, and resulting in vasodilation (Vorstrup et al., 1986). The ease of implementation with a single dose injection has made it an attractive CVR method. Limitations of this approach include high individual variability as well as high risk of adverse side effect, such as dizziness, light-headedness and headaches (Ringelstein et al., 1992).

Carbon dioxide is another potent stimuli used in CVR measurement. Although both hypocapnia
and hypercapnia have been proposed, Ringelstein et al. demonstrated that the CVR response to hypocapnia was reduced compared to the hypercapnia (Ringelstein et al., 1988). Hypercapnia is achieved by increasing PaCO$_2$ in the blood causing a decrease in the perivascular pH level. Cerebrovascular smooth muscles, sensitive to the levels of pH, react with a decrease in cerebrovascular resistance leading to vessel dilation and increase in CBF (R. F. Leoni et al., 2008). Two hypercapnia-inducing methods are commonly employed in CVR measurements, CO$_2$ inhalation and BH. Advantages and limitations of both are discussed in more detail next.

**Breath-hold challenge**
BH challenge is one of the earliest techniques used to induce hypercapnia. It requires no additional hardware and is well tolerated by most individuals, which makes it well-suited for clinical applications. The main limitation in the use of BH for CVR measurement is the high inter-individual variability in the resultant PaCO$_2$. The PaCO$_2$ during BH increases gradually with time, and therefore, the final PaCO$_2$ depends on BH duration, as well as other factors including metabolic rate, lung capacity and participant compliance (Fierstra et al., 2013). Several techniques has been proposed to reduce PaCO$_2$ variability during BH, such as the use of paced respiration following BH (Scouten & Schwarzbauer, 2008), use of end-expiration BH to reach higher PaCO$_2$ (Kastrup et al., 1998; Scouten & Schwarzbauer, 2008) and collecting partial end-tidal CO$_2$ (P$_{ET}$CO$_2$) traces to be included in the model of vascular response (Murphy et al., 2011).

**CO$_2$ Inhalation**
Direct CO$_2$ inhalation produces more robust PaCO$_2$ levels compared to BH, but often requires an elaborate set up limiting its implementation for clinical use. Several techniques of CO$_2$ delivery has been employed in the past including inspiration of fixed fractional concentration of CO$_2$ (2%, 5% or 7%) (Ringelstein et al., 1988; Ringelstein et al., 1992), rebreathing of the exhaled gas (Read, 1967) and, more recently, the use of prospective targeting of P$_{ET}$CO$_2$. RespirAct$^\text{TM}$ device is an example of the latter (Slessarev et al., 2007). It has an advantage of being able to independently control the P$_{ET}$CO$_2$ and P$_{ET}$O$_2$ levels, and the P$_{ET}$CO$_2$ were shown to closely correspond to the PaCO$_2$ measures from arterial blood sampling (Ito et al., 2008). Although there are clear advantages in the use of prospective P$_{ET}$CO$_2$ targeting for CVR measurement, its implementation in clinical populations is still a challenge. BH and CO$_2$ inhalation using RespirAct$^\text{TM}$ device will be used in the studies described Chapters 2 and 3, respectively.
1.7.5 fMRI analysis

Best practice guidelines for neuroimaging CVR data analysis are still a topic of debate. Among the most common approaches is the use of general linear model (GLM) on a voxel-by-voxel basis, similar to task-based fMRI analysis.

*General linear model*

The GLM is a statistical framework that has a widespread use in many fields of science, notably in neuroimaging; the GLM assumes that the data (e.g. a measured BOLD signal over time) is composed of a linear combination of explanatory variables and an error term, see Equation (18) (Kiebel & Holmes, 2003)

\[ Y_j = x_{j1} \beta_1 + \ldots + x_{jl} \beta_l + \ldots + x_{jL} \beta_L + \varepsilon_j \]  

(18)

\( Y \) - measured data variable, i.e. fMRI time-series from a single voxel
\( j=1,\ldots, J \) – index of data variable (i.e. time points or volumes)
\( x \) – a set of explanatory variables, also referred to as design matrix
\( l=1,\ldots, L \) – index of explanatory variables
\( \beta_l \) – parameter estimate corresponding to each \( x_{jl} \)
\( \varepsilon_j \) – error term

Several methods are available to estimate the values of \( \beta \), the simplest of which, ordinary least squares, calculates the optimal parameters by minimizing the sum of squared residuals (difference between observed \( Y \) and predicted \( X\beta \)). The ordinary least squares method relies on three assumptions: 1) errors for all observations are independently and identically distributed, 2) regressors in \( X \) are independent of the error, deterministic and known, and 3) no regressor can be expressed as a linear combination of the other regressors (Monti, 2011). Of note, fMRI is composed of time-series data, therefore, the correlations between residuals of the successive time-points may violate the 1st assumption, as the common sources of noise, related to hardware or physiological fluctuations, can introduce serial correlations. Several approaches, including temporal smoothing, “pre-whitening” (estimating and removing the autocorrelation) and explicit noise modeling have been used to address serial correlations (Monti, 2011).

A standard GLM example is the linear regression with a single continuous explanatory variable
$X_j$ measured for each observation. In this case $\beta$ represents a simple regression slope. A two-sample t-test is another common case of GLM in fMRI experiments. For example, comparing patients and control groups. FMRI software packages such as FSL FMRI Expert Analysis Tool (FEAT) (http://fsl.fmrib.ox.ac.uk/fsl/) can be used to analyze both individual subject data and to perform group analysis. Accurate modeling of BOLD response to an external stimulus is one of the challenges when performing an fMRI analysis. The BOLD signal is proportional to CBF changes induced by task stimuli, however, those changes do not occur instantaneously and may vary depending on the individual and the type of stimulus. In-vivo optical imaging demonstrated a dispersion of hemodynamic response following visual stimuli in monkeys that resulted in a series of activity-related changes: first, highly localized oxygen delivery was noted 200-400ms after the onset on neuronal activity, followed by an increased in blood volume at 300-400ms and, finally, a significant rise in oxyhemoglobin after 1000ms (Frostig et al., 1990). Early fMRI experiments by Bandettini et al. support this findings with the relative oxygenation found to reach its maximum at 4-10s following neuronal activity (Bandettini, 1993; Friston, Jezzard et al., 1993). The delay and dispersion of hemodynamic response can introduce a substantial bias in fMRI analysis if not properly accounted for. A hemodynamic response function (HRF) attempts to capture this physiological response. Several models of the HRF have been proposed with single and double gamma variate function being the most widely used (Handwerker et al., 2004). A convolution of stimulus model with an HRF is one way to improve the model fit, others include introduction of response delay and inclusion of physiological factors such as head motion, heart rate and respiration as covariates.

*CVR analysis*

Murphy et al. compared nine different GLM models for BH BOLD response. The best prediction model included partial P$_{\text{ET}}$CO$_2$ traces convolved with HRF and incorporating P$_{\text{ET}}$CO$_2$ temporal derivative (Murphy et al., 2011). P$_{\text{ET}}$CO$_2$ traces, however, are not typically collected during the BH challenge, therefore, a commonly used approach for BH modeling is the use of box-car model convolved with HRF and including an additional delay to account for hemodynamic latency (Kastrup et al., 2001). The use of P$_{\text{ET}}$CO$_2$ traces as a voxel-wise regressor in the GLM is more common in the CO$_2$ inhalation analysis than BH. Leoni et al., however, demonstrated that the response to hypercapnia is not uniform across the brain. Authors suggested that the characteristics of the signal may differ in various regions of the brain based on the underlying
vasculature in that area (R. F. Leoni et al., 2008). This suggests that the use of a single regressor across the entire brain may introduce a bias in the CVR estimate, e.g. in the regions with a delayed CVR response. An alternative method for CVR analysis that accounts for regional response variability is proposed in Chapter 3.

1.8 Resting state functional connectivity

Resting-state functional connectivity refers to synchronized blood flow fluctuations in the functionally related brain regions in the absence of a prescribed task. This technique will be used in Chapter 3 of this thesis and described in detail next.

The concept of functional connectivity was explored early on using electrophysiology (Gochin et al., 1991) and PET (Friston, Frith et al., 1993). In the early days of BOLD fMRI, however, neuronal activation was mainly probed by having participants perform a task, i.e. finger tapping or administering a stimulus. Spontaneous fluctuations of BOLD signal were reported by several groups in 1992 (Biswal et al., 1992; Weisskoff et al., 1992) but were regarded as “noise” prompting the development of post-processing methods to reduce their contribution to measured activation. Then, in 1995 Biswal et al. used resting-state fMRI (RS-fMRI) to show a strong correlation between BOLD time-course from the region in sensorimotor cortex in one hemisphere with the time-course of the corresponding region in the opposite hemisphere, attributing high correlation to functional connectivity of the regions (Biswal et al., 1995). Similar inter-hemispheric correlations were later observed by Lowe et al. in the motor, visual and limbic regions (Lowe et al., 1998). Since then, a set of highly reproducible RS networks (RSNs) has been identified (Damoiseaux et al., 2006). The early notion attributing spontaneous BOLD fluctuations to noise was also addressed by examining the contribution of temporal frequencies in the patterns seen in spontaneous BOLD. The power spectrum of the random noise is expected to be flat (all frequencies are contributing to an equal degree). The frequencies observed in the spontaneous BOLD fluctuation, however, follow 1/f distribution (increasing power at lower frequencies) (M. D. Fox & Raichle, 2007). Furthermore, Cordes et al. demonstrated that only frequencies below 0.1 Hz contribute to the regionally specific BOLD correlations while higher frequencies relate to cardiac and respiratory factors (Cordes et al., 2001). Notably, the networks identified during rest persist during task performance (Smith et al., 2009), although, in some instances, with modified characteristics, i.e. DMN is deactivated during cognitive tasks.
1.8.1 Clinical relevance

A major focus of RS-fMRI studies is now on the correlation between functional connectivity and clinical and behavioral outcome measures. Studies of numerous patient groups revealed abnormalities in the RS functional connectivity that often correlated with cognitive and behavioral symptoms (Castellanos et al., 2008; Kennedy & Courchesne, 2008; Zhou et al., 2007). Although, multiple networks have been implicated in neurological conditions, one, in particular, appears to be vulnerable to a wide range of neurological disorders, including SVD discussed in Section 1.4, and is commonly referred to as the DMN.

1.8.2 The Default Mode Network

The concept of a default mode network in the brain was first introduced by Raichle et al. who used PET imaging to examine the oxygen extraction fraction during rest (Raichle et al., 2001). Shortly after, Geicuis et al. used RS-fMRI to demonstrate functional connectivity in a set of brain regions with high BOLD signal during rest but attenuated signal during cognitive tasks. It is for this reason that the DMN is thought of as the “mind wandering” network in the brain. DMN includes precuneus/posterior cingulate cortex, medial prefrontal cortex, and medial, lateral and inferior parietal cortices (Greicius et al., 2003). Posterior cingulate cortex is involved in continuous assessment of internal and external environments, the attenuation of which facilitates attention focus during the transition to the task-specific activity (Raichle et al., 2001). Medial prefrontal cortex has been implicated in social cognition and is thought to mediate dynamic interplay between cognitive function and emotional processes. Both posterior cingulate and medial prefrontal cortices govern the introspective processes, which are attenuated when attention is directed towards an external task (Broyd et al., 2009; Gusnard & Raichle, 2001). DMN dysfunction can manifest in a number of different forms and has been identified in several neurological disorders. For example, altered anti-correlation between DMN and the “task positive” networks were seen in patients with schizophrenia (Zhou et al., 2007) and autism spectrum disorder (Kennedy & Courchesne, 2008), where anti-correlations were increased, and attention deficit hyperactivity disorder, with decreased anti-correlation (Castellanos et al., 2008). Authors postulate that these correlation abnormalities may help explain common symptoms associated with the disorders, such as over-mentalization and deficits in attention control in schizophrenia (Zhou et al., 2007), social withdrawal in autism (Kennedy & Courchesne, 2008).
and attentional lapses in attention deficit hyperactivity disorder (Castellanos et al., 2008). Changes in functional connectivity between DMN regions and between DMN and other resting networks are also commonly observed (Kennedy et al., 2006; Wang et al., 2006). Decreased connectivity between right hippocampus and components of the DMN including medial prefrontal cortex, ventral anterior cingulate cortex, middle temporal gyrus and posterior cingulate cortex likely relates to the dysfunction in the working-memory and attention among adults with AD (Wang et al., 2006). Finally, atypical patterns of DMN activity were seen to correlate with symptom severity in schizophrenia (Garrity et al., 2007), autism (Kennedy et al., 2006) and depression (Greicius et al., 2007), and helped differentiate AD and MCI patients (Rombouts et al., 2005).

System-specific RSNs have been widely explored using BOLD-based fMRI. Recall that the BOLD fMRI signal reflects hemodynamic changes via deoxyhemoglobin concentration in voxels and not neuronal activity directly (see Section 1.6.2 for more detail on BOLD). Several studies used EEG and magnetoencephalography to confirm BOLD-based RS patterns demonstrating a good overlap between modalities. Chapter 3 of this thesis will examine a possible link between spatial patterns of the RS networks and variability of vascular hemodynamics across the brain.

1.8.3 Resting-state fMRI analysis
Resting-state fMRI involves BOLD imaging for 5 to 10 minutes while the participant is resting with eyes open and without any external stimuli or task. This technique has gained wide appeal because of its ease of implementation, participant compliance and demonstrated sensitivity. How to analyze RS-fMRI, i.e. “best practice guidelines”, has been the topic of extensive research and of wide debate. Analysis methods include: model-based (often referred to as seed-based) and data-driven approaches. The seed-based analysis identifies areas of the brain that are functionally connected with predefined region of interest, i.e. the “seed”. The data-driven approaches, on the other hand, require no a priori hypothesis and identify multiple independent, functionally connected networks. The most widely used data-driven method for RS-fMRI is independent component analysis (ICA). The seed-based and ICA approaches are examined in more detail in the following sections.

Seed-based analysis
In a seed-based analysis a BOLD time-course is extracted from a “seed” region, and the temporal
correlations are computed between the extracted signal and time-courses in every voxel of the brain. The voxel-wise correlation maps are then thresholded at the predefined statistical value producing a network of regions connected with the “seed”. This approach was used in the original RS-fMRI manuscript (Biswal et al., 1995) and is still widely used today due to its simplicity of implementation, ease of interpretation and sensitivity. The limitations, however, include the dependence of the results on the definition of a seed region and inability to examine multiple systems at once (M. D. Fox & Raichle, 2007).

**Independent component analysis**

ICA uses blind source separation technique to decompose the spatial-temporal BOLD data into spatial components that are maximally statistically independent (McKeown et al., 1998). Each independent component corresponds to a spatial map, some of which represent neuronal systems while others may reflect spatial patterns associated with structured noise (Damoiseaux et al., 2006; Kelly et al., 2010; Smith et al., 2009). This section will review application of ICA for fMRI analysis along with the dual-regression (DR), a statistical technique commonly used in conjunction with ICA, and that will be used in this thesis. Depending on the particular goals of the study, ICA can be used to maximize statistical independence in either spatial or temporal domains. A spatial ICA implemented using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) software (Beckmann & Smith, 2004) is used in this thesis and described in detail below.

First, the original BOLD data are demeaned and normalized to have a unit noise variance (Beckmann, 2012). This step ensures a uniform false-positive detection rate across the brain by removing the spatial bias towards the high-variance voxels (i.e. within cerebrospinal fluid). Variance normalization requires a prior knowledge of the signal to estimate the noise that would be forced to have the same variance across the brain. To achieve this, an iterative probabilistic principal component analysis is used to split the total data into the noise and signal sub-spaces. The initial noise estimate is then used to refine the normalization step. Following the variance normalization, temporal data prewhitening is applied using the initial noise estimate from principal component analysis step (Woolrich et al., 2001). Finally, the data are decomposed into a set of independent components (IC) and each IC map is transformed to a voxel-wise Z-statistic by dividing the raw IC map by the standard deviation of the residuals from principal component analysis step. To identify the regions significantly modulated by the component time-course a
threshold is applied to the Z-maps. The threshold is determined by fitting a Gaussian/Gamma mixture model into the component’s histogram (Beckmann, 2012). Figure 1.7 depicts a schematic representation of spatial ICA.

**Figure 1.7. Schematic illustration of data decomposition performed by spatial ICA. Adapted from (Beckmann, 2012)**

One of the limitations of ICA, and the topic of much debate, is determining the appropriate number of components to be extracted from the data. Early ICA studies extracted as many components as was required to account for a predefined proportion of variability in the data. In the MELODIC the number of components is estimated based on the analysis of the eigen spectrum of the data covariance matrix (Beckmann, 2012). Although it is hard to estimate the optimal number of components, recent work by Smith demonstrated that decomposition across a wide range of dimensionalities might have a biological interpretation, i.e. higher dimensionality with 70 components describes the same systems as 20 components, except they split the systems into its subcomponents (Smith et al., 2009). Another limitation that is worth noting is that since ICA decomposition is achieved through iterative optimization the results may differ somewhat for each run of the analysis on the same data. Attempts have been made to reduce this variability by introducing a stringent convergence criteria (Himberg et al., 2004). ICA has many advantages over the use of traditional GLM approaches. As mentioned earlier, ICA is a data-driven approach, therefore, it is not biased by the use of an *a priori* model. Furthermore, the blind separation of the data allows for the identification of artifacts and noise components, which could subsequently be removed from the data (Kelly et al., 2010).

A common practice for this type of analysis is to concatenate data across individuals to generate robust group networks. Once the group-level network maps are acquired, the DR approach is used to produce participant-specific maps. Dual-regression method performs two linear regressions. The first regression uses group-level networks as spatial regressors in a GLM on
individual BOLD datasets to produce a matrix of participant-specific time-series for each group map. In the second GLM, participant-specific time-series serve as temporal regressors against the same BOLD datasets producing a participant-specific map of parameter estimates for each group-level network (Filippini et al., 2009). Once participant-specific maps are generated, statistical tests can be performed to determine patient/control group differences or the correlation of functional connectivity measures with behavioral/physiological parameters.

VRF-related changes in brain vasculature and neuronal function are often accompanied by structural changes (Alosco et al., 2013; Brundel et al., 2012; Last et al., 2007). The next section will describe structural MRI techniques and introduce common imaging biomarkers used to examine brain atrophy.

1.9 Structural brain imaging

T1-weighted and T2-weighted high-resolution images are most commonly used for structural brain imaging and an example of a typical T1-weighted MRI sequence for structural assessment was discussed in Section 1.6.1.

1.9.1 Brain structure

The brain is comprised of two major tissue types: GM and WM. Grey matter consists primarily of neuronal cell bodies, while WM contains long myelinated axons and serves to connect different parts of the cortex. The cortex takes the form of the folded surface of GM that is wrapped around the WM with the estimated surface area of approximately 2000-2500 cm\(^2\) and 2-3 mm thickness (Griffin, 1994). The complex folding structure of the brain is suggested to reflect the underlying connectivity and fulfill an evolutionary need for the surface area increase without the increase in the intracranial size. The cortical structure is composed of inward and outward folds (sulci and gyri). The outward fold forms between two strongly interconnected regions. This configuration allows the reduction in the distance between the opposite sides of the fold. The inward folds, on the other hand, tend to occur between more loosely connected regions (Van Essen, 1997). The process of cortical folding begins as early as 10 weeks of fetal life and by the third trimester, the cortical surface resembles closely the morphology of the adult cortex. Cortical complexity continues to develop throughout childhood and adolescence particularly in prefrontal regions (Mangin et al., 2010).
1.9.2 Imaging biomarkers

Several structural metrics have been developed to examine the effects of aging and neurodegenerative conditions on brain structure, including GM density, GM volumes and cortical thickness using manual, automated and semi-automated methods. Voxel-based morphometry is one example of a fully automated method that is used to estimate voxel-wise differences in GM density, i.e. between patient and control groups (Ashburner & Friston, 2000). A semi-automated brain region extraction (SABRE) algorithm has also been successfully applied to extract regional volumetric data by combining powerful computing techniques with user input of few easily identifiable landmarks (Dade et al., 2004). In this thesis, cortical thickness will be used to examine structural effects of VRFs. The methodology of CThk estimation is discussed in more detail next.

Cortical thickness

Cortical thickness can provide important information on the progression of neurodegenerative diseases and have been shown to be more sensitive compared to volumetric measures (Pereira et al., 2011). Measuring CThk can be challenging, since thickness not only varies between individuals but also by brain regions. Early measurements were done manually at autopsy and were very time consuming. The two most commonly used techniques to determine the distance between WM and the surface of the brain involved using a probe or examining individual brain slices. The first technique involved direct insertion of the probe through the outer surface of the brain and measuring the distance to the WM along a chosen angle. The second technique examined slices of the cortex measuring the distance between the GM/WM interface and the surface of the brain. Both techniques are highly susceptible to CThk overestimation if the chosen angle of measure (technique 1) or the angle of the slice cut (technique 2) are not orthogonal to the cortical surface (Lerch & Evans, 2005).

With advances in MR imaging and the development of analysis software, high-resolution images can now be obtained that permit automated measurements of CThk in various regions of the brain. Computer-based software is now capable of conducting thickness calculation on the entire brain within a few hours and with limited demand on the user input. Cortical thickness measures have since been used to study numerous neurological disorders such as schizophrenia (Kuperberg et al., 2003), multiple sclerosis (Sailer et al., 2003) as well as normal aging (Salat et al., 2004).
1.9.3 Cortical thickness analysis

*Surface reconstruction and CThk measurements with FreeSurfer*

One of the widely used software for CThk calculations, and the one used in this thesis, is FreeSurfer. It was developed at the Martinos Center for Biomedical Imaging and includes a set of tools for automated cortical surface reconstruction and CThk estimation based on high-resolution T1-weighted images. Surface reconstruction is a multistep process. First, T1-weighted images are motion corrected and averaged across multiple volumetric images (Reuter, Rosas, & Fischl, 2010). All non-brain structures are then removed using a “skull-stripping” algorithm based on watershed/surface deformation procedure (Ségonne et al., 2004), and the skull-stripped images are transformed into Talairach space (a standardized three-dimensional coordinate system of the human brain developed by Jean Talairach and Gabor Szikla (Talairach & Szikla, 1980)).

Next, the subcortical WM and deep GM structures (i.e. hippocampus, amygdala, caudate, putamen, etc.) are segmented and intensity normalization is applied (Fischl et al., 2002; Sled, Zijdenbos, & Evans, 1998). The GM/WM boundary, from the segmentation step, is then covered with triangular tessellation and a deformation algorithm is applied to produce a final representation of the boundary between GM and WM as well as the GM/CSF boundary. CThk is computed for each point on the surface by measuring the distance from the pial surface to the closest point on WM/GM interface and from identified point on WM/GM interface back to the pial surface. CThk is calculated as the average of the two measurements (Dale et al., 1999).

*Group Analysis*

Combining individual structural metrics into a group analysis is accomplished either by registering individual data to a standard template and running a vertex-wise GLM analysis or by extracting average values from pre-defined regions and running a univariate or multivariate analyses to examine differences between patient and control groups or associations of regional brain measures with continuous metrics, i.e. behavioral scores. Analyses using cortical surface models have several advantages. First, cortical models allow for a better visualization of the structural changes compared to the single slice view provided by voxel-based morphometry. Second, for single subject analysis, statistical methods can benefit from excluding the non-GM structures. Furthermore, smoothing along the cortical surface results in a superior resolution and sensitivity compared to rectilinear methods. Finally, surface-based registration to a common template during group analysis is more advantageous than the 3D Talairach registration as it
employs inter-individual alignments based on the patterns of sulci and gyri (Hagler Jr. et al., 2006). As with most neuroimaging analysis, the results of surface-based analysis must be corrected for multiple comparisons. Multiple comparisons correction refers to the need to adjust significance levels for the number of statistical tests. In the voxel-wise and vertex-wise analyses, the number of tests can range between 10,000 and 100,000, corresponding to the number of voxels/vertices. With such a large number of tests, some of them will give significant results purely by chance (i.e. false positives). Several methods are available to reduce the number of false positive results: bonferroni correction, false discovery rate, random field theory, permutation testing and simulation (cluster analysis). In Chapter 2, a simulation approach was used to correct CVR and CThk group difference maps. Monte Carlo simulation approach is based on the assumption of similar behavior between the neighboring voxels and calculates the likelihood of obtaining different cluster sizes (Forman et al., 1995). The calculated cluster size thresholds are then applied to the statistical maps in combination with the single-voxel threshold to achieve global probability error of p< 0.05.

An alternative multivariate method for CThk analysis is proposed in Chapter 4, which is called partial least squares (PLS) analysis and discussed in more detail next.

**Partial least squares analysis**

PLS is a multivariate statistical analysis that identifies spatially distributed patterns of brain changes related to a specific task or condition (Addis et al., 2004; Luk et al., 2010). PLS generates orthogonal latent variables that best explain the covariance between the design and data matrices (McIntosh & Lobaugh, 2004). The design matrix contains exogenous variables of interest, such as task, behavior or group, while the data matrix is generated using measured data (e.g. MRI measures). Figure 1.8 provides a schematic illustration of the PLS analysis steps. First, a cross-correlation matrix (S) is computed between the design matrix (X) and data matrix (Y). Next, singular value decomposition is applied to the correlation matrix to generate latent variables (LVs). Each LV contains a pair of singular images and the corresponding singular values (d). A singular image pair is composed of: 1) a matrix of singular vectors of the design matrix (A) that represents a linear combination of design contrasts that contributed optimally to the cross-correlation matrix, and 2) a matrix of saliences for data matrix (B) that determine how
Figure 1.8. A schematic illustration of PLS analysis. Adapted from (McIntosh et al., 1996).

strongly each voxel contributes to the identified LV. Singular values (d) represent the covariance between the singular image pair and are used to compute the proportion of cross-correlation accounted for by this LV. Finally, the brain scores are computed as the dot product of the salience matrix and original data (McIntosh et al., 1996; McIntosh et al., 2004).

Statistical assessment of the LV is done using permutation testing for the LV significance and bootstrap resampling for region localization. In the first case, permutations are used to establish if any/or all of the LVs are statistically significant by randomly reordering the rows of the design matrix and computing a new set of LVs each time. The singular values of these LVs are then compared to that of the original LV and the probability is assigned to the original LV value based on the number of times the statistic from the permuted LVs exceeds the original value (McIntosh et al., 1996). The advantage of this method over the conventional parametric statistical tests is that it does not rely on any distributional assumptions. To determine the reliability of the saliences for each voxel characterizing the LV pattern a bootstrap resampling is
used on the salience matrix, estimating the standard error of voxel saliences within that LV. The ratio of saliences to standard errors is referred to as the bootstrap ratio (BSR) (McIntosh et al., 1996). Since both permutation and bootstrapping are performed on the entire LV pattern no additional multiple comparison correction is required (McIntosh et al., 2004). PLS is economical and efficient, from a statistical point of view and may improve characterization of disease impact on the brain in conditions where effects are expected to involve multiple cortical and subcortical areas distributed throughout the brain by examining multiple brain regions at the same time (Luk et al., 2010; Khedher et al., 2015). In Chapter 4 this technique will be used to examine the effects of summative VRF index on regional CThk measures in cognitively intact older controls (NC) and MCI individuals.

1.10 Statistical analysis

Statistical analysis is a critical part of neuroimaging experiments. Several statistical techniques, pertaining to specific MRI measures have already been discussed in the previous sections, including: GLM, ICA and PLS. Several other techniques will also be used in this thesis and are discussed in more detail next.

*Parametric vs. Nonparametric tests*

Statistical analysis techniques used in neuroimaging can be broadly classified as parametric or nonparametric. Parametric tests rely on the assumptions that data in all groups are normally distributed and that distributions in all groups have the same variance. In contrast, nonparametric tests have no assumptions about population distribution. Nonparametric procedures generally have less power for the same sample size, therefore, parametric tests are often favored if the data assumptions are satisfied. Parametric tests used in this thesis include: unpaired t-test (comparing 2 independent groups), analysis of variance (ANOVA: comparing >2 independent groups) and analysis of covariance (ANCOVA: comparing ≥2 independent groups, while controlling for the effects of other continuous variables) are all examples of the GLM discussed in Section 1.7.5. For parameters with nonnormal distribution, a Mann-Whitney (comparing 2 independent groups) and Kruskal-Wallis (comparing >2 independent groups) tests were used.

*Multiple comparison corrections*

In addition to the Monte-Carlo simulation approach for multiple comparisons, discussed in Section 1.9.3, Bonferroni and False Discovery Rate (FDR) corrections were used in Chapters 3
and 4, respectively, to account for the number of tested regions. Bonferroni correction assumes that all tests are independent and, therefore, the probability of obtaining one or more false positive is \((1-p)^N\), where \(N\) is the number of test. To achieve a global error probability of \(p < 0.05\), the significance level of each individual test must be divided by the number of tests. Bonferroni correction ensures that the number of false remains low, however, it may be too stringent for use with MRI analyses with large number of voxels that have similar covariation. MRI measures of neighboring regions often show similar patterns, therefore, the tests are not truly independent. FDR uses a different approach by controlling the number of false positive voxels among a subset of voxels labeled as significant (Benjamini & Hochberg, 2000). This method may be more appropriate when a large number of related measures are tested.

### 1.11 VRFs – Remaining questions

Previous sections provide valuable insight on the factors that can degrade brain health.

Neuroimaging has played a key role in our understanding of VRF impact on the brain, as described in Section 1.3. Many questions, however, still remain unanswered. For example, HTN is the most common VRF and has a negative impact on the brain, it is still unclear, however, whether having additional VRFs contribute unique deleterious effects to brain health. The challenge in this field of research is the need for the empirical evidence to align with the proposed mechanisms. For instance, we still do not know whether the effects of multiple VRFs are cumulative in a linear or non-linear manner and the precise linkages and co-dependencies between the factors. The lack of studies targeting multiple VRF impact is of particular concern given the prevalence of their co-occurrence. In the large population study (\(N=2.1\) million) almost 50% of older adults (age >65) with VRFs had more than 1 VRF (Selby et al., 2004), see Figure 1.9.

To help address some of these important questions, we first pose a fundamental question: “does
the number of VRFs matter?” or “is there a ceiling effect of VRFs on the brain?” Answering these questions would not only reinforce the importance of reducing VRF burden but would emphasize the need for better understanding of different VRF combinations’ impact on the brain. The prevalence of VRF combinations differs, as demonstrated in Figure 1.9, which may present a challenge in recruiting study participants. Increased VRF number may also increase the rate of other complications, such as coronary artery disease that have an additional impact on the brain (MacIntosh et al., 2015). In this thesis two approaches were used for participant selection. Chapter 2 used a strict inclusion/exclusion criteria to examine the associations between HTN+T2DM and HTN and vascular and structural brain measures. Chapters 3 and 4, on the other hand, did not exclude other comorbidities and classified groups based on the overall VRF index. Although this approach may potentially reduce specificity of identified group differences, it provides a better representation of the general population living with VRFs. VRFs were considered as a binary diagnosis as opposed to the continuous measures of HBA1C, BP etc. This choice was made to facilitate an easy translation of the findings to the clinical settings. It will also serve as a “stepping stone” for future studies that could examine the evolution of VRFs from the onset to the most severe forms by considering continuous measures.

1.12 Thesis hypothesis

The main hypothesis of this thesis is that higher number of VRFs will be associated with specific effect(s) on brain health compared to lower VRF number, and that these effect(s) could be identified using multimodal MRI. To address this overarching hypothesis we consider study groups that are composed of NC older adults as well as two study groups with common old-age neurodegenerative disorders, i.e. SVD and MCI. The hypotheses for the individual chapters are as follows:

I. NC adults with HTN + T2DM will show reduced regional CThk and CVR relative to the group with HTN only (Chapter 2).

II. Older adults with WMH and multiple VRFs will have altered regional CVR responses and functional connectivity relative to those with WMH and few or no VRFs (Chapter 3).

III. VRF index will help explain CThk heterogeneity in older adults and its effect will vary between NC and MCI groups (Chapter 4).
1.13 Thesis Outline

The goal of Chapter 2 is to demonstrate that having two VRFs, in particular, HTN and T2DM, among older NC adults contributes to alteration in brain structure and vasculature compared to HTN alone. Chapter 2 was published in 2014 (Tchistiakova et al., 2014). Chapters 3 and 4 focus on the VRFs in two common old-age neurodegenerative disorders: 1) SVD (Chapter 3) and 2) MCI (Chapter 4). Chapter 3 examines the association between VRF burden and CVR and functional connectivity in older adults with SVD. Chapter 3 also introduces a new CVR analysis approach designed to characterize spatial and temporal CVR patterns, reminiscent of resting state networks. Chapter 3 was published in 2015 (Tchistiakova et al., 2015). In Chapter 4 PLS is used to establish the relationship between summative VRF index and regional CThk measures in NC and MCI adults that participated in the multi-center Alzheimer’s Disease Neuroimaging Initiative (ADNI). The results of this chapter were presented as an abstract and a manuscript is under review. Finally, Chapter 5 discusses potential clinical implications of thesis findings and makes recommendations on neuroimaging strategies for studying VRF impact in elderly population.

1.14 Contribution

Studies described in Chapters 2 and 3 are a collaborative work of several labs at Baycrest’s Rotman Research Institute and Sunnybrook Health Sciences Centre. This section describes my contribution to data presented in Chapters 2-4.

Participant recruitment, neuropsychological assessment and MRI data collection for Chapter 2 were performed at the Baycrest’s Rotman Research Institute. I assisted in participant recruitment and performed all structural and functional MRI data analysis.

For Chapter 3 study participant recruitment and MRI were performed at Sunnybrook Health Sciences Centre. I assisted with participant recruitment, MRI data collection and performed all MRI data analysis.

In Chapter 4, preprocessed MRI data were obtained from ADNI database. I conducted VRF classification of ADNI participants based on available medical history and medication records and performed statistical analysis on regional MRI measurements.
Chapter 2

2  Associations between combined effects of type 2 diabetes and hypertension and measures of cortical thickness and cerebrovascular reactivity relative to hypertension alone in older adults*

2.1 Introduction

Type 2 diabetes mellitus is amongst the most common conditions that affect individuals over the age of 65 years (Wu et al., 2013). Vascular complications associated with diabetes have profound effects not only on peripheral organs, but also on the brain (described in Chapter 1). Vascular impairment increases the risk of neurological events, as seen by the increased risk of TIA and stroke by 2-5 fold in diabetic individuals (Baird et al., 2002; B. J. Kim et al., 2008). Along with vascular impairment, T2DM is associated with loss of GM and WM tissue in excess of that seen in normal aging (Bresser et al., 2010; Last et al., 2007). Regions most susceptible to diabetic damage include prefrontal (Bruehl et al., 2009), hippocampal (Bruehl et al., 2009) and occipito-parietal areas (Last et al., 2007). These structural and vascular changes are thought to contribute to increased impairment in speed of information processing, memory and executive function commonly reported in T2DM (Brands et al., 2007; Manschot et al., 2007).

There are still many gaps in our understanding on the impact of T2DM on the brain, one of which is the contribution of HTN, a common comorbidity reported in individuals with T2DM (Colosia et al., 2013), as HTN itself contributes to brain vascular (Hajjar et al., 2010) and structural (Den Heijer et al., 2005) damage through mechanisms that both overlap with and are

independent of T2DM (Meusel et al., 2012). A recent study demonstrated that individuals with T2DM have decreased brain tissue volumes and CVR compared to healthy age-matched controls (Last et al., 2007). However, given the lack of control for the effects of HTN, the specific contributions of T2DM remain unclear. Poor hypertensive control was found to exacerbate macro- and micro-vascular T2DM complications (Turner et al., 1998), therefore, it is critical to not only establish individual effects of HTN and T2DM on the brain but to also address the combined effects of the two conditions. As a first step towards understanding the combined effects of T2DM and HTN, several studies contrasted normotensive and hypertensive diabetics and reported lower CVR (Last et al., 2007) and global volumetric measures (Schmidt et al., 2004) in those with both conditions, suggesting that HTN worsens vascular and structural abnormalities in the face of T2DM.

This chapter attempts to advance this field of research, by specifically investigating the associations between combined effects of HTN and T2DM (HTN+T2DM) and measures of GM CThk and CVR, relative to the effects of HTN alone. CThk has demonstrated high structural sensitivity in previous neuroimaging studies (Hutton et al., 2009; Pereira et al., 2011) and CVR is an established measure of vascular health (Riecker et al., 2003). Although controlled hypercapnia using CO₂ gas inhalation is closer to a gold standard, a BH technique for CVR assessment was selected for this study because of its ease of implementation.

We hypothesized that individuals with HTN+T2DM will show reduced regional CThk and reduced CVR relative to an HTN group. In addition, secondary objectives used measures of executive function, processing speed and memory to determine whether there are significant cognitive associations with CThk and CVR in this population (Nandipati et al., 2012; Takeuchi et al., 2012).

2.2 Methods

2.2.1 Participants

The study protocol was approved by Research Ethics Boards at Baycrest Centre and Sunnybrook Health Sciences Centre and experiments were conducted at Baycrest’s Rotman Research Institute. Informed consent was obtained from all participants. Eighteen HTN+T2DM participants and twenty-two HTN participants were recruited through an internal participant database and via newspaper and community center advertisement. Participants completed a
demographic and medical questionnaire via telephone. Those who scored within the dementia range on the Telephone Interview for Cognitive Status (Brandt et al., 1988) or who self-reported having hepatic disease, recent coronary heart disease, other significant medical or psychiatric disorders affecting cognition (e.g., stroke, major depressive disorder), taking medications that act on the central nervous system (e.g. depression, sleep disorders, migraine headaches), hormone replacement therapy, major inflammatory disorders (e.g. arthritis), inflammatory bowel disease, rheumatological disorders, heart failure and chronic lung disease were excluded. Diabetic individuals were invited to participate as long as their disease, based on self report, did not include the following complications: retinopathy, nephropathy and neuropathy. Furthermore, controlling for diabetes through diet and/or hypoglycemic medication were inclusions, whereas use of insulin injections and T2DM diagnosis for less than 2 years were exclusions. Individuals recruited to the HTN group had to have fasting blood glucose levels less than 6.1 mmol/l on two consecutive testing days. Participants in both groups had a history of HTN for at least 2 years, which was controlled by long-acting antihypertensive medications. Participants with MRI-incompatible metal implants, pacemakers and stents were excluded. On the first day of testing, participants provided a fasting blood sample and underwent neuropsychological testing to assess processing speed, memory and executive function. Blood pressure was measured during the same session using a blood pressure monitor (BpTRU Medical Devices), taken as the average of the last 5 of 6 readings, after participants had been sitting quietly for 5-10 minutes. During a second session, structural and functional brain MRI were performed. The time interval between neuropsychological testing and MRI was within 3 months (average 30.5 days; range 0 to 80 days) with the exception of one participant who was scanned after 372 days.

2.2.2 MRI

MR images were acquired on the 3T Magnetom Trio Siemens system with 12-channel head coil. Anatomical imaging included T1-weighted 3D MPRAGE (TR/TE/TI=2000/2.63/1100ms, matrix=256x192, FOV=256x192 mm$^2$, slice thickness=1mm, number of slices=160, flip angle=9°, total duration=6m30s).

Cerebrovascular reactivity was measured as the change in BOLD signal during a series of breath holds (TR/TE=2000/30ms, matrix=64x64, FOV=200x200 mm$^2$, slice thickness=5mm, number of slices=32, flip angle=90°, with 156 volumes, total duration=5m20s). The BH challenge consisted of six breath holds lasting 15s each following 3s expiration period with intermittent 30s
periods of normal breathing. The BH instructions were projected on the computer screen and included the start and end of the expiration period and the BH countdown. Fluid attenuation inversion recovery (FLAIR) images were also obtained to assess WMH volumes (TR/TE/TI=9000/96/2500ms, matrix=256x212, FOV= 224x186mm$^2$, slice thickness=5mm, number of slices=32, flip angle=165°, total duration=3m38s).

2.2.3 Image Analysis
Compliance with BH instructions was monitored using respiratory below traces recorded during the task. Participants with poor compliance (N= 2 for the HTN+T2DM and N=1 for the HTN groups) were excluded from subsequent analysis. CVR analysis was conducted using tools available through FMRIB Software Library (FSL, version 4.1, http://fsl.fmrib.ox.ac.uk/).
Preprocessing for BOLD images included: motion correction (Jenkinson et al., 2002), spatial smoothing with Gaussian kernel of 5mm FWHM, slice-time correction and high-pass temporal filtering with 100s cutoff. Statistical analysis was carried out using GLM, i.e. a box-car paradigm was convolved with double-gamma HRF to model the response to BH challenge. Motion parameters were added as covariates of non-interest. A participant specific delay in hemodynamic response was estimated for each participant and incorporated into the model. Delay was computed by averaging the time difference between BOLD signal peaks in GM and the end of the corresponding BH. This procedure for estimating a response delay is valid for low to moderate hypercapnia conditions where signal increases are linear with PaCO$_2$ (Tancredi & Hoge, 2013). To avoid bias related to initial compliance and baseline signal drift due to hyperventilation, for example, the first BH was discarded and analysis was performed on the remaining five BH trials. A cerebrovascular reactivity (%BOLD change) map for a representative HTN participant is shown in Figure 2.1. CVR maps were generated for each participant and resampled to an average surface-based reference template. Surface-based analysis of the CVR data was chosen so as to match the geometry for the CThk analysis (described below). It also has the advantages of improved inter-subject alignment and reduced partial volume influence when compared to the 3D analysis. Furthermore, a 2D representation of the cortex employed in the surface based analysis is thought to provide a more accurate anatomical description than a 3D volume-based analysis as it incorporates cortical folding information (Oosterhof et al., 2011; Tucholka et al., 2012).
Cortical surfaces for CVR registration and CThk measurements were generated for each participant using a freely available automated procedure in FreeSurfer (V 5.1.0, http://surfer.nmr.mgh.harvard.edu/), described in Chapter 1. Cortical thickness maps and surface-
registered CVR maps were computed for each participant separately and later registered to a spherical atlas, applying a 10mm FWHM smoothing kernel, to facilitate inter-subject comparison (Fischl et al., 1999). FLAIR images were used in conjunction with in-house software, a fuzzy lesion extractor (Gibson et al., 2010) that is designed to segment WMH.

2.2.4 Correlation with cognitive scores
Three cognitive tests corresponding to cognitive domains implicated in previous research on T2DM (Nandipati et al., 2012; Takeuchi et al., 2012) were selected to examine the correlations with CVR and CThk. These measures included: 1) Trail Making Test A (time to complete) to assess processing speed, 2) California Verbal Learning Test (total number of words remembered over 5 trials) to examine memory function and 3) Wisconsin Card Sorting Test (number of

Figure 2.1. Top: a CVR map for a representative HTN participant. Bottom: a mean BOLD time course for the same participant averaged across the GM.
categories achieved) to measure executive function.

2.2.5 Statistical Analysis
Demographics, laboratory measurements and cognitive scores that were normally distributed were compared between the groups using an unpaired t-test in SPSS (v.21, IBM Corp., IBM SPSS Statistics for Mac, Armonk, NY). A non-parametric Mann-Whitney test (SPSS) was used for group comparison of total cholesterol, C-reactive protein levels, BMI, WMH volumes, and executive scores that were not normally distributed. Unpaired two-group t-tests were performed on the cortical surface, i.e. vertex-wise, for CVR and CThk measures (using mri_glmfit, a GLM analysis within FreeSurfer). Furthermore, per subject average CThk and CVR values were extracted from respective ROIs identified by the group comparison and examined for correlation with clinical measures and cognitive scores. A step-wise linear regression (SPSS) was used to identify clinical factors that contribute to observed CThk and CVR changes. These measures included gender, age, total cholesterol, SBP and WMH volume. Associations between average CThk/CVR, extracted from respective ROIs, and cognitive scores were examined using partial correlation, controlling for the effects of age and education. Finally, vertex-wise correlations of the brain measures with cognitive scores were also conducted, with age and education and diagnosis as covariates. The vertex-wise group and regression analyses included correction for multiple comparisons using a Monte-Carlo simulation method (Hagler Jr. et al., 2006) with vertex p-value threshold of 0.05 and cluster-wise threshold of P=0.025 (P=0.05/2, to account for separate analysis of 2 hemispheres).

2.3 Results
2.3.1 Demographics
Demographic characteristics, laboratory measures, cognitive scores and WM volumes were compared between HTN+T2DM and HTN groups (Table 2.1) and revealed that the groups were matched for age and sex. The HTN+T2DM group had higher hemoglobin A1C (P<0.0001) and fasting blood glucose (P<0.0001). Systolic blood pressure (P=0.02), LDL cholesterol (P<0.0001) and total cholesterol (P<0.0001), on the other hand, were higher in the HTN group. Cognitive scores and WMH volumes were not significantly different between the two groups (P>0.2).
Table 2.1. Participant demographics. Data are means ± SD unless specified otherwise. Blood pressure measurements were not available for 3 participants (2 from HTN+T2DM and 1 from HTN groups).

2.3.2 Group differences in cerebrovascular reactivity and cortical thickness

CVR was significantly lower in the HTN+T2DM group in: 1) Bilateral - ligual gyrus, cuneus and superior parietal areas; 2) Right - lateral occipital, inferior parietal and precuneal regions; 3) Left - pericalcarine cortex (P<0.025), relative to the HTN group. Cortical thickness was lower in the HTN+T2DM group, compared to the HTN group, in the right lingual and fusiform gyri.

<table>
<thead>
<tr>
<th></th>
<th>HTN group</th>
<th>HTN+T2DM group</th>
<th>Between-group comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (women/men)</td>
<td>12/10</td>
<td>8/10</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>73.4±6.2</td>
<td>71.8±5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>NA</td>
<td>10.9±6.6</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension duration (years)</td>
<td>10.4±6.9</td>
<td>10.3±7.3</td>
<td>NS</td>
</tr>
<tr>
<td>HBA1C %</td>
<td>5.7±0.3</td>
<td>6.9±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.3±0.3</td>
<td>7.2±1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>58.8±19.3</td>
<td>65.0±37.4</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.9±15.8</td>
<td>125.1±15.9</td>
<td>0.02</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75.1±10.7</td>
<td>70.6±9.2</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.7±0.4</td>
<td>1.5±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.0±1.0</td>
<td>1.9±0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.3±1.1</td>
<td>3.9±0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein (mmol/l)</td>
<td>2.1±1.4</td>
<td>3.6±7.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>26.3±2.7</td>
<td>27.3±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>WMH volume (cc)</td>
<td>3.9±7.4</td>
<td>2.8±4.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Cognitive scores**

<table>
<thead>
<tr>
<th></th>
<th>HTN group</th>
<th>HTN+T2DM group</th>
<th>Between-group comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive function (Num. of categories)</td>
<td>5.1±1.4</td>
<td>4.4±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Processing speed (s)</td>
<td>35.2±9.9</td>
<td>35.5±12.0</td>
<td>NS</td>
</tr>
<tr>
<td>Memory function (Num. of words)</td>
<td>42.6±10.2</td>
<td>41.8±12.2</td>
<td>NS</td>
</tr>
</tbody>
</table>
(P<0.025). CVR and CThk results are both shown in Figure 2.2, illustrating the spatially overlapping findings.

Post-hoc analyses of the vertex-wise correlations between CThk/CVR and HBA1C (a measure of glucose control), and between CThk/CVR and C-reactive protein level (a measure of inflammation), both commonly present at higher than normal levels in T2DM and HTN, showed no significant associations (P> 0.05, data not shown).

Figure 2.2. Blue - regions of decreased CVR in HTN+T2DM group compared to HTN group; Orange - region of decreased CThk in HTN+T2DM group; Yellow - overlapping region of decreased CVR and CThk. Inset highlights the region of CVR and CThk overlap which was overlaid on the inflated surface for enhanced visualization.
2.3.3 Correlation with clinical measures

Clinical measures could not explain between-subject differences in CThk and CVR, using functionally relevant ROIs (described in 2.3.2) and a step-wise linear regression (P>0.05).

2.3.4 Correlation with cognitive scores

Average CThk in the right lingual gyrus ROI identified by the group comparisons was subsequently found to be significantly associated with executive function ($F_{33,1}=4.24$, $P=0.048$), after adjustment for age and education, whereas processing speed and attention were not (P>0.05). No significant associations were found between average CVR and cognitive function (P>0.05). Results of the vertex-vise analysis were similar to that of the ROI analysis. Only executive function was significantly correlated with CThk (P<0.025), after adjustment for age, education and diagnosis. Better executive function performance was associated with higher CThk in the: 1) Bilateral - isthmus and posterior cingulate, precuneus, superior frontal, medial and lateral orbito-frontal regions; 2) Left –middle and inferior temporal gyri and 3) Right – rostral middle frontal areas. 

![Cortical thickness vs. executive function](image)

*Figure 2.3. Highlighted regions (red, orange and yellow) showed significant correlation between higher CThk and better executive function.*
2.4 Discussion

In this chapter we demonstrated that a combination of T2DM and HTN is associated with decreased CThk and CVR in a spatially overlapping region of the occipital lobe when compared to adults that had HTN alone. As further support for our hypothesis, we found that CVR was significantly reduced in brain regions that extended beyond the occipital finding, which included bilateral occipito-parietal areas. Our secondary cognitive finding demonstrated that higher executive function was associated with preserved CThk in posterior cingulate, precuneus as well as temporal and frontal regions.

Diabetes leads to perfusion abnormalities, as reported in studies that compare patients and age-matched healthy controls (Kaplar et al., 2009; Last et al., 2007). Several studies also report reduced CVR compared to healthy individuals (Kaplar et al., 2009; Last et al., 2007). Yet in many instances, it is unclear whether T2DM per se, or commonly comorbid HTN, or a combined impact of the two, underscores these vascular changes. Findings from this chapter suggest that presence of both conditions, T2DM and HTN, contribute to cerebrovascular and structural abnormalities and that these changes are in excess of those apparent in older adults with HTN alone. Specifically, the combined effects of T2DM and HTN were associated with a deleterious impact on both CVR and CThk in the occipito-parietal areas. Our CVR findings are regionally more localized compared to a previous report (Last et al., 2007), which may be due to the study design that included HTN in both study and control groups.

A regional decrease in CThk was detected in right occipital region in the HTN+T2DM group compared to the HTN group. The region of reduced CThk was smaller in spatial extent but overlapping with the CVR results, which is a novel finding relative to the literature that has primarily focused on CThk in T2DM (Ajilore et al., 2010; Brundel et al., 2010; Z. Y. Chen et al., 2013; Leritz et al., 2011; Seo et al., 2012) and HTN separately (Seo et al., 2012; Vuorinen et al., 2013). A previous study involving older adults demonstrated that blood glucose levels and BP were both associated with cortical thinning in occipital regions, among others (Leritz et al., 2011). These metrics of metabolic and hypertensive control identify regions that may be preferentially impacted by T2DM and HTN. Current findings are in agreement with these earlier reports and emphasize that occipital regions show greater impairment among adults with both T2DM and HTN conditions. This localized impact could be due to preferential impairment of
posterior circulation. For example older adults with T2DM are more likely to develop infratentorial infarcts (Kameyama et al., 1994) and have a higher degree of vertebral stenosis (Iwase et al., 1998) than non-T2DM. Diabetes induced structural changes are often attributed to chronic exposure to hyperglycemia (Korf et al., 2006), inflammation (Novak et al., 2011), as well as direct and indirect effects of insulin dysregulation on the brain (Craft & Watson, 2004; Korf et al., 2006). Furthermore, hyperglycemia as well as its underlying oxidative stress result in the release of proinflammatory cytokines that contribute to endothelial dysfunction (Monnier et al., 2006), reduce production of vasodilator nitric oxide (Brownlee, 2005; Kameyama et al., 1994) and increase concentration of vasoconstrictor endothelin-1 (Kalani, 2008). When it comes to chronic exposure, such as in T2DM, these factors can lead to diminished vessel wall elasticity and impaired CVR (Last et al., 2007).

Cognitive impairment associated with T2DM (Brands et al., 2007; Manschot et al., 2007) and HTN (Dahle et al., 2009; Gifford et al., 2013) are well established, although what continues to be debated is whether structural and/or vascular abnormalities play a mediating role. We observed a significant association between executive function and CThk in the superior and middle frontal gyri, middle and inferior temporal gyri and parietal regions. The findings of this chapter are novel because they involved an examination of cognitive function and regional CThk specifically in older adults with T2DM and HTN. A study on a more general population, however, identified similar regions of association between CThk and executive function, namely in lateral prefrontal and parietal cortices (Burzynska et al., 2012). Others have also observed a negative correlation between subcortical atrophy and executive function and subcortical and cortical atrophy and information processing speed in individuals with T2DM (Manschot et al., 2006). Finally, in a recent large scale T2DM study, Moran et al. demonstrated that global GM atrophy can mediate differences in cognitive performance between individuals with and without T2DM, particularly in visuospatial memory and cognitive speed domains (Moran et al., 2013).

Although both T2DM and HTN are known to impact brain hemodynamics, no studies to date reported on the correlation between regional CVR and cognitive function in this population. In the earlier T2DM studies global CVR (Brundel et al., 2012) and global CBF (Brundel et al., 2012; Tiehuis et al., 2008) were examined for correlations with cognitive function. Similarly to the findings of this chapter, no correlation was detected between CVR and cognition (Brundel et al., 2012), although CBF was associated with executive function (Brundel et al., 2012; Tiehuis et
al., 2008) and processing speed (Brundel et al., 2012). Current results in conjunction with this earlier work suggest that CBF but not CVR may act as a hemodynamic mediator on cognitive function in adults with T2DM and HTN, but further research is required.

2.5 Conclusion

The detrimental impact of VRFs on brain health has been reported in a number of previous studies, however, few have attempted to quantify the impact of multiple VRFs, i.e. T2DM and HTN, relative to one VRF, i.e. HTN. CVR and CCThk measures provided independent and converging evidence of the adverse effects of combined T2DM and HTN, principally in the occipital lobes. Cortical thickness was correlated with executive function performance, which argues for the ‘real world’ relevance of cortical thinning in these groups. As such it may serve as a useful imaging biomarker of cognitive decline in populations with HTN and T2DM.

The goal of Chapter 2 was to establish if the combination of T2DM and HTN has additional detrimental effects on brain health above and beyond the effects of HTN alone. The novelty of this study is in the choice of HTN as a control group, which was chosen to distinguish T2DM effects from the contribution of commonly co-occurring HTN. Chapter 2 examined associations between VRFs and brain health in a group of otherwise healthy older adults. The prevalence of comorbid neurological disorders in aging population, however, is high, with SVD and MCI being among the most common. Therefore, it is also imperative to establish the effect that the presence of VRFs may have in conjunction with these conditions. This will be the topic of Chapters 3 and 4.
Chapter 3

3 Vascular risk factor index correlates with cerebrovascular reactivity but not resting state co-activation in the default mode network in older adults with WMH*

3.1 Introduction

Cerebral small vessel disease is associated with impaired vascular regulation and is a major risk factor for stroke and dementia (Vermeer et al., 2003). Currently available clinical imaging techniques are able to probe SVD indirectly by identifying parenchyma lesions such as WMH of presumed vascular origin (Pantoni, 2010). WMH lesions tend to occur in periventricular and deep WM regions, and are thought to be caused by demyelination and/or microvascular ischemic events (Debette & Markus, 2010; Pantoni, 2010). WMH lesion volume is associated with poor cognitive performance, particularly in executive function, processing speed and memory domains (O'Brien et al., 2002; Vermeer et al., 2003) as well as a reported doubling the risk of dementia (Debette & Markus, 2010; Vermeer et al., 2003) and tripling the risk of stroke (Debette & Markus, 2010). Recent studies linked WMH to impaired brain hemodynamics, i.e. reduced CBF and CVR (Isaka et al., 1994; Marstrand et al., 2002) and alteration of cognitive networks (Zhou et al., 2013), as discussed in Chapter 1.

Systemic VRFs such as hypertension, diabetes and hypercholesterolemia are thought to contribute towards SVD pathology. Several early studies show an increase in WMH prevalence in individuals with T2DM and HTN relative to controls (De Leeuw et al., 2002; Murray et al., 2005), while others reported only a modest effect of VRFs on WMH volume (Wardlaw et al.,

Reports on the association of hypercholesterolemia and WMH are also controversial (Murray et al., 2005; Ohwaki et al., 2013). VRFs contribute to endothelial dysfunction by decreasing the availability and/or activity of vasodilatory agent nitric oxide (Brunner et al., 2005) impacting vascular function throughout the body and brain (Bakker et al., 2000; Hajjar et al., 2010; Last et al., 2007).

High prevalence of both VRFs and WMH in the elderly adults prompts the need for a better understanding of their potential interactive effects, particularly, as it pertains to cerebrovascular GM dysfunction, associated with stroke risk, and functional measures linked to cognitive decline. The primary hypothesis of this chapter is, therefore, that individuals with WMH and higher VRF index will have altered vascular and neuronal functions relative to individuals with WMH and lower VRF index or no VRFs. Two measures are used as primary outcome measures: CVR, assessed by inhalation of a carbon dioxide enriched gas mixture, and resting state co-activation (RS co-activation), measured as the correlation of spontaneous brain activity between brain regions. These two techniques have wide clinical utility (Greicius et al., 2004; Hajjar et al., 2010; Last et al., 2007) and are well suited for accessing the impact of WMH on the function of remote GM regions.

The primary objective of this chapter was to examine the association between VRF index and RS co-activation and CVR in three relevant brain networks; namely, the DMN, sensory-motor and the medial-visual networks. The DMN is chosen because of its sensitivity to cognitive decline, particularly in the episodic and working memory domains pertaining to VRF and WMH impact (Greicius et al., 2004; Meusel et al., 2014; Vermeer et al., 2003). The sensory-motor and medial-visual networks are chosen because they are highly vascularized primary sensory regions that may be sensitive to vascular disturbances as shown in previous VRF studies (Last et al., 2007; Tchistiakova et al., 2014). A secondary analysis examines the correlation between CVR and RS co-activation within the same networks.

3.2 Methods

3.2.1 Participants

Twenty-nine elderly adults participated in this study. Participants were recruited to the study if they were suspected of having WMH based on the presence of one or more of the following criteria: 1) the individual reporting a memory complaint to their Sunnybrook neurologist, 2)
evidence of WMH findings on a previous MRI that was independent of the current study, 3) a
history of VRFs. Exclusion criteria were: cortical infarcts, age < 50, genetic SVD and known
severe carotid stenosis. Individuals were also excluded if they had contraindications to MRI.
Cognitive status was obtained from medical charts. Presence of VRFs (HTN, T2DM and
hypercholesterolemia) was ascertained based on self-report as corroborated by medical notes and
current medications. Participants were assigned to one of three VRF index sub-groups based on
the number of VRFs (i.e. 0, 1, ≥2). Detailed information on VRF characteristics for each sub-
group is included in Table 3.1. Participants completed a Montreal cognitive assessment
(Nasreddine et al., 2005) and an MRI session that included an RS-fMRI scan and a CO₂
inhalation challenge. A written informed consent was obtained from all participants. The study
was approved by the Sunnybrook Research Ethics Board.

3.2.2 MRI

MRI was performed on a 3T Philips Achieva system using a body coil for transmission and an 8-
channel receiver head coil. CO₂ inhalation challenge (Mutch et al., 2012; Slessarev et al., 2007)
was used to measure CVR. T2*-weighted BOLD EPI was performed throughout the CO₂
inhalation epochs with the following parameters: TR/TE=2000/30ms, matrix=64x64, voxel
size=3.6x2.9x3mm³, number of slices= 40, flip angle=90°, first 4 dynamics discarded. BOLD
acquisitions for RS-fMRI and CVR were identical except for scan durations (RS-fMRI: 6min 8s,
CVR: 8min 38s). T1-weighted images were acquired for image registration and GM
segmentation, TR/TE/TI=9.5/2.3/1400ms, matrix=256x164, voxel size=1x1x1.2mm³, number of
slices=140, flip angle=8° and scan duration=8min 56s. Fluid attenuation inversion recovery
images were acquired to determine the WMH volume with the following parameters:
TR/TE/TI=9000/125/2800ms, matrix=240x217, voxel size=1x1.1x3mm³, number of slices=52,
scan duration = 4min 48s.

3.2.3 Hypercapnia challenge

In this chapter a CO₂ inhalation method was selected for CVR assessment over the BH
challenge, used in Chapter 2, due to the higher prevalence of vascular and cognitive impairment
in the selected cohort, which may impact CVR estimate, i.e. in case of poor BH compliance.
Moderate hypercapnia was induced through administration of a gas mixture using a RespirAct™
breathing circuit with participants breathing via a tight fitted mask (Thornhill Research, Toronto,
Canada). RespirAct was implemented in accordance with previously described reports (Mutch et
al., 2012; Slessarev et al., 2007) that involved increasing $P_{ET}CO_2$ levels by 10 mmHg while keeping $P_{ET}O_2$ levels constant. Elevated CO$_2$ levels were delivered 45s after the start of scanning for a period of 45s, followed by 90s of normocapnia. The second CO$_2$ period was 2 min in duration followed by 3 min 30s of normocapnia. CO$_2$ values were measured using a gas analyzer at the exhalation. This is a passive hypercapnia method that requires no participant input, which makes it particularly useful for clinical populations.

3.2.4 Image Analysis

**Vascular risk factors sub-group comparison**

*Resting state co-activation analysis*

Imaging data were analyzed using tools available through the FMRIB Software Library (FSL, version 4.1, [http://fsl.fmrib.ox.ac.uk/](http://fsl.fmrib.ox.ac.uk/)). Pre-processing of RS-fMRI images included motion correction, spatial smoothing with a Gaussian kernel of 5mm FWHM and a band-pass filter (0.01<$f<$0.08Hz) to reduce high-frequency respiratory and cardiac noise and very low-frequency fluctuations (Cordes et al., 2001). Participants with relative motion >1.5mm during the RS-fMRI scan were excluded from subsequent analyses. A brain extraction technique was used to remove non-brain structures (Smith, 2002). Registration of BOLD images to the common template involved initial linear transformation to anatomical images using 7 degrees of freedom, followed by a secondary linear transformation to a group template using 12 degrees of freedom. The template was generated from 29 high-resolution scans from older adults using the Advanced Normalization Tool (Avants et al., 2008) and down-sampled to 4x4x4mm$^3$ resolution. Multivariate Exploratory Linear Optimized Decomposition into Independent Components (Beckmann & Smith, 2004) was used to run a group temporally-concatenated ICA (see Chapter 1 for more details) that automatically extracted a total of 21 group level components. Ten of these components corresponded to established RSNs (Smith et al., 2009), while six were clearly artefacts consistent with head motion and non-neuronal physiological effects (Kelly et al., 2010; Smith et al., 2009). The remaining five components were in proximity to large blood vessels and not considered further. Participant-specific RSNs were derived using a DR method (Filippini et al., 2009), see Chapter 1 for details. The last step converted the parameter estimates to variance-normalized % BOLD change maps:

$$v.n. \text{%BOLD change map} = \frac{\text{Parameter estimate map} \times \text{temporal regressor height}}{\text{mean image}} \times 100\%$$  \hspace{1cm} (19)
which reflect how closely the fluctuations of BOLD signal at each voxel resemble the component time-series and referred to as RS co-activation (Figure 3.1).

DMN, sensory-motor and medial-visual RSNs from group ICA were chosen based on their established spatial patterns (Smith et al., 2009), and thresholded using an alternative hypothesis testing approach with threshold level of $r>0.5$ to produce the networks of interest (NOIs). NOI masks were used to calculate an average RS co-activation value for each participant.

*Cerebrovascular reactivity analysis: Dual-regression*

CVR-BOLD image pre-processing was the same as RS-fMRI except a temporal high-pass filter with 250s cut-off was used instead of a band-pass filter. Following pre-processing and registration, DR was carried out on the CVR-BOLD data using the RS-fMRI RSNs as spatial inputs. Extracted CVR time-series are specific for each RSN and were subsequently used in the second linear model (Figure 3.1). The results of the DR were CVR patterns corresponding to RSNs, which were tabulated as a % BOLD change value as in Equation 19 and referred to as DR-based CVR. Average DR-based CVR was calculated for three NOIs.

*Cerebrovascular reactivity analysis: Model-based*

CVR was also computed using the $P_{ET}CO_2$ trace in a GLM. Similarly to the DR-based CVR, resulting maps were converted to variance-normalised % BOLD change, averaged within NOIs and are further referred to as model-based CVR.

*RSN co-activation vs. CVR*

Pearson’s correlations between average RS co-activation and average DR-based CVR were calculated for each NOI using SPSS.

*Contribution of grey matter and WMH lesions to NOIs*

T1-weighted images were segmented using FSL FAST (Zhang et al., 2001) to create GM partial volume maps, which were registered to group template space and thresholded to only include voxels with $\geq 50\%$ GM contribution. NOI masks (DMN, sensory-motor and medial-visual) generated during group ICA were used to calculate percentage of GM in each network by dividing GM volume within NOI by the total NOI volume.
Figure 3.1. Flow chart for data-driven analysis approach on RS-fMRI and CVR-BOLD data. The model-based CVR analysis is not included. Abbreviations: DR=dual regression, RS-fMRI- BOLD data collected during rest, CVR-BOLD - BOLD data collected during hypercapnia challenge, ICA – independent component analysis, Part. 24 – representative data from participant 24, RSNs- resting state networks, NOIs – networks of interest, VRFs – vascular risk factors. Stage 1 refers to the first linear model fit in dual regression using RSN spatial inputs. Stage 2 refers to the second linear model fit that relies on a temporal input that is defined by time-series data from voxels ascertained in Stage 1. The results generated from this analysis procedure are shown in grey for reference.
FLAIR images were processed using in-house software, i.e. a two-class fuzzy C-means clustering technique (Gibson et al., 2010), to segment voxels as WMH. The segmentation was confirmed by visual inspection and the total volume of the segmented WMH regions was calculated after normalizing intracranial volume to the average head size of 1300ml (Sanfilipo et al., 2004). A WMH probability map was generated using the binary WMH masks for all participants, as a means to compare the WMH locations and the NOIs. The WMH masks were combined into a single probability map for visualization. In addition to a qualitative comparison of WMH distribution and NOI locations, a percentage of WMH in each NOI was calculated by dividing WMH volume within NOI by the total NOI volume.

**Statistical analyses**

ANCOVA was used to test the differences between VRF index sub-group in RS co-activation, DR-based CVR and model-based CVR measures. VRF index sub-group comparisons were carried out for each NOI with age and GM percentage covariates using SPSS. Values of p< 0.05 were considered statistically significant. Post-hoc, analyses were re-run after removing the potential outlier effects from participants with a probable AD diagnosis. In addition, contribution of motion to VRF index sub-group differences was examined using ANOVA on relative mean motion measurements during RS-fMRI and CVR-BOLD (obtained from the motion correction step).

3.3 Results

3.3.1 Demographics

Four participants were excluded due to head motion greater than 1.5mm during RS-fMRI scan. The remaining 25 participants were assigned to VRF sub-groups depending on their number of risk factors. WMH volumes, age, gender ratio, and MoCA scores were normally distributed and did not differ significantly between the VRF index sub-groups (see Table 3.1).
<table>
<thead>
<tr>
<th></th>
<th>No VRFs</th>
<th>VRF index 1</th>
<th>VRF index ≥2</th>
<th>VRF index sub-group comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (women/men)</td>
<td>4/4</td>
<td>5/5</td>
<td>¾</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>73.4±7.8</td>
<td>72.4±10.5</td>
<td>71.5±9.9</td>
<td>NS</td>
</tr>
<tr>
<td>WMH (CCs)</td>
<td>20.4±19.3</td>
<td>27.3±21.5</td>
<td>17.4±19.5</td>
<td>NS</td>
</tr>
<tr>
<td>MoCA</td>
<td>25.75±3.5</td>
<td>22.0±6.2</td>
<td>21.0±5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Vascular Cognitive Impairment</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.1. Participant demographics. Data are means ± SD. In case of diagnostic conditions, the number represents the number of participants with this condition in the group. MoCA scores were not available for two participants from VRF index 1 sub-group.

### 3.3.2 VRF index sub-group comparison

NOIs used for VRF index sub-group comparisons are shown in Figure 3.2. Sensory-motor NOI included postcentral and precentral gyri as well as regions in parietal operculum cortex and superior temporal gyrus; medial-visual NOI consisted of occipital pole, bilateral lingual gyri, intracalcarine and cuneal cortices; and DMN NOI encompassed precuneus, posterior cingulate and paracingulate gyri, bilateral: hippocampus, angular, postcentral gyri, as well as middle temporal and medial frontal regions.
Group comparison of the RS co-activation showed no significant differences in any of the three networks (DMN: p=0.31; sensory-motor: p=0.44; medial-visual: p=0.88), as shown in Figure 3.3a. DR-based CVR group differences were significant after adjustment for age and GM percentage in the DMN ($F_{20,2}=5.17$, p=0.015) but not sensory-motor (p=0.16) nor medial-visual networks (p=0.13), as presented in Figure 3.3b. These DMN findings remained significant after correction for multiple comparisons ($p_{corrected} = 0.05/3 = 0.017$) and after excluding 4 participants with AD ($F_{16,2}=6.22$, p=0.01). A post-hoc analysis demonstrated that DR-based CVR in the DMN in VRF index $\geq 2$ sub-group was significantly reduced compared to participants in No VRF sub-group (p=0.025) and VRF index of 1 sub-group (p=0.007). No significant difference was detected between No VRF and VRF index of 1 sub-groups.
No significant group differences were detected, when using the model-based analysis of the CVR data (DMN: p=0.15; sensory-motor: p=0.15; medial-visual: p=0.32, data not shown).

3.3.3 RS co-activation vs. CVR

Significant correlation between RS co-activation and DR-based CVR was detected in the DMN ($r^2=0.28$, $p=0.006$) but not in sensory-motor nor medial-visual NOIs ($p>0.05$), as shown in Figure 3.4.
Correlations were performed across the VRF sub-groups, however, VRF classification of each participant is included in the scatter plots as: ● for No VRFs; ■ for VRF index of 1; ◆ for VRF index ≥2.

3.3.4 Influence of WMH and head motion

Figure 3.5 shows a probability map of WMH distribution in the current population. Contribution of WMH to NOIs was minimal (DMN: 0-1.2%; sensory-motor: 0-2.9%; medial-visual: 0-4.6%, percentage refers to the proportion of NOI occupied by WMH).

Figure 3.5. Probability of each voxel being classified as WMH. Probability map was generated using individual's WMH segmentation and is shown overlaid on the anatomical template.

Head motion during RS-fMRI and CVR-BOLD scans was not significantly different between the VRF index sub-groups (p=0.73 and p=0.10, respectively).
3.4 Discussion

In this cohort of older adults at-risk for stroke and cognitive decline, VRF index did not contribute to the WMH volume but was associated with decreased regional CVR. Individuals with VRF index of 2 or more had reduced CVR in the DMN when compared to those without VRFs or with only one. This CVR difference by VRF index sub-group was observed as a result of the data-driven analysis approach, as opposed to the model-based approach that used the P_{ET}CO_{2} traces. VRF index sub-group did not significantly influence the average RS co-activation in any of the NOIs, however, a correlation was observed between average RS co-activation and DR-based CVR when considering the DMN, which suggests that these two methods share common neurovascular information.

A hypercapnia CVR paradigm has previously been used to detect vascular impairment in T2DM (Last et al., 2007; Tchistiakova et al., 2014), HTN (Hajjar et al., 2010; Tchistiakova et al., 2014) and hypercholesterolemia (Ohwaki et al., 2013) studies. A decrease in global and regional CVR was also seen in individuals with high WMH volumes (Isaka et al., 1994; Marstrand et al., 2002). The results of this chapter add to this literature by identifying regions vulnerable to increasing VRF index in the presence of WMH. The findings of CVR impairment in the DMN are in part supported by the results of a study by Last et al. showing that the presence of multiple VRFs, i.e. T2DM and HTN, associates with decreased CVR in the temporal lobe relative to diabetes alone (Last et al., 2007).

Regions showing significant vulnerability to VRFs were those associated with DMN. Similar regions showed a decrease in glucose metabolism on FDG-PET scans of AD and MCI cohorts and were strongly associated with characteristic cognitive decline in these groups (Landau et al., 2011). Increased WMH volume is a common neuroimaging finding in AD and MCI patients (Yoshita et al., 2006). Vascular damage associated with SVD may directly contribute to AD pathology by reducing Aβ clearance, as discussed in Chapter 1 (Grimmer et al., 2012). Grimmer et al. detected a positive correlation between WMH volume and amyloid deposition over time in parieto-occipital regions (Grimmer et al., 2012). VRFs are also known to increase the risk and accelerate the onset of AD, as well as other dementias (Meusel et al., 2014; Whitmer et al., 2005), mediated by both vascular and metabolic changes. For example, peripheral hyperinsulinemia, a hallmark of T2DM, indirectly promotes Aβ accumulation and neurofibrillary
tangles formation by down-regulating the enzymes responsible for their clearance (Meusel et al., 2014). Furthermore, evidence from several longitudinal studies suggest that mid-life hypertension and cholesterol levels are associated with increased risk of dementia later in life (Whitmer et al., 2005).

No correlations between MoCA scores and RS co-activation nor CVR brain measures were detected, which could be because MoCA is designed to obtain a global measure of cognitive status (Nasreddine et al., 2005). More specialized tests targeting DMN functions should be used in the future to examine the link between cognition and CVR/RS co-activation in this network.

Whereas CVR changes have been reported in sensory-motor network as a function of the adult lifespan (Riecker et al., 2003), no significant association was detect with VRF index subgrouping in this network. In Chapter 2, CVR differences were also observed in several regions of the visual network, namely bilateral lingual gyrus and cuneal cortex, in individuals with HTN+T2DM compared to HTN only (Tchistiakova et al., 2014). Here, however, CVR was averaged across a larger area of visual cortex, which may have reduced group differences and weakened potential associations. Furthermore, CVR variability within the VRF index sub-groups was higher in sensory-motor and medial-visual NOIs compared to DMN, which may also explain the lack of significant findings and suggests an impact of other factors on CVR in these networks that were not accounted for in the model.

In the secondary analysis a positive correlation was detected between CVR and RS co-activation in the DMN. This is consistent with previously reported association between whole brain CVR and RS co-activation (Liu et al., 2013). A study examining resting state CBF also showed a significant correlation between baseline CBF and functional connectivity in the DMN (Khalili-Mahani et al., 2014), further supporting the importance of examining the vascular integrity of functional networks. Authors of the study suggested that the close correlation between CBF and RS co-activation in the DMN might be due to a tight coupling between metabolic and hemodynamic mechanisms required to support regulation of states of consciousness (Khalili-Mahani et al., 2014).

CVR group differences were detected using a DR approach but not when the global $P_{ET}CO_2$ signal was used as an input in a single-regression (model-based approach). Dual-regression has the advantage of accounting for regional variation in brain $CO_2$ response, which can contribute to
improved sensitivity. Others suggested this is an important methodological consideration and identified anatomical location (Rostrup et al., 2000) and/or vascular territories (R. F. Leoni et al., 2008) as potential sources of CO₂ response variance across the brain. The DR approach, used in this study, further advances our understanding of cerebrovascular function by demonstrating there is a close correspondence between regions with similar vascular response and functional RSNs.

3.5 Conclusion

This chapter examined the associations between VRF index and CVR and RS co-activation in individuals with WMH, a common comorbidity in elderly adults. This neurological group was chosen because WMH-based small vessel disease is fundamentally a vascular ischemic disease that may predispose individuals to additional VRF-related cerebrovascular impairment. A novel method for CVR analysis was developed, which allowed for the incorporation of a priori networks of interest and was found to have additional sensitivity to VRF status when compared to the model-based method. CVR was reduced in the DMN in the group with VRF index ≥ 2. No significant associations were detected between VRF index and RS co-activation, although a positive correlation between CVR and RS co-activation in the DMN suggests a link between the two metrics. Future confirmation of this new analysis approach in a larger population is needed.

Chapters 2 and 3 used CVR, a highly sensitive and specialized MRI technique for cerebrovascular assessment. CVR provides a valuable measure of cerebrovascular health, however, the set-up required for CVR experiment may limit its application in the clinical settings, particularly in individuals with comorbid neurological impairments. In Chapter 4, a T1-weighted high-resolution imaging was chosen to examine the associations between the summative VRF index and structural brain measures in older adults with MCI. Structural imaging is common in clinical and research practice, which makes it ideal for large population studies. Chapter 4 used data from ADNI database, with over 1400 structural scans available for NC and MCI.
Chapter 4

4 Associations between summative vascular risk factor effects and cortical thinning in mild cognitive impairment

4.1 Introduction

Alzheimer’s disease is a progressive neurodegenerative age-related disease characterized by increased accumulation of fibrillar β amyloid in neuritic and nonneuritic plaques in the cortex and striatum (Edison et al., 2008), high prevalence of neurofibrillary tangles formed by hyperphosphorylated tau (Selkoe, 2001), extensive brain atrophy (Thompson et al., 2003), decreased glucose metabolism (Devanand et al., 2010), and debilitating cognitive decline (Becker et al., 1988). Amnestic MCI is considered to be a prodromal stage of AD (Petersen et al., 1999), however, not all individuals with MCI go on to develop AD. Consequently, several recent studies identified MCI sub-groups that are at a higher risk of converting to AD using univariate and multivariate treatment of structural, cognitive and CSF measurements (Nettiksimmons et al., 2014; Spulber et al., 2013). These findings have prompted new questions about the underlying causes of MCI heterogeneity. VRFs are known to increase the risk of AD as well as other dementias, by acting adversely upon vascular and metabolic pathways synergistic with AD-related neurodegeneration (Clerici et al., 2012; Kivipelto et al., 2001; Luchsinger et al., 2005), discussed in details in Chapter 1. Furthermore, non-demented individuals with VRFs have

* Tchistiakova, E., & MacIntosh, B. J. (2015). Cortical volume and perfusion are influenced by vascular risk factors in addition to cognitive status- new insight made available from the ADNI study. Proceedings of Annual Meeting - International Society for Magnetic Resonance in Medicine

demonstrated changes in brain structure in regions that are closely related to AD (Hajjar et al., 2010; Last et al., 2007). The impact of VRFs on brain structure in individuals at risk for AD, however, is still unclear.

The primary objective of this chapter was to investigate the associations between the number of VRFs and CThk in individuals with MCI and NC older adults. Previous studies demonstrate an association between individual VRFs, T2DM, HTN and history of smoking, and cortical thinning in multiple brain regions (Brundel et al., 2010; Gonzalez et al., 2015; Leritz et al., 2011). In the current study we combined these VRFs into a summative VRF index (i.e. having 1, 2 or 3 VRFs) that was used as a primary measure of interest. Whereas the literature on smoking, T2DM and HTN is unambiguous for their deleterious effects on brain structure, the same may not be the case for hypercholesterolemia since some report positive (Leritz et al., 2011; van Velsen et al., 2013) and others report negative (Walhovd et al., 2014) associations with CThk; therefore, it was not included in VRF index.

The association between VRFs and CThk was assessed using multivariate PLS analysis with the summative VRF index as an ordinal variable. PLS is often more sensitive than univariate techniques when dependent variables are correlated (McIntosh et al., 2004), i.e. in the case of structural measures. Primary PLS analysis was conducted on a group of MCI participants and older NC group. In the secondary analysis PLS findings were used to examine cognitive group by VRF index interaction effects. Inter-regional CThk correlations were also computed within-VRF index sub-groups and compared between sub-groups. Lastly, CThk regions identified in baseline PLS analysis were examined for correlation with VRF index using a 1-year follow-up MRI. The primary hypothesis of this chapter is that it will be possible to identify a network of regions with decreased CThk related to the summative VRF index. The secondary hypotheses are that the effects of VRF index will be different between diagnostic categories, and that inter-regional CThk correlations will be influenced by the VRF index.

4.2 Methods

4.2.1 Participants

The imaging data analyzed in this chapter were downloaded from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.adni.loni.usc.edu). ADNI is a multi-center project launched in 2003 by the National Institute on Aging, the National Institute of Biomedical
Imaging and Bioengineering, the Food and Drug Administration and private pharmaceutical companies with a goal to identify MRI, PET and other biological markers, and clinical and neuropsychological measures of MCI and early AD progression (see Appendix A for more details on ADNI). Participants were included in the current analysis if they were between 55-90 years old, spoke English or Spanish as their first language, completed at least 6 years of schooling, were classified as NC or MCI by ADNI investigators using established criteria (G. McKhann et al., 1984; G. M. McKhann et al., 2011; Petersen et al., 1999) and had a baseline MRI scan. MRI data were collected on 1.5T and 3T MRI scanners in accordance with a standardized protocol (Jack Jr. et al., 2008). A typical protocol for T1-weighted 3D MPRAGE sequence included: TR/TE/TI=2300/2.9/900ms, matrix=256x240x192, voxel size=1x1x1.2mm³, number of slices=160 and flip angle=9°. Follow-up MRI data were also obtained at a one year for a subset of MCI participants.

In addition to imaging data, participants’ demographics including SBP, DBP, fasting blood glucose levels and ApoE genotype, as well as composite score on memory and executive function and education were obtained. Participants were included in the present work if they had at least one VRF (T2DM, preHTN/HTN or history of smoking) and were assigned into sub-groups based on the summative VRF index (i.e. 1, 2 or 3). Presence of VRF was established based on the medications associated with the treatment of that VRF and/or the condition was listed in the medical notes of the recent medical history. Additionally, measures of BP (SBP≥130 mmHg and/or DBP≥85 mmHg) (Chobanian et al., 2003; Haight et al., 2015) and fasting blood glucose (≥126 mg/dL) (American Diabetes Association, 2011) were used as part of the classification for preHTN/HTN and T2DM, respectively, to account for potential undiagnosed VRFs. Smoking was classified as “yes” if participants had a history of smoking at any time based on the previous evidence of the effects of mid-life smoking on brain structure (Debette et al., 2011).

4.2.2 Image pre-processing

T1-weighted high-resolution images from ADNI database were pre-processed by the University of California, San Francisco Medical Centre using FreeSurfer software (V5.1, http://surfer.nmr.mgh.harvard.edu/), described in details in Chapter 1. CThk was computed for 34 regions for right and left hemispheres (Fischl & Dale, 2000) and available for download from the ADNI website. A visual quality control of automated FreeSurfer segmentation was also
performed with segmentation rated as “Pass”, “Fail”, “Hippocampus-only” and “Partial”, which indicates a failure in one or more of 8 regions: frontal, temporal, insula, parietal, occipital, cerebral WM, basal ganglia and ventricles. Only CThk measures for images that passed segmentation quality control in frontal, temporal, parietal, occipital lobes and insula were included in the analyses described in this chapter.

4.2.3 Identifying associations between summative VRF index and CThk with PLS
Comparison of VRF index sub-groups was performed using PLS on 68 CThk regions produced by FreeSurfer segmentation for MCI and NC groups separately. Prior to PLS analysis, a linear model was used to regress the effects of age, gender, ApoE status (0 = no ε4 alleles, 1 = one or more ε4 alleles) and MRI field strength for each cortical region. This strategy of adjusting CThk values was designed to restrict PLS analysis to structural differences driven by VRF index and has been previously used in multivariate analyses (He et al., 2008). The PLS pipeline was developed at Baycrest hospital Rotman Research Institute, implemented using Matlab software and is described in detail in Chapter 1 (McIntosh et al., 1996; McIntosh & Lobaugh, 2004).

Adjusted regional CThk measures were formatted as 2D images making data conducive to the PLS software, namely, adjusted CThk values were assigned to 68 voxels in an image for each participant. Individuals’ data were then combined into a single matrix for each VRF index sub-group, where each row corresponded to a single participant and each column contained regional adjusted CThk measures. Next, PLS was performed to produce a set of latent variables (LVs), thereby identifying inherent association patterns between CThk and VRF index. Permutation testing with 1500 permutations (p<0.05 for significance) was performed to determine the significance of each LV, followed by a bootstrap resampling with 500 bootstraps to identify brain regions that consistently showed the significant LV pattern (BSR ≥2.0 for significance) and to generate a 95% confidence intervals (CIs) for VRF index sub-group comparison.

4.2.4 Cognitive group x VRF index interaction
In a secondary analyses main effect of VRF index across the cognitive cohorts and cognitive group x VRF index interaction in the regions identified by PLS were examined using ANCOVA in SPSS. These analyses included both NC and MCI groups. CThk was adjusted for age, gender, ApoE status and MRI field strength. Correction for multiple comparisons was performed using FDR method (q<0.05 for significance).
4.2.5 Inter-regional CThk correlations

Inter-regional correlation matrices were computed for each VRF index sub-group by calculating correlation coefficients between CThk measures for every pair of regions identified by PLS. Correlation coefficients were then converted into z-values using Fisher’s transform and VRF index sub-groups were compared in a pairwise manner. Results were adjusted for multiple comparisons using FDR (q<0.05 for significance). Similarly to PLS analysis, residuals were used to substitute for the raw CThk values in the correlation analysis.

4.2.6 Analysis of one-year follow-up MRI

Effects of VRF index on CThk were examined in the regions identified in baseline PLS findings using the subset of MCI participants with available one-year follow-up. An ANCOVA model that included age, gender, ApoE4 status and MRI field strength was used in SPSS. Results were adjusted for multiple comparisons using FDR (q<0.05 for significance).

4.2.7 Statistical analysis

Statistical comparison of VRF index sub-groups in terms of demographics and clinical characteristics was performed using SPSS. Age, SBP, DBP, fasting blood glucose, BMI, WMH volumes and cognitive measures were not normally distributed in at least one VRF index sub-group and were, therefore, compared using non-parametric Kruskal-Wallis test. Categorical variables were compared using χ² test.

4.3 Results

4.3.1 Participants

T1-weighted MRI data were pre-processed for 1437 participants (532 NC and 905 MCI), of which 383 and 672 passed segmentation quality control for NC and MCI respectively (see Figure 4.1). For NC, 354 out of 383 had a VRF index of 1 or more. For MCI, 604 out of 672 had a VRF index of 1 or more. Since ApoE status was included as a covariate in all analyses, 11 NC and 28 MCI participants with missing genotype data were excluded.
Participant demographics are summarized in Table 4.1. Fasting blood glucose levels were significantly different between VRF index sub-groups in both NC and MCI (p=0.002 and p<0.001, respectively), with a higher VRF index being associated with higher fasting glucose. SBP and BMI were also significantly different between VRF index sub-groups in MCI (p=0.01 and p<0.001, respectively). No significant differences were detected between VRF index sub-groups in any other measures.

4.3.2 Associations between summative VRF index and CThk

PLS analysis of CThk data in the MCI cohort produced one significant LV (p=0.01) that explained 95.3% of the covariance between adjusted CThk and the VRF index. Contributions of each group to this pattern and pairwise sub-group differences were determined using 95% CI bars from bootstrap testing. All three VRF index sub-groups contributed significantly to the identified pattern (i.e. CIs did not overlap with zero). The LV showed a stepwise pattern with increasing VRF index, i.e. higher VRF index was associated with lower brain scores (which in this case correspond to weighted CThk measures) (see Figure 4.2a).
<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>MCI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>187</td>
<td>311</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>72.9</td>
<td>72.7</td>
</tr>
<tr>
<td></td>
<td>(56.2, 89.6)</td>
<td>(55, 87.8)</td>
</tr>
<tr>
<td><strong>Race (%W)</strong></td>
<td>90.4</td>
<td>94.5</td>
</tr>
<tr>
<td></td>
<td>89.3</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>93.8</td>
<td>93.5</td>
</tr>
<tr>
<td><strong>Gender (%F)</strong></td>
<td>52.9</td>
<td>43.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>16 (6, 20)</td>
<td>16 (6, 20)</td>
</tr>
<tr>
<td></td>
<td>16 (7, 20)</td>
<td>16 (9, 19)</td>
</tr>
<tr>
<td><strong>Memory (a.u)</strong></td>
<td>0.96 (-0.3, 3.2)</td>
<td>0.17 (-1.4, 2.0)</td>
</tr>
<tr>
<td></td>
<td>0.88 (-0.5, 2.6)</td>
<td>0.09 (-1.4, 2.0)</td>
</tr>
<tr>
<td></td>
<td>0.90 (0.0, 2.2)</td>
<td>0.12 (-1.5, 1.6)</td>
</tr>
<tr>
<td><strong>Executive function (a.u)</strong></td>
<td>0.77 (-0.9, 2.6)</td>
<td>0.22 (-1.8, 2.6)</td>
</tr>
<tr>
<td></td>
<td>0.66 (-0.7, 2.4)</td>
<td>0.11 (-2.0, 2.4)</td>
</tr>
<tr>
<td></td>
<td>0.45 (-0.3, 1.6)</td>
<td>0.31 (-1.9, 1.9)</td>
</tr>
<tr>
<td><strong>ApoE (ε4≥1 allele)</strong></td>
<td>54</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>135 (93, 201)</td>
<td>134 (90, 184)</td>
</tr>
<tr>
<td></td>
<td>137.5 (104, 196)</td>
<td>139 (98, 201)</td>
</tr>
<tr>
<td></td>
<td>136 (112, 150)</td>
<td>138 (108, 175)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>74.0 (50, 100)</td>
<td>76 (50, 103)</td>
</tr>
<tr>
<td></td>
<td>76 (54, 100)</td>
<td>76.5 (54, 107)</td>
</tr>
<tr>
<td></td>
<td>77 (59, 88)</td>
<td>76 (54, 97)</td>
</tr>
<tr>
<td><strong>Fasting blood glucose (mg/dL)</strong></td>
<td>96 (55, 180)</td>
<td>93 (65, 158)</td>
</tr>
<tr>
<td></td>
<td>98 (70, 200)</td>
<td>97 (61, 220)</td>
</tr>
<tr>
<td></td>
<td>126 (79, 206)</td>
<td>114 (84, 413)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>26.6 (15.8, 39.1)</td>
<td>28.6 (21.5, 40.9)</td>
</tr>
<tr>
<td></td>
<td>26.8 (20.1, 51.5)</td>
<td>28.6 (21.5, 40.9)</td>
</tr>
<tr>
<td></td>
<td>28.9 (20.5, 38.8)</td>
<td>28.6 (21.5, 40.9)</td>
</tr>
<tr>
<td><strong>WMH ICV norm (cc)</strong></td>
<td>0.69 (0, 19.9)</td>
<td>0.94 (0, 66.7)</td>
</tr>
<tr>
<td></td>
<td>1.0 (0, 47.4)</td>
<td>1.2 (0, 23.5)</td>
</tr>
<tr>
<td></td>
<td>0.19 (0.04, 28.5)</td>
<td>1.1 (0.04, 31.7)</td>
</tr>
<tr>
<td><strong>DM (%)</strong></td>
<td>3.8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>31.4</td>
<td>64</td>
</tr>
<tr>
<td><strong>preHTN/HTN (%)</strong></td>
<td>80.2</td>
<td>253</td>
</tr>
<tr>
<td></td>
<td>97.1</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td><strong>Smoking history (%)</strong></td>
<td>16.0</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>71.4</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 4.1. Participant demographics. Data are medians (range) or percentages. Race: W-white, B-black, O-other. DM - Diabetes Mellitus, HTN- hypertension.
* Fasting blood glucose measures were missing for: 28 CN (VRF index 1: 8, VRF index 2: 19 and VRF index 3: 1) and 10 MCI (VRF index 1: 7, VRF index 2: 1 and VRF index 3: 2)
**BMI measurements were missing for : 4 CN (VRF index 1: 2, VRF index 2:2)
***WMH volumes were missing for: 19 CN (VRF index 1: 5 and VRF index 2: 14) and 11 MCI (VRF index 1: 5, VRF index 2: 5 and VRF index 3: 1)

Significant sub-group differences were observed between VRF index 1 and VRF index 3 sub-groups and VRF index 2 and VRF index 3 sub-groups (i.e. non-overlapping CIs), but not VRF index 1 and VRF index 2 sub-groups. This LV pattern was reliably detected (i.e. BSR≥2.0) in the 1) bilateral - entorhinal cortex, pars orbitalis and lateral orbitofrontal regions; 2) right – parahippocampal, inferior temporal, medial orbitofrontal, rostral middle frontal and frontal pole.
regions; and 3) left – insula and temporal pole (see Figure 4.2b). Our secondary PLS analysis on the NC group did not produce any significant LVs related to VRF index differences (p=0.4).

![Brain scores chart](image)

**Figure 4.2.** a) Significant latent variable pattern for PLS on CThk vs. summative VRF index in MCI cohort. Error bars represent 95% confidence intervals generated using bootstrap testing, b) Regions exhibiting the LV pattern.

### 4.3.3 Cognitive group x VRF index interaction

The results of the ANCOVA analysis revealed a main effect of VRF index across two cognitive groups in the right frontal pole (p=0.01), left lateral orbitofrontal (p=0.02) and right pars orbitalis (p=0.04). An interaction between cognitive diagnosis and VRF index was observed in the right medial orbitofrontal (p=0.001) and right parahippocampal (p=0.03) regions, where the effect of

![Brain scores chart](image)

**Figure 4.3.** Main effects of VRF index (*<0.05, **< 0.01) and cognitive group x VRF index interaction (#<0.05, ##< 0.01, ###≤0.001). Also shown are the post-hoc between - VRF index sub-group comparisons. For regions showing a significant interaction, the post-hoc test compares the CThk between VRF index sub-groups within the cognitive cohorts. OF – orbitofrontal.
VRF index was seen in the MCI but not NC group. Results are summarized in Figure 4.3. Only the interaction term in the right medial orbitofrontal region survived FDR correction.

4.3.4 Inter-regional CThk correlations

The inter-regional CThk correlation matrices for PLS-identified regions in the MCI group are shown in Figure 4.4 (Top). Groups with VRF index of 1 and 2 had positive inter-regional correlations between all examined regions. By contrast, the group with a VRF index of 3 had several negative correlations, primarily with right and left entorhinal cortices. Pairwise comparisons revealed significant (p<0.05 FDR-corrected) between-VRF index sub-group differences (see Table 4.2 and Figure 4.5 (Bottom)).

<table>
<thead>
<tr>
<th>Region 1</th>
<th>Region 2</th>
<th>VRF index 1 (r)</th>
<th>VRF index 2 (r)</th>
<th>VRF index 3 (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. Entorhinal</td>
<td>R. Rostral middle frontal</td>
<td>-</td>
<td>0.30</td>
<td>-0.33</td>
<td>0.001</td>
</tr>
<tr>
<td>R. Entorhinal</td>
<td>L. Pars orbitalis</td>
<td>-</td>
<td>0.31</td>
<td>-0.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R. Entorhinal</td>
<td>R. Rostral middle frontal</td>
<td>0.22</td>
<td>-</td>
<td>-0.33</td>
<td>0.004</td>
</tr>
<tr>
<td>R. Entorhinal</td>
<td>L. Pars orbitalis</td>
<td>0.17</td>
<td>-</td>
<td>-0.39</td>
<td>0.003</td>
</tr>
<tr>
<td>L. Entorhinal</td>
<td>R. Rostral middle frontal</td>
<td>0.25</td>
<td>-</td>
<td>-0.34</td>
<td>0.002</td>
</tr>
<tr>
<td>L. Entorhinal</td>
<td>R. Medial orbitofrontal</td>
<td>0.22</td>
<td>-</td>
<td>-0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L. Entorhinal</td>
<td>R. Lateral orbitofrontal</td>
<td>0.31</td>
<td>-</td>
<td>-0.30</td>
<td>0.002</td>
</tr>
<tr>
<td>L. Entorhinal</td>
<td>R. Inferior temporal</td>
<td>0.44</td>
<td>-</td>
<td>-0.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4.2. Inter-regional correlation coefficients for brain regions where CThk was found to correlate with the VRF index by PLS. Correlation coefficients (r) represent the strength of the correlation between CThk in region 1 and region 2. The unadjusted p-values represent the pair-wise t-tests between VRF index sub-groups after r to Z transform (see Methods). The r and p-values displayed only for regions that had significant correlation differences between VRF index sub-groups after FDR correction.

4.3.5 Analysis of one-year follow-up MRI

One year follow-up MRI data were available for three hundred and twenty eight MCI participants, 54.3% of the original sample (VRF index 1: 178, VRF index 2: 138, VRF index 3: 12). Baseline demographics and clinical characteristics of this subgroup did not differ significantly (p>0.05) from the original MCI group. Of the thirteen regions identified in the
baseline PLS findings, three showed significant association with VRF index at follow-up: right inferior temporal region (p=0.001), right entorhinal cortex (p=0.02) and right parahipocampal (p=0.05). In the right inferior temporal region this effect remained significant after FDR correction. A post-hoc analysis revealed significant decrease in CThk in all 3 regions in the group with VRF index of 3 compared with VRF index of 1 (right inferior temporal: p<0.001, right entorhinal: p=0.02, right parahipocampal: p=0.04) and 2 (right inferior temporal: p=0.001, right entorhinal: p=0.006, right parahipocampal: p=0.02), but not between VRF index of 1 and 2 (p> 0.05).

Figure 4.4. Top: CThk region-by-region correlation coefficient maps shown for each of the VRF index sub-groups; Bottom: pairwise VRF index sub-group comparisons. White squares represent regions where the Z-transformed correlation values were significantly higher in the VRF sub-group with the lower index (p<0.05, FDR-corrected). Black squares show non-significant VRF index sub-groups differences. OF – orbitofrontal, MF – middle frontal
4.4 Discussion

Summative VRF index showed significant association with CThk in the temporal and frontal regions among older adults with MCI that had at least one VRF. No such VRF patterns of cortical thinning were seen when the NC group was treated separately; however, a main effect of VRF index across the cognitive cohorts was found in right pars orbitalis, left lateral orbitofrontal and right frontal pole regions and a significant group by VRF index interaction effect was observed in the right medial orbitofrontal and right parahippocampal regions. Increased VRF index was also associated with differences in inter-regional CThk correlations between left and right entorhinal cortices and several frontal regions. Follow-up CThk data in a subset of the original MCI sample confirmed the impact of increasing VRF index on CThk in three temporal regions identified at baseline.

In this chapter PLS was used to identify regions where reduced CThk was associated with summative VRF index. In those with MCI, these regions included temporal structures that are commonly affected by AD (Thompson et al., 2003), as well as frontal regions, related to individual VRF effects (Brody et al., 2004; Gonzalez et al., 2015; Leritz et al., 2011). These findings are also consistent with earlier studies that showed negative associations between Framingham Cardiovascular Risk Profile and temporal (Cardenas et al., 2012; Villeneuve et al., 2014) and frontal (Villeneuve et al., 2014) CThk in a combined group of older NC and MCI adults.

VRF index was also found to influence inter-regional CThk correlations in the MCI cohort. Negative correlations were observed between bilateral entorhinal cortices and several frontal regions in the group with 3 VRFs. Changes in the inter-regional CThk correlations were previously observed in the AD individuals compared to NC (He et al., 2008), where negative correlations between brain regions were attributed to regional disconnectivity and/or localized degeneration (Alexander-Bloch et al., 2013).

This data add to a growing literature showing that the impact of VRFs on the brain is multifactorial, including structural degeneration of GM and WM, cerebrovascular dysfunction and functional network reorganization (Hajjar et al., 2010; Last et al., 2007; Leritz et al., 2011; Musen et al., 2012). These processes are often interconnected, such that impaired perfusion decreases the supply of oxygen and nutrients to brain tissues, contributing to cortical thinning.
(Alosco et al., 2014), while degeneration of connecting white matter fibers correlates with the decrease in gray matter volumes (Choo et al., 2010). CThk was chosen for the current analysis as it has been shown to be a sensitive structural biomarker for detecting the effects of VRF as well as early signs of AD (Brundel et al., 2010; Leritz et al., 2011; Sabuncu et al., 2011). Other metrics such as perfusion and white matter tractography may provide additional information on the mechanisms that could contribute to these structural changes. For example, a recent study by Maillard et al. demonstrated a significant impact of VRFs on white matter fractional anisotropy, which was found to be reduced in individuals with ≥ 2 VRFs compared to those with only 1 VRF in a range of white matter tracts including the cingulum and uncinate fasciculus that connect temporal and frontal brain regions (Maillard et al., 2015). Unlike the structural measures, however, white matter and perfusion imaging are rarely used in clinical protocols, and have only recently been added to ADNI, limiting the number of available data. These measures were, therefore, not considered in the current chapter but could be explored in future work.

The multivariate PLS analysis identified a set of VRF sensitive regions within an MCI but not the NC cohort, which may be explained by several factors. First, the VRF index may have a larger effect size in MCI because this group is more susceptible to neurodegeneration. Second, there may be inherent biases in the selection of MCI and NC, namely the breakdown of VRF index sub-groups. No attempts were made to match cognitive groups since the primary objective was to characterize the impact of VRF index in MCI. Third, VRFs may act synergistically with AD pathology in the MCI group, which served to reinforce VRF index/CThk associations. A post-hoc analysis on the selected PLS regions across the two cohorts showed a significant cognitive group by VRF index interaction in the right medial orbitofrontal and right parahippocampal regions. These results are consistent with a study on the interaction of VRFs and Aβ deposition (a hallmark of AD) that observed thinner fronto-temporal cortical regions in individuals with higher number of VRFs (Villeneuve et al., 2014). Finally, analysis on the follow-up data produced mixed results in the sense that only a subset of regions identified by PLS at baseline showed as association with VRF index at follow-up and included right entorhinal, right parahippocampal and right inferior temporal areas. This may be due to the smaller sample available at follow-up, i.e. 328 samples vs. 604 baseline samples. Lower effect size may also be due to the different effects of specific combination of VRFs on CThk, which were not tested in this study. Of note, the follow-up data findings were specific to the right
temporal lobe, which may indicate a persistent vulnerability of these brain regions to the VRF index. These temporal findings are consistent with previous studies on HTN and T2DM (Brundel et al., 2010; Leritz et al., 2011). The current findings add on to this literature by demonstrating the detrimental effect of summative VRFs on CThk in these regions. Although the design of this experiment does not allow to determine the exact nature of VRF interaction, one potential explanation is that the impact of metabolic dysregulations, that are know to preferentially impact temporal structures in T2DM (Brundel et al., 2010), are exacerbated by cerebrovascular deficits, caused by HTN and smoking (Beason-Held et al., 2007; Rogers et al., 1985).

4.5 Conclusion

This chapter demonstrates the utility of PLS as a means to identify VRF-sensitive brain regions. A higher VRF index was associated with reduced CThk in the MCI cohort. No VRF index effect on CThk was detected in the NC group, although a main VRF index effect was seen in several frontal regions when the two cognitive groups were combined. Inter-regional CThk correlations changes between temporal and frontal brain regions were observed in the sub-group with VRF index of 3 compared to VRF index of 1 and 2 sub-groups. Overall, the findings of this chapter suggest that the use of a summative VRF index is a well-suited strategy for characterization of the influence of comorbid vascular risk factors on neurodegeneration in the MCI cohort.
Chapter 5

5 Thesis Discussion

After two decades of research on VRFs, the evidence clearly shows that VRFs increase the risk of developing stroke and dementia (Kivipelto et al., 2001; P. A. Wolf et al., 1991). The literature to date has, however, primarily focused on studying individual VRF effects on the brain, which may limit the generalizability of these findings in the context of the comorbid effects of VRFs on neurodegeneration. The findings of the current thesis add to this research by providing evidence of the detrimental effect of increasing VRF number on brain structure and vasculature in NC older adults as well as individuals with comorbid old-age neurodegenerative disorders. The following section provides a brief synopsis of the main findings, followed by a discussion on potential clinical implications and recommendation for future directions for neuroimaging studies.

5.1 Summary and Conclusions

Hypothesis I posited that it will be possible to detect CThk and CVR differences between HTN+T2DM and HTN only groups in NC older adults. Findings from Chapter 2 confirmed this hypothesis by demonstrated that there are several regions in occipito-parietal cortex where CVR is reduced in HTN+T2DM compared to HTN only group. CThk differences were found to be less widespread than CVR but overlapped within right occipital areas (Tchistiakova et al., 2014). To our knowledge this is the first study that compares the effects of these two commonly co-occurring comorbidities to an HTN only group.

Hypothesis II postulated that increasing VRF number could exacerbate the impact of old-age neurodegenerative conditions, such as SVD, on brain hemodynamics and neuronal function. In Chapter 3 CVR, but not RS co-activation was found to be reduced in older adults with WMH and ≥ 2 VRFs compared to those with WMH and none or 1 VRF. A new technique for CVR assessment was developed in this chapter, which incorporated a priori functional networks to help account for heterogeneity of hypercapnia response across the brain. This method showed
additional sensitivity to VRF status when compared to a model-based method (Tchistiakova et al., 2015).

Hypothesis III proposed that MCI heterogeneity, observed in several previous studies (Nettiksimmons et al., 2014; Spulber et al., 2013), may be related to the presence of comorbid VRFs. This was confirmed in Chapter 4 where regional CThk was found to be sensitive to the summative VRF index in the MCI group, namely in the frontal and temporal regions. Results also indicated that VRF index effects vary between NC and MCI groups, suggesting a potential interaction between VRF and AD-related neurodegenerative processes. Unlike the findings from Chapter 2, no significant associations between VRF index and CThk were detected in the NC group in Chapter 4. This is likely due to fact that Chapter 2 focused on a specific combination of VRFs, i.e. HTN+T2DM vs. HTN, while Chapter 4 used an overall summative index. Together, these findings suggest that CThk integrity is influenced not only by the number of VRFs but also by their specific combination.

Analysis tools developed in this thesis, including DR-based CVR analysis (Chapter 3) and multivariate PLS on regional CThk data (Chapter 4), helped improve characterization of VRF relationship with brain health measures. It is important to note, however, that these methods could also be applied to a wide range on neurological conditions. Namely, the use of DR-based CVR assessment may be particularly advantageous in populations with compromised cerebral vasculature, where the use of a single model across the entire brain may lead to CVR underestimation or introduce potential biases in the interpretation of CVR data. The use of PLS technique may also improve sensitivity when examining conditions with dispersed effects on the brain, as well as in experiments with no a priori knowledge of the condition effect on the brain. Although the early applications of PLS in neuroimaging focused on task-based fMRI (McIntosh et al., 1996), it has since been extended to other imaging modalities (i.e. PET and EEG), and, as demonstrated in Chapter 4 of this thesis, can also be adopted for regional brain measures.

5.2 Clinical implications

According to the Employment and Social Development Canada, seniors make up the fastest growing age group, which is estimated to reach 22.8% by 2031 compared to 14.4% in 2011 (www.hrsdc.gc.ca). Advancing age and sedentary life-style rapidly increases the prevalence of VRFs and neurodegenerative disorders posing a major strain on Canada’s health care system.
With 1.4 million Canadians estimated to develop AD by 2031 (Alzheimer Society Canada), the precedence is high to develop new treatments combatting this disease. Although the clinical trials for potential treatments are ongoing, few have shown positive results (Kelley, 2015). Presence of mixed pathologies and comorbidities may be a contributing factor in the limited success of treatment development simply because new molecular entities are designed to ‘attack’ the main pathological substrate, such as the Aβ-plaques, and may not be effective when other neurological or vascular burdens are present. VRFs are common in the elderly, and have been shown to contribute to AD onset. Their effects on the brain, however, are potentially modifiable. Effective management of VRF symptoms, for example, was shown to have a beneficial effect on brain health (Firbank et al., 2007; Korf et al., 2004; Manschot et al., 2006). The findings of this thesis identified brain regions where structural and/or vascular deficits are associated VRF presence and may therefore benefit the most from an effective treatment. In particular, Chapter 4 demonstrated a high degree of overlap between regions commonly impacted in AD and VRF-related CThk heterogeneity in MCI. A strict VRF treatment may help reduce structural deterioration of these regions and potentially slow down the progression of MCI to AD.

The discovery of common mechanisms of neurodegeneration between VRFs and AD, discussed in Chapter 1, lead to the investigation of potential uses of VRF medications for AD treatment. Diabetic medications are of particular interest due to the evidence of insulin dysregulation in AD (Craft & Watson, 2004). Figure 5.1 describes the pathway connecting insulin resistance with AD pathology. Binding of insulin to the insulin receptor causes activation of the insulin receptor substrate (IRS-1), a starting point in the IRS 1-AKT pathway, and inhibition of the glycogen synthase kinase (GSK-3) (Yarchoan & Arnold, 2014). In the healthy brain, presence of insulin increases phosphorylation of insulin receptor-β subunit, IRS-1, AKT and other insulin-signaling proteins. In the AD brain, however, this response is reduced, resembling insulin resistance in the peripheral tissue and resulting in the abnormal GSK-3 activation increasing neurofibrillary tangles and Aβ-plaques formation (Yarchoan & Arnold, 2014).

Metformin, is an oral, glucose lowering medication commonly prescribed for T2DM treatment. In the periphery, metformin improves glucose metabolism by increasing organs’ insulin sensitivity (Yarchoan & Arnold, 2014). Less, however, is known on the effects of metformin in the cerebral nervous system. Evidence from rodent studies demonstrate that metformin can cross the blood-brain barrier, activating the AMP-activated protein kinase (AMPK), see Figure 5.1,
increasing neuronal sensitivity to insulin and reducing AD pathology (Labuzek et al., 2010; Nath et al., 2009), i.e. tau protein phosphorylation (Kickstein et al., 2010). Memory function was also shown to improve following metformin treatment, albeit only in females (DiTacchio et al., 2015). Another study, however, detected an increase in Aβ-amyloid production when metformin was used on its own, but a decrease during combined insulin/metformin treatment (Y. Chen et al., 2009). A human pilot clinical study on effects of metformin in MCI was recently completed (ClinicalTrials identifier: NCT00620191) and results are pending, while another trial is ongoing (ClinicalTrials identifier: NCT01965756). The results of these two trials will greatly improve the understanding of metformin impact on AD pathology, and its potential use as part of AD treatment regimen.

Insulin has also been proposed for AD treatment. In order to bypass the blood-brain-barrier and avoid peripheral hypoglycemia, an intranasal insulin administration has been used in several clinical trials (Craft et al., 2012; Reger et al., 2008). Among 104 older adults with MCI or AD, administration of intranasal insulin resulted in significant improvement of memory function and increase in glucose uptake on PET scans, although no changes were found in the Aβ levels in CSF (Craft et al., 2012). Some also observed different insulin response based on the genetic risk, namely, only those with negative ApoE ε4 status showed memory improvements (Reger et al., 2008). To date, evidence of beneficial insulin effects are only available from the

**Figure 5.1.** Impact of diabetic drugs (insulin and metformin) on the relationship between brain insulin resistance and AD through the IRS–1 → AKT pathway (pink) in MCI and AD, adopted from (Yarchoan & Arnold, 2014)
short-term trials. A potential concern for long-term trials, is that a chronic hyperinsulinemic state may lead to the development of brain insulin resistance that could exacerbate AD pathology. To further the understanding of insulin effect on cognition the U.S. National Institute of Health launched “The Study of Nasal Insulin in the Fight Against Forgetfulness (SNIFF)” in 2013 (ClinicalTrials identifier: NCT01767909), which will recruit 250 MCI/AD participants and randomize them to receive intranasal insulin or placebo for 12 months. Two of the three clinical trials (NCT01965756 and NCT01767909) described above were designed to include brain MRI to measure structural and perfusion (NCT01965756) changes following the treatment. MRI biomarkers play an important role in clinical studies as they are often more sensitive in detecting subtle effects of the treatments than the conventional neuropsychological tests and are, therefore, of particular use for developing pharmaceutical agents to combat AD.

The high prevalence of VRF in neurodegenerative disorders may not only exacerbate the disease but may also lead to erroneous study results. Namely, the lack of proper control for VRF effects in clinical trials can attribute VRF-related brain changes to other conditions and/or obscure the effects of treatments on the studied condition. For example, of the 258 papers published on the structural MRI data from ADNI database only 10 included VRFs (T2DM, HTN, coronary artery disease, history of smoking) or associated clinical measures (BMI, BP, cholesterol levels) (see Appendix B for the details on systematic review of VRF in ADNI publications). The primary objective of ADNI, as stated in its mandate, is the “determination of sensitive and specific markers of very early AD progression, intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness”. Chapter 4 of this thesis demonstrated a significant association of VRFs with regional CThk heterogeneity within the MCI cohort. Failure to account for this variability may decrease the specificity of identified structural biomarkers to AD processes.

5.3 Limitations and future directions

Previous chapters of this thesis demonstrated an association between VRFs and deficits in cerebrovascular function and structural integrity of the brain. These findings are of particular importance as they emphasize the need for better awareness of VRF effects in neuroimaging studies. Chapter 2 demonstrated that structural and vascular deficits overlapped in the group with HTN+T2DM. This cross-sectional study precludes our ability to determine the causality of these effects, although observed CVR changes were more spatially extensive, which may suggest that
they precede structural deficits. Future studies would benefit from a longitudinal design to study vascular and structural changes.

Chapters 3 and 4 adopted a cumulative measure of VRF number to examine the impact of multiple VRFs. The decision to rely on a cumulative index was driven by the even larger sample sizes that would be required for a full factorial model. Large multi-center studies such as ADNI and Coronary Artery Risk Development in Young Adults (CARDIA) provide a unique research opportunity to develop a better understanding of the effects of conditions, such as VRFs, on the brain as well as develop disease specific biomarkers, which may not be possible in the single-site studies due to their limited sample size. Future work could combine the data from these two studies to identify and compare the additive/interactive effects of specific VRFs.

MRI measures such and CVR and CThk may be compromised in the older adults in general and, even more so, in adults with comorbid SVD and MCI. Namely, vascular abnormalities associated with age and/or disease can lead to underestimation of CVR measurements, while decreased tissue contrast may cause segmentation errors during CThk estimation. Potential contributions of these two sources of error are examined in the “Data quality considerations” section below.

5.3.1 Data quality considerations
First, the two hypercapnia methods used in Chapters 2 and 3 each have their strengths and challenges. Passive CO₂ inhalation provides a robust and reproducible signal independent of the participant’s compliance. Additional set-up, equipment and designated personal required for this procedure, however, make it unlikely to be implemented in larger clinical studies. The BH challenge can be easily added on to the clinical protocols as it requires no additional set-up, although, its validity in the elderly and impaired populations have not been confirmed. To examine the data quality when using either of these approaches, an analysis was conducted to directly compare CVR measures from BH and CO₂ inhalations challenges in a group of older adults with WMH. The hypothesis is that regional CVR will not be influenced by the hypercapnia method (i.e. BH challenge vs. CO₂ inhalation).
CVR study: BH challenge vs. CO\textsubscript{2} inhalation

Methods

Participants
This pilot study was conducted on a sub-group (N=8, mean age 67.0±11.0 years) of older adults with WMH from Chapter 3.

Hypercapnia challenges
CO\textsubscript{2} inhalation challenge was the same as described in Chapter 3. RespirAct\textsuperscript{TM} breathing circuit was used to increase PetCO\textsubscript{2} levels by 10 mmHg from participant’s baseline for two subsequent periods of 45s and 2 min with 90s of normocapnia in between and a 3 min 30s of normocapnia at the end. Following the CO\textsubscript{2} challenge, the gas mask was removed for the sake of comfort and participants performed six breath holds lasting 15s each following 3s expiration period with intermittent 30s periods of normal breathing. Participants started and stopped BH by a tactile cue on the leg by one of the experimenters. To achieve compliance, participants practiced the BH task prior to commencing of the imaging session. MRI protocol for BOLD imaging was the same for CO\textsubscript{2} inhalation and BH challenge (described in Chapter 3), except for the scan duration (CO\textsubscript{2} inhalation: 8 min 38s; BH challenge: 5min 18s).

Image processing
BOLD images were motion corrected using MCFLIRT (Jenkinson et al., 2002) and spatially smoothed with Gaussian kernel of 5mm FWHM. A high pass temporal filter with 100s cutoff for BH and 250s for CO\textsubscript{2} was also applied. The different high pass filters were chosen to account for the paradigm differences between the two tasks. Brain extraction technique (Smith, 2002) was used to remove non-brain structures. PetCO\textsubscript{2} traces from RespirAct\textsuperscript{TM} were used as the GLM model for CO\textsubscript{2} inhalation analysis. Analysis of the BH CVR was similar to that described in Chapter 2, where box-car model was convolved with double-gamma hemodynamic response function and individual response delay was estimated for each participant.

Statistical analysis
Percent BOLD change maps were calculated for each participant for each task. The maps were then registered to the common template and a Pearson’s correlation was used to calculate the correlation between two hypercapnia methods on the voxel-wise bases. T1-weighted images
were segmented using FSL FAST (Zhang et al., 2001) to create GM and WM partial volume maps and used as masks to examine the correlation coefficients in different tissue types.

**Results**

A correlation map and R-value distributions in GM, WM and full brain are shown in Figure 5.2. Correlation between the two hypercapnia tasks was moderate and did not vary between tissue types. Regions with poor correlation (R<0.5) included superior frontal gyrus, anterior cingulate, frontal pole, as well as areas within the superior and middle temporal gyri, lateral occipital cortex and occipital pole.

![Correlation map and R-value distributions in GM, WM and full brain.](image)

**Figure 5.2. CVR correlation map for CO₂ inhalation vs. BH challenge. Also shown are the R-value distribution curves for GM, WM and full brain.**

**Discussion and Conclusion**

In this section, two hypercapnia challenges, commonly used for CVR assessment were compared in a group of older adults with WMH. CVR measurements based on CO₂ inhalation and BH challenge showed moderate correlation across the brain with several clusters of low correlations. The discrepancy in the CVR measures may be due to the larger PetCO₂ differences achieved during CO₂ inhalation. If this were the case, however, we might expect a more uniform increase
in BOLD signal across all brain regions, given that both tasks produce moderate hypercapnia (Tancredi & Hoge, 2013; Zande et al., 2005). Regional disparity in CVR likely reflects underlying differences between the hypercapnia challenges. For example, anterior cingulate cortex has been previously implicated in perception of dyspnea (i.e. shortness of breath) during hypercapnia (K. C. Evans et al., 2002). The CO$_2$ inhalation periods were longer than the breath holds, therefore, differences may reflect participants’ discomfort during the CO$_2$ experiment. Kastrup et al. have previously demonstrated a high degree of correlation between the two methods in healthy young adults (Kastrup et al., 2001), which may suggest that age and cerebrovascular impairment (i.e. presence of WMH) may play a role in the correspondence between the two tasks. These findings caution against comparing study results with different hypercapnia challenges and draws attention to the need of standardization for CVR assessment. It is unlikely that the choice of CVR methods played a significant role in the thesis findings since only a small region in the lateral occipital cortex overlapped between detected group differences in Chapter 2 and low CO$_2$/BH correlations. Future studies, however, should be mindful of the potential CVR measurement differences related to the hypercapnia experimental methods.

**Segmentation quality control**

Aging and disease contribute to the decline in brain volumes, tissue density and CThk. Furthermore, they can also have a significant effect on the tissue signal properties, such as T1 relaxation, discussed in Chapter 1, and signal intensity. Salat et al., demonstrated age-related changes in GM and WM tissue properties, as well as a decrease in GM/WM contrast, mostly due to a decrease in WM signal (Salat et al., 2009). Changes were most apparent in a large portion of frontal regions, inferior parietal, superior temporal, precuneus and retrosplenic regions. These findings are consistent with several earlier studies (Jernigan et al., 1991; Magnaldi et al., 1993; Raz et al., 1990). The mechanisms contributing to the signal changes are still unclear, with some attributing T1 relaxation changes to iron deposition (Ogg & Steen, 1998), increased water content in WM and neuronal loss in GM (Magnaldi et al., 1993) or regional changes in myelination (Salat et al., 2009). Changes in tissue contrast can impact the quality of segmentation algorithms and cause misclassification of the brain tissues. In this thesis, FreeSurfer was used for segmentation of high-resolution T1-weighted images. The automated segmentation was followed-up by a visual inspection rating the segmentation as “Pass”, “Fail”, “Hippocampus-Only” and “Partial”, which indicates a failure in one or more of 8 regions:
frontal, temporal, insula, parietal, occipital, cerebral WM, basal ganglia and ventricles. Only images that passed segmentation quality control in frontal, temporal, parietal, occipital lobes and insula were included in the CThk analyses. Figure 5.3 provides an example of FreeSurfer segmentation failure in the temporal region. Besides physiological factors, technical aspects, such as magnetic field inhomogeneities may impact tissue contrast.

Analyses in Chapter 4 included imaging data from over 50 research sites. To ensure that the results were not biased by low image quality in a particular site, a distribution of CThk for a sample region was examined in NC and MCI adults, see Figure 5.4.

![Figure 5.3. Example of FreeSurfer segmentation fail in the temporal region.](image)

![Figure 5.4. Distribution of L. Entorhinal CThk in NC and MCI groups by collection site.](image)

No systematic differences were observed in CThk measures across different sites. Strict segmentation quality control described above makes it unlikely that structural findings of this thesis were influenced by segmentation errors.
5.3.2 Characterization of VRF impact on brain perfusion with ASL

In this thesis BOLD imaging during hypercapnia challenge was used to examine cerebrovascular dysfunction associated with VRFs. BOLD, however, is an indirect measure of perfusion, as discussed in Section 1.7.3, an alternative method for perfusion imaging, arterial spin labeling (ASL), is gaining popularity. ASL is a non-invasive MRI technique that uses magnetically labeled arterial blood water as an endogenous tracer and allows quantitative measurement of perfusion in the tissues by acquiring pairs of ASL images, with and without magnetic labeling. ASL can provide a valuable addition to the CVR BOLD measurements by examining the effect of VRF on the resting cerebral blood flow. Furthermore, implementation of a recently developed vessel-selective ASL (Wong, 2007) that uses selective labeling of blood vessels could help identify specific regions of brain vasculature impaired by VRFs. The work of this thesis provides indication of some novel brain targets that could be explored by this method. Arterial transit time is another ASL technique of interest when considering VRF effects. ATT represents the time it takes for magnetically labeled blood water to travel from the tagging plane to the imaging slice (MacIntosh et al., 2010). ATT measurement is a key parameter in calculation of absolute cerebral blood perfusion, but it can also provide information about hemodynamic impairment because prolonged transit time is thought to indicate vascular pathology and/or recruitment of secondary pathways of collateral perfusion to compensate for insufficient blood supply through primary pathways (Bokkers et al., 2008).

5.3.3 Multimodal imaging

Finally, multimodal imaging should also be considered for future studies of VRF effects on the brain, such as by combining the CVR/perfusion measures from MRI and amyloid/tau PET imaging. VRFs contribute to amyloid deposition and tau-phosphorylation directly (i.e. trough the reduction in insulin degrading enzyme in T2DM) and through cerebrovascular dysfunction, which hinders Aβ clearance. Combining vascular and amyloid/tau imaging can be used to further our understanding of the link between the two processes as well as be used as a powerful tool in tracking the progression of the disease.
5.4 Conclusions

As the average life span continues to increase with an estimated 79 years for men and 83 for women by 2017 (Statistics Canada), the burden of neurodegenerative diseases will be more apparent. As the former Surgeon General of the United States, Dr. Richard Carmona, stated, “the good news, is that many of us are going to live to 90, the bad news is that people are outliving their brains”. Deleterious effects of HTN and T2DM on the peripheral organs have been recognized for decades with extensive research devoted to treatment development. The mechanisms by which these conditions impact the brain, however, are still poorly understood. Neuroimaging has become a powerful tool that plays a key role in investigating the VRF effects on brain health. Several MRI methods were used in this thesis to characterize the associations between multiple VRFs and brain structure and vasculature. Presented findings have two important implications. Firstly, they demonstrated an exacerbated effect of multiple VRFs on structural and vascular brain health. Secondly, they emphasized the need for proper control for VRFs in neurological studies by demonstrating the contribution of VRFs to structural and vascular brain deficits in neurological disorders. Many more questions on the mechanisms of VRF-induced neurodegeneration remains unanswered, however, the growing awareness of VRF impact on the brain is a good start towards development of successful management/treatment strategies.
Summary of thesis dissemination

Publications


Abstracts


Appendices

Appendix A. Alzheimer’s Disease Neuroimaging Initiative

The Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (www.adni.loni.usc.edu) was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies and non-profit organizations as a 5-year private-public partnership. The primary goal of ADNI has been to test whether serial MRI, PET and other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco.

ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. Diagnostic classification is made by ADNI investigators using established criteria for normal controls (NC), early MCI (eMCI), late MCI (lMCI) or early stages of AD (G. McKhann et al., 1984; G. M. McKhann et al., 2011; Petersen et al., 1999). Controls had mini-mental status examination (MMSE) scores between 24-30 and no significant memory concerns. MCI adults had MMSE scores between 24-30; memory complaint; objective memory loss as quantified by the Wechsler Memory Scale Logical Memory II test; a Clinical Dementia Rating (CDR) score of 0.5; lack of cognitive impairment in other domains such as executive function, visuospatial function and language; relative sparing of activities of daily living; and absence of frank dementia. Data collection and sharing for this project was funded by the Alzheimer’s Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer’s Association; Alzheimer’s Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company...
Genentech, Inc.; Fujirebio; GE Healthcare; ; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.
Appendix B.  Systematic review of ADNI publications for VRF inclusion

PubMed and Scopus databases were searched for publications based on MRI data collected for ADNI between 2003 (launch date) and August 2015 using the search terms [“ADNI” and “magnetic resonance imaging”] and [“ADNI” and “mri”]. Across the two databases, the search produced 380 results, of which 258 were original articles published in the peer-reviewed journal that used structural T1-weighted imaging as their primary measure of interest. The demographics were reviewed for each paper identifying the studies that included either categorical VRF classification (i.e. T2DM, HTN), clinical measures related to these VRFs (i.e. BMI, BP, cholesterol levels) or any other measures reflecting cardiovascular health (i.e. Framingham risk factor score). Based on these criteria, 10 studies were identified and are summarized below.

<table>
<thead>
<tr>
<th>Study</th>
<th>VRF criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Authors</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
</tr>
</tbody>
</table>
References


Studies included in systematic review on ADNI


alzheimers disease: Implications for sequence of pathological events in alzheimers

and its application to hippocampal segmentation and brain parcelation. *Medical Image
Analysis, 17*(6), 671-684. doi:10.1016/j.media.2013.02.006

94. Kantarci, K., Gunter, J. L., Tosakulwong, N., Weigand, S. D., Senjem, M. S., Petersen, R.

functionals based brain to ventricle index for analysis of AD progression in MR

algorithm to measure changes in medial temporal lobe volume in alzheimer

Coulson, E. J. (2014). Basal forebrain atrophy correlates with amyloid β burden in

98. Khan, W., Westman, E., Jones, N., Wahlund, L. -, Mecocci, P., Vellas, B., . . . Simmons,
A. (2014). Automated hippocampal subfield measures as predictors of conversion from
mild cognitive impairment to Alzheimer's disease in two independent cohorts. *Brain
Topography, 28*(5), 746-759. doi:10.1007/s10548-014-0415-1

of alzheimer's disease based on partial least squares, principal component analysis and
support vector machine using segmented MRI images. *Neurocomputing, 151*(P1), 139-150. doi:10.1016/j.neucom.2014.09.072

100. Kim, A., Fagan, A. M., Goate, A. M., Benzinger, T. L. S., Morris, J. C., & Head, D.
(2015). Lack of an association of BDNF Val66Met polymorphism and plasma BDNF with

Johnson, S. C. (2014). Multi-resolutional shape features via non-euclidean wavelets:
Applications to statistical analysis of cortical thickness. *NeuroImage, 93*(P1), 107-123. doi:10.1016/j.neuroimage.2014.02.028


associated with vascular damage. *Psychology and Aging, 28*(1), 191-201. doi:10.1037/a0031063


