Effects of Hypertension and Type 2 Diabetes on Cerebrovascular and Cognitive Function in Older Adults

by

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Department of Nutritional Sciences
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Abstract

The present research examined the effects of hypertension and type 2 diabetes on cerebrovascular and cognitive function in order to better understand how these disorders may contribute to cognitive aging in older adults. Participants provided physiological and medical data, completed neuropsychological tests, and performed a breath hold task in an MRI. The results of group comparisons indicated three clusters across which older adults with comorbid hypertension and type 2 diabetes had lower cerebrovascular reactivity than older adults with hypertension alone. Older adults with comorbid hypertension and type 2 diabetes also had lower global cognitive function than older adults with hypertension alone or control participants. Finally, higher HbA1c predicted lower cerebrovascular reactivity, while both higher HbA1c and higher Framingham Risk Scores predicted lower global cognitive function. These findings underscore the importance of maintaining glucose control (HbA1c) and managing vascular risk factors (Framingham Risk Scores) in older adults.
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List of Abbreviations

ACEI = angiotensin converting enzyme inhibitor
AD = Alzheimer’s disease
AFNI = Analysis of Functional NeuroImages
ANOVA = analysis of variance
ASL = arterial spin labelling
BMI = body mass index
BOLD = blood oxygenation level dependent
fMRI = functional magnetic resonance imaging
CBF = cerebral blood flow
CON = normal aging (control)
COWAT = Controlled Oral Word Association Task
CRP = C-reactive protein
CVLT-II = California Verbal Learning Test II
CVR = cerebrovascular reactivity
DBP = diastolic blood pressure
DEX = Dysexecutive Questionnaire
DM = diabetes mellitus (Type 1 or Type 2)
DMN = default mode network
DRAMMS = Deformable Registration via Attribute Matching and Mutual-Saliency Weighting
EEG = electroencephalography
FEAT = fMRI Expert Analysis Tool

FG = fasting glucose

FOV = field-of-view

FRS = Framingham Risk Score

FSL = Oxford Centre for Functional MRI of the Brain Software Library

HADS = Hospital Anxiety and Depression Scale

HbA1c = glycated hemoglobin

HDL = high-density lipoprotein

HOMA-IR = homeostasis model assessment of insulin resistance

HPTN = hypertension

HPTN+T2DM = comorbid hypertension and type 2 diabetes mellitus

IFG = impaired fasting glucose

LDL = low-density lipoprotein

LNS = Letter-Number Sequencing

MAP = mean arterial pressure

MATLAB = Matrix Laboratory

MNI = Montreal Neurological Institute

MPRAGE = magnetized prepared rapid acquisition gradient echo

MR = magnetic resonance

NCCEA = Neurosensory Center Comprehensive Examination for Aphasia
pCASL = pseudo-continuous ASL

PET = positron emission tomography

PP = pulsatile pressure

SART = Sustained Attention to Response Task

SAS = Statistical Analysis System

SBP = systolic blood pressure

SNK = Student-Newman-Keuls

SNR = signal-to-noise ratio

SPSS = Statistical Package for the Social Sciences

SSS = superior sagittal sinus

T2DM = type 2 diabetes mellitus

TC = total cholesterol

TE = echo time

TG = triacylglycerides

TI = inversion time

TMT–A = Trail Making Test Part A

TMT–B = Trail Making Test Part B

TR = repetition time

WAIS = Wechsler Adult Intelligence Scale

WAIS–R = Wechsler Adult Intelligence Scale–Revised
WASI = Wechsler Abbreviated Scale of Intelligence

WCST = Wisconsin Card Sorting Task

WMH = white matter hyperintensity

WMS = Wechsler Memory Scale

WMS-R = Wechsler Memory Scale Revised
Chapter 1
Introduction

1 Introduction

1.1 Background

1.1.1 Purpose of the Present Study

Hypertension (HPTN) and type 2 diabetes mellitus (T2DM) are potential risk factors for cognitive decline in older adults (Feinkohl, Price, Strachan, & Frier, 2015; Iadecola et al., 2016). Although both HPTN and T2DM are associated with cerebrovascular dysfunction (Pires, Dams Ramos, Matin, & Dorrance, 2013; Ryan, Fine, & Rosano, 2014), the extent to which these disorders contribute to cognitive aging in older adults remains unclear. Underreporting of HPTN and T2DM in studies, and failing to adjust interpretations of neuroimaging indicators in terms of the potential vascular effects of these disorders, are significant issues in the literature that add to this lack of clarity. A recent systematic review, for example, indicated that many neuroimaging studies of cognitive aging do not consider HPTN or T2DM in their selection criteria (22% and 34%, respectively), and only 29% discuss the potential effects of interparticipant vascular variability on blood-oxygen-level-dependent (BOLD) or positron emission tomography (PET) signals (Meusel et al., 2014). Without screening for HPTN and T2DM, it is difficult to establish the negative consequences of these disorders beyond the changes associated with normal aging. Finally, individuals often have comorbid HPTN and T2DM (HPTN+T2DM; Bretzel, 2007; Colosia, Palencia, & Khan, 2013), making it challenging to determine whether changes in indicators of cerebrovascular function, brain health, or cognitive function are related to HPTN, T2DM, or HPTN+T2DM. The purpose of the present research was to address some of these challenges by examining the effects of HPTN and HPTN+T2DM on cerebrovascular and cognitive function by comparing the neuroimaging data and cognitive task performance of older adults with HPTN or HPTN+T2DM to that of older adults without these conditions (CON). The relationship between cerebrovascular function and cognitive function, and the explanatory variables associated with each of these relationships, were also explored (Figure 1).
Figure 1. Conceptual map of the relationships of interest in the present study.
1.1.2 Thesis Organization

This thesis consists of four chapters (Introduction, Methods, Results, and Discussion). Chapter 1 begins with a discussion of the role of cerebrovascular function in brain health and cognitive function. This is followed by a review covering what is known about the effects of HPTN, T2DM, other vascular risk factors, and comorbid vascular risk factors on cerebrovascular function, brain health, and cognitive function in older adults. The chapter ends with an overview of the present study and a statement of four hypotheses informed by the literature review. Chapter 2 describes the methodology of the present study. Chapter 3 reports the results for the descriptive statistics and for the tests of each hypothesis. Chapter 4 begins by summarizing the findings at an integrative, global level. The chapter ends with a general discussion of the results followed by a consideration of the strengths and limitations of the present study and possible future directions.

1.2 Literature Review

Normal cerebrovascular function supports brain health and cognitive function (Kalaria, 2012). With normal cerebrovascular function, sufficient cerebral blood flow (CBF) is maintained through cerebral autoregulation, allowing for the nourishment of brain tissue and removal of metabolic waste products (Paulson, Strandgaard, & Edvinsson, 1990). In the context of aging or disease, however, CBF may become insufficient due to impaired cerebrovascular function (Yang, Sun, Lu, Leak, & Zhang, 2016), particularly in cerebral small vessels (Pantoni, 2002). Compromised CBF in cerebral small vessels over an extended period of time may lead to suboptimal nourishment, lingering waste products, and brain damage (Wardlaw, Smith, & Dichgans, 2013). Thus, indicators of cerebrovascular function are of much interest, since changes in CBF associated with aging or disease likely contribute to changes in brain health and cognitive function.

Cerebrovascular reactivity (CVR), the capacity of cerebral blood vessels to dilate in order to accommodate increases in CBF, is an established indicator of cerebrovascular function. A breath hold task, in which participants alternate between periods of breath holding and normal breathing, is a common method of assessing CVR. Breath holding elicits a transient hypercapnia condition in which CO₂ levels in the blood are abnormally elevated (MacIntosh, Klassen, & Menon, 2003; Pillai & Mikulis, 2015). The hypercapnia induced by the breath hold results in a
compensatory global increase in CBF intended to improve oxygen delivery (Kastrup, Krüger, Glover, Neumann-Haefelin, & Moseley, 1999). Given that the cerebral blood vessels must dilate in order to accommodate these increases in blood flow, the increase in CBF following a hypercapnic challenge serves as an indicator of CVR. This increase in CBF, when measured by the BOLD response, is highly reproducible with breath holds longer than 9 s (Magon et al., 2009; Murphy, Harris, & Wise, 2011). Although there are brain regions (e.g., brainstem) that are responsible for breath regulation, a breath hold task is considered a valid technique to produce a global, vascular-only response.

There is evidence to suggest that several diseases are associated with reductions in CVR. A recent systematic review assessing CVR during a breath hold task indicated that CVR is typically higher in control participants than in participants with medical conditions, including HPTN and diabetes mellitus (DM; Urback, MacIntosh, & Goldstein, 2017). Similarly, a systematic review and meta-analysis of studies using BOLD and/or arterial spin labelling (ASL) functional magnetic resonance imaging (fMRI) to assess CVR in patients with steno-occlusive vascular disease or stroke found reduced CVR in the same hemisphere of the brain as the disease (Smeeing, Hendrikse, Petersen, Donahue, & de Vis, 2016). There is also evidence of CVR dysfunction in a number of neurodegenerative diseases, such as Alzheimer’s disease (AD), Parkinson’s disease, and multiple sclerosis (Smoliński & Członkowska, 2016). Beyond its potential roles in cognitive aging and neurodegenerative diseases, compromised CVR has also been associated with a greater risk of mortality, even after adjusting for incident stroke (Portegies, de Bruijn, Hofman, Koudstaal, & Ikram, 2014). The following sections review findings across a substantial body of literature that suggest HPTN, T2DM, other vascular risk factors (e.g., aging, dyslipidemia, sex, smoking), and comorbid vascular risk factors likely have negative effects on cerebrovascular function, brain health, and cognitive function.

1.2.1 Effects of HPTN on Cerebrovascular Function, Brain Health, and Cognitive Function

HPTN, a condition characterized by chronic high blood pressure, likely has negative effects on cerebrovascular health (Birns & Kalra, 2009; Iadecola, 2014). Normally, arteries are flexible and strong; blood flows easily and tissues are supplied with the nutrients and oxygen they require. HPTN, however, often reflects compromised vascular function. Over time, HPTN can lead to the development of arteriosclerosis, a thickening and stiffening of the arteries that reduces risk of
rupture but decreases lumen diameter (Iadecola et al., 2016). Once the lining of the blood vessels is damaged, fat in the blood stream can lodge itself within the arterial walls and decrease lumen diameter, a condition referred to as atherosclerosis (Alexander, 1995). In addition to these structural changes, HPTN is also associated with neurovascular signaling changes, such as increases in the production of the vasoconstrictor endothelin-1 and reductions in the bioavailability of the vasodilator nitric oxide (Dunn & Nelson, 2014). As a result of these structural and functional changes, blood flow to the brain and other vital areas of the body can become progressively obstructed.

Examining animal models of HPTN can provide important insights into how HPTN may affect cerebrovascular function, since they allow for greater experimental control than studies with human participants. In studies with human participants, there are more potential confounding variables than in animal studies, and HPTN status is necessarily a quasi-independent variable as it cannot be assigned. The results of many mouse and rat studies indicate similar relationships between HPTN and cerebrovascular function as in human studies. In a rat model of aging and chronic HPTN, CVR was reduced relative to control rats (Tamaki, Nakai, Yokota, & Ogata, 1995). Similarly, Fujishima, Ibayashi, Fujii, and Mori (1995) found HPTN was associated with impaired CBF and lower cognitive function in both human participants and rats. In a mouse model of AD, midlife HPTN was associated with decreased CBF (Wiessman et al., 2015). The cerebral arteries of rats with HPTN have also shown increases in arterial resting tone and reduced responsiveness to nitric oxide (González et al., 2008). However, the results of some rat studies suggest cerebrovascular function could be preserved in the context of treated HPTN (e.g., Clozel, Kuhn, & Hefti, 1989; Harper, 1987). Thus, more animal research is needed to understand the effects of HPTN on cerebrovascular function, whether these effects are attenuated by treatment of HPTN, and whether these effects are generalizable to humans.

In humans, HPTN is likely associated with reductions in CBF across several brain regions (Beason-Held, Moghekar, Zonderman, Kraut, & Resnick, 2007; Dai et al., 2008; Hajjar, Zhao, Alsop, & Novak, 2010; Nobili et al., 1993). Reductions in CVR associated with HPTN may also signal structural damage to the brain. The results of a case-control study of patients with lacunar infarction and control participants indicated that CVR in response to hypercapnia was significantly lower in the patient group than the control group (Molina et al., 1999). There was a trend towards a negative correlation between CVR and the number of lacunar infarctions, while
CVR and history of HPTN were identified as significant, independent predictors of first lacunar infarction. Thus, reductions in CVR likely contributed to the development of first lacunar infarct and to increases in the number of lacunar infarcts, while HPTN also likely contributed to the development of first lacunar infarct. In a cross-sectional study of patients with small vessel disease, indicators of higher blood pressure were associated with higher white matter hyperintensity (WMH) volume in analyses adjusted for age, sex, and other cardiovascular risk factors, including DM (Gons et al., 2010). These indicators of higher blood pressure were also associated with lower fractional anisotropy in both normal appearing white matter and WMHs and higher mean diffusivity in WMHs, findings considered to reflect compromised structural integrity of white matter. Thus, in participants with small vessel disease, HPTN was a probable contributor to the development of WMHs and lower structural integrity in normal appearing white matter. HPTN in older adults has also been associated with more rapid progression of whole brain atrophy and WMHs (Firbank et al., 2007), while temporal and occipital brain regions may be particularly vulnerable to atrophy (Strassburger et al., 1997).

HPTN and indicators of high blood pressure are associated with lower cognitive function. Harrington and colleagues (2000) found that older adults with untreated HPTN performed significantly worse than older adults with normotension on a broad range of cognitive domains, including attention, verbal memory, visuospatial memory, and working memory. Similarly, the results of a meta-analysis indicated that HPTN was associated with lower global cognitive function, attention, episodic memory, and a trend towards lower language abilities (Gifford et al., 2013). When present in midlife, HPTN and indicators of HPTN have also been found to predict lower cognitive function later in life. In a prospective cohort study, Debette and colleagues (2011) found that participants with midlife HPTN had more rapid increases in WMH volume with aging and decreases in executive function. Similarly, in a cohort study of men, Kilander, Nyman, Boberg, Hansson, and Lithell (1998) found high diastolic blood pressure (DBP) at age 50 predicted lower cognitive performance at age 70.

Measures of blood pressure may differ in their ability to predict cognitive outcomes. In a sample of middle-aged and older adults, Elias, D'Agostino, Elias, and Wolf (1995) found three blood pressure variables (systolic blood pressure [SBP], DBP, and percentage of blood pressure measurements within the HPTN range over 8–10 years) were all negatively associated with visual and verbal memory scores. However, in a longitudinal study of men at midlife, old age,
and over a follow-up period, Freitag and colleagues (2006) found midlife SBP was a better predictor of dementia in old age than DBP, mean arterial pressure (MAP), or pulsatile pressure (PP). Finally, in a sample of otherwise healthy middle-aged adults, increased arterial stiffness was the best predictor of declines in executive function, memory, and working memory, independently of HPTN status, SBP, or DBP (Hajjar, Goldstein, Martin, & Quyyumi, 2016). A possible explanation for this finding is that some individuals with HPTN may have high blood pressure and even partially occluded arteries, but retain enough arterial flexibility to accommodate CBF. Thus, arterial stiffness might serve as a more specific indicator of vascular problems (e.g., reduced CVR) that may contribute to deficits in cognitive performance.

HPTN may also be associated with compensatory increases in the CBF of other brain regions to support cognitive performance. In a sample of older adults with untreated HPTN and control participants, Jennings and colleagues (1998) found the HPTN and control groups did not significantly differ in their performance on a continuous performance task and an auditory free recall task. However, relative to the control group, the HPTN group showed lower regional CBF in right hemisphere areas and higher compensatory regional CBF in homologous areas of the left hemisphere. In a sample of older adults with HPTN and older adults with normotension, Jennings and colleagues (2005) found participants with HPTN performed more poorly than control participants on some measures of memory, but the two groups did not significantly differ on measures of perceptual, motor, and executive function. Participants with HPTN, relative to control participants, showed task-induced compensatory regional CBF in the amygdala and hippocampus, which was correlated with task-associated prefrontal regional CBF.

Treatment may or may not mitigate some of the negative effects of HPTN on cerebrovascular function, brain health, and cognitive function. The results of an early prospective study indicated that medical treatment of mild HPTN in older adults was associated with increases in CBF at 6, 12, and 24 months (Meyer, Rogers, & Mortel, 1985). Molina and colleagues (1999) found that untreated or irregularly treated patients with HPTN had a higher risk of first lacunar infarct than patients with regularly treated HPTN. In a sample of older men, Brady, Spiro III, and Gaziano (2005) found participants with uncontrolled HPTN (i.e., high blood pressure, medication), relative to participants with normal blood pressure (i.e., normal blood pressure, no medication), had larger age deficits on executive function and memory. However, participants with controlled HPTN (i.e., normal blood pressure, medication) and untreated HPTN (i.e., high blood pressure,
no medication) did not show larger age deficits on any of the tasks than participants with normotension. Even controlled HPTN may be associated with increases in brain atrophy and declines in cognitive performance (Raz, Rodrigue, & Acker, 2003). A possible explanation for these mixed findings could be related to when treatment of HPTN starts. For example, a recent systematic review concluded there was insufficient evidence to support the contention that treatment of HPTN reduces cognitive decline in older adults with dementia (Beishon et al., 2014). Thus, early management or prevention of HPTN may be associated with better outcomes than management of HPTN later in life.

HPTN may also signal cerebrovascular changes in midlife. In a sample of middle-aged adults, Haight and colleagues (2015) examined the relationship between vascular risk factors and CVR in the default mode network (DMN). The DMN, a network of brain regions that are typically less active during attention-demanding cognitive tasks (Anticevic et al., 2012; Fox et al., 2005), is of interest to many researchers, as abnormal behaviour in the DMN (e.g., reduced deactivation during attention-demanding cognitive tasks) is theorized as a potential marker of brain disorders such as AD (Koch et al., 2012). In the participants with pre-HPTN/HPTN, relative to the participants with normotension, Haight and colleagues (2015) found reduced CVR in the posterior cingulate/precuneus, anterior cingulate, and medial frontal lobe. In another study of middle-aged participants, mean CBF velocity in response to hypercapnia did not significantly differ at any time point between the HPTN group and the control group (Ficzere et al., 1997). However, cerebrovascular reserve capacity (% change in mean CBF velocity) was lower in the HPTN group at 5 and 10 minutes.

### 1.2.2 Effects of T2DM on Cerebrovascular Function, Brain Health, and Cognitive Function

T2DM, a condition characterized by insulin dysregulation and hyperglycemia, also likely has negative effects on cerebrovascular health (Messier, 2005; Meusel et al., 2012). Normally, insulin is an effective regulator of blood glucose levels; changes in blood sugar are shadowed by similar changes in insulin release by the pancreas. Insulin binds to cell receptors, inducing an intracellular signal that causes the translocation of glucose transporters to the plasma membrane (Zaid, Antonescu, Randhawa, & Klip, 2008). These transporters bring glucose into the cell, thereby lowering blood glucose levels. The early stages of the development of T2DM, however, are associated with decreased sensitivity to insulin, and increasing concentrations of insulin are
required before glucose transporters will translocate to the plasma membrane. The pancreas compensates for this reduced sensitivity by releasing more insulin, but eventually this mechanism can fail to curb rises in blood glucose. For individuals diagnosed with T2DM, levels of blood glucose and insulin typically remain high for extended periods of time. This state of hyperglycemia and hyperinsulinemia is associated with deleterious outcomes (Luchsinger, Tang, Shea, & Mayeux, 2004). Hyperglycemia is accompanied by increased intracellular formation of advanced glycation end-products and protein kinase C activation, while hyperinsulinemia can lead to brain insulin deficiency, a condition that may contribute to the development of the amyloid plaque and neurofibrillary tangles associated with AD (Heni et al., 2013). T2DM is also associated with endothelial dysfunction and microvascular complications that may impair CBF (Meusel et al., 2012).

The results of several studies examining animal models of DM indicate that DM is associated with changes in brain health. Four months after streptozocin-induced DM, rats showed decreased glucose metabolism in the basal ganglia and white matter, although CBF did not change significantly in any region (Jakobsen, Nedergaard, Aarslew-Jensen, & Diemer, 1990). The cerebral tissue of rats with streptozocin-induced DM, relative to control rats, also contained lower levels of occludin, a tight junction structural protein, which suggests DM could increase permeability of the blood-brain barrier (Chehade, Haas, & Mooradian, 2002). Indeed, Huber, VanGilder, and Houser (2006) found streptozocin-induced DM rats showed increases in permeability of the blood-brain barrier, particularly in the midbrain. Interestingly, like Jakobsen and colleagues (1990), Huber and colleagues (2006) did not find reductions in CBF in streptozocin-induced DM rats. Deficits in cognitive performance for rats with DM have also been noted in a number of studies (e.g., Kamal, Biessels, Duis, & Gispen, 2000; Popović, Biessels, Isaacson, & Gispen, 2001). Taken together, these results suggest DM in rats is associated with reductions in brain health and cognitive function, while CBF may remain intact. More research is needed to explore potential mechanisms responsible for these differences and the relevance of these mechanisms to human beings.

In humans, T2DM, like HPTN, is associated with reductions in CVR. In an early study of patients with DM and control participants, Dandona, James, Newbury, Woollard, and Beckett (1978) compared CBF before and after hypercapnia. Both groups showed a significant decline in CVR with age; however, the hypercapnic challenge produced significant increases in CBF in 24
of the 28 controls participants and in only 23 of the 59 patients with DM. In another early study, Griffith, Saimbi, Lewis, Tolfree, and Betteridge (1987) found 19 of 20 control participants showed an increase in CBF in response to hypercapnia while only 12 of 22 patients with DM responded normally. More recent research has found similar relationships between T2DM and CVR. Fülesdi and colleagues (1999) compared patients with T2DM and control participants on CVR and cerebrovascular reserve capacity (maximal percent increase of blood flow velocity) in response to hypercapnia. Both control participants and patients diagnosed with T2DM for < 10 years showed elevated CVR for a longer time than patients diagnosed with T2DM for ≥ 10 years, and cerebrovascular reserve capacity was negatively correlated with T2DM duration. These findings suggest, perhaps not surprisingly, that longer durations of T2DM are associated with larger deficits in cerebrovascular function. The contribution of HPTN to these findings of reduced CVR is unclear, since HPTN status or blood pressure variables were not reported in these papers. However, more recent studies controlling for HPTN have found even pre-diabetic status in middle-aged adults may be associated with reductions in cerebrovascular function. In a sample of lean controls, overweight/obese participants with insulin resistance and overweight/obese participants without insulin resistance, insulin resistance was independently associated with lower CVR (Frosch et al., 2017). Similarly, in a sample of control participants, participants with insulin resistance and participants with T2DM, participants with insulin resistance showed lower CBF in gray matter than control participants (Rusinek et al., 2015). Interestingly, CBF was not lower in participants with T2DM, a finding the authors attributed to medical management of blood glucose, cholesterol, and blood pressure in the participants with T2DM.

T2DM likely has negative effects on brain health and cognitive function in both middle-aged and older adults. Gold and colleagues (2007) found that middle-aged adults with T2DM and older adults with T2DM performed more poorly than the control participants on hippocampus-dependent memory and learning tasks. Participants with T2DM also had significantly smaller hippocampal volumes than the control participants. Glycated hemoglobin (HbA1c) was the only significant predictor of hippocampal volume, which suggests that the glucose dysregulation of T2DM was the primary factor associated with the observed differences in hippocampal volume. Interestingly, HbA1c was negatively correlated with hippocampal volume both across all participants and within the T2DM group alone, which implies that this relationship was not
driven solely by differences in glucose regulation between non-T2DM status and T2DM status. Last and colleagues (2007) found that participants with T2DM had lower CBF, lower white and gray matter volume, and higher CSF volume than control participants. Individuals with T2DM also have higher risk of lacunes and hippocampal atrophy (Korf, White, Scheltens, & Launer, 2006).

There is also evidence that elevated blood sugar in older adults without T2DM could have negative effects on brain health and cognitive function. In a sample of older adults without DM, Convit, Wolf, Tarshish, and de Leon (2003) found that, independent of age, higher blood glucose levels two hours after glucose administration were associated with lower memory scores and lower hippocampal volumes, a finding similar to the relationships observed by Gold and colleagues (2007) in participants with T2DM. In a cross-sectional study of individuals ages 55 and older, Cukierman-Yaffe and colleagues (2009) found lower global cognition scores in participants with DM than in participants without DM. Across the sample, higher fasting glucose (FG) was associated with lower global cognition scores. The relationship between higher FG and lower global cognition scores remained significant after controlling for DM status and a variety of demographic variables, previous cerebrovascular disease, and vascular risk factors, including HPTN status. These results suggest that increasing glucose dysregulation, in individuals with and without DM, is associated with decreases in cognitive function that are independent of HPTN status.

In a sample of older adults without DM, Di Bonito and colleagues (2005) found impaired fasting glucose (IFG) was associated with a greater risk of cognitive impairment. Interestingly, IFG only predicted cognitive impairment when participants were categorized according to the 1997 criteria for IFG (FG = 110–125 mg/dl) rather than the 2003 criteria (FG = 100–125 mg/dl). This distinction suggests that more severe IFG may be required to negatively affect cognitive function. Although over half of the participants had HPTN, HPTN was not associated with cognitive impairment. Thus, it is likely that disruptions in glucose metabolism were the primary driver of the relationship between IFG and cognitive impairment. Similarly, the results of a systematic review indicated strong evidence for relationships between both impaired glucose tolerance and lower glucose tolerance, within the clinically defined normal range, and verbal memory deficits (Lamport, Lawton, Mansfield, & Dye, 2009). However, there was little evidence to support the contention that impaired glucose tolerance negatively affects other
cognitive domains, which the authors suggested may be due in part to the common usage of limited measures of global cognitive function in the reviewed studies.

The brain health decrements associated with T2DM likely contribute to deficits in cognitive function in older adults. In a sample of older adults with DM, WMHs in the parietal lobe and hyperintensities in the thalamus were negatively correlated with performance on processing speed and global cognitive function, respectively (Akisaki et al., 2006). This relationship persisted after adjusting for age, education, and SBP. Subcortical atrophy adjacent to the lateral ventricles was negatively associated with lower scores for verbal memory and processing speed. In a longitudinal cohort study of older adults with DM and without DM, Arvanitakis, Wilson, Bienias, Evans, and Bennett (2004) found participants with DM, relative to participants without DM, had a 65% higher risk of developing AD. At baseline, DM was also associated with lower performance on global cognitive function, episodic memory, semantic memory, working memory, and visuospatial ability. However, it is unclear the extent to which HPTN or differences in blood pressure measures may have contributed to these outcomes, since they were not addressed in the paper. Midlife T2DM may also contribute to brain aging and cognitive decline in later life. Debette and colleagues (2011) found that participants with midlife T2DM had more rapid increases in temporal horn volume, an indicator of hippocampal atrophy. However, participants enrolled in an intensive glycemic therapeutic strategy targeting HbA1c levels below 6% over a 40 month period maintained total brain volume better than the standard group, but showed no difference in cognitive performance (Launer et al., 2011). More research is needed to better understand the relationships between glucose dysregulation, brain health, and cognitive function.

In summary, findings across the literature suggest that HPTN and T2DM are associated with deficits in cerebrovascular function, brain health, and cognitive function. The effects of other vascular risk factors are considered in the following section.

1.2.3 Effects of Other Vascular Risk Factors on Cerebrovascular Function, Brain Health, and Cognitive Function

In addition to HPTN and T2DM, there are several other vascular risk factors that may have negative effects on cerebrovascular function, brain health, and cognitive function. Thus, the effects of these vascular risk factors need to be considered in order to assess the effects of HPTN
and T2DM. Indeed, a number of these vascular risk factors have been included in measures of overall vascular burden. For example, the Framingham Risk Score (FRS) provides an estimate of an individual’s probability of developing cardiovascular heart disease over a 10-year period, and its calculation typically includes sex, treatment of SBP, SBP, age, TC concentration, HDL concentration, smoking status, and T2DM status (Wilson et al., 1998). The following sections review previous findings regarding the effects of some of these vascular risk factors (i.e., aging, dyslipidemia, sex, and smoking).

**Aging**

A large body of studies have found that aging is associated with cognitive declines across a range of domains (Craik & Salthouse, 2011). Interestingly, aside from using brief questionnaires to screen for dementia or other conditions with potential effects on neurological function (e.g., stroke), many studies have not considered the cognitive effects of HPTN, T2DM, dyslipidemia, or other conditions that are more commonly found in older adults than younger adults. Thus, the results of many of these studies could reflect changes in cognitive function with normal aging (i.e., statistically typical aging) rather than healthy aging (i.e., aging in the absence of these conditions). By this standard, relatively few studies have contrasted younger adults and healthy older adults, especially given that the terms normal aging and healthy aging have often been used interchangeably in the literature, primarily to refer to the absence of dementia. However, even under the best circumstances, healthy aging is associated with declines in brain health and cognitive function (Hedden & Gabrieli, 2004). The effects of normal or healthy aging on cerebrovascular function are less clear (Salthouse, 2011). In terms of brain health and cognitive function, Bennett, Madden, Vaidya, Howard, and Howard (2010) found that healthy older adults, relative to younger adults, had significantly lower structural integrity of white matter, particularly in frontal white matter, and performed more poorly on measures of processing speed, cued recall, and free recall. In summary, while more research is needed to contrast the effects of normal and healthy aging, it is likely that both have negative effects on brain health and cognitive function.

**Dyslipidemia**

Cognitive function in older adults may be negatively affected by dyslipidemia. In a sample of older adults with HPTN or T2DM, Meusel and colleagues (2017) assessed the relationships
between measures of cardiovascular risk (Framingham Risk Score [FRS], LDL), DMN deactivation, and working memory. According to the dominant theoretical view on the function of the DMN (Anticevic et al., 2012; Fox et al., 2005), given that working memory tasks are attention-demanding, the DMN should show deactivation during their performance, thereby implying reduced DMN deactivation reflects abnormal activity that may negatively impact performance. The results indicated that higher LDL, not FRS, predicted both poorer working memory and reduced DMN deactivation. Reduced DMN deactivation also predicted poorer working memory. Interestingly, these results suggest the potential existence of a mechanism through which higher LDL specifically, rather than overall cardiovascular burden (FRS), may lead to reduced DMN deactivation, resulting in poorer performance during working memory tasks. Similarly, Kivipelto and colleagues (2001) found that older adults with elevated cholesterol at midlife had a greater risk of MCI in later life than control participants. However, a small number of studies have also reported positive relationships between LDL and cognitive function in middle-aged and older adults (Leritz, McGlinchey, Salat, & Milberg, 2016; West et al., 2008). It is possible that the participants in these studies had intact cerebrovascular function, or that dyslipidemia only affects cerebrovascular function for some individuals. More research is also needed to determine the extent to which the effects of dyslipidemia on cognitive function are age-dependent.

Medical management of lipid levels seems to have variable effects on vascular function. In a sample of patients with T2DM, intensive lipid lowering through atorvastatin did not improve vasoreactivity in forearm resistance arteries (van Etten et al., 2002), which could suggest that hyperglycemia has more important effects than dyslipidemia on vasoreactivity in individuals with T2DM. In a sample of patients with controlled hypertension and elevated LDL, Forteza and colleagues (2012) found atorvastatin only had a positive effect on CVR in a subgroup of patients with baseline CVR impairment. Sterzer and colleagues (2001) also found pravastatin improved CVR in patients with subcortical small vessel disease. Finally, a review examining the relationship between statins and cerebral hemodynamics similarly found that statins were most beneficial after long-term administration in patients with impaired vascular function or vascular disease (Giannopoulos, Katsanos, Tsivgoulis, & Marshall, 2012). Thus, these findings suggest that statins may be more likely to provide a benefit to cerebrovascular function in patients with vascular disease, while providing less or little benefit to individuals who may have elevated lipid
levels without negative effects on their cerebrovascular function.

Sex

Vasoreactivity in response to hypercapnic stimuli may differ as a function of sex. The results of a study of healthy participants indicated that CVR in response to hypercapnia only decreased with aging in women (Kastrup, Dichgans, Niemeier, & Schabet, 1998). Interestingly, in a sample of younger men and women, women showed a stronger vasodilatory response than men to hypercapnia (Kastrup, Thomas, Hartmann, & Schabet, 1997). However, Kastrup et al.’s (1998) finding of no decrease in CVR in men with aging is not likely explained by younger men starting with a weaker vasodilatory response than younger women (Kastrup et al., 1997), since the relationship between aging and CVR response for the men in Kastrup et al.’s (1998) study was far from significant. However, in a more recent study of healthy participants, age was associated with reductions in mean CBF in the middle cerebral artery in response to hypercapnia and with increased arterial stiffness for the common carotid artery (Zavoreo & Demarin, 2010). Further research is needed to determine whether sex has any consistent effect on CVR with normal or healthy aging, and whether these effects are consistent across different types of hypercapnic challenges.

The results of several studies indicate HPTN and T2DM may have different effects on brain health and cognitive function in men than in women. For example, in a sample of men and women without T2DM, Gianaros, Greer, Ryan, and Jennings (2006) found higher SBP in men predicted lower gray matter volumes in the supplementary motor area, superior frontal gyrus, anterior cingulate cortex, and middle temporal gyrus. Also in men, lower gray matter volume in the supplementary motor area predicted poorer performance on executive control and working memory. In contrast, no significant relationships were found between SBP, regional brain tissue volume, and cognitive performance in women. Similarly, Jongen and colleagues (2007) found that only female participants with T2DM, relative to control participants, had lower gray matter volume, greater lateral ventricle volume, greater WMH volume, and trended towards greater CSF volume. Finally, Hempel, Onopa, and Convit (2012) found that hippocampal atrophy was more pronounced in female participants with T2DM than in male participants with T2DM, despite better glucose control in the female participants.

Smoking
The negative effects of smoking on a range of health outcomes are well established (U.S. Department of Health and Human Services, 2014). The results of a systematic review also indicated that current tobacco use was among the factors most strongly associated with greater risk of cognitive decline later in life (Plassman, Williams, Burke, Holsinger, & Benjamin, 2010). Midlife smoking in another study was associated with increases in temporal horn volume, decreases in total brain volume, and increases in WMH volume (Debette et al., 2011). The evidence regarding the relationship between smoking and cerebrovascular function is somewhat less clear. A systematic review of studies using breath hold challenges to assess CVR (Urback et al., 2017) reported one study (Friedman et al., 2008) in which smokers had higher CVR than control participants. However, the results of the other two studies with smokers included in the review found smoking was associated with lower CVR, as measured by transcranial Doppler (Neu, Schlattmann, Schilling, & Hartmann, 2004; Silvestrini, Troisi, Matteis, Cupini, & Bernardi, 1996). Further research is needed to more fully elucidate the likely negative effects of smoking on cerebrovascular function, but its negative effects on brain health and cognitive function are well supported by findings in the literature.

1.2.4 Effects of Comorbid Vascular Risk Factors on Cerebrovascular Function, Brain Health, and Cognitive Function

The previous sections have provided a review of the evidence regarding the effects of HPTN, T2DM, and other vascular risk factors on cerebrovascular function, brain health, and cognitive function. However, a number of findings in the literature also indicate that comorbidities of these vascular risk factors are common, and that comorbid vascular risk factors are associated with greater deficits. For example, HPTN+T2DM may be associated with greater deficits in cerebrovascular function, brain health, and cognitive decline than HPTN or T2DM alone. In a sample of older adults with HPTN+T2DM or HPTN, CVR was significantly lower in the HPTN+T2DM group than the HPTN group in bilateral (lateral occipital, cuneus, superior parietal), right lateral (lateral occipital, inferior parietal, precuneal), and left lateral (pericalcarine cortex) regions (Tchistiaikova, Anderson, Greenwood, & McIntosh, 2014). Cortical thickness was also lower in the HPTN+T2DM group, relative to the HPTN group, in the right lingual and fusiform gyrus. Similarly, Last and colleagues (2007) found that participants with HPTN+T2DM, relative to participants with HPTN and normotension, had higher CSF volume and lower grey matter volume across the brain, but greater grey matter volume in frontal regions.
Certain networks within the brain may be more sensitive to the effects of comorbid vascular risk factors than to those vascular risk factors in isolation. In a sample of older adults, Tchistiakova and colleagues (2015) examined the associations between number of vascular risk factors, CVR in response to hypercapnia, and resting state coactivation in participants with WMHs. Based on whether they had 0, 1, or 2 or 3 of three vascular risk factors (HPTN, T2DM, or hypercholesterolemia) believed to contribute to small vessel disease, participants were assigned to three separate groups: VRF 0, VRF 1, and VRF ≥ 2. CVR (model-based and dual-regression-based) and resting state coactivation in three networks of interest (DMN, sensory-motor network, and medial-visual network) were compared amongst these vascular risk factor groups. No differences were found between the groups for resting state coactivation or model-based CVR. However, after adjustment for age and gray matter percentage in the DMN, dual-regression-based CVR in the DMN was significantly lower in the VRF ≥ 2 group than in the VRF 0 and VRF 1 groups. Finally, for the three networks of interest, resting state coactivation was only positively correlated with dual-regression-based CVR in the DMN.

The comorbidity of multiple vascular risk factors may also be associated with cortical thickness losses in brain areas implicated in AD. In a sample of participants with MCI and control participants over the age of 55, Tchistiakova and MacIntosh (2016) examined the relationship between number of vascular risk factors (DM, pre-HPTN/HPTN, history of smoking) and cortical thickness. The results indicated that having all three vascular risk factors in the MCI group was associated with lower cortical thickness than having one or two of the vascular risk factors, but having two vascular risk factors was not associated with lower cortical thickness than having just one vascular risk factor. The brain regions in which number of vascular risk factors was associated with lower cortical thickness were (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. However, number of vascular risk factors was not associated with differences in cortical thickness for the control participants. A possible explanation for this finding could be that older adults in which vascular risk factors are associated with reductions in cortical thickness tend to be the older adults with MCI, while older adults without MCI are showing resilience in the context of these vascular risk factors, possibly due to other lifestyle or genetic factors offsetting the negative effects of these vascular risk factors. These effects may also have been smaller and
more difficult to detect in the control group, as suggested by the results of an ANCOVA in which number of vascular risk factors was associated with lower cortical thickness in the right frontal pole and left lateral orbitofrontal region across the MCI and control groups.

1.3 The Present Study

There is a reasonable body of evidence to suggest that HPTN, T2DM, and other vascular risk factors likely have negative effects on cerebrovascular function, brain health, and cognitive function, and that these effects are more pronounced in the context of comorbid vascular risk factors (e.g., HPTN+T2DM). However, the effects of HPTN and T2DM on cognitive aging still remain unclear, perhaps mostly due to insufficient screening, reporting, or consideration of HPTN and T2DM status in many neuroimaging studies of cognitive aging (Meusel et al., 2014). Even in studies examining HPTN that exclude participants with T2DM and studies examining T2DM that exclude participants with HPTN, there are challenges related to analysis and interpretation. For example, many of the participants included in these studies may not have HPTN or T2DM but still express HPTN-like (e.g., elevated SBP) or T2DM-like (e.g., elevated levels of blood glucose and insulin) characteristics within the normal range, which may interact with the condition of HPTN or T2DM status that was of primary interest in the study. There are also a range of vascular risk factors other than HPTN or T2DM that may obscure the relationships of these disorders with cerebrovascular function, brain health, and cognitive function.

The purpose of the present research was to address some of these challenges by clarifying the effects of HPTN and HPTN+T2DM on cerebrovascular and cognitive function in older adults. To explore these effects, three groups of older adults with characteristics considered representative of CON, HPTN, and HPTN+T2DM were recruited. These participants provided their medical history and physiological data, and they completed a battery of neuropsychological tests and an fMRI breath hold task. Four hypotheses informed by the literature were tested. Hypothesized relationships, explanatory variables, and operationalizations of variables for this study are summarized in Figure 2.

Hypothesis 1: CVR and global cognitive function would be lower in the HPTN and HPTN+T2DM groups than in the CON group, and lower in the HPTN+T2DM group than in the HPTN group (HPTN+T2DM < HPTN < CON).
Hypothesis 2: In brain areas that significantly differed on CVR between the groups, lower CVR would predict lower global cognitive function.

Hypothesis 3: Higher values for FRS, or for indicators of HPTN or T2DM, would predict both lower CVR in brain areas that significantly differed on CVR between the groups and lower global cognitive function.

Hypothesis 4: The relationship between HPTN or HPTN+T2DM status and lower global cognitive function would be partially mediated by CVR, and the relationship between lower CVR and lower global cognitive function would be partially mediated by FRS or indicators of HPTN or T2DM.
Figure 2. Hypothesized relationships and explanatory variables for the conceptual map presented in Figure 1. Colours indicate the relationships and explanatory variables relevant to Hypothesis 1 (gold), Hypothesis 2 (purple), Hypothesis 3 (blue), and Hypothesis 4 (brown). Indicators of HPTN were operationalized as indicators of blood pressure, indicators of T2DM were operationalized as indicators of glucose/lipid dysregulation, vascular risk factors were operationalized as FRS, cerebrovascular function was operationalized as BOLD-based CVR in response to the breath hold task, and cognitive function was operationalized as global cognitive function composite score.
2 Methods

2.1 Participants

Seventy-five older adults (ages 65-85) were recruited through the participant database at Baycrest and through advertisements at the University of Toronto, its affiliated teaching hospitals, and in newspapers. On the basis of screening measures, these older adults were placed in the CON group ($n = 24$), HPTN group ($n = 33$), or HPTN+T2DM group ($n = 18$). In order to explore the effects of increasing glycemic dysregulation on cerebrovascular and cognitive function in participants with HPTN but not T2DM, we had planned to recruit twice as many participants for the HPTN group than the CON and HPTN+T2DM groups ($CON = 25$, $HPTN = 50$, $HPTN+T2DM = 25$). However, this was not possible due to insufficient recruitment of eligible participants during data collection.

2.1.1 Inclusion Criteria

CON participants had a mean SBP $\leq 140$ mmHg, and a mean DBP $\leq 90$ mmHg, as measured using a BpTru™ blood pressure monitor; no history of antihypertensive medication use; and a FG $\leq 6.1$ mmol/L, as assessed by finger-prick blood, using a One Touch Basic Meter™ glucometer. Participants with HPTN had been using antihypertensive medication under physician orders for a minimum of two years, with current blood pressures within a normal or HPTN range. Selection of participants with HPTN was limited to those who were using long-acting antihypertensive medications (e.g., ACEIs, angiotensin II receptor blockers, diuretics) in order to capture the most commonly prescribed medications. In addition to the criteria for the participants with HPTN, participants with HPTN+T2DM had a physician diagnosis of T2DM with a duration of at least two years, were controlling their T2DM through diet or hypoglycemic medication alone, and were free of major T2DM complications as defined in the exclusion criteria. A duration of at least two years for HPTN and T2DM was chosen in order to allow sufficient time for disease-associated disturbances in cerebrovascular and cognitive function to become apparent.
2.1.2 Exclusion Criteria

Participants were excluded from entering the study if they met any of the following criteria: (1) a score ≤ 31 on the Telephone Interview for Cognitive Status – modified version (Welsh, Breitner, & Magruder-Habib, 1993) in order to exclude participants with possible dementia; (2) the use of insulin to treat T2DM; (3) the presence of T2DM complications, based on self-report, including clinically significant gastroparesis, retinopathy, nephropathy, neuropathy, hepatic disease, or a recent coronary heart disease event as determined by a physician; (4) other significant medical or psychiatric disorders affecting cognitive function, such as stroke (self-report or evidence from structural scans) and major depressive disorder; (5) current or recent use of central nervous system-active medications, including those for the treatment of depression, sleep disorders, and migraine headaches; (6) major inflammatory disorders, heart failure, and chronic lung disease; or (7) hormone replacement therapy in female participants.

2.2 Experimental Procedures

Participants attended three sessions lasting approximately 7 hours in total.

2.2.1 Screening Session

During the first session, participants were screened to ensure group status (CON, HPTN, HPTN+T2DM) and completed a brief medical questionnaire, which included questions about the use and duration of all medications and duration of HPTN and T2DM. Participants provided a fasting blood sample for measurement of hematocrit, lipid profile (triacylglycerides [TG], TC, LDL, and high-density lipoprotein [HDL]), CRP, glucose, insulin, and HbA1c. All blood analyses were completed at the Mount Sinai Hospital Biochemistry Laboratories. Blood pressure, weight, height, and waist circumference were also measured. These measurements were followed by a practice session of the breath hold task in an MRI simulator to ensure that the participant was comfortable with the fMRI scanning protocol. Participants were asked to continue their usual diet, medications, and activity level for the duration of their involvement in the study.

2.2.2 Neuropsychological Session

During the second session, participants completed a battery of neuropsychological tests. The Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) was used to assess
mood. The Shipley Institute of Living Scale (Shipley, 1940) served as a measure of vocabulary, and Animal Fluency (Rosen, 1980) served as a measure of semantic fluency. Declarative memory measures included the California Verbal Learning Test II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000), the Logical Memory and Faces I subtests of the Wechsler Memory Scale III (WMS-III; Wechsler, 1997a), and the Visual and Verbal Paired Associates tasks from the WMS revised (WMS-R; Wechsler, 1987). Executive function measures included the Controlled Oral Word Association Task (COWAT; Ruff, Light, Parker, & Levin, 1996), 64-item version of the Wisconsin Card Sorting Task (WCST; Heaton, Chelune, Talley, Kay, & Curtiss, 1993), and Trail Making Tests A and B (TMT-A and TMT-B; Reitan & Wolfson, 1985). Verbal fluency was assessed using the F-A-S Test, a subtest of the Neurosensory Center Comprehensive Examination for Aphasia (NCCEA; Spreen & Benton, 1977). Everyday signs of executive dysfunction were assessed using the Dysexecutive Questionnaire (DEX; Wilson, Alderman, Burgess, Emslie, & Evans, 1996). Visual-spatial function was assessed using Matrix Reasoning from the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). Attention and cognitive speed were assessed using Digit Span and Digit Symbol from the Wechsler Adult Intelligence Scale III (WAIS III; Wechsler, 1997b), Letter-Number Sequencing (LNS) from the WMS III (Wechsler, 1997a), the Sustained Attention to Response Task (SART; Robertson, Manly, Andrade, Baddeley, & Yiend, 1997), and Reading Span (Daneman & Carpenter, 1980). Frontal measures included Mental Control from the WMS III (Wechsler, 1997a) and Arithmetic from the WAIS revised (WAIS-R; Wechsler, 1981).

2.2.3 fMRI Session

During the third session, participants completed the fMRI breath hold task. For the task, participants entered the magnetic resonance (MR) suite, changed into a gown, and were fitted for MR-compatible glasses if necessary. A block design was adopted for the breath hold task, based on previous work by Tchistiakova and colleagues (2014). Each breath hold was 15 s in duration followed by 32 s of rest. Visual stimuli were used as breathing cues. The words on the screen switched from “rest” for 30 s to “breathe out” for 2 s followed by “hold breath” for 15 s. This sequence was repeated six times to allow an average breath hold response to be calculated, which provided an indicator of CVR. The first breath hold was deleted for each participant to avoid bias related to initial compliance and baseline signal drift. Cognitive tasks were also performed in the
MRI during this session, but the present research focused on the BOLD data for the breath hold task.

2.3 Neuroimaging Procedures

2.3.1 BOLD and ASL Imaging

BOLD fMRI, the most widely used functional imaging technique, measures dynamic changes in blood oxygenation during task performance (Ogawa et al., 1992). When neurons increase their metabolic activity (e.g., in response to the demands of a cognitive task), they cause an increase in regional CBF, cerebral blood volume, and metabolic oxygen consumption, all of which contribute to an increase in the BOLD signal (Buxton, 2010). BOLD’s limitations relate to its specificity: it relies on relative, not absolute, changes in signal intensity. A conventional BOLD approach may assume an intact neurovascular unit and normal CVR, which could be compromised in the context of aging and disease (D’Esposito, Deouell, & Gazzaley, 2003). Indeed, the BOLD signal can be compromised and difficult to interpret in those with severe cerebrovascular disease (Roc et al., 2006). BOLD is also influenced by the competing effects of oxygen consumption, blood volume, and blood oxygenation. Thus, dynamic changes could occur simultaneously in these parameters without significantly altering the direction or magnitude of the BOLD signal (Buxton, 2010). Finally, BOLD has relatively poor temporal resolution compared to other neuroimaging techniques, such as electroencephalography (EEG). However, with these limitations in mind, the BOLD signal is sensitive to subtle changes in task demands, and it can give estimates of event-related, neurovascular responses such as CVR throughout the entire brain.

ASL, like BOLD, is a noninvasive imaging technique. Relative to BOLD, the primary strengths of ASL are its greater specificity, as it is a direct indicator of change in CBF, and its lower interparticipant variability (Detre & Wang, 2002). The primary weaknesses of ASL, again relative to BOLD, are poorer sensitivity due to weaker signal-to-noise ratio (SNR), decreased temporal resolution, and smaller volume coverage. When BOLD and ASL imaging are run concurrently, as they were for this study, the higher sensitivity of BOLD compensates for the lower sensitivity of ASL and the higher specificity of ASL compensates for the lower specificity of BOLD. Also, if analyses drawing on each technique found the same pattern of results for CVR, there would be more confidence in those results. For these reasons, we had planned to
compare the results obtained with BOLD and ASL imaging. However, for the purposes of the present research, only the BOLD data were processed and analyzed.

2.3.2 Neuroimaging Parameters

Imaging was conducted on a research-dedicated 3.0 Tesla MRI system at Baycrest using a 32-channel matrix head phased-array coil. Anatomical imaging included three-dimensional T1-weighted imaging (magnetized prepared rapid acquisition gradient echo [MPRAGE], field-of-view [FOV] = 256 mm, 192 x 256 acquisition matrix, 1.0 mm³ voxels, bandwidth = 200 Hz/Pixel, inversion time (TI)/echo time (TE)/repetition time (TR) = 1100/2.63/2000 ms, flip angle = 9 degrees, 160 slices, averages = 1, 1 concatenation, scan duration 6:26) to provide detailed anatomical images for functional co-registration. For the functional imaging, simultaneous pseudo-continuous ASL (pCASL) and BOLD were conducted with T2*-weighted echo planar imaging (FOV = 220 mm, 220 x 220 acquisition matrix, 3.4 x 3.4 x 6.0 mm voxels, bandwidth = 2790 Hz/Pixel, TE1/TE2/TR = 9.1/25/4000 ms, flip angle = 90 degrees, 16 slices, averages = 1, 1 concatenation, scan duration 5:24). The breath hold task and scanning were synchronized according to trigger pulses sent by the scanner. Refractory errors up to ± 6 diopters were corrected in the scanner using MR-compatible corrective lenses. Each participant’s head was restrained using a vacuum pillow that fit inside the head coil. Processing and analyses of the imaging data were performed using the Analysis of Functional NeuroImages (AFNI; Cox, 1996; Cox & Jesmanowicz, 1999) and Oxford Centre for Functional MRI of the Brain Software Library (FSL; Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) software packages.

2.3.3 Preprocessing of Neuroimaging Data

Prior to analysis, the BOLD data needed to be isolated from the ASL data, and the effects of the tagging pulses of the ASL scanning on the BOLD data needed to be removed. The BOLD data were first isolated using AFNI’s 3dTcat. The BOLD data were then split into even and odd TRs using FSL’s fslsplit. Outlier fractions were computed for each volume using AFNI’s 3dToutcount, and the functional data was aligned to the anatomical scan using AFNI’s 3dcalc and warped to Montreal Neurological Institute (MNI) space using AFNI’s align_epi_anat.py. The even and odd images were reconcatenated using AFNI’s 3dAFNItoNIFTI and FSL’s fslsplit and fslmerge. To remove the effects of the tagging pulses of the ASL scanning on the BOLD data, each BOLD TR was averaged with the next TR using FSL’s fslroi, fslmerge, and fslmaths.
A low-pass filter of 0.03 Hz was applied to reduce noise using AFNI’s 3dFourier. Finally, FSL’s fslmeants was used to extract the mean time series across the whole brain for estimation of the individualized breath hold delay value in Matrix Laboratory (MATLAB). AFNI’s 3dretroicor and physiological data recorded during the scan were used to generate BOLD data that were corrected for noise associated with physiological motion (i.e., heartbeat, respiration). However, the uncorrected data were used in the analyses; the physiologically corrected data removed too much of the BOLD signal of interest, as the physiological motion was closely correlated temporally with the breath hold task.

2.3.4 Optimization of Model Fit

In order to optimize the fit of the BOLD signal’s time course to the timing of the breath hold task, a fixed delay value was incorporated to accommodate the time required for blood flow to peak in response to the hypercapnic stimulus provided by the breath hold (Murphy et al., 2011). First, individual-level whole brain CVR maps were generated using FSL. These analyses were conducted in standardized MNI anatomical space using the T1-weighted anatomical scans as participant reference images. Using FSL’s fMRI Expert Analysis Tool (FEAT), spatial smoothing of 5 mm was applied to improve SNR, and the BOLD time course for each voxel was fit with: (1) a double-gamma hemodynamic response function regressor representing the task’s block model of 15 s of breath hold followed by 32 s of rest, and (2) a fixed delay value. Four delay values were tested: (1) 0 s (no delay), (2) 9 s, (3) 11 s, and (4) individualized. Zero seconds was tested in order to provide an indication of how well the timing of the breath hold blocks alone predicted the BOLD signal time course. For younger adults, a delay of 9 s is commonly used (Murphy et al., 2011), and a delay of 11 s has been used to accommodate the slower CVR response in older adults (Raut, Nair, Sattin, & Prabhakaran, 2016). The individualized delay, determined in MATLAB, was the average amount of time required for the BOLD response to peak before or after the offset of each breath hold (Tchistiakova et al., 2014). Overall, a delay value of 11 s was the best fit (i.e., it resulted in activation in the largest number of voxels in the individual-level whole brain CVR maps) for 41 participants, and was the second best fit for 14 participants. Thus, the delay value of 11 s was selected, as it appeared to produce the best overall fit between the BOLD response and the timing of the breath hold task for most participants.
2.4 Determination of Final Sample

2.4.1 Missing Data

Due to technical issues, or to participant discomfort during scanning, there were no breath hold data for six participants (1 CON, 3 HPTN, 2 HPTN+T2DM). For the full sample, FRS could not be calculated for two participants (1 CON, 1 HPTN+T2DM), as these participants did not indicate whether they currently smoke. For the final sample, one of these two participants (CON) was retained; the other participant was removed for poor compliance on the breath hold task. Neither participant was included in any analyses involving FRS as a predictor.

2.4.2 Participant Compliance

The degree to which participants complied with the task instructions was assessed prior to group analyses. Compliance was determined by comparing participants’ performance across three compliance measures: (1) whole brain CVR maps, (2) superior sagittal sinus (SSS) CVR, and (3) respiratory graphs.

*Whole Brain CVR Maps*

In addition to being used earlier to optimize the delay, the individual-level whole brain CVR maps produced in FSL were used as a measure of compliance. The breath hold task provides a hypercapnic stimulus (MacIntosh et al., 2003) that increases whole brain CBF (Kastrup et al., 1999). Thus, high levels of activation in the whole brain CVR maps would suggest high levels of compliance provided the participants have adequate cerebrovascular function. The CVR maps were ordered from lowest number of activated voxels to highest number of activated voxels, and then broken into three tertiles: (1) low, (2) moderate, and (3) high.

*SSS CVR*

BOLD signal change within the SSS can serve as an indicator of a whole brain cerebrovascular response to the breath hold challenge (Bandettini & Wong, 1997; Haight et al., 2015). Thus, if participants are breathing at the appropriate times and they have adequate cerebrovascular function, the time course of activation in the SSS should be closely related to the time course of the breath hold regressor. The SSS analyses were conducted in AFNI in native space rather than MNI space. The BOLD data were otherwise preprocessed in the manner previously described.
To assess SSS CVR, a SSS template from the Jakob atlas (Haight et al., 2015) was warped to each participant’s functional scan in native space using the Deformable Registration via Attribute Matching and Mutual-Saliency Weighting (DRAMMS; Ou, Sotiras, Paragios, & Davatzikos, 2011) software package. TRs associated with excessive head motion, which was defined as those exceeding a Euclidean norm of 2.0, were censored from participant-level data prior to analysis. Analyses were conducted using AFNI’s 3dDeconvolve in which the BOLD time course for each voxel was fit with: (1) a double-gamma hemodynamic response function regressor representing the task’s block model of 15 s of breath hold followed by 32 s of rest, and (2) a fixed delay of 11 s. AFNI’s 3dROIstats was used to extract a percent signal change value from the SSS for each participant. These SSS CVR values were ordered from lowest to highest, and then broken into three tertiles: (1) low, (2) moderate, and (3) high.

Respiratory Graphs

In order to record breathing patterns over the course of the breath hold task, participants wore a respiratory belt that tracked abdominal movement. Respiratory graphs of this movement over time were assessed for compliance with the visual breathing prompts provided during the task. Compliance was determined qualitatively by whether the participant’s abdomen was relatively active during the designated breathing periods and relatively still during the designated breath hold periods, as reflected in the respiratory graphs as periods of high and low amplitudes, respectively. These respiratory graphs were ranked in order of increasing compliance, and then categorized into three tertiles: (1) low, (2) moderate, and (3) high. Compliance was determined by consensus of two raters (Alain Carlson and Dr. Liesel-Ann Meusel), and any lack of consensus was resolved by a third rater (Dr. Carol Greenwood).

Participants with Poor Compliance

Individuals who fell into the lowest tertile across all three compliance measures (whole brain CVR maps, SSS CVR, and respiratory graphs) were excluded from group analyses. The basis for this rule was that poor compliance on just one of the measures may not indicate true poor compliance. For the respiratory graphs measure, a participant could be breathing and holding their breath at the correct times, but the presence of other issues (e.g., chest breathing or a poor belt fit) could result in a graph that suggests poor compliance. For the SSS CVR and whole brain CVR indicators, low CVR could indicate either poor compliance or poor cerebrovascular
function. Thus, removing only those participants with poor compliance across all three measures helped to ensure participants were removed for poor compliance rather than for unrelated issues or poor cerebrovascular function. Ten participants (2 CON, 3 HPTN, 5 HPTN+T2DM) who fit this definition were excluded.

2.4.3 Final Sample

The final sample included 59 participants after removing participants with no breath hold data or with poor compliance during the breath hold task. See Figure 3 for the participant flow diagram.
Figure 3. Participant flow diagram from full sample to final sample.

Full sample (n = 75)
24 CON, 33 HPTN, 18 HPTN+T2DM

Excluded (n = 16)
- No breath hold scan (n = 6)
  1 CON, 3 HPTN, 2 HPTN+T2DM
- Poor breath hold compliance (n = 10)
  2 CON, 3 HPTN, 5 HPTN+T2DM

Final sample (n = 59)
21 CON, 27 HPTN, 11 HPTN+T2DM
2.5 Assessment of Cognitive Function

Neuropsychological test scores that reflected the measurement of related variables were collapsed into composite scores intended to capture specific cognitive domains. First, participant z-scores were generated in the Statistical Package for the Social Sciences (SPSS) software package from raw scores in reference to the mean and standard deviation of the full sample. The full sample, rather than the final sample, was used to ensure that determination of the domain and global cognitive function composite sample z-scores drew on as much data as possible. Where needed, z-scores were reversed to ensure higher z-scores reflected higher levels of performance. These scores were then organized into four cognitive domains according to the theoretical basis for each test: (1) working memory, (2) processing speed, (3) memory, and (4) executive function (Table 1). Correlation matrices were generated across all of the tests and within each of these domains. Scores that were highly correlated within each domain and normally distributed were used to form the composite scores for each domain. Each composite was calculated by averaging the z-scores of the component tests within each domain. The working memory composite was formed from five test scores: (1) Digits backward total, (2) LNS, (3) Arithmetic, (4) Mental Control, and (5) Reading Span total correct. The processing speed composite was formed from two test scores: (1) Digit Symbol total, and (2) TMT–A. The memory composite was formed from six test scores: (1) Digit Symbol Pairing, (2) CVLT II short delay free recall, (3) Logical Memory I, (4) Visual Paired Associates I, (5) Verbal Paired Associates I, and (6) Faces I. The executive functioning composite was formed from four test scores: (1) F-A-S, (2) TMT–B, (3) WCST total errors, and (4) DEX. A correlation matrix was generated to evaluate the relationships between the domain composites in the full sample (Table 2). All four domain composites (working memory, processing speed, memory, executive function) were significantly correlated with each other, so they were averaged together to form a global cognitive function composite.
Table 1.

*Organization of neuropsychological test sample z-scores into cognitive domains*

<table>
<thead>
<tr>
<th>Working memory</th>
<th>Processing speed</th>
<th>Memory</th>
<th>Executive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digits forward span score</td>
<td>Digit Symbol total</td>
<td>Digit Symbol implicit learning %</td>
<td>F-A-S</td>
</tr>
<tr>
<td>Digits forward longest</td>
<td>TMT–A</td>
<td>Digit Symbol free recall %</td>
<td>TMT–B</td>
</tr>
<tr>
<td>Digits backward span score</td>
<td></td>
<td>CVLT A total</td>
<td>CVLT free recall intrusions</td>
</tr>
<tr>
<td>Digits backward longest</td>
<td></td>
<td>CVLT B</td>
<td>CVLT cued recall intrusions</td>
</tr>
<tr>
<td>LNS</td>
<td></td>
<td>CVLT short delay free recall</td>
<td>CVLT perseverations</td>
</tr>
<tr>
<td>Arithmetic</td>
<td></td>
<td>CVLT short delay cued recall</td>
<td>WCST categories</td>
</tr>
<tr>
<td>Mental Control</td>
<td></td>
<td>CVLT long delay free recall</td>
<td>WCST total errors</td>
</tr>
<tr>
<td>Reading Span absolute</td>
<td></td>
<td>CVLT long delay cued recall</td>
<td>WCST % perseverative errors</td>
</tr>
<tr>
<td>Reading Span total correct</td>
<td></td>
<td>CVLT discriminability</td>
<td>WCST failure to maintain set</td>
</tr>
<tr>
<td>Reading Span longest span</td>
<td></td>
<td>Logical Memory I</td>
<td>DEX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Logical Memory II</td>
<td>SART correct go %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Visual Paired Associates I</td>
<td>SART correct nogo %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Visual Paired Associates II</td>
<td>SART go correct reaction time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verbal Paired Associates I</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verbal Paired Associates II</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Faces I</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.

_Correlation matrix of domain composite scores in full sample_

<table>
<thead>
<tr>
<th></th>
<th>Working memory</th>
<th>Processing speed</th>
<th>Memory</th>
<th>Executive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working memory</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td><em>r</em> = .508**</td>
<td><em>X</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td><em>r</em> = .534**</td>
<td><em>r</em> = .442**</td>
<td><em>X</em></td>
<td></td>
</tr>
<tr>
<td>Executive function</td>
<td><em>r</em> = .306**</td>
<td><em>r</em> = .266*</td>
<td><em>r</em> = .276*</td>
<td><em>X</em></td>
</tr>
</tbody>
</table>

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).
2.6 Assessment of Vascular Risk Factors

To assess vascular risk factors, each participant’s FRS was calculated in the Statistical Analysis System (SAS) software package using a FRS Cox model (Wilson et al., 1998) that included sex (male/female), treatment of SBP (yes/no), SBP (mmHg), age (years), TC (mg/dL), HDL (mg/DL), smoking (yes/no), and T2DM (yes/no). FRS estimates each participant’s probability of developing cardiovascular heart disease over a 10-year period. As described in Hypotheses 3 and 4, respectively, it was expected that higher values for FRS, or for indicators of HPTN or T2DM, would predict lower CVR in brain areas that significantly differ between the groups and lower global cognitive function, and that the relationship between lower CVR and lower global cognitive function would be partially mediated by FRS or indicators of HPTN or T2DM. Thus, FRS, and the vascular risk factors it represented, was one of the primary predictors of interest.

2.7 Assessment of Insulin Resistance and MAP

Insulin resistance was assessed using the Homeostasis Model of Insulin Resistance (HOMA-IR; Matthews et al., 1985), which was calculated by dividing the product of blood glucose concentration and insulin concentration by 22.5. MAP was calculated using the following formula: MAP = 1/3 * SBP + 2/3 * DBP.

2.8 Descriptive Statistics

Descriptive variables for participants in the final sample are summarized in Table 3. Analysis of variance (ANOVA) in SPSS was used to test for group differences on descriptive variables. Significant ANOVAs were followed up with Student-Newman-Keuls (SNK) post hoc tests to determine which groups were significantly different from each other. The HPTN and HPTN+T2DM groups had higher SBP and MAP than the CON group. The HPTN+T2DM group had higher FRS and indicators of glucose dysregulation (FG, HbA1c, HOMA-IR) than the CON and HPTN groups. Lipid levels (TC and LDL) were higher in the CON and HPTN groups than the HPTN+T2DM group. No other comparisons reached significance. Relative to the final sample, the participants excluded from the full sample had significantly higher HbA1c (p = .010) and trended towards lower TC (p = .077). This finding was likely driven by the fact that several of the participants excluded (7 of 16 participants) were from the HPTN+T2DM group. Otherwise, the excluded participants did not significantly differ from the final sample.
Table 3.

Descriptive variables for participants in final sample

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HPTN</th>
<th>HPTN+T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>21</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Sex (women/men)</td>
<td>10/11</td>
<td>19/8</td>
<td>8/3</td>
</tr>
<tr>
<td>Age</td>
<td>70.5 ± 3.6</td>
<td>71.4 ± 4.6</td>
<td>71.4 ± 3.4</td>
</tr>
<tr>
<td>T2DM duration (years)</td>
<td>NA</td>
<td>NA</td>
<td>11.8 ± 4.1</td>
</tr>
<tr>
<td>HPTN duration (years)</td>
<td>NA</td>
<td>11.6 ± 8.1</td>
<td>12.9 ± 6.4</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>119.5 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131.1 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134.1 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>103.4 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.5 ± 13.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114.2 ± 12.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 ± .3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± .6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FG (mmol/l)</td>
<td>5.1 ± .6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.1 ± .9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± .7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.7 ± .5</td>
<td>1.9 ± 0.6</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.0 ± .7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRP (mmol/l)</td>
<td>2.3 ± 2.6</td>
<td>2.2 ± 2.2</td>
<td>2.0 ± 2.5</td>
</tr>
<tr>
<td>FRS (%)</td>
<td>12.4 ± 7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.8 ± 9.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.4 ± 13.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HADS anxiety (sample z)</td>
<td>-.10 ± 1.1</td>
<td>.21 ± 1.02</td>
<td>-.8 ± 0.82</td>
</tr>
<tr>
<td>HADS depression (sample z)</td>
<td>.00 ± 1.10</td>
<td>.07 ± 1.04</td>
<td>.33 ± 1.09</td>
</tr>
<tr>
<td>Shipley (sample z)</td>
<td>.01 ± 1.20</td>
<td>.12 ± 0.74</td>
<td>-.19 ± 1.21</td>
</tr>
</tbody>
</table>

Data are means ± SDs unless specified otherwise.

Groups with different letters are significantly different from each other at p < .05, according to results of SNK post hoc test.
2.9 Hypothesis Testing

The following sections detail how each hypothesis was tested statistically.

2.9.1 Hypothesis 1

It was hypothesized that CVR and global cognitive function would be lower in the HPTN and HPTN+T2DM groups than in the CON group, and lower in the HPTN+T2DM group than in the HPTN group (HPTN+T2DM < HPTN < CON). Group-level analyses of CVR were conducted in FSL’s FEAT, using the whole brain CVR maps produced by the individual-level analyses, in order to test Hypothesis 1. An $F$-test for the effect of group (CON, HPTN, HPTN+T2DM) and six follow-up pairwise contrasts (CON > HPTN, HPTN > CON, CON > HPTN+T2DM, HPTN+T2DM > CON, HPTN > HPTN+T2DM, HPTN+T2DM > HPTN) were produced in FSL, and were corrected using cluster-extent based thresholding. A threshold of $z = 3.10$ ($p < .001$) or more is recommended to improve spatial specificity of cluster-extent based thresholding and to reduce the risk of inaccurate family-wise error rate correction (Woo, Krishnan, & Wager, 2014). However, due to the relatively small sample size of the HPTN+T2DM group in the final sample ($n = 11$), a less conservative threshold of $z = 2.58$ ($p < .005$), also common in the literature (Woo et al., 2014), was selected to increase spatial specificity and reduce family-wise error while preserving clusters that might not survive a more stringent primary threshold. For clusters that significantly differed between groups, AFNI’s 3dROIstats was used to extract percent signal change values from each cluster for each participant.

One-way ANOVAs in SPSS were used to test for group differences on the domain and global cognitive function composites, in both the full and final samples. Significant ANOVAs were followed up with SNK post hoc tests to determine which groups were significantly different from each other.

For testing of Hypotheses 2–4, additional exploratory analyses were conducted in which analyses excluded, or were constrained to, the HPTN+T2DM group. The purpose of these analyses was to determine whether explanatory variables of CVR or cognitive function continued to be significant across the CON and HPTN groups or within the HPTN+T2DM group alone. Particularly for indicators of glycemic control, this would assess whether disruption, within the normal or T2DM range, was associated with lower CVR or cognitive function.
2.9.2 Hypothesis 2

It was hypothesized that, in brain areas that significantly differed on CVR between the groups, lower CVR would predict lower global cognitive function. Robust regressions in SAS were conducted to test Hypothesis 2. Robust regression was utilized in order to preserve sample size while reducing the weighting of outliers. The p-values for each regression were adjusted using the Benjamini-Hochberg (1995) procedure to control the increases in false discovery rate associated with conducting an increasing number of tests. Due to shared variance, and to reduce the number of tests, the four domain cognitive function composite scores were not used as outcome variables in the regression analyses. To further ensure the observed relationships were not driven by outliers, sensitivity analyses were conducted in which outliers were removed. Residual CVR values for each cluster with the variance associated with age removed were used for each regression involving CVR; however, sensitivity analyses were also conducted in which the CVR values were not adjusted for age. These analyses were conducted only with the final sample, as CVR values were not generated for each participant of the full sample due to missing data or poor compliance.

2.9.3 Hypothesis 3

It was hypothesized higher values for FRS, or for indicators of HPTN or T2DM, would predict both lower CVR in brain areas that significantly differ between the groups and lower global cognitive function. FRS, SBP, MAP, FG, HbA1c, TC, LDL, and HOMA-IR were evaluated as predictors of CVR and global cognitive function because they significantly differed between the groups. Although CRP did not significantly differ between the groups, it was also evaluated as a predictor of global cognitive function and CVR because elevated CRP may negatively affect vascular health (Ridker, Buring, Shih, Matias, & Hennekens, 1998; van Wijk et al., 2013). To examine correlations between the predictors, a correlation matrix was generated for these variables in the final sample (Table 4).
Table 4.

Correlation matrix of predictors of global cognitive function and CVR in final sample

<table>
<thead>
<tr>
<th></th>
<th>FRS</th>
<th>SBP</th>
<th>MAP</th>
<th>FG</th>
<th>HbA1C</th>
<th>TC</th>
<th>LDL</th>
<th>HOMA-IR</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRS</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.42**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
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<td></td>
<td>.43**</td>
<td>.98**</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>FG</td>
<td></td>
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<td>.56**</td>
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<td>HbA1c</td>
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<td>X</td>
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<td>.25</td>
<td>-.23</td>
<td>X</td>
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<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>-.26**</td>
<td>-.11</td>
</tr>
<tr>
<td>CRP</td>
<td>.13</td>
<td>.19</td>
<td>.26*</td>
<td>-.07</td>
<td>-.07</td>
<td>.16</td>
<td>.25</td>
<td>.12</td>
<td>X</td>
</tr>
</tbody>
</table>

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).
Several of the predictors were highly correlated with each other. For each such instance, one of the variables was selected on a theoretical basis. HbA1c was chosen rather than FG because HbA1c is a better indicator of chronic glycemia (American Diabetes Association, 2014). HbA1c reflects average blood glucose levels over a period of 2–3 months. In contrast, FG is an indicator of acute glycemia that can be affected by participant compliance, since fasting is necessary to obtain an accurate measure of FG. MAP was chosen over SBP because MAP was considered to be a more complete measure of blood pressure, as MAP also incorporates DBP into its calculation. LDL was chosen over TC, as TC does not take into account the relative proportions of LDL and HDL. Generally, high LDL levels are considered to have negative effects on vascular health while moderate HDL levels are considered to be protective (Stone et al., 2014). FRS was selected instead of HOMA-IR because FRS was the operationalization of vascular risk factors in this study, and FRS was considered to be a better measure than HOMA-IR of the diverse factors that may negatively affect cerebrovascular health. Finally, the predictors were set into two models: (1) MAP, HbA1c, and LDL, and (2) FRS.

Robust regressions in SAS were conducted to test the two models. Since the first model had multiple predictors, a robust version of the Wald test ($R^2_A$) was used to assess significance of the overall model (Hampel, Ronchetti, Rousseeuw, & Stahel, 1986). P-value adjustment and sensitivity analyses were conducted in the same manner as described under Hypothesis 2. The CVR analyses were conducted only with the final sample, as CVR values were not generated for each participant of the full sample due to missing data or poor compliance. The global cognitive function analyses were conducted on both the full and final samples, as values for MAP, HbA1c, LDL, FRS, and global cognitive function were available for all participants.

2.9.4 Hypothesis 4

It was hypothesized that the relationship between HPTN or HPTN+T2DM status and lower global cognitive function would be partially mediated by CVR, and the relationship between lower CVR and lower global cognitive function would be partially mediated by FRS or indicators of HPTN or T2DM. Any mediation tests involving CVR were conducted with the final sample, since CVR values were only available for participants in the final sample. Mediation tests were conducted using 95% confidence intervals of the indirect effect derived from Monte Carlo estimation using the PROCESS macro in SPSS (Preacher & Selig, 2012).
Chapter 3
Results

3 Results

3.1 Hypothesis 1

It was hypothesized that CVR and global cognitive function would be lower in the HPTN and HPTN+T2DM groups than in the CON group, and lower in the HPTN+T2DM group than in the HPTN group (HPTN+T2DM < HPTN < CON).

3.1.1 CVR

The HPTN > HPTN+T2DM contrast produced three clusters, indicating regions across which the HPTN group showed higher CVR than the HPTN+T2DM group (Figure 4). Cluster 1 fell mostly within the cingulate gyrus and paracentral lobule; cluster 2 fell mostly within the precuneus, cuneus, and posterior cingulate cortex; and cluster 3 fell mostly within the temporal gyrus, insula, and basal ganglia. No other pairwise contrasts produced significant clusters.
Figure 4. Clusters 1 (green), 2 (orange), and 3 (red) for HPTN > HPTN+T2DM contrast with cluster-extent based thresholding, z = 2.58 (p < .005).
3.1.2 Cognitive Function

Domain and global cognitive function scores for the full and final samples are summarized in Table 5. In the full sample, there was a significant group effect on working memory, $F(2, 74) = 5.84, p = .004$. The CON and HPTN groups, which did not differ from each other, scored higher than the HPTN+T2DM group. There was also a significant group effect on processing speed, $F(2, 74) = 3.33, p = .042$. The CON group scored higher (faster processing speeds) than the HPTN+T2DM group, while the HPTN group did not differ from either the CON or HPTN+T2DM groups. The group effect on memory approached significance, $F(2, 74) = 2.70, p = .074$. The CON group scored higher than the HPTN+T2DM group, while the HPTN group did not differ from either the CON or HPTN+T2DM groups. There was no effect of group on executive function, $p = .402$. Finally, there was a significant group effect on global cognitive function, $F(2, 74) = 5.22, p = .008$. The CON and HPTN groups, which did not differ from each other, scored higher than the HPTN+T2DM group.
Table 5.

*Domain and global cognitive function sample z-scores for full and final samples*

<table>
<thead>
<tr>
<th></th>
<th>Full sample</th>
<th></th>
<th></th>
<th>Final sample</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HPTN</td>
<td>HPTN+ T2DM</td>
<td>CON</td>
<td>HPTN</td>
<td>HPTN+ T2DM</td>
</tr>
<tr>
<td>Working memory</td>
<td>.20 ± .66</td>
<td>.10 ± .59</td>
<td>-.43 ± .66</td>
<td>.18 ± .69</td>
<td>.17 ± .56</td>
<td>-.51 ± .76</td>
</tr>
<tr>
<td>Processing speed</td>
<td>.19 ± .62</td>
<td>-.04 ± .74</td>
<td>-.42 ± .95</td>
<td>.18 ± .66</td>
<td>.03 ± .72</td>
<td>-.36 ± .81</td>
</tr>
<tr>
<td>Memory</td>
<td>.23 ± .86</td>
<td>.04 ± .84</td>
<td>-.38 ± .93</td>
<td>.21 ± .91</td>
<td>.08 ± .77</td>
<td>-.17 ± .69</td>
</tr>
<tr>
<td>Executive function</td>
<td>.15 ± .69</td>
<td>-.06 ± .71</td>
<td>-.12 ± .68</td>
<td>.15 ± .68</td>
<td>-.07 ± .71</td>
<td>.18 ± .59</td>
</tr>
<tr>
<td>Global cognitive function</td>
<td>.19 ± .48</td>
<td>.01 ± .54</td>
<td>-.34 ± .58</td>
<td>.18 ± .50</td>
<td>.05 ± .52</td>
<td>-.22 ± .54</td>
</tr>
</tbody>
</table>

Data are means ± SDs of participants’ full sample z-scores. Within each sample, groups with different letters are significantly different from each other at p < .05, according to results of SNK post hoc test.
In the final sample, as in the full sample, there was a significant group effect on working memory, $F(2, 58) = 5.01, p = .010$. Also as in the full sample, the CON and HPTN groups, which did not differ from each other, scored higher than the HPTN+T2DM group. The groups did not significantly differ on processing speed, memory, executive function, and global cognitive function.

### 3.2 Hypothesis 2

It was hypothesized that, in brain areas that significantly differed on CVR between the groups, lower CVR would predict lower global cognitive function. Lower CVR did not predict lower global cognitive function for any of the three clusters in the final sample, or when analyses excluded, or were constrained to, the HPTN+T2DM group (all $p s ≥ .378$).

### 3.3 Hypothesis 3

It was hypothesized higher values for FRS, or for indicators of HPTN or T2DM, would predict both lower CVR in brain areas that significantly differ between the groups and lower global cognitive function.

#### 3.3.1 Predictors of CVR

For cluster 1, the model of MAP, HbA1c, and LDL did not predict CVR in the final sample ($p = .102$). However, higher HbA1c predicted lower CVR within the model, $\chi^2 (1) = 6.27, p = .012$. A follow-up robust regression, with HbA1c as the sole predictor, found HbA1c accounted for 8% of the variance in CVR, $\chi^2 (1) = 6.51, p = .011$ (Figure 5). When analyses excluded, or were constrained to, the HPTN+T2DM group, the model of MAP, HbA1c, and LDL did not predict CVR ($p = .237$ and $p = .492$, respectively). FRS did not predict CVR in the final sample, or when analyses excluded, or were constrained to, the HPTN+T2DM group (all $p s ≥ .237$).
Figure 5. Higher HbA1c predicts lower age-adjusted CVR for cluster 1 in final sample.
For cluster 2, neither model (MAP, HbA1c, and LDL or FRS) predicted CVR in the final sample, or when analyses excluded, or were constrained to, the HPTN+T2DM group (all $p$s $\geq .118$).

For cluster 3, the model of MAP, HbA1c, and LDL did not predict CVR in the final sample ($p = .104$). However, higher HbA1c predicted lower CVR within the model, $\chi^2 (1) = 5.79, p = .016$. A follow-up robust regression, with HbA1c as the sole predictor, found HbA1c accounted for 9% percent of the variance in CVR, $\chi^2 (1) = 6.65, p = .010$ (Figure 6). When analyses excluded, or were constrained to, the HPTN+T2DM group, the model of MAP, HbA1c, and LDL did not predict CVR ($p = .520$ and $p = .855$, respectively). FRS did not predict CVR in the final sample, or when analyses excluded, or were constrained to, the HPTN+T2DM group (all $p$s $\geq .520$). In summary, higher HbA1c predicted lower CVR in clusters 1 and 3, but not in cluster 2. The removal of outliers, or the use of CVR values that were not adjusted for age, did not change the statistical significance of these analyses.
Figure 6. Higher HbA1c predicts lower age-adjusted CVR for cluster 3 in final sample.
3.3.2 Predictors of Global Cognitive Function

In the full sample, MAP, HBA1c, and LDL predicted global cognitive function, $R^2 = 11.61, p = .009, R^2 = .13$. However, higher HbA1c was the only significant predictor within the model, $\chi^2 (1) = 10.21, p = .001$. A follow-up robust regression, with HbA1c as the sole predictor, found HbA1c accounted for 12% of the variance in global cognitive function, $\chi^2 (1) = 11.45, p < .001$; Figure 7. Higher FRS also predicted lower global cognitive function, $\chi^2 (1) = 8.55, p = .008, R^2 = .10$; Figure 8. When analyses excluded, or were constrained to, the HPTN+T2DM group, the model of MAP, HbA1c, and LDL did not predict global cognitive function ($p = .428$ and $p = .600$, respectively). Higher FRS predicted lower global cognitive function when analyses excluded the HPTN+T2DM group [$\chi^2 (1) = 5.73, p = .034, R^2 = .09$; Figure 9], but not when analyses were constrained to the HPTN+T2DM group ($p = .600$).
Figure 7. Higher HbA1c predicts lower global cognitive function in full sample.

Figure 8. Higher FRS predicts lower global cognitive function in full sample.
Figure 9. Higher FRS predicts lower global cognitive function in full sample when HPTN+T2DM group is excluded.
In the final sample, the model of MAP, HbA1c, and LDL predicted global cognitive function, $R^2_n(3) = 9.65, p = .022, R^2 = .14$. However, higher HbA1c was the only significant predictor within the model, $\chi^2(1) = 9.01, p = .003$. A follow-up robust regression, with HbA1c as the sole predictor, found HbA1c accounted for 13% of the variance in global cognitive function [$\chi^2(1) = 9.52, p = .002$; Figure 10]. Higher FRS also predicted lower global cognitive function [$\chi^2(1) = 7.74, p = .010, R^2 = .12$; Figure 11]. When analyses excluded, or were constrained to, the HPTN+T2DM group, the model of MAP, HbA1c, and LDL did not predict global cognitive function ($p = .509$ and $p = .402$, respectively). FRS did not predict global cognitive function when analyses excluded, or were constrained to, the HPTN+T2DM group ($p = .114$ and $p = .402$, respectively). In summary, higher HbA1c and FRS predicted lower global cognitive function in both the full and final samples. The relationship between higher FRS and lower global cognitive function was maintained in the full sample when the HPTN+T2DM group was excluded. The removal of outliers did not change the statistical significance of these analyses.
Figure 10. Higher HbA1c predicts lower global cognitive function in final sample.

Figure 11. Higher FRS predicts lower global cognitive function in final sample.
3.4 Hypothesis 4

It was hypothesized that the relationship between HPTN or HPTN+T2DM status and lower global cognitive function would be partially mediated by CVR, and the relationship between lower CVR and lower global cognitive function would be partially mediated by FRS or indicators of HPTN or T2DM. However, these relationships failed to reach significance. In place of these planned analyses, exploratory analyses were conducted to determine whether: (1) CVR mediated relationships between higher HbA1c and lower global cognitive function, (2) CVR mediated relationships between higher FRS and lower global cognitive function, and (3) HbA1c mediated relationships between higher FRS and lower global cognitive function. The CVR values for the three clusters produced by the HPTN > HPTN+T2DM contrast were averaged together to form one CVR value for each participant in order to reduce the number of mediation tests. For the final sample, CVR did not mediate the relationships between higher HbA1c and lower global cognitive function (95% CI: -5.40, 5.99) or between higher FRS and lower global cognitive function (95% CI: -0.12, 0.32). Also, HbA1c did not mediate the relationship between higher FRS and lower global cognitive function (95% CI: -1.36, 0.01). For the full sample, the relationship between higher FRS and lower global cognitive function was partially mediated by HbA1c (95% CI: -1.27, -0.09; Figure 12). When the HPTN+T2DM group was excluded in the full sample, the relationship between higher FRS and lower global cognitive function was not mediated by HbA1c (95% CI: -0.005, 0.003). In summary, CVR did not mediate the observed relationships between HbA1c and global cognitive function or between FRS and global cognitive function. HbA1c partially mediated the relationship between FRS and global cognitive function when the full sample was considered, but this relationship was not maintained in the final sample or when the HPTN+T2DM group was excluded in the full sample.
Figure 12. Partial mediation of relationship between higher FRS and lower global cognitive function by HbA1c in full sample.
Chapter 4
Discussion

4 Discussion

4.1 Global Findings and Significance

The purpose of the present study was to clarify how HPTN and HPTN+T2DM affect cerebrovascular and cognitive function in older adults. Several analyses in the present research indicated relationships between HPTN+T2DM and lower CVR and cognitive function (Figure 13). In the final sample, older adults with HPTN+T2DM, relative to older adults with HPTN, had lower CVR across three clusters. Higher HbA1c predicted lower CVR in two of the three clusters. When the full sample was considered, global cognitive function was also lower in older adults with HPTN+T2DM than older adults with HPTN and CON participants. Finally, higher HbA1c and FRS predicted lower global cognitive function in both the full and final samples. In contrast, HPTN alone did not appear to have negative effects on CVR or cognitive function.

Overall, these findings underscore the importance of maintaining glucose control (HbA1c) and managing vascular risk factors (FRS) in older adults. Thus, the present research has helped to clarify how HPTN and HPTN+T2DM affect cerebrovascular and cognitive function in older adults. Research like this is a necessary first step in developing strategies to minimize the adverse impact of hyperglycemia, independent of HPTN, on cerebrovascular and cognitive function with aging.
Figure 13. Relationships and explanatory variables supported by the data in the present study. Colours are used to indicate where the data supported Hypothesis 1 (gold) and Hypothesis 3 (blue). Hypothesis 2 and Hypothesis 4 were not supported by the data.
4.2 Effects of HPTN and Indicators of HPTN on Cerebrovascular and Cognitive Function

One of the purposes of this thesis was to explore the effects of HPTN and indicators of HPTN on cerebrovascular and cognitive function, where it was hypothesized that CVR and global cognitive function would be lower in the HPTN group than the CON group. It was also hypothesized that higher values for indicators of HPTN would predict lower CVR and global cognitive function.

The finding of no significant differences in CVR between the CON and HPTN groups runs contrary to previous findings of lower CVR/CBF in the context of HPTN or elevated indicators of HPTN (Beason-Held et al., 2007; Dai et al., 2008; Haight et al., 2015; Hajjar et al., 2010; Nobili et al., 1993; Urback et al., 2017). It is unlikely that differences in SBP between studies account for this finding. The mean SBP for the HPTN group was 8 to 10 mm Hg lower than the mean SBP of participants with HPTN in two of the studies that found HPTN was associated with deficits in CVR/CBF (Beason-Held et al., 2007; Dai et al., 2008). However, the mean SBP for the HPTN group was comparable to the mean SBP of the HPTN group in the study conducted by Hajjar and colleagues (2010), which could suggest that impairments in CVR/CBF could occur at the levels of SBP in the present study. Similarly, Haight and colleagues (2015) found SBP $\geq$ 130 mm Hg was associated with reduced CVR in the DMN, an SBP cut-off similar to the mean SBP of the HPTN group of the present study. It is possible that the findings of Haight and colleagues were driven by participants with SBPs much higher than the SBP cut-off, but this is not possible to assess since the mean and standard deviation of SBP in the pre-HPTN/HPTN group were not reported. It is also possible that Haight and colleagues found these differences in CVR because they focused on specific brain regions of the DMN, allowing for greater statistical power to detect group differences. The present study assessed CVR using a whole brain approach, which may have failed to pick up subtle differences in CVR between the HPTN and CON groups.

The findings of no significant differences in cognitive function between the HPTN and CON groups, and of the poor predictive value of MAP, in the present study also run contrary to findings of lower cognitive function in the context of HPTN or elevated indicators of HPTN (Debette et al., 2011; Elias et al., 1995; Gifford et al., 2013; Hajjar et al., 2016; Harrington et al., 2000; Kilander et al., 1998; Raz et al., 2003). As in the case of the lack of CVR differences,
differences in SBP between studies are unlikely to account for these findings. Harrington and colleagues (2000) found cognitive deficits in a HPTN group with a substantially higher mean SBP (i.e., 164 ± 9 mm Hg) than the HPTN group in the present study. However, the mean SBP for the HPTN group was only 4 to 6 mm Hg lower than the mean SBP of participants with HPTN in two of the studies that found HPTN was associated with deficits in cognitive function (Hajjar et al., 2016; Raz et al., 2003). Debette and colleagues (2011) and Elias and colleagues (1995) conducted regression-based analyses rather than group-based analyses, so it is unclear whether their findings were driven by participants with higher SBPs than those of the HPTN group in the present study.

It is possible that treatment of HPTN minimized disruption of cerebrovascular and cognitive function, an interpretation supported by the findings of some previous studies (Brady et al., 2005; Meyer et al., 1985; Molina et al., 1999). Finally, arterial stiffness could serve as a more direct measure of structural or functional changes underlying CVR disruption, and thus could be a better predictor of declines in CVR, brain health, and cognitive function than HPTN or other indicators of HPTN. Hajjar and colleagues (2016) found increased arterial stiffness was a better predictor of declines in cognitive function than were HPTN status, SBP, or DBP.

4.3 Effects of HPTN+T2DM, FRS, and Indicators of T2DM on Cerebrovascular and Cognitive Function

Another purpose of this thesis was to explore the effects of T2DM and indicators of T2DM on cerebrovascular and cognitive function, where it was hypothesized that CVR and global cognitive function would be lower in the HPTN+T2DM group than in the CON and HPTN groups. It was also hypothesized that higher values for indicators of T2DM would predict lower CVR and global cognitive function.

The finding of lower CVR in the HPTN+T2DM group than the HPTN group corresponds with a previous finding of our research group (Tchistiakova et al., 2014). However, the lack of differences in CVR between the HPTN+T2DM and CON groups runs contrary to previous findings of lower CVR/CFB in participants with prediabetes, DM, or T2DM (Dandona et al., 1978; Frosch et al., 2017; Griffith et al., 1987; Last et al., 2007; Rusinek et al., 2015; Urback et al., 2017). It is unclear why the HPTN+T2DM and CON groups did not differ on CVR. The CON group had lower indicators for blood pressure (SBP and MAP), vascular risk factors (FRS),
and glucose dysregulation (FG, HbA1c, HOMA-IR) than the HPTN+T2DM group. However, likely due to medical management of lipid dysregulation in the HPTN+T2DM group, the CON and HPTN groups had higher TC and LDL than the HPTN+T2DM group. It is possible that these higher lipid levels in the CON and HPTN groups had negative effects on CVR, rendering differences in CVR associated with glucose dysregulation for the CON > HPTN+T2DM contrast nonsignificant while failing to reduce the differences in CVR for the HPTN > HPTN+T2DM contrast to nonsignificance. However, while dyslipidemia is considered a vascular risk factor, more research is needed to clarify the effects of dyslipidemia on CVR.

The findings of lower global cognitive function in the HPTN+T2DM group than in the HPTN and CON groups, in both the full and final samples, correspond with previous findings of lower cognitive function in the context of T2DM or elevated blood sugar (Arvanitakis et al., 2004; Convit et al., 2003; Cukierman-Yaffe et al., 2009; Di Bonito et al., 2005; Gold et al., 2007; Lamport et al., 2009). The results of exploratory analyses examining the cognitive domains that comprised the global cognitive function score similarly indicated lower processing speed, memory, and working memory in participants with HPTN+T2DM in the full sample. In contrast, a previous study conducted by our lab found no significant differences in the domains of executive function, processing speed, and memory between participants with HPTN and participants with HPTN+T2DM (Tchistiakova et al., 2014). There were also no significant group differences in processing speed or memory in the final sample of the present study. These findings suggest that the larger sample size of the full sample in the present study was needed to observe the group differences in processing speed and memory. Overall, the findings of the present study suggest HPTN+T2DM was associated with negative impacts on cognitive function. Working memory may have been particularly compromised by HPTN+T2DM, since the HPTN+T2DM group was found to have significantly poorer working memory even in the lower powered final sample. Executive function seemed relatively unaffected by HPTN+T2DM, since there were no significant group differences on executive function in the full or final samples.

It was found that higher HbA1c, an indicator of T2DM, predicted lower CVR in clusters 1 and 3, and lower global cognitive function in both the full and final sample. This finding suggests that the glycemic dysregulation component of HPTN+T2DM was largely responsible for these relationships, especially given that higher glycemic dysregulation in the HPTN+T2DM group was the primary difference between the HPTN and HPTN+T2DM groups. It was also possible
that higher HbA1c simply served as an indicator of the presence of several comorbid vascular risk factors. However, there is strong evidence to suggest that HbA1c was the main driver of these relationships. FRS, an indicator that would be sensitive to comorbid vascular risk factors, was not a significant predictor of CVR for any of the three clusters. Although higher FRS was correlated with lower global cognitive function in both the full and final samples, the results of exploratory analyses indicated that HbA1c partially mediated the relationship between FRS and global cognitive function in the full sample. HbA1c’s partial mediation of this relationship suggests that part of the relationship between FRS and global cognitive function is accounted for by HbA1c. That being said, HbA1c only partially mediated this relationship, so FRS may be picking up on other vascular risk factors that may affect this relationship (e.g., sex, smoking). It is unclear why neither HbA1c nor FRS were significant predictors of CVR in cluster 2, but this result does suggest at the very least that CVR across the brain regions of cluster 2 was less vulnerable to the effects of glycemic dysregulation.

4.4 Effects of CVR on Cognitive Function

Given the intimate relationship between the perfusion of brain tissue and cognitive function, it was hypothesized that, in brain areas that significantly differed on CVR between the groups, lower CVR would predict lower global cognitive function. However, lower CVR in any of the three clusters did not predict lower global cognitive function. The results of exploratory analyses indicated that CVR did not mediate the relationships between HbA1c and global cognitive function or between FRS and global cognitive function, which is not surprising given that CVR did not predict global cognitive function. Jennings and colleagues (1998, 2005), finding little evidence of significant differences in cognitive performance between participants with HPTN and CON participants, posited that increases in CBF to homologous areas of the brain supported the cognitive performance of participants with HPTN. Thus, it is possible that compensatory increases in CBF in homologous regions may have obscured the relationship between lower CVR within the three clusters and lower global cognitive function. Differences in the stimuli provided by the breath hold task and a cognitive task may also partially explain the lack of relationship between CVR and global cognitive function. First, the breath hold task may not stimulate blood flow in the same way as cognitive activity. The breath hold stimulus produces a global increase in CBF (Kastrup et al., 1999), while cognitive activities increase CBF in particular regions. Thus, while the breath hold stimulus may produce a weak global increase in
CBF for a participant, it is possible that CBF may increase adequately in the regions that support performance of a cognitive task. Second, analysis of the breath hold task focused on optimizing the fit of the time course of the BOLD signal and the task regressor. Thus, since analysis of the breath hold task focused on optimizing model fit, it is possible that a participant could be labelled as having low CVR, but the brain’s tissues are being adequately perfused outside that time window. Even within this study, 11s was not the ideal delay value when optimizing model fit for 18 of the 59 participants in the final sample. Given these differences, it is possible that participants’ brain tissues are adequately perfused during cognitive tasks despite having a poor CVR response to the breath hold task.

4.5   Strengths and Limitations

4.5.1 The Sample

The recruitment approach and inclusion/exclusion criteria ensured the participants in the sample were representative of individuals from the community with the conditions of interest (CON, HPTN, HPTN+T2DM) rather than of a specialized population. However, there were a couple of challenges related to sample size. In order to address the question of how cerebrovascular and cognitive function were affected by increasing glucose dysregulation in participants with HPTN but not T2DM, we had intended to divide the HPTN group into a higher HbA1c group and a lower HbA1c group. Unfortunately, the HPTN group was not large enough to support this strategy due to difficulties in recruitment and loss of data. The HPTN+T2DM group was also smaller than planned, which may have contributed to some of the nonsignificant results. Despite these challenges, the groups were large enough to identify a number of significant differences and trends, which can be further investigated in future studies.

Some characteristics of the sample posed interpretive challenges. First, participants in the HPTN+T2DM group had comorbid HPTN and T2DM, not T2DM alone. While the effects of T2DM in the absence of HPTN are theoretically interesting, it was impractical to try to recruit a sample of older adults with T2DM alone given the high comorbidity of HPTN and T2DM. Second, despite the dyslipidemia associated with T2DM, TC and LDL were higher in the CON and HPTN groups than the HPTN+T2DM group. This finding was likely attributable to medical management of lipid levels through medications (e.g., statins). A previous investigation by our research group also found higher TC and LDL in participants with HPTN than participants with
HPTN+T2DM (Tchistiakova et al., 2014). Finally, the variety of medications used to treat HPTN and T2DM could introduce additional interpretive challenges, since it is conceivable that some of these medications could affect cerebrovascular function.

4.5.2 Breath Hold Task

There were also challenges concerning the breath hold task. For the breath hold task regressor, a fixed delay of 11s was used to accommodate the time required for blood flow to peak in response to the hypercapnic stimulus provided by the breath hold (Murphy et al., 2011). The strength of this approach was that it allowed for the determination of each participant’s peak blood flow response to the breath hold task. However, though this value was the best fit for the majority of participants, it was not the best fit for all participants. Also, while peak blood flow is certainly interesting, the timing of the response may have functional relevance. Another difficulty encountered in this study was the assessment of compliance. It is possible that the respiratory graph did not accurately assess breathing in some of the participants. It is also possible low SSS CVR or low whole brain CVR maps could be an indicator of low CVR or poor compliance. However, a rigorous approach was applied in which participants who fell into the lowest tertile across all three compliance measures were removed, which helped to ensure participants were removed for poor compliance rather than low CVR. Interestingly, recent studies have addressed the problems related to compliance and regressor modeling by using direct gas inhalation or a nasal cannula. It was not possible to apply these methods to the present research, since the data had already been collected.

4.5.3 BOLD and ASL Data

One of the primary strengths of this study was the collection of BOLD and ASL data. For the purposes of this thesis, only the BOLD data were processed and analyzed. However, the ASL data have been collected, so they can be examined at a later time. Comparing the results obtained with the ASL data to the results obtained with the BOLD data may yield some interesting insights, regardless of whether the results are convergent or divergent. If the same pattern of results is found with the ASL data, there would be more confidence in the results obtained with the BOLD data. If a different pattern of results is found with the ASL data, it could be related to a number of factors, such as the greater specificity or lower sensitivity of ASL relative to BOLD.
4.5.4 Correlational Nature of Results

These results were correlational. Causation cannot be inferred, since the quasi-independent variable of group was latent not assigned. However, the results are interesting, and efforts were made to control the potential for false-positives. The Benjamini-Hochberg (1995) procedure was used to adjust the p-values of the robust regressions, the number of outcome variables were reduced by focusing on global cognitive function, and the number of mediation tests were reduced by averaging together the three CVR cluster values into one overall CVR value for each participant. Finally, the numerous tests that comprised the neuropsychological battery were collapsed into domain cognitive scores and then a global composite score, which further reduced the number of tests.

4.5.5 Whole Brain Assessment of CVR

These results could not speak to brain regions with high specificity. For the three clusters produced by the HPTN $>$ HPTN+T2DM contrast, it was tempting to speculate on brain-behaviour relationships. However, it is important to note, for clusters produced through cluster-extent based thresholding, that inferences about structures and brain-behaviour relationships cannot be more anatomically specific than the spatial extent of the clusters, although such inferences are commonplace in the literature (Woo et al., 2014). If a cluster spans more than one region, inferences can only be made reliably about CVR across the spatial extent of the cluster, not about any specific region within the cluster. Thus, given the diffuse clusters produced by the HPTN $>$ HPTN+T2DM contrast, not much can be said with confidence about these differences in CVR beyond the fact that they spanned several brain regions. Greater spatial specificity would be required to speculate on specific neural structures or networks. Given the small sample size of the HPTN+T2DM group, the higher threshold recommended to optimize spatial specificity ($z = 3.1, p < .001$; Woo et al., 2014) would likely have rendered these clusters nonsignificant. Moreover, if the purpose was to speculate on brain-behaviour relationships, it would have been more appropriate to make a priori predictions about regions or networks and limit analyses to these regions. The whole brain approach employed in this study was intended to identify broad differences in CVR across the brain rather than within regions or networks of interest. The results did highlight whole brain patterns and predictive relationships that deserve further scrutiny, patterns and relationships that may have been missed with an approach focused on specific regions or networks.
4.6 Future Studies

Future studies could build on the findings of the present study. First, it could be helpful to contrast the findings of the present study with those of a similar experiment in which participants are treating their HPTN and T2DM through lifestyle alone. Second, direct gas inhalation or a nasal cannula could be used to address issues related to compliance and regressor modeling. Finally, in contrast to the whole brain approach to CVR assessment in the present study, a future study with a larger sample could test *a priori* predictions about specific brain regions or networks. By focusing on specific regions or networks of the brain, it may be possible to detect group differences that were not found in the present study. Although the present study found no differences in CVR between the HPTN and CON groups, the findings of previous research suggests pre-HPTN/HPTN may be associated with reduced CVR in regions of the DMN (Haight et al., 2015). Focusing on specific brain regions or networks would also allow for speculation on brain-behaviour relationships, which could have clinical importance for individuals with HPTN or HPTN+T2DM. For example, if HPTN+T2DM was associated with both worse performance on a cognitive task and with reduced CVR in brain regions that support performance on that task, it could suggest reduced CVR contributed to the poorer performance of the participants with HPTN+T2DM.

4.7 Conclusions

Overall, the present work has made important contributions to the literature related to the cerebrovascular and cognitive effects of HPTN, T2DM, and other vascular risk factors. In isolation, HPTN status and indicators of HPTN did not predict differences in cerebrovascular and cognitive function. As discussed earlier, it is unlikely that the values for indicators of HPTN (e.g., SBP) were too low in this study to find negative effects on cerebrovascular and cognitive function. However, it is possible that an approach focusing on specific areas of the brain may reveal subtle deficits in CVR that could be missed using a whole brain approach like that employed in the present study. Another possibility that deserves further research is that arterial stiffness may serve as a more direct measure of the changes in structure and/or function that underlie deficits in cerebrovascular and cognitive function in the context of HPTN. In the present study, older adults with HPTN+T2DM had lower CVR and cognitive function, and higher HbA1c predicted lower CVR and global cognitive function. Higher FRS also predicted lower
global cognitive function. Thus, the present research draws attention to the importance of maintaining glucose control and controlling vascular risk factors in older adults.
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