Understanding Glucose-induced Neuronal Activation During Executive 2-back Task Performance In Hypertensive Otherwise Healthy Older Adults: A Functional Magnetic Resonance Imaging Study

by

William Yuen

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Abstract

The primary objective of this research was to explore the impact of glucose ingestion on 2-back task performance (accuracy, discrimination, and reaction times (RT) to target), its relationship to neural activation, using functional magnetic resonance imaging, and potential modulation by insulin resistance (IR) and low density lipoprotein (LDL) in hypertensive but otherwise healthy older adults. While there was no effect of glucose ingestion on task performance or task-relevant neural activation patterns, this study uniquely observed that IR and LDL associated with all 3 measures of 2-back performance and task-relevant neural activation patterns. The left and right precuneus, left cingulate, and left insula were identified as task-associated regions according to our specific target minus nontarget contrast. Of particular importance was the task activation in the right precuneus as it both showed sensitivity to IR and predicted task RTs to targets, suggesting it plays a modulatory role linking IR to task performance.
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List of Abbreviations

ADA = American Diabetes Association
ANCOVA = analysis of covariance
BBB = Blood Brain Barrier
BMI = Body Mass Index
BOLD = blood-oxygenation-level dependent response
CBF = cerebral blood flow
CHD = coronary heart disease
CRP = C-reactive protein
CVLT II = California Verbal Learning Test, Second edition
CVR = cerebrovascular reactivity
ERP = event-related potential
fMRI = functional magnetic resonance imaging
FSL = FMRIB Software Library
gAUC = glucose area under the curve
HAROLD = hemispheric asymmetry reduction in the old
HbA1c = hemoglobin A1c
HC = hippocampus
HDL = high density lipoprotein
IFG = impaired fasting glucose
IR = insulin resistance
ISI = inter-stimulus interval
LDL = low density lipoprotein
MMSE = Mini-Mental State Exam
MR = magnetic resonance
PFC = prefrontal cortex
rCBF = regional cerebral blood flow
ROI = regions-of-interest
RT = reaction time
SILS = Shipley Institute of Living Scale, Revised
T2DM = type 2 diabetes mellitus
T-NT = target minus nontarget activation contrast
WAIS-III = Wechsler Adult Intelligence Scale III
WASI = Wechsler Abbreviated Scale of Intelligence
WHO = World Health Organization
WMH = white matter hyperintensities
WMS-III = Wechsler Memory Scale III
WMS-R = Wechsler Memory Scale Revised
Chapter 1
Introduction

1 Introduction

In North America, the aging population is a rapidly expanding. Age-associated cognitive decline is a burden on personal, societal, and healthcare system levels. To further understand cognitive decline, health factors cannot be ignored. Studies have shown that obesity, poor gluco-regulation, lipid status, and vascular health accelerated cognitive decline beyond the effects of normal aging (Bruehl et al., 2009, Manschot et al., 2007, Mortimer et al., 2010). Since 53.2% of elderly Canadians are hypertensive (Wilkins, 2010), to obtain study results that are relatively generalizable to North Americans and to a population that may potentially be more susceptible to various degrees of cognitive impairment, this thesis focuses on older adults with hypertension.

Many studies of cognition have shown that glucose ingestion, a carbohydrate mainly used for energy in the brain and as metabolic backbone for neural substrates (i.e. neurotransmitters), can boost cognitive function in older adults. This is often referred to as glucose facilitation effect. Evidence for this effect on memory has been explored since the 1980s (Messier, 1998). Since relatively little glucose can be stored in the brain, the brain is reliant on a continuous supply of glucose from the periphery delivered centrally through the blood brain barrier (BBB). Peripherally, glucose ingestion raises blood glucose levels, and insulin facilitates the uptake of glucose into cells. While not dependent on insulin for glucose uptake, certain cognitive functions have been shown to be responsive to changes in blood glucose levels, although the exact mechanism of glucose’s actions and its brain regional specificity are unknown. For example, most studies show that performance on cognitive tasks associated with the hippocampus (HC) is effectively improved with glucose intake (Sunram-Lea, 2002), while performance on tasks of working memory involving the prefrontal cortex (PFC) shows a much less consistent response in terms of glucose facilitation. The degree to which the diverging response of the HC and PFC represents brain
regional specificity in its response to glucose versus inherent differences in cognitive task complexity is still under debate.

With a rise in the aging population (Jackson and Howe, 2008), it becomes necessary to better understand the susceptibility of the aging brain to hypertension in relation to cognitive decline. This can be done through functional magnetic resonance imaging (fMRI), which has the ability to monitor brain activation patterns while cognitive tasks are being performed. In general, even completely healthy older adults demonstrate poorer cognitive abilities compared to healthy younger adults (Drag and Bieliauskas, 2009; Raz, 2000), and hypertension appears to worsen cognitive abilities (Glodzik et al., 2013). One of the major cognitive functions that is affected by aging is executive functioning (Drag and Bieliauskas, 2009; Raz, 2000) associated with the PFC (Tanji and Hoshi, 2001). By understanding how the PFC is activated through task performance and its interactions with glucose supply in a hypertensive, aging population, it may be possible to ameliorate some of the cognitive deficits associated with hypertension and aging through a more targeted therapeutic intervention.

To date, how older people with controlled hypertension respond to glucose administration during cognitive task performance has not yet been systematically explored. Exploring whether glucose drink administration alters performance and brain activity associated with task forms the basis of the thesis. It was predicted that prefrontal cortical brain activation patterns and performance in a working memory task would be altered with glucose administration.
Chapter 2
Background

2 Background

2.1 Hypertension

2.1.1 Vascular health in relation to cognition and aging

Other than brain atrophy, vascular disorders (such as those associated with hypertension) have been shown to impair cognitive function particularly in executive function and visual organization (Stavitsky et al., 2010). Vascular damage resulting from hypertension can affect multiple organs in the body, leading to various complications such as retinopathy, nephropathy, and coronary or peripheral vascular diseases (de Bresser J et al., 2010). Within the brain, macro- and microvascular pathologies result in inadequate regulation of cerebral blood flow (CBF), which in turn disrupts nutrient and oxygen delivery necessary for metabolic demands. Aging vasculatures are also less responsive to dilatation signals to accommodate increased blood flow demand such as when doing cognitive tasks (Gianaros et al., 2005; Jennings et al., 2005). Indeed, adults with hypertension show decreases in regional cerebral blood flow (rCBF), particularly in frontal regions (Last et al., 2007), and decreased blood vessel dilation in response to vasodilatory stimuli (i.e., CO$_2$ inhalation). This is likely a consequence of both reduced cerebrovascular reactivity (CVR) and disrupted neural-vascular responses (Girouard and Iadecola, 2006).

2.1.2 Structural and functional changes with hypertension

Results from imaging studies exploring rCBF and cerebrovascular dilation in individuals with hypertension support a disruption to CVR, a measure of vasodilation (Molina et al., 1999; Ficzere et al., 1997). Furthermore, structural studies report that hypertensive patients are more likely to have whole brain (Wiseman et al., 2004) and regional atrophy in areas including the HC (den Heijer et al., 2005), the superior frontal and middle temporal gyrus, the anterior cingulate cortex, and the supplementary motor area (Gianaros et al., 2006). A study of type 2 diabetes (T2DM) showed that those individuals with hypertension are at risk
of prefrontal atrophy even when successfully treated with medication. Furthermore, this study showed a link specifically between prefrontal cortex and cardiovascular disease risks (Bruehl et al., 2009).

2.1.3 Mechanisms of Hypertension-Induced Damage

A number of mechanisms through which hypertension may have a negative impact on cognition have been suggested. This includes vascular remodeling, impaired cerebral autoregulation, cerebral microbleeds, white matter hyperintensities (WMH), unrecognized lacunar infarcts, and Alzheimer-like changes such as amyloid angiopathy, and cerebral atrophy (Manolio et al., 2003). As such, vascular health cannot be ignored when looking at cognitive performance in older adults. This thesis however is not focused on the effect of hypertension on cognitive performance per se. Instead, we are controlling for the effects of hypertension by recruiting only older adults with medication-controlled hypertension, and adjusting data analysis for CVR to accommodate potential heterogeneity in study participants’ vascular health; thereby allowing us to make inferences on neuronally mediated brain activities, independent of underlying vascular disorders.

2.2 Brain region of interest in relation to cognitive function

2.2.1 Prefrontal cortex

The PFC plays an important part in executive functioning to generate purposeful cognitive function. It is the site where neural circuits integrate currently available memory to generate information necessary to perform an action (Tanji and Hoshi, 2001). Anatomically, the PFC is the anterior portion of the frontal lobes. It is in the front of the motor and premotor areas. Its function in particular involves every aspect of the cognitive processes necessary for behavioural planning (Tanji and Hoshi, 2001), personality expression, decision making, and moderating acceptable social behaviour (Miller et al., 2002). Some of these cognitive processes performed by the PFC are thought to involve associative learning (Tanji and Hoshi, 2001), working memory (Curtis and D’Esposito, 2003), active retrieval and encoding of information (Petrides, 2005). PFC activity is typically evaluated through performance on
neuropsychological task like the n-back task which tests working memory involving attention, problem solving, and verbal reasoning.

2.3 Healthy aging, changes in cognition, structure, and patterns of brain activation

2.3.1 Healthy aging changes in cognition
Healthy aging is associated with decline in many cognitive domains including attention, verbal memory, processing speed, executive function, language, speech, and visuospatial functioning (Drag and Bieliauskas, 2009; Raz, 2000). The general trend is that older adults perform poorer on a variety of cognitive tasks compared to younger adults. This is reflected by Anderson et al. (2000) where they demonstrated that younger adults showed stronger performance compared to older adults on measures of verbal memory, processing speed, executive function, and fluid intelligence. Cabeza et al. (2000) also demonstrated that younger adults had better executive function and verbal memory than older adults; however, the older adults had better vocabulary skills.

Other than task challenges, other factors such as environmental distractions causing division in attention can also contribute to lower scores on cognitive tasks in older adults. A negative impact on declarative memory is observed when older adults are placed in stressful environments, such as being required to give a speech (Lupien et al., 1997) and when they experience the confinement and noise stress of a Magnetic Resonance (MR) environment (Gutchess and Park, 2006). This distracting stress exacerbates decrements in performance in healthy older adults compared to younger adults during a long-term memory task but not necessarily during a passive working memory task (Gutchess and Park, 2006).

2.3.2 Age related changes in PFC brain structure and patterns of activation
The decline in PFC-dependent cognitive functions associated with healthy aging is likely due to decrements in brain structure and altered patterns of neuronal activation. Even healthy aging is associated with age-related grey and white matter atrophy, especially in the HC and
frontal lobe regions (Salat et al., 2005; Sullivan et al., 2006). However, studies involving both positron emission tomography (PET) and fMRI have revealed reduction of activation between brain regions in older adults compared to younger even after adjusting for atrophy (Anderson and Grady, 2004; Cabeza, 2001).

Several fMRI studies on healthy older adults showed changes in PFC activations relative to young adults (Daselaar et al., 2003; Morcom et al., 2003; Park et al., 2003). These changes may be linked to well established changes in HC activity in normal aging since the HC and PFC are known to be integrated in the neural circuitry and may be susceptible to the negative impacts of aging (Hedden and Gabrieli, 2004). Also, studies have found age-related reductions in left inferior PFC activation during encoding (Anderson et al., 2000; Cabeza et al., 2001, 1997; Grady et al., 2002; Stebbins et al., 2002), which is consistent with results from cognitive studies showing reduced semantic processing during encoding in older compared to younger adults (Craik et al., 1995). Although less consistently observed, age-related reductions in activation of right PFC regions during episodic retrieval have been reported (Anderson et al., 2000; Schacter et al., 1996). It was shown that persistent activation in the lateral PFC increases performance on the n-back task, a cognitive task related to PFC, possibly through sustained attention and increasing working memory (Ball et al., 2011). The same task was shown to have common patterns of bilateral fronto-parietal activation, especially in the dorsolateral prefrontal cortex in both young and older participants. Typically, with the negative impacts of aging on the PFC, it is expected that elderly will perform poorer on tasks of executive functioning or working memory compared to younger adults. The n-back task will be described in more detail later, as it is a task used in this thesis.

2.3.3 Compensatory mechanisms

Though it is established there are age-related reductions in neuronal activation, a PET study by Cabeza et al. (2002) further demonstrated that there are some functional differences between cognitively normal high performing older adults and poorer performing older adults. The study explored word list recall and paired associates (Cabeza et al., 2002). There were differences in neuronal activation patterns between high performing and poorer
performing older adults independent of brain volume. The differences were attributed to the high performing older adults’ ability to initiate compensatory brain activation pattern responses, helping to sustain cognitive function in the face of aging. So, it is important to take these discrepancies into account when exploring a group of older adults’ brain activation patterns during task performance.

Not only do compensatory mechanisms show more areas of activation, older adults demonstrate bilateral hippocampal activation compared to younger adults (i.e. more left hippocampal activation during retrieval of autobiographical event memories) (Maguire and Frith, 2003). Older adults also tend to display more bilateral activation of the PFC during working memory (Grady et al., 2008; Mattay et al., 2006; Reuter-Lorenz et al., 2001) and episodic memory tasks, both during encoding (Stebbins et al., 2002; Anderson et al., 2000; Logan et al., 2002) and retrieval compared to younger adults (Grady et al., 2002; Cabeza et al., 2000; Backman et al., 1997; Duarte et al., 2008; Madden et al., 1999; Morcom et al., 2007). Cabeza et al. (2002) has coined this collective finding as the “hemispheric asymmetry reduction in the old” (HAROLD). In short, better performance by high functioning older adults compared to poorer functioning older adults is correlated with the extent of bilateral activation that is attributed to a HAROLD compensatory mechanism (Duverne et al., 2009; Miller et al., 2008). This compensatory mechanism allows adults to maintain their levels of cognitive function as they age (Cabeza et al., 2002). Therefore, it is important to understand contributors that enhance or impair these compensatory responses.

However, another school of thought is that having more activation to maintain task performance may temporarily benefit performance, but in the long run is damaging to the brain as neurons are being overworked. In other words, the brain is becoming less efficient at processing cognitive tasks and requires hyper-activation to do the same work as seen in individuals with mild cognitive impairment (Faraco et al., 2013). Furthermore, older adults with the apolipoprotein E gene ε4 allele (risk allele for Alzheimer’s Disease) were found to activate more compared to those without the risk allele to achieve similar performance (Bookheimer et al., 2010). So, it is unclear whether the compensatory mechanism is beneficial throughout the lifetime of elderly people.
2.4 Health measures and cognition and aging

Age, gender, and years of education have been shown to be good predictors of cognition (Kivipelto et al., 2006). But more importantly, many lifestyle-associated factors, including suboptimal health, contribute to accelerated cognitive loss with aging. While only briefly highlighted here, more details and orientation to mechanisms explaining the relationship to health measures can be found in a review, of which I was a co-author (Meusel et al., 2012) or elsewhere (Gadzinski et al., 2010; Mortimer et al., 2010; Bruehl et al., 2009; Manschot et al., 2007).

Generally, poorer lipid status, including higher low density lipoprotein (LDL) cholesterol, lower high density lipoprotein (HDL) cholesterol, and higher triglycerides, are associated with poorer age-adjusted cognitive performance (Vikarunnessa et al., 2013). Since LDL does not cross the BBB, how LDL directly affects brain function is not known. Nevertheless, current evidence points to an indirect mechanism, secondary to enhanced oxidative stress and inflammation. For example, elevated blood LDL cholesterol is associated with higher oxidation, leading to more reactive oxidative species which can be damaging to vascular and tissue health (Hui et al., 2012; Vikarunnessa et al., 2013). Similarly, increased inflammatory responses are also associated with poor lipid status and cognitive decline. In turn, oxidative species, inflammatory markers, and chemical signals to regulate LDL in vivo synthesis are known to cross the BBB. Much evidence supports this as there were many confirmed association of higher LDL with poorer brain function performance (Vikarunnessa et al., 2013; Gonzales et al., 2011; Bhat et al., 2013).

A wealth of studies showed that gluco-regulation is significantly associated with cognitive performance using tasks of declarative verbal memory including episodic memory (but not semantic memory) and that this effect of gluco-regulation is independent of vascular factors (Riby et al., 2006; Riby et al., 2004) in the elderly. In particular, there is strong evidence for the utility of gluco-regulatory control measures as indicators of cognitive decline in the elderly. A study using a task of modified Wechsler Memory Scale, a test of auditory, visual working memory, immediate memory, and delayed memory, found that gluco-regulation in
individual subjects predicted memory in the elderly (Hall, 1989). In addition, evidence suggested that poorer gluco-regulation, such as in diabetics, is correlated with poorer scores on neuropsychological measures (eg. visuospatial tasks, attention, reaction time) compared to seniors who are non-diabetic. Specifically, the cognitive areas of working memory, executive function, processing speed were negatively affected by poorer glucose regulation. These are also areas affected by subcortical and white matter changes (Messier et al., 2003) perhaps through dysregulation of insulin. Better gluco-regulation is generally associated with better age-adjusted cognitive performance, especially on tasks dependent on HC functions (Meusel et al., 2012). Fasting glucose and insulin levels, even within the normal range, associate with poorer cognitive performance and increased risk of dementia (Mortimer et al., 2010; Manschot et al., 2007; Cherbuin et al., 2012).

While hypertension, elevated LDL and insulin resistance (IR) are all associated with obesity, obesity, in and of itself, appears to affect brain activation and contribute to poor cognitive function. For example, a significant negative correlation was found between body mass index (BMI) and metabolic activity in PFC at rest, but not during cognitive stimulation (a numerical calculation task), where these analyses adjusted for hypertension, hyperlipidemia, and IR (Volkow et al., 2008). Thus BMI may influence brain activity, but the relationship between BMI and brain activity becomes less clear during cognitive stimulation and its effect on performance (Volkow et al., 2008). In contrast, the relationships of hypertension, IR, and LDL during cognitive performances are better established. There are however speculations that impaired performance reported in healthy obese individuals on some cognitive tests of executive function (Gazdzinski, et al., 2009; Volkow et al., 2008) may be secondary to higher levels of inflammation (e.g. C-reactive protein (CRP) often measured as an indicator of inflammation). The metabolic syndrome and other associated factors work in a complex web of interaction to affect cognition and brain metabolism; with oxidative burden, inflammation and impaired vascular function are likely being key factors contributing to the brain insults observed.
2.5 Glucose and cognitive function

Glucose intake is used as a way to explore normal age-related changes in patterns of brain neuronal activation and cognitive function.

2.5.1 Why glucose as a treatment?

The brain uses glucose as its primary fuel. In recent years, a wealth of literature has developed from both human and animal studies indicating that exogenous administration of glucose can facilitate cognitive functioning (Scholey, 2009; Meikle et al., 2005; Scholey et al., 2001; Messier et al., 2004). This phenomenon has been termed the glucose facilitation effect. Other sugars (ie fructose) and macronutrient (ie protein and fat) have been tested and yielded conflicting outcomes (Kaplan et al., 2000). Glucose is by far the most consistent substance to demonstrate a facilitative effect in cognitive performance in both attention and memory (Messier et al., 2004).

2.5.2 Dose and ingestion timing

Interestingly, glucose improvement of memory appears to involve two optimal doses in animals (100 mg/kg and 2 g/kg) that may correspond to two distinct physiological mechanisms underlying glucose’s effects on memory. In humans, lower doses of glucose (25 g) appeared to be more effective in younger adults while higher doses (50–75 g) were found to improve memory in older adults (Messier, 2004). Also, not only the dose but the length of the fasting interval prior to a glucose load is crucial. For example, a higher glucose dose (60 g) increased working memory performance following an overnight fast, whereas a lower dose (25 g) enhanced working memory performance following a 2-hour fast (Owen et al., 2011) in older adults. Furthermore, Riby et al. (2009) demonstrated an interaction between glucose ingestion and task delay in both healthy older adults and older adults with mild cognitive impairment (MCI). The results suggested that the longer the delayed time point of an episodic memory task, the larger the enhancing effect of glucose. Focusing on the timing of glucose administration, cognitive testing can either occur before or after administration of glucose. Post-testing administration of glucose is thought to reflect an action of glucose on
memory processes that take place after learning a new task; pre-testing glucose administration is thought to interact with attentional, perceptual and other cognitive processes which take place before learning a task. Pre-testing glucose administration appears to be more effective at improving cognitive performance; with the greatest benefits being observed when task are commenced after blood glucose levels have reached their peak level (e.g. 45 min after administration) (Meikle et al., 2005; Scholey et al., 2001). Thus, the glucose dose (60g) and timing of cognitive tasks relative to drink ingestion (60 to 130 minutes), used in this thesis, were chosen to optimize the effect of glucose intake on cognitive performance.

2.5.3 Comparing glucose facilitation in younger and older adults

While both younger and older adults appear to benefit from glucose ingestion, the ability to observe cognitive changes is somewhat dependent on the choice of cognitive tasks and their complexity. For example, glucose facilitation on episodic memory tasks is often observed in older, but not younger, adults (Sommerfield et al, 2004). Nevertheless, when task difficulty is increased and includes divided attention, glucose facilitation of performance has been reported in younger adults (Scholey, 2009; Meikle, 2006). One explanation for the age-associated difference in response to glucose administration is that younger adults are already performing close to ceiling on many episodic memory tasks, leaving little room to observe an effect of glucose. As task difficulty is increased and performance in younger adults is not at ceiling, there is a greater likelihood of observing benefits associated with glucose ingestion. Yet, when memory and tracking tasks were administered concurrently, unexpectedly, glucose administration only improved tracking, but not memory, in younger adults, suggesting a non-memory specific nature of glucose’s benefits in younger adults (Scholey, 2009). One explanation is that glucose simply allows greater allocation of attentional resources in younger adults, instead of having a specific effect on memory. In addition, some studies, especially in younger adults, show that glucose only benefits more difficult tasks that may require additional cognitive ‘fuel’ (Meikle, 2005). As cognitive load increases, greater allocation of attentional resources is required so performance does not
suffer (Scholey, 2009). In fact, increased energy expenditure and glucose utilisation have been shown during more intense cognitive processing (Scholey, 2009).

By contrast, in older adults, the benefits of glucose ingestion are more consistently observed on episodic memory tasks, with results on non-memory tasks being highly variable (Riby, 2004; Riby et al., 2009). Based on rodent studies monitoring extra-neuronal glucose levels during cognitive tasks relying on the HC (McNay et al., 2001), it has been argued that hippocampal glucose levels decline to a greater extent in older, relative to younger, rats and that this is associated with age-related differences in task performance. Glucose ingestion prior to cognitive task performance prevented the extra-neuronal decline in hippocampal glucose levels in the older rats and enhanced performance close to that observed in the younger animals. This suggests that with aging, the blood supply of glucose to the HC may not be adequate to support optimal cognitive function, but that this can be overcome by glucose intake prior to task performance. Whether similar relationships between extra-neuronal glucose levels, their recovery with glucose ingestion, and task performance are observed in non-HC regions has not been assessed. Consequently, it is not known whether this contributes to the highly variable effects of glucose ingestion on non-HC cognitive tasks.

Others have suggested that the lack of benefit of glucose ingestion in older adults on non-memory tasks may reflect that fact that they were already working at their cognitive limits and beyond any facilitative effect of glucose or that they are less efficient at allocating cognitive resources to support non-HC tasks. For example, older individuals were less able to effectively track a moving target while concurrently encoding or retrieving information (Scholey, 2009). Whether glucose’s benefits are confined to specific tasks involving specific regions in older adults or its benefits are based purely on task difficulty is unknown. The latter may suggest that glucose might have a global effect on the brain to improve cognitive performance and is not necessarily involving specific task-related regions of the brain (seen in younger adults). Though it is unknown how glucose acts to improve performance, it may act as a cognitive remedy to support specific functions in older adults.
2.5.4 Molecular mechanism of glucose facilitation effect

Skeptics argue that glucose may produce a pleasurable effect thereby inducing neural activations as opposed to an effect in and of itself; however, this is unlikely since only glucose, and not saccharin, improves memory function. In most studies, a saccharin drink serves as a placebo drink and was designed to taste similar to the glucose drink. So, the facilitation effect is likely to be valid and unique to glucose. Assuming glucose does have a direct effect on brain, it is important to consider both central and peripheral mechanisms since they work in conjunction as blood glucose does cross the BBB.

*Central acting: Cholinergic system*

Centrally, it is thought that most of the increases in neuronal glucose uptake during neuronal activation contribute to increased synaptic function (Atwell *et al.*, 2010). Synapses generally have higher energy requirements because of the activity of ionic pumps involved in neurotransmission particularly in the post-synaptic terminal. In addition, several key neurotransmitters in the brain are directly dependent on exogenous glucose for their synthesis. This includes two of the main excitatory neurotransmitters: glutamate and acetylcholine (Ach) and an inhibitory transmitter, gamma-aminobutyric acid (GABA). The synthesis of neurotransmitters may also require a small portion of energy from glucose. One hypothesis put forward is that glucose acts mainly to alleviate memory deficits associated with impaired glucose regulation in older adults (Messier, 2004). An underlying assumption for this is that peripheral glucose administration may directly increase central glucose availability and brain metabolism (Manning, 1992). Both glucose and cholinergic drugs have been shown to benefit primacy effect (recalling the 1st 5 words of a word list) in a word list learning task (Messier, 1998, Manning, 1992) suggesting a similar cholinergic pathway. Mechanistically, glucose may affect the hippocampal cholinergic system through release of Ach. Further evidence showed that scopolamine, a competitive cholinergic antagonist, reduces the primacy effect and glucose ameliorated this effect of the drug indicating there is crosstalk between the mechanisms of action (Messier, 1998, Manning, 1992).
To address the issue of why glucose selectively enhances more difficult tasks that involve interference (found in younger adults), a hypothesis is that peripheral glucose injections could alleviate localized deficits in extracellular glucose via changes in glucose transporters to allow more glucose uptake in the brain during more difficult tasks (Scholey, 2009). Also, because certain neurotransmitters like Ach are directly dependent on the glucose supply for their synthesis, glucose is thought to facilitate neurotransmitter synthesis under certain circumstances (i.e. interference). To explain the dose-response, it is possible that glucose-sensitive neurons in the brain or the periphery may serve as glucose sensors that function at a particular glucose threshold and eventually produce neural changes (i.e. recruiting more brain regions or more synapsis) that would facilitate memory processing (Messier, 2004; Owen, 2011).

Peripheral acting glucose metabolism

Though cognition is centrally regulated, cognitive demand is associated with arousal, including increased heart rate and somatic energy expenditure by the musculature to generate a response (Scholey, 2001). Consequently, a peripheral mechanism cannot be ignored. These processes will cause peripheral depletion of glucose. To deal with the issue of arousal which may enhance encoding of tasks, studies which showed that glucose enhanced memory even when administered after encoding (e.g. 24 hrs), suggest that the beneficial effects observed on memory may not be related to arousal during encoding (Manning, 1992). But peripheral and central actions may be connected as Hall (1989) proposed that peripheral systems could interact with central systems to direct neuronal innervations via vagal afferents. In addition, glucose may have peripheral effects on memory performance through mechanism such as hormone regulation (i.e. neuroendocrine) (Best et al., 2008). For example, regulation of neuroendocrine hormones such as epinephrine release from the adrenal medulla or promotion of cholinergic function (Allen et al., 1996; Meikle et al., 2005) are all known to affect brain activity. There may be an advantage to develop an integrated mechanism that includes both peripheral and central actions of glucose (Messier, 2004) because they likely work in conjunction with each other.
2.6 Functional Magnetic Resonance Imaging

Brain imaging techniques can provide indices of atrophy, lesions, resting cerebral perfusion, and brain activity in response to task performance. Functional magnetic resonance imaging (fMRI), the imaging technique used in this study, is itself a fairly new neuroimaging technique that was developed based on blood-oxygenation-level dependent (BOLD) contrast. BOLD fMRI is now a widely used technique, and it monitors functional changes in brain activity (Huettel et al., 2004) by measuring dynamic changes in blood oxygenation during a cognitive challenge (Ogawa et al., 1992). fMRI is increasingly popular because of its non-invasiveness and relatively high-resolution images. BOLD fMRI’s strengths relative to PET imaging include its superior sensitivity, temporal resolution, and large volume coverage enabling whole brain inquiry (Huettel et al., 2004). In addition, BOLD signals are relatively sensitive to subtle changes in task demands and can give estimates of event-related neural responses over the entire brain (Huettel et al., 2004).

2.6.1 How does BOLD work?

The BOLD signal monitors local increases in CBF that are associated with neural activity which provides an indirect but relatively high resolution measure of brain activity (Harris et al., 2011). The mechanism starts with ATP expenditure by neural activity (i.e. ions pumped out from neurons for neurotransmitter synaptic activity or action potentials). In turn, this increases oxygen consumption to replenish ATP and is followed by a large increase of blood flow to the active area. Oxygen is carried in the blood by hemoglobin (Hb). The basis of BOLD signal is formed from differences in magnetic properties of haemoglobin molecules. During an increase in oxygen consumption, oxygen is taken from diamagnetic oxygenated-Hb (oxyHb), leaving behind paramagnetic deoxygenated-Hb (deoxyHb) which decreases the BOLD signal. The subsequent increase of blood flow (overlaps with the increase of O2 consumption) gradually converts to a decrease of deoxyHb by the delivery of fresh oxygenated blood (oxyHb), thus increasing BOLD signal. The BOLD fMRI signal is mainly reflected of this delayed decrease of deoxyHb due to the increase in blood flow. The magnitude of the BOLD fMRI signal is determined by the difference between the amount of
blood flow increase (increases the signal) and the use of O$_2$ by neurons (decreases the signal) (Harris et al., 2011).

### 2.6.2 Interpretation of the BOLD signal

Atwell and Ladecola (2002) argue that most brain energy is used for postsynaptic currents and action potentials, so the haemodynamic BOLD response can be thought of as neurotransmitter-related signalling. It is thought that BOLD signals could reflect the neural processing occurring within a brain area. Thus, changes in processing but no net change of energy usage could lead to a BOLD signal and vice versa. Energy use does not directly increase blood flow, so BOLD response is a function of signalling, not a function of energy. It is thought that the BOLD signal is driven locally by glutamate-mediated signalling processes, and more globally by amine- and ACh-mediated neural systems. The view that the haemodynamic response is coupled to signalling processes represents a conceptual shift from the traditional idea that the energy demands of the tissue directly determine the flow increase associated with neural activation.

Note that brain activation patterns may differ between genders. Males in general have greater mean task associated activation than females while engaged in a working memory task in the right superior parietal gyrus and right inferior occipital gyrus, and a greater BOLD magnitude occurring in the left inferior parietal lobe (Bell et al., 2006). There was, however, no difference in cognitive performance.

### 2.6.3 Glucose and fMRI studies

The studies of glucose facilitation on task performance using fMRI are, at present, confined to younger adults and draw on episodic memory. A study by Parent and others (2011) showed that fMRI is sensitive enough to monitor changes in brain activities induced by 50 g of glucose ingestion in various regions of interest. Furthermore, elevation of blood glucose was found to be associated with successful encoding of emotional images and increase in functional coupling between the activity of the hippocampus, prefrontal cortex, amygdala, and other network of brain regions (Parent et al., 2011). These data suggest we can also see
glucose induced BOLD changes in working memory tasks associated brain regions. Our study is the first to use of BOLD fMRI to explore neurological events occurring in the brain regions during a working memory task in hypertensive but otherwise healthy older adults following glucose ingestion.

2.6.4 Concerns using BOLD fMRI

One of BOLD’s limitations relates to its lack of specificity since it relies on relative and not absolute changes in signal intensity of the MR images (Huettel et al., 2004). Moreover, the BOLD signal is influenced by the competing effects of oxygen consumption, blood volume, and blood oxygenation (Huettel et al., 2004). Regional perfusion is mediated by both neuronal and astrocytic enzymes that regulate blood vessel diameter, astrocyte morphology, and hence the coupling of neuronal activity to blood flow changes. All these alter neural activity. These changes in neurovascular coupling mechanisms (and energy use) may compromise the power of BOLD imaging to detect relevant changes in neuronal information processing (Harris et al., 2011). Thus, dynamic changes could occur in several parameters but ultimately not be reflected in the BOLD signal in a meaningful direction or magnitude (Buxton, 2010). In order to separate each component, additional measures are required. Although sites of increased neural activity often co-localize with areas of increased metabolism, CBF and energy utilization can be dissociated (Atwell and Ladecola, 2002). To address this issue, this study includes a breath hold task, to obtain an estimate of CVR which is used as a covariate to adjust for vascular reactivity effects that are known to alter the BOLD signal but are independent of neural signalling.

With regards to glucose ingestion, one study showed that increases in blood glucose, within physiologic limits, using a hyperglycemic clamp, did not have a significant effect on the BOLD fMRI signal. These data argue that it is unlikely that the modest blood glucose elevations caused by the treatment used in this thesis will independently alter BOLD or confound the results. By contrast, others have argued that manipulations of blood glucose (Anderson et al., 2006) and insulin (Seaquist et al., 2007) levels in healthy volunteers can influence the BOLD signal independent of performance induced neuronal activation. These
authors argue that BOLD fMRI measures made in isolation following glucose ingestion may not be appropriate for older adults with impaired fasting glucose (IFG) and other endocrine complications (Seaquist et al., 2007). To help account for this potential confound, participants in this study completed the breath hold task following both the placebo and glucose drinks, such that non-neuronal changes in BOLD signal following glucose ingestion would be accounted for in the CVR estimates.

A concern specific to our within-subject cross over design is that there is large variability involved in repeated measures BOLD signalling. When using fMRI and a repeated measures crossover design, it is important to consider the experimental effect, the reliability of the measurements, and the number of subjects. A study explored BOLD signal stability through a within-subject crossover design using a motor task (Zanbelt et al., 2008). Task performance, spatial activation patterns, and group-wise BOLD signal changes were stable across sessions. However, there were substantial within-subject fluctuations (up to half the size of the group mean activation level) in activation levels (Zanbelt et al., 2008). Within-subject sources of variation could include stress, practice effects and physiological noise across sessions. For example, it is known that the stress and anxiety in the MR decreases over time, and so do BOLD signals, thus contributing to large variation across sessions. We attempted to minimize some of this variation by 1) having individuals enter a MR simulator several days before actual MR scanning to become familiar with the environment, 2) having participants practice the cognitive tests in the simulator both on the day so that they were becoming familiar with the environment and again just before entering the scanner and 3) accounting for a ‘session’ effect in the analyses. Nevertheless, all these potential variations may dilute significant findings. Because of this, others have recommended that for intervention studies, a priori region of interest analyses might yield more significant results since whole brain voxel-wise analysis might be too stringent after multiple comparison (Zanbelt et al., 2008). Despite knowing it may be difficult to obtain significant results in this thesis, because of the novelty of our study, we still adopted a group whole-brain voxel analysis, in large part because of the large number of regions involved in the task performance and the inability to define specific regions of interest within the PFC with current anatomical maps.
2.7 Cognitive and Neuropsychological Tasks

To assess the cognitive abilities of our participants, a number of cognitive and neuropsychological tasks were used.

2.7.1 Cognitive Tasks

Cognitive tasks, such as tasks of working memory, allow assessment of cognitive performance both at the behavioural and neuronal level in an MR environment. We wanted to see if the 2-back task of working memory, related to PFC, can be enhanced by glucose ingestion. The 2-back task was modified to suit imaging purposes and behavioural measures. A breath hold task was also included so that analyses could be adjusted for between subject differences in CVR and within subject differences in CVR following the placebo and glucose drinks.

2-back task

The 2-back test assesses executive functioning in terms of working memory and reaction times (Kirchner, 1958). It is one of the most popular experimental paradigms for functional neuroimaging studies of working memory (Owen et al., 2005). Working memory can be described as a cognitive system for the temporary storage and manipulation of remembered information (Baddeley, 1992) that is active and only relevant for a short period (Fuster, 1995; Goldman-Rakic, 1995). In the 2-back task, the subject is required to monitor a set of stimuli (i.e. words or letters) and to respond whenever a repeated stimulus is presented, or it appears in the same position, as the one presented 2 trials previously. Regardless of the version of the n-back task being used, consistent activation of frontal and parietal cortical regions are observed as reported in a meta-analysis (Owens et al., 2005), making n-back usage an ideal choice to study prefrontal cortical activation. In that meta-analysis, in all verbal, non-verbal, and spatial n-back identifications, the brain regions that had significant activity included premotor cortices, dorsolateral and ventrolateral PFC, frontal poles, and medial and lateral posterior parietal cortices. These regional activities are also relatively consistent throughout developmental stages of a person (Thomas et al., 1999). In particular, single unit recordings of prefrontal neurons have demonstrated that there is sustained activity
in dorsolateral regions during the memory delay interval of a working memory paradigm. In sum, 2-back is a working memory task that induces specific and robust brain activations.

Performance of older and younger adults on n-back

In a study that explored varying the difficulty of the n-back task, older subjects performed as well as the younger subjects at 1-back, but performed worse than the younger subjects in the more demanding 2-back and 3-back (Mattay et al., 2006). Interestingly, in the 1-back task, older adults showed greater prefrontal cortical activity bilaterally when performing similarly compared to younger adults. At higher working memory loads, however, older adults performed worse than the younger subjects and showed relatively reduced activity in these prefrontal regions. Overall, however, there was a similar distribution of cortical activity between younger and older subjects at all task levels (Mattay et al., 2006). These data suggest that, within capacity, compensatory mechanisms, such as additional prefrontal cortical activity, are called upon to maintain proficiency in task performance (Mattay et al., 2006), perhaps when showing a decrease in neural efficiency (Reuter-Lorenz et al., 2001). As one ages, more neural units would be recruited to supply the required capacity for a task even at lower levels of computational complexity. When the task is beyond capacity, there is relatively decreased frontal activity (Mattay et al., 2006). Thus, older adults reach the limits of resource reserves more rapidly than the younger adults. This understanding contributed to our use of 2-back, rather than the more demanding 3-back in this thesis.

Breath-hold task

The breath hold fMRI task serves as measure of CVR. CVR is generally assessed in highly vascularized brain regions (i.e. visual or motor cortex). The breath hold task is a well-established method to induce a transient, hypercapnic condition that causes a global increase in CBF (Kastrup et al., 1999). The result of the task is highly reproducible when monitored by the BOLD response with breath holds longer than 9 s within a healthy population (Murphy et al., 2010). Although some regions in the brain (i.e. brainstem) may regulate breathing, the effects are negligible in a short breath hold task. Hence, it is considered a valid estimate of the global, vascular-only effect independent of neuronal physiological actions (Buxton et al., 2010; Magnon et al., 2009). Importantly, the breath hold task is less invasive.
compared to other traditional hypercapnic methods which deliver gas via a tight fitting mask. In contrast, fMRI BOLD responses associated with task performance reflects both the neural-vascular response and CVR. Using the CVR response following a breath hold as a covariate in the analyses will help account for between individual cerebrovascular differences and provide a better estimate of the neural-vascular response, independent of underlying cerebrovascular deficits related to hypertension. By monitoring the fMRI BOLD response to the breath hold task following both the placebo and glucose drinks, it should also capture, and ultimately adjust for, none neural-specific changes in BOLD responses associated with increases in blood glucose and/or insulin levels post glucose drink intake.

2.7.2 Neuropsychological battery task

Neuropsychological tasks assess global cognitive function and have been used to quantify specific mental functions such as episodic memory, executive functioning, speech, language, phonemic and semantic fluency, intellectual functioning, motor and visuospatial functioning, overall intelligence, mood, and independence. These standardized neuropsychological measures can be used to describe cognitive abilities of an individual, to diagnose mental conditions, and to provide valuable information about the location of certain disorders in the brain. We utilized a set of standardized tests during the screening session to assess baseline cognitive function and exclude individuals who scored with ranges consistent with either dementia or mild cognitive impairment.

To assess for declarative memory function, tasks included: the California Verbal Learning Test II (Delis et al., 2000), Wechsler Memory Scale III Logical Memory, Faces I Recognition (WMS-III, Wechsler, 1997), Wechsler Memory Scale Revised Verbal, and Visual Paired Associates subtests (WMS-R, Wechsler, 1987). The California Verbal Learning Test II is composed of a list of words that are first heard and learned and subsequently recalled through free or cued recall immediately after learning and after longer delay periods. The WMS-III Logical Memory task uses short stories that are first heard and learned then recalled immediately after learning and following a long delay period. The WMS-III Faces I Recognition presents a series of photos of people’s faces to be learnt and recognized immediately after. Finally, the WMS-R Verbal Paired Associates subtest uses
pairs of nouns that are first heard and learned. The test requires participants to recall the paired word after being presented the first word of the pair, both immediately and following a long delay. Similarly, the WMS-R Visual Paired Associates subtest uses colours paired to figures. These colours are then recalled when given the figures, both immediately and at a later time.

To assess executive functioning, tasks included: the Wisconsin Card Sorting Task (Heaton et al., 1993) and the Trail Making Test Parts A and B (Corrigan and Hinkeldey, 1987). The Wisconsin Card Sorting Task requires matching of the cards in a deck to four key cards that are supposed to represent characteristics which include colour, feature, and number presented. The participant needs to determine what characteristic is currently being requested through trial and error, with the matching characteristic changing throughout the task. The Part A Trail Making Test requires drawing lines from a number to the next number in consecutive order, and Part B test requires drawing lines from a number to a letter to a number to a letter and so on in consecutive order for both the numbers and the letters as fast as possible. Tasks that assess phonemic and semantic fluency include the FAS and Animals task (Tombaugh et al., 1999). In FAS test, participants are required to generate as many words they can think that begins with the letters F, A, and S in one minute duration per letter. In Animals, participants are required to generate as many names of any animals as possible in one minute.

To assess intellectual functioning, participants completed the Wechsler Abbreviated Scale of Intelligence Matrix Reasoning subtest (Wechsler, 1999), and Shipley Institute of Living Scale (SILS; Shipley, 1940). To assess general cognitive function, Wechsler Adult Intelligence Scale III Digit Span, Mental Control, and Arithmetic subtests (Wechsler, WAIS-III, 1997) were tested. The Wechsler Abbreviated Scale of Intelligence Matrix Reasoning subtest is a test of matching and pattern observation skills, and it requires correctly identifying a missing piece from a picture from five choices given. The Shipley Institute of Living Scale (SILS; Shipley, 1940) has both verbal and abstract subtests and these serve as a quick estimate of intelligence. The SILS verbal subtest assesses vocabulary skills and the abstract subtest assesses the ability to identify patterns. The WAIS-III Digit Span subtest requires immediate repetition, either forward or backward, of a set of digits that are heard.
The WAIS-III Mental Control subtest test the reaction time of accessing basic knowledge such as counting 1-20 and stating the alphabet, the days of the week, the months of the year, etc. The WAIS-III Arithmetic subtest tests reaction time in solving arithmetic problems verbally.

Finally, neuropsychological measures of mood and daily independence were included. For mood assessment, the Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983) was used which assesses the level of anxiety and depression based on statements for which subjects identify how they have been feeling for the past week. Lastly, to assess subjects’ independence in activities of daily living, the Physical Self-Maintenance Scale and the Instrumental Activities of Daily Living questionnaires (Lawton and Brody, 1969) were used.

2.8 Summary and Thesis Orientation

We have chosen a hypertensive patient group that are free of other health complications to test the effect of glucose ingestion, relative to placebo, on measures of neural activation and 2-back task performance. Hypertension is known to contribute to cognitive decline beyond effects of normal aging, and glucose may help ameliorate the decline. The goal was to identify the underlying mechanisms facilitating and/or enhancing cognitive function in this patient group when given glucose. The PFC, a brain region related to executive functioning, was of particular interest as previous research has been inconsistent in showing that this region is sensitive to glucose ingestion during task performance in older adults. The main focus was to use fMRI to explore how patterns of neuronal activation and cognitive function differ in brain regions associated with a 2-back working memory task due to effects of glucose versus placebo (sweetener) consumption. We also explored how adverse health measures like LDL and IR associate with performance and brain activation. This is the first study to explore cognitive function in hypertensive older individuals under basal (placebo) and glucose conditions using fMRI. These findings will shed light on the contributions of glucose ingestion and underlying differences in LDL and IR, to variability in performance and brain neural activity during a 2-back working memory task.
Chapter 3
Hypotheses and Aims

3 Hypothesis and Aims

Pure glucose (60g) and carbohydrate intake can enhance cognitive functions in older adults; however, less is known about the neural mechanism(s) and neural networks underlying this cognitive enhancement. This thesis undertook an experiment to address the hypotheses that:

1) Participants will perform better on 2-back task when provided with a glucose drink compared to a placebo drink.
2) There will be greater changes in task-induced neuronal activation in brain regions associated with the 2-back task (ie PFC) following glucose ingestion compared to placebo.
3) Higher insulin resistance (IR) and higher dyslipidemia (LDL) will be associated with poorer cognitive performance and lower levels of task associated BOLD responses under basal (placebo drink) conditions and greater brain response to glucose drink ingestions, relative to placebo, in turn leading to better performance.

Specific aims:
(1) Identify differences in the patterns and magnitude of regional neuronal activation associated with 2-back task performance by comparing the fMRI BOLD signals following glucose and placebo drink consumption.
(2) Examine differences on measures (i.e. scores) of 2-back task performance between treatments.
(3) Explore the association between treatment-induced changes in fMRI BOLD signal and treatment-induced changes in performance
(4) Conduct a pilot exploration on the effect of participants’ IR and blood LDL levels on task performance and brain activation patterns under placebo and glucose conditions.
4 fMRI Study

Understanding Glucose-induced Neuronal Activation During Executive 2-back Task Performance In Hypertensive Otherwise Healthy Older Adults: An FMRI Study

4.1 Abstract

Background: Aging and age-associated chronic disorders insulin resistance (IR), hyperlipidemia (measured by low density lipoprotein (LDL)) and hypertension, contribute to cognitive decline. By contrast, glucose intake enhances cognitive performance in older adults. Yet the underlying neural mechanism(s) associated with this enhancement in performance and the effect of underlying chronic disorders on treatment response have yet to be identified.

Hypothesis: Using BOLD fMRI during a 2-back working memory task, we expect that glucose ingestion 1) improves 2-back task performance 2) increases task-associated neuronally-mediated BOLD activity, and 3) that IR and LDL influence the changes in performance and activations.

Method: Within-subject cross-over design in 12 healthy hypertensive older adults (mean age: 75 ± 7 years; 7 F/ 5M). Day 1: Collected clinical data and conduct neuropsychological battery to exclude mild cognitive impairment or dementia. Days 2&3: Subjects were counterbalanced to receive either a glucose (60g) or placebo drink first, then took the alternate drink in the second session. A 2-back and a breath-hold task were performed in a 3.0 Tesla MRI scanner post drink.

Results: Task performance and task associated activations did not differ between glucose and placebo drinks. The left and right precuneus, left cingulate, and left insula were identified to
be significant in target minus nontarget (T-NT) %BOLD task associated activations, where nontarget activations were greater compared to target activations (p_{cluster-corrected} < 0.05). Greater nontarget activations in the left ($r^2=0.63$, $p<0.01$) and right precuneus ($r^2=0.34$, $p=0.04$) activations predicted faster RTs while greater nontarget activations in the left precuneus activations predicted better accuracy ($r^2=0.56$, $p<0.01$) and discrimination ($r^2=0.68$, $p<0.01$), relative to target. Higher IR and lower LDL predicted better performance in all 3 measures ($p$’s <0.05). IR was exponentially and negatively associated with T-NT %BOLD activations in the right precuneus ($r^2=0.47$, $p=0.02$), and LDL was linearly and negatively associated with T-NT % BOLD ($r^2=0.37$, $p=0.03$) activations in left cingulate. Only left precuneus’ activity predicted accuracy and discrimination, yet it was not associated with either IR or LDL.

**Conclusion:** While no effect of glucose ingestion on task performance or task-relevant neural activation patterns were observed, this study uniquely observed that IR and LDL, even within a relatively healthy clinical range, associated with measures of 2-back performance and task-relevant neural activation patterns. Of particular importance was the task activation in the right precuneus as it showed both sensitivity to IR predicted task RTs to targets.

### 4.2 Introduction

Both population aging and age-associated chronic disorders, including insulin resistance, hyperlipidemia and hypertension, are prominent in North American and both contribute to cognitive decline. Aging itself is associated with decrements in a variety of cognitive domains, but especially hippocampal episodic memory and prefrontal executive functioning (Drag and Bieliauskas, 2009; Raz, 2000). These decrements are associated with diminished neural responses during cognitive task performance (Daselaar *et al.*, 2006; Cabeza *et al* 2001, 2004, Cabeza *et al*., 2000; Grady *et al*., 2002). While it is acknowledged that aging affects the functioning of both the hippocampus and prefrontal cortices, the prefrontal executive functioning is the focus on this study and draws on the n-back task (Owen *et al*., 2005) as a measure of working memory. Executive functioning is integrating memory to generate information to perform an action which involves working memory, attention, problem solving, and task switching, relying on multiple brain regions. The n-back task is
relatively robust in terms of regions the task recruits, including the precuneus, inferior parietal lobules, bilateral premotor cortex, dorsal cingulate, medial premotor cortex, and various regions of the prefrontal cortex (Owen et al. 2005).

Many chronic health conditions which are common in older adults are associated with further decrements in working memory, over and above that associated with age per se. Though hypertension, lipid levels, and IR may be interrelated physiologically, as evident in the metabolic syndrome, it is likely that they initially affect brain neural responses and cognitive performance by somewhat independent mechanisms; yet ultimately contributing to changes in both neurons and cerebrovasculature.

Hypertension alters brain vasculature leading to inadequate regulation of cerebral blood flow (CBF) which in turn disrupts nutrient and oxygen delivery and compromises neuronal health and function (Meusel et al., 2012). For example, on working memory tasks, poorer processing speed and recognition memory are associated with higher systemic blood pressure (Pase et al., 2013; Gianaros et al., 2006). From a neuroanatomical perspective, people with hypertension have a greater risk of frontal white matter hypertensities (WMH) (Raz et al., 2003; Gianaros et al., 2006) and lower regional grey matter volumes within the anterior cingulate and middle temporal gyrus (Gianaros et al., 2006). Lower volumes in these regions are in turn associated with poorer Stroop processing and working memory independent of other risk factors (Pase et al., 2013; Gianaros et al., 2006). It is likely that both the neuroanatomical changes, themselves, in combination with impaired nutrient and oxygen delivery necessary to support the metabolic demands during task performance (Gianaros et al., 2005; Jennings et al., 2005), contribute to the cognitive deficits.

Insulin resistance (IR) is also highly prevalent in older adults (Imamura et al., 2013) and is associated with increased risk of cognitive decline and dementia, even in those within the borderline high range (>2.6) (Qu et al., 2011, Bruehl et al., 2010; Ganzelas et al., 2010). Structural imaging studies show that people with poorer gluco-regulation have greater atrophy in structures relevant to aging and neurodegenerative processes, particularly within the hippocampus and amygdala (Cherbuin et al., 2012). These structures are known to be
involved in episodic memory, declarative memory, and emotional processing (Cherbuin et al., 2012). The mechanism of how IR contributes to poorer performance was first proposed by Craft (2007), where she and colleagues suggested that peripheral hyperinsulinemia may cause central nervous system inflammation and dysregulation of amyloid beta processing. Their work showed that higher plasma insulin levels were associated with increased cerebral spinal fluid levels of amyloid beta and inflammatory agents, both of which contribute to the neuropathology of Alzheimer’s disease. It is now recognized that other metabolic disturbances, heightened oxidative burden and impaired vascular function are also important contributors to brain dysfunction in those with IR and overt type 2 diabetes mellitus (T2DM) (Meusel et al., 2012; Gadzinski et al., 2010; Mortimer et al., 2010; Bruehl et al., 2009) and have been argued to play a causal role in the development of Alzheimer’s disease and vascular dementia.

Lastly, low density lipoprotein (LDL) cholesterol status is consistently associated with the level of cognitive function and indicators of brain health (Lesser et al., 2011; Oliveira et al., 2011; Vikarunnessa et al., 2013; Hui et al., 2012). Elevated LDL levels (>3.4 mmol/L) contribute to cardiovascular risk and oxidation and may in turn disrupt signalling in the brain and contribute to dementia associated neuropathology. In animal studies, a high cholesterol diet, relative to a basal diet (5% fat with no added cholesterol) led to greater hyperphosphorylation of tau, a hallmark of Alzheimer disease neuropathology, and poorer performance on working memory tasks (Bhat et al., 2012). In a functional magnetic resonance imaging (fMRI) study of a 2-back working memory task, serum total cholesterol associated with extent of task-related activation. Mean task associated activation intensity in the left inferior parietal lobe, right superior frontal gyrus, and right middle frontal gyrus, known for their contribution to working memory function, were lower in people with higher total cholesterol, relative to those with normal total cholesterol. Furthermore, lower activation in the left inferior parietal lobe was associated with poorer task performance and slower RT (Gonzales et al., 2011; Macander et al., 2011). These data suggest that high serum cholesterol levels decrease performance on tests of executive function and may do this through altering brain activation. Whether the differences in task-related brain activation relates specifically to elevations in LDL, or whether the altered fMRI responses were
secondary to changes in cerebro-vascular, rather than neuronal, responses have yet to be determined.

In summary, there is robust data demonstrating that the presence of hypertension, IR and elevated LDL contribute to brain structural abnormalities and deficits in performance on executive function tasks. However, there is a paucity of literature looking directly at whether these disorders predict changes in patterns of neural activation in association with cognitive task performance – although admittedly, their presence has been used as a covariate in a limited number of studies exploring the independent effect of age on neural activity and cognitive function.

The search for strategies to ameliorate cognitive deficits in the elderly has been around for decades. Treatments, especially through natural methods such as nutrition, to improve cognition in elderly are popular (Ferry et al., 2013). One of the most cited treatments is to ingest glucose. Glucose ingestion in older adults has been associated with enhanced learning and memory (Gold, 1995), especially in tasks involving episodic memory. Less consistently, some studies have shown that glucose may also benefit tasks of working memory and executive function (Messier et al., 2010). Which cognitive domains glucose acts on is still unclear and the effect of glucose ingestion may differ in magnitude in different age groups. For example, behavioural studies in younger adults showed glucose ingestion benefited non-domain specific aspects of task, such as task difficulty, indicating that glucose simply allows greater allocation of attentional resources by acting as additional cognitive fuel instead of having a specific effect on memory (Meikle et al., 2005). In contrast, behavioural studies in older adults consistently show glucose benefiting domain-specific episodic memory (Scholey, 2009). Given that glucose consumption generally improves hippocampal-dependent tasks in older adults (Kaplan et al., 2000; Kaplan et al., 2001) and less consistently is associated with improvements in prefrontal-dependent executive tasks, it suggests older adults do have a greater need for additional extra fuel to be provided during task performance – something which has also been demonstrated in animal studies (McNay et al., 2001). In sum, glucose facilitation is evident in both younger and older adults, where it seems to provide additional cognitive fuel to meet cognitive task demands.
Only one study explored task performance specific to episodic memory following glucose ingestion using fMRI in younger adults. This study demonstrate that fMRI is sensitive enough to monitor glucose-induced changes in brain activities in various regions of interest (Parent et al., 2011). For example, Parent et al found that glucose intake, relative to placebo, increased functional coupling between the activity of the hippocampus, amygdala, and other brain networks during an emotional picture encoding task, a task of episodic memory involving the amygdala and hippocampus. Whether these observations extend to an older population and how the presence of chronic disease conditions such as hypertension, IR and LDL may affect the neuronal response to glucose ingestion remains unexplored.

This study explores working memory in response to glucose in otherwise healthy medication-controlled hypertensive older adults. We used BOLD fMRI during a 2-back task to test the hypotheses that glucose ingestion 1) improves 2-back task performance on reaction time (RT), accuracy, and discrimination 2) increases task-associated neuronally-mediated BOLD activity, and 3) that the changes observed are influenced by physiologic factors specifically IR and LDL.

To ensure that changes in BOLD activation were not confounded by decrements in vascular health in our hypertensive participants, data were adjusted for cerebrovascular reactivity (CVR), measured during a breath hold task. By adjusting for CVR, our fMRI BOLD activations analysis can be attributed to neuronal induced activity solely. Additionally, overall associations with white matter hyperintensity (WMH) burden and overlap of task-relevant regions and WMH locations were explored to exclude the contribution of WMH to the observed results. Due to the novelty of fMRI technology and a lack of fMRI studies examining the effects of acute glucose ingestion on working memory function, this study was the first to assess the neural effect of glucose intake and its association with executive functioning in hypertensive elderly. Understanding these are first steps towards the development of effective strategies for the retention and recovery of cognitive function with aging.
4.3 Methods

4.3.1 Participants

All procedures were approved by the Research Ethics Board at Baycrest. In total, 20 participants were recruited into the study, with 8 being eliminated from the analyses because of poor breath hold compliance (n=3), excess movement resulting in poor physiological data (n=1), scoring within the mild cognitive impairment range (n=3), and an overly high BOLD activation that was more than 2 standard deviations above the mean (n=1). A total of 12 participants (mean age 75±7 years, 7 females and 5 males) were included in our final analyses. The inclusion criteria included being diagnosed with medication controlled hypertension for at least two years. Hypertension medications were restricted to long acting anti-hypertensives (i.e. angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), and diuretics). This captured the most common medications used in this patient population, did not restrict ourselves to a specific pharmacologic study (if only one medication was allowed) and eliminated possible fMRI changes that could be attributed to medication ‘withdrawal’ if shorter acting anti-hypertensives were included. Also, participants needed to have a fasting blood glucose within the normal range of ≤ 6.1 mmol/L and score within a cognitively normal range (screened first by Telephone Interview for Cognitive Status (TICS) (Desmond et al, 1994) and later confirmed to be within a normal range on the neuropsychological battery described below). The exclusion criteria included participants having hepatic disease, recent coronary heart disease, other significant medical or psychiatric disorders affecting cognition (e.g., stroke, major depressive disorder), taking medications that act on the central nervous system (e.g., depression, sleep disorders, migraine headaches), hormone replacement therapy, major inflammatory disorders (e.g. arthritis), inflammatory bowel disease, rheumatological disorders, heart failure, chronic lung disease, and having non-MRI safe device (e.g. implantation of metal devices). Participants enrolled in a prior study that used similar neuropsychological battery were also excluded. High cholesterol and hypo-or hyper-thyroidism were not considered as exclusion criteria if medically controlled.
4.3.2 Sessions and conditions

Participants visited the testing site at 9 a.m. on three separate occasions, one week apart and each following an overnight fast (10-14 hours to eliminate any possible meal related effects). A light breakfast was provided at the end of each session. Antihypertensive medications were allowed prior to testing and participants took their medications according to their normal schedule.

4.3.3 Day 1 Neuropsychological session

The first session entailed collection of biological and anthropometric measures, a neuropsychological assessment, exposure to an MR simulator machine, and practice of all experimental tasks. A fasting blood sample for measurement of hematocrit, cholesterol profile (total, LDL and high density lipoprotein (HDL) cholesterols), CRP, fasting glucose, fasting insulin and HbA1c was collected. All blood measures were conducted at the biochemistry laboratory of Mt Sinai Hospital, Toronto, ON. HOMA-IR was calculated based on fasting glucose and insulin levels as an estimate of IR (Matthews et al, 1985). Blood pressure (using a BpTRU Blood Pressure Monitor for a regular adult), weight, height and waist circumference were also taken. Participants then completed a neuropsychological battery to describe patient groups on the basis of performance measures and exclude those scoring within a mild cognitive impairment range. Tasks included tests of declarative memory, executive functioning, and intellectual functioning which are explained as follows:


*Executive functioning:* Wisconsin Card Sorting Task (Heaton et al., 1993) and the Trail Making Test Parts A and B (Corrigan and Hinkeldey, 1987), FAS, Animals task (Tombaugh et al., 1999).

*Intellectual and general cognitive functioning:* Wechsler Abbreviated Scale of Intelligence Matrix Reasoning subtest (Wechsler, 1999), Shipley Institute of Living Scale (SILS;
Shipley, 1940), Wechsler Adult Intelligence Scale III Digit Span, Mental Control, and Arithmetic subtests (Wechsler, WAIS-III, 1997).

4.3.4 Days 2 & 3 Experimental sessions
During the experimental sessions, participants entered the MR suite, changed into a gown, and were fitted with MR-compatible glasses if necessary (eye sights of refractory errors up to ±6 diopters were corrected). The participants then received either a glucose drink or placebo drink. Drink assignment was counterbalanced such that half of the enrolled subjects received the glucose drink and half received the placebo drink in the first fMRI session. The alternate drink was then provided on the second experimental session. Participants then practiced the experimental tasks in the MR simulator.

The glucose drink contained 71 g of dextrose powder (equivalent of 60 g of glucose), 2 teaspoons (10 ml) of lemon juice for flavour, and 290 ml of cold bottled water. The placebo drink was similar to the glucose drink, except the dextrose powder was replaced with 23.7 g of sodium saccharin.

4.3.5 Blood glucose measurements
During the two scanning sessions, blood glucose levels were measured at three intervals: the start of session 0 minute (before drink ingestion), 30 minutes post-drink ingestion and before entering the scanning room to begin fMRI set-up, and the end of the session, roughly at 120 minutes. Each measure was taken by pricking the participant’s finger with a disposable Unistik 3 normal lancet. A drop of blood was collected on the test strip and the blood-glucose level was measured using a One Touch Ultra 2 glucometer (LifeScan, Inc.). These measures were used to check participants’ fasting glucose for any abnormalities and to monitor changes in the participants’ blood glucose levels following the different drinks.

4.3.6 MR Scanning
The experimental scanning session protocol and timeline is outlined in Table 4.6.1. Roughly 45 minutes following drink ingestion, the participants were scanned in a 3.0 Tesla MRI scanner. After the brain localizer and reference scans, the 2-back working memory task commenced (approximately at 60 minutes after drink ingestion). There were 2 versions of
the 2-back task, and assignment of 2-back task versions within the MR session was counterbalanced across sessions, so participants would receive different task versions at each session to avoid familiarity. After completing the 2-back task, participants then completed a wordlist learning task (data not presented). Participants were then scanned for T1 structural scans, the breath-hold task, and FLAIR images. Following this, participants were removed from the scanner.

2-back task

A mixed block and event-related task design commenced approximately at 60 minutes post drink ingestion. The 2-back task consisted of 80 trials split into five blocks of 16 trials with a 9 second crosshair for participants to rest between blocks. On each trial, selected letters from A to Z (chosen to be perceptually distinct) were shown on an MR compatible screen using a 50-point black Arial font either in upper or lower case centred against a white background for 1500 ms followed by a jittered 500 or 2500 ms crosshair inter-stimulus interval (ISI). Participants were instructed to press one button if the current letter was the same as the letter seen two before, or press the other button if it was not. Throughout the task, 30 (6 per block) letter presentations were target trials (the letter was presented 2-back). The remaining were nontargets which included 40 (8 per block) letter presentations which were 0-back lures (the current letter did not match the preceding one, two, or three trials), 5 (1 per block) letter presentations which were 3-back lures (the current trial matched the letter three trials back), and 5 (1 per block) letter presentations which were 1-back lures. Button press responses were made with the index fingers of each hand, with assignment of left or right hand to response (to respond ‘yes’ or ‘no’ to 2-back) counterbalanced across sessions. The task took approximately 5 minutes.

For behavioural data collection of the 2-back task, button press responses were recorded using a Fibre-Optic Response Pad System (Current Designs Inc., 4 buttons available per hand). E-prime (Psychological Software Tools, Inc., Pittsburgh, PA) was used to control stimulus delivery and record responses and response time (reaction time) for each button press. Task accuracy for proportion of correctly identified targets and nontargets was subsequently determined using E-prime. Discrimination was assessed as Hit Rate (HR)
which is proportion of correctly identified targets, minus False Alarm Rate (FAR) which is proportion of incorrectly identified targets over the complete trial (HR-FAR). Reaction time (RT) was defined as the response time to 2-back targets. Task performance is a combination of accuracy, discrimination, and RT to targets.

Breath hold task

The breath hold task was used to obtain an average estimate of CVR for each subject. Participants performed six breath holds lasting 15 seconds each following 3 seconds expiration period with intermittent 30 seconds periods of normal breathing. The breath hold task is an established method to elicit a transient hypercapnic condition, resulting in a global increase in CBF (Katstrup, 1999) and is highly reproducible when monitored by the BOLD response with breath holds longer than 9 s in healthy populations (Magon et al., 2009; Murphy and Harris, 2010; MacIntosh et al., 2003). The results of the breath-hold were used as a covariate in our analysis.

White Matter Hyperintensities (WMH)

Small vessel disease burden was assessed based on the volume of WMH on the FLAIR image segmented using Fuzzy Lesion Extractor (FLEX) (Gibson E et al, 2010). The average WMH volume was obtained for each participant. WMH maps were also obtained to allow for visual inspection of WMH locations versus task-relevant brain regions.

4.3.7 Imaging parameters and equipment

Brain imaging was conducted in a 3.0 Tesla MRI scanner on a research-dedicated system at Baycrest (Magnetom Trio, 32-channel “matrix” configuration with Total Imaging Matrix, Syngo MR 2006T vb15 software, Siemens, Canada) through a head phased-array coil. Anatomical imaging (in addition to initial localizer scan) included: 1) a three-dimensional T1-weighted imaging [MPRAGE, field-of-view (FOV)=256 mm, 192 x 256 acquisition matrix, 1.0 mm³ voxels, bandwidth=200 Hz/Pixel, TI/TE/TR=1100/2.63/2000 ms, flip angle=9 degrees, 160 slices, averages=1, 1 concat, ipat/ref lines = 3/48, scan duration 4:16] to provide detailed T1 anatomical images for functional co-registration; 2) fluid-attenuated
inversion recovery (FLAIR) [field-of-view (FOV)=256 mm, 256 x 256 acquisition matrix, 1 x 1 x 3 mm voxels, TI/TE/TR=2500/94/13790 ms, 48 slices, Turbo factor=2, flip angle=150 degrees, fat saturation. Anatomical scans #1 and #2 were used in combination to calculate the total brain volume to quantify brain atrophy and detect WMH.

FMRI BOLD signal was obtained through a gradient echo T2*-weighted echo planar imaging (FOV=224mm, 64 x 64 acquisition matrix, 3.5 x 3.5 x 5.0 mm voxels, bandwidth = 2232 Hz/Pixel, TE/TR = 30/2000, flip angle = 70 degrees, 30 slices). The BOLD scans were aligned to the same orientation to facilitate co-registration. The time to repetition (TR) was 2 seconds for BOLD data. Behavioural tasks and fMRI was synchronized according to trigger pulses sent by the scanner. The participant’s head was restrained using a vacuum pillow that fits snugly inside the head coil.

CVR BOLD MRI response to a breath hold challenge was obtained with TR/TE=2000/30ms, matrix=64x64, FOV=200x200 mm², slice thickness=5mm, number of slices= 32, flip angle=90°, with 156 volumes, and lasting for an approximate total duration of 5 minutes and 20 seconds.

4.3.8 Statistics

Image analysis

Individual 2-back functional data preprocessing including correction for head motion, detrending of baseline fluctuations, spatial smoothing, deconvolution of hemodynamic responses, and transformation of activation maps to standardized anatomical space through 3dDeconvolve were done using standard Analysis of Functional Neural-Images (AFNI) procedures (Cox, 1996). The whole-brain group analysis was conducted using mixed effects linear model 3dLME (Chen, 2013) exploring a model of Task by Drink interaction while covarying for the effects of CVR and session. Cluster-size correction for multiple comparisons was carried out using 3dClusts to determine significance of each identified cluster based on averaged blur values of each individual’s data, a group mask created from
group average functional data, and the uncorrected thresholded p-value of the mask. The mean %BOLD change of each main effect of task regions (target minus nontarget) were extracted from the outputs of the 3dLME model using 3dROIstats in AFNI.

Individual CVR data analysis was conducted using FMRIB Software Library (FSL) fMRI expert analysis tool (FEAT). Pre-processing included motion and slice time correction for interleaved acquisition and a spatial smoothing with a Gaussian kernel of FWHM=5mm. Statistical analysis of the time-series was carried out using general linear modelling (GLM). An assumption was made, that in the absence of a response delay, the highest BOLD signal change corresponds to the highest arterial PaCO2 in the blood, which occurs at the end of the breath hold. The hemodynamic response delay was estimated as the time difference between the end of the breath hold and the peak of the BOLD signal following that breath hold.

**Blood Glucose and Task Performance Measures**

Repeated measures analysis of variance was used to compare differences in blood glucose levels at each time point (0 minutes (prior to drink ingestion), 30 minutes post drink, and 120 minutes post drink) within and across sessions using PROC MIXED in SAS (version 9.2). Student t-tests were then run to compare the effect of time, within each drink condition. The main effect of drink, after adjusting for session, on task accuracy, discrimination, and reaction time was determined using repeated measures in PROC MIXED in SAS (version 9.2).

**Regression analysis to explore relationships between LDL, brain activation, and performance**

The %BOLD change, behavioural performance, and metabolic data were then analyzed using SAS or Systat, to explore the relationships amongst brain activation, task performance, LDL, and IR through pearson r correlation while covarying for CVR. Data were subsequently analysed using PROC REG in SAS to explore possible exponential, rather than linear, relationships.
Sample size justification

The study was first powered to observe an effect of glucose on cognitive task performance and subsequently it was determined if this was a sufficient sample size to uncover changes in the BOLD signal. Estimation of statistical power in fMRI requires knowledge of the expected percent signal change between two conditions and estimates of variation (inter- and intra-subject). Using the 2-back working memory fMRI task on a 3 Tesla scanner, it has been shown that the minimum detectable change (MDC) of the percent increase in the BOLD signal from baseline within the right and left dorsal-lateral PFC for two sessions is between 0.3 and 0.6% (Goodyear and Douglas, 2008). This BOLD MDC is at the level of changes that we expect from our placebo vs. glucose condition. A percent signal change of 0.5% at a single voxel level required at least 12 participants to ensure a power of 0.80 at an alpha of 0.05, but this calculation was calculated assuming a block design (rather than event-related design), and a simple, within-subject comparison of two task conditions (Desmond and Glover, 2002). A more sensitive event-related analysis for group level fMRI, incorporating temporal autocorrelations and time of each block, indicates that at least 18 participants are needed to yield 80% power (Mumford and Nicholas, 2008). We initially recruited 20 subjects, allowing for a 5% loss of subjects due to unsuitable fMRI data; nevertheless we had to drop more than the anticipated number of subjects, leading to under powering. Given fMRI’s novelty in terms of examining glucose’s effect, no adequate data were available to allow for direct powering based on glucose-related BOLD responses during n-back task performance.

4.4 Results

4.4.1 Demographics

Five males and 7 females completed the study and were included in the analysis. Anthropometric measures including BMI, systolic and diastolic pressure, gender, waist circumference, height, and weight were recorded (Table 4.6.2). Blood measurements were also taken which included fasting glucose, HbA1c, fasting insulin, total cholesterol, HDL cholesterol, LDL cholesterol, and CRP. All blood measures of the study population were close to normal clinical ranges as identified by American Heart Association (Robbins et al, 2013; Germino, 2009), World Health Organization (Berman, 2010) or American Diabetes
Association (Diabetes Care, 2012). Values were also obtained for average CVR % BOLD change and WMH burden volume (Table 4.6.3). In all domains of the neuropsychological assessment, participants were within the normal range of cognitive functioning (Table 4.6.4), consistent with the inclusion criteria.

4.4.2 Blood glucose measurement during drink sessions

There was a significant drink by time interaction (F=19.32; p < 0.01) on blood glucose levels. Following the placebo drink, blood glucose levels stayed consistent and were not significantly different throughout the 3 time points (0, 30, 120 minutes) (t’s < 2.19; p’s>0.05). Following glucose consumption, blood glucose levels rose from 0 to 30 minutes post drink ingestion (t=6.74 p<0.01) and stayed consistent relative to the 30 minutes time point till the end of the scanning session at 120 minutes (t= 0.3 P=0.78) (Figure 4.7.1).

4.4.3 2-back behavioural performance

There was no effect of glucose ingestion, relative to placebo, on measures of A) Accuracy (proportions of correctly identified targets and nontargets) (F=1.1; P=0.4) B) discrimination (proportions of HR - FAR) (F= 1.8, P=0.17), or C) RTs to targets (F=0.86; P=0.59) (Figure 4.7.2).

4.4.4 Image analysis using AFNI

2-back whole brain voxel-wise analysis

Group BOLD data were analyzed with a task (target vs nontarget) by drink (glucose vs placebo) interaction model while adding CVR and session as covariates using 3dLME. All the resultant whole-brain statistical maps shown were thresholded at p\text{uncorrected} < 0.02. A significant main effect of task was observed in the left (BA 7; 81 voxels; p\text{cluster-corrected} < 0.01) and right precuneus (BA 31; 124 voxels; p\text{cluster-corrected} < 0.01), the left cingulate (BA 31/24; 66 voxels; p\text{cluster-corrected} < 0.01), and left insula (BA 13; 62 voxels; p\text{cluster-corrected} = 0.02) (Figure 4.7.3). For all of the identified brain regions, the nontarget event BOLD values were greater than target BOLD values.
A drink main effect was identified in the right inferior parietal lobule (BA 40; 67 voxels; p_{cluster-corrected} = 0.02) where glucose showed greater activation compared to placebo (Figure 4.7.4). There were no significant regions identified in the drink by task interaction.

To account for the potential contribution of between subject differences in CVR, each individual's whole brain average % BOLD change during the breath hold task was used as a covariate in the analyses. The data were subsequently visually examined to determine if regional specificity could be influencing outcome. CVR main effects (not shown) did not overlap with task or drink main effect regions. A similar approach was used to account for the potential contribution of between individual differences in overall WMH volume and/or location. WMH volumes were obtained from T1 structural white matter maps. As with CVR, the WMH map for each individual did not overlap with main effect task regions identified. Thus, our interpretation of BOLD data can be safely attributed to neural responses free of CVR or WMH effects.

4.4.5 2-Back – task regional analyses

To explore the inter-relationships amongst task activation, task performance and their association with IR and LDL, data were collapsed across drink conditions. Mean %BOLD change activations were extracted from brain regions identified in the task main effect (left and right precuneus, left cingulate, and left insula) using 3dROIstats for both placebo and glucose conditions separately and then averaged across drinks. Through regression analysis, we first explored if BOLD activations predicted performance (accuracy, discrimination, and RTs). Then we explored if LDL or IR predicted BOLD activations. Lastly, we explored if LDL or IR predicted performance.

2-Back reaction times (RT) to targets

Within the right precuneus, target minus nontarget (T-NT) % BOLD activation was linearly and positively associated with target RTs (Figure 4.7.5 A, r^2=0.34, p=0.04), while a similar,
positive, exponential association was observed within the left precuneus (Figure 4.7.5 A, \( r^2=0.63, p<0.01 \)). In both the left and right precuneus, lower T-NT % BOLD activation predicted faster RTs which were indicative of better performance. No associations were found between target RT with activation in left cingulate or insula.

With regards to blood measures, IR was exponentially and negatively associated with T-NT % BOLD activation in the right precuneus (after removing an outlier; Figure 4.7.5 B, \( r^2=0.47, p=0.02 \)). IR was also exponentially and negatively associated with target RTs (Figure 4.7.5D, \( r^2=0.34, p=0.05 \)). This indicated that the right precuneus’ sensitivity to IR may play a mediating role in the relationship between IR and target RTs. Other task associated region activations did not associate with IR.

LDL showed a linear negative relationship with T-NT % BOLD activation in the left cingulate (Figure 4.7.5 C, \( r^2=0.37, p=0.03 \)). LDL also had a positive exponential relationship with RTs to target (Figure 4.7.5 D, \( r^2=0.36, p=0.04 \)). However, there was no relationship between left cingulate activations and target RTs (not shown; \( r^2=0, p=1 \)); consequently it could not help to directly explain the slower target RTs in those with higher LDL. Other task associated region activations did not associate with LDL.

2-back accuracy and discrimination

Within the left precuneus, the T-NT %BOLD activation was exponentially and negatively associated with accuracy (Figure 4.7.6 A, \( r^2=0.56, p<0.01 \)) and discrimination (Figure 4.7.6 B, \( r^2= 0.68; p<0.01 \)). However, there was no relationship between LDL (not shown; \( r^2=0.01, p=0.38 \)) or IR (not shown; \( r^2=0.12, p=0.13 \)) with the T-NT %BOLD activation within left precuneus. No other task associated brain regions associated with accuracy or discrimination.

IR was associated with accuracy and discrimination. IR had a significant exponential and positive association with accuracy (Figure 4.7.6 C, \( r^2=0.45, p=0.02 \)) and an exponential positive association with discrimination (Figure 4.7.6 D, \( r^2=0.32, p=0.04 \)). Unexpectedly, these indicate that higher IR was associated with better performance.
Similarly, LDL was also associated with accuracy and discrimination. LDL had a significant exponential and negative association with accuracy (Figure 4.7.6 C, $r^2=0.62$, $p<0.01$) and discrimination (Figure 4.7.6 D, $r^2=0.56$, $p<0.01$). These indicate that lower LDL was associated with better task performance.

In sum, while lower LDL and higher IR were associated with better task performance, we were unable to identify a task associated brain region which showed both a relationship between task performance and sensitivity to either LDL or IR.

### 4.5 Discussion

The primary objective of this research was to explore the effect of glucose ingestion on 2-back performance (accuracy, discrimination, and RTs to target), its relationship to neural activation and potential modulation by physiologic factors, specifically IR and LDL. While no effect of glucose ingestion on task performance or task-relevant neural activation patterns was observed, this study uniquely observed that IR and LDL associated with all 3 measures of 2-back performance and task-relevant neural activation patterns. Of particular importance was the task activation in the right precuneus as it showed both sensitivity to IR and was predictive of task RTs to targets.

*Regions associated with task*

Task related activation, identified through a T-Nt %BOLD contrast, was observed in the left and right precuneus, left cingulate, and left insula. Not surprisingly, all of these regions are associated with working memory, and more specifically, an n-back task (Owen *et al.*, 2005). Our results suggest that these regions are responsible for differentiating between targets and nontargets.

Unexpectedly, the activation was greater in nontargets relative to targets. This could represent changes in attentional demands during the task and the relative proportion of target versus nontarget conditions. Specifically, there were more nontargets than targets; consequently participants were pressing the nontarget button more frequently and needing to
attend to nontargets more often than targets. Perhaps having more nontarget events required longer sustained attention for that condition, and hence required more activation than a rarer target event. This would result in lower T-NT %BOLD activation. Other studies supporting this notion found that persistent activations in a brain region associated with a n-back task were related to sustained attention and increasing working memory function (Ball et al., 2011).

Given that the 2-back working memory task involves attention, temporary storage, and manipulation of remembered information (Owen et al., 2005), it was anticipated that PFC regions would be identified in the task analysis (Tanj and Hoshi, 2001; Curtis and D’Esposito, 2003). The lack of PFC identification may reflect the nature of the event contrast in our study. Indeed, PFC activation was apparent in other fMRI studies where a target activation was contrasted to a baseline (ie. without task demand) (Owen et al., 2005). This is in stark contrast to our event related design where participants were engaged in the attentional demands and manipulation of remembered information of the task during both the target and nontarget events; only their ability to discriminate between and respond differentially to targets and nontargets was contrasted. Our results suggest that discriminating between and responding differentially to targets and nontargets does not heavily involve the PFC, or that the PFC is involved to the same extent during the target and nontarget events.

*Cerebrovascular Reactivity (CVR) and White Matter Hyperintensities (WMH)*

Between individual heterogeneity in the CVR and burden of WMH were predicted to occur in hypertensive older adults and could alter measures of %BOLD change and consequently confound any neural-only interpretation of the BOLD data. In an attempt to remove the contribution of these measures, the average drink-specific CVR, obtained from the breath-hold task, during both placebo and glucose drink conditions, was used as a covariate in the analysis. Additionally, regions identified in the main effect of CVR did not overlap with those associated with the 2-back task activation. Furthermore, average WMH volume was not correlated with any of our variables of interest and the regional distribution of WMH, within individuals, did not overlap with any of the n-back task associated brain regions.
Therefore, it is unlikely that either CVR or WMH affected the interpretation of the analysis since they do not specifically contribute to the task associated regions and overall between individual differences, at least in CVR, were accounted for by using it as a covariate in the analyses.

*Task related activations associated with performance*

The left and right precuneus are known to be involved in consciousness and work in conjunction with the posterior cingulate for motor-coordination (Vogt and Laureys, 2005). Greater activation in nontargets, relative to targets, in both the left and right precuneus predicted faster RTs. Likewise, but only in the left precuneus, the same activation pattern predicted higher accuracy and discrimination. Perhaps having more nontargets events consecutively one after another, relative to target events, required sustained attention which elicited a higher neural activation response. Our results suggest that the greater the activation of nontargets, the longer the sustained attention which translates to better performance. This is consistent with the notion that persistent activation in working memory associated brain regions (ie, PFC) increases performance on the n-back, through sustained attention and increased working memory function (Ball et al., 2011).

*Task related activations not associated with performance*

The left mid/posterior cingulate and the left insula were identified as 2-back task associated regions, but their activity did not associate with any performance measures. However, the left cingulate could be working in conjunction with other brain regions such as the precuneus to benefit performance. For example, the cingulate, precuneus and retrosplenial cortices (not identified in our task activation) regions make up the neural network correlates of consciousness (NNCC) (Vogt and Laureys, 2005) which is highly involved in cognitive processing and awareness. These regions are known have the highest level of brain glucose metabolism and cytochrome c oxidase activity. The anterior insula is known to be involved in task control and focal attention (Xin et al., 2013). All these functions are relevant to 2-back associated abilities. Whether they contributed to task performance through a more indirect route, not identified in the current analyses, is unknown.
Biological variables that affect activation or performance

Glucose in relation to task-activation and task performance

Our findings suggest no effect of glucose ingestion, relative to placebo, on either measures of 2-back task performance or task associated activation patterns. This lack of effect of glucose on measures of task performance was observed, despite literature suggesting that glucose benefits working memory and PFC function (Owen et al., 2011). It is plausible that our 2-back task may not be demanding enough or sensitive enough for glucose to have a visible effect in terms of task performance. Indeed, our participants were performing well already under the placebo condition. Furthermore, it is important to recognize that results from studies exploring the effect of glucose ingestion on working memory task performance are inconsistent. Indeed, our own previous studies failed to show a benefit of consuming 50g carbohydrate on an executive function task, Trail Making Test Part B, in older adults (Age = 63±9; Greenwood et al., 2003).

Despite not seeing a task-associated effect, glucose induced greater activations in the right inferior parietal lobule compared to placebo. This region has been consistently identified in an n-back memory task (Owen et al., 2005). Ravizza and others (2004) found that this region may support executive processing of phonological encoding—central to a variety of language tasks. Specifically, they suggest that the dorsal inferior parietal cortex may be important for phonological encoding, retaining temporal information in verbal, spatial, and working memory tasks. Given the region’s sensitivity to glucose ingestion, it would be interesting to explore neural activation patterns and task performance following glucose ingestion drawing on tasks more reflective of the right inferior parietal lobule’s function (i.e. phonological awareness task) in future experiments.

IR relationship with activation and performance

We showed higher IR predicted better accuracy, discrimination, and faster RTs. This was unanticipated since many studies showed an association between high IR and poorer task performance, throughout the continuum of IR through to overt type 2 diabetes (Qu et al., 2011, Bruehl et al., 2010; Ganzelas et al., 2010). One possible explanation for this unanticipated association relates to the fact that participants were tested under fasting
conditions and those with higher IR also had higher fasting glucose. Indeed, in our data, associations between fasting glucose, task performance, and neural activity were similar to those reported here for IR (data not shown). Thus, it is possible that higher blood glucose (within normal range) enabled participants to overcome demands of a task during a fasting state.

Our study is the first to explore the relationship between IR and functional activation. Higher IR was associated with a greater nontarget activation compared to target in the right precuneus, and in turn faster RTs. This activation pattern and performance relation is consistent with the notion of persistent activation during sustained attention leading to better performance (Ball et al., 2011). Our finding suggests that sensitivity of the right precuneus to IR (or fasting glucose as argued above) may play a contributory role to explain the relationship between IR and RTs. We however did not identify a region that could explain the relationship between IR and accuracy or discrimination. It is important to note that most of our participants’ IR levels were within a normal clinical range of less than 2.6 (Qu et al., 2011) and hence not reflecting any adverse effect of pre- or overt diabetes.

**LDL relationship with activations and performance**

Higher LDL predicted slower RTs and poorer accuracy and discrimination. Elevated LDL has long been known to associate with cognitive deficits in working memory (Vikarunnessa et al., 2013). Past studies have consistently shown that adverse clinical ranges of LDL contribute to cardiovascular risks, oxidation, and dementia (Gonzales et al., 2011; Hui et al., 2012; Vikarunnessa et al., 2011). Interestingly, though our study sample had a relatively normal range of LDL (most had <3.4 mmol/l), even higher LDL within this range predicted poorer performance in our hypertensive elderly population. Our results suggest that within this patient group, the brain may be exquisitely sensitive to blood LDL levels, perhaps reflecting a dual brain insult when combined with hypertension. Whether a similar association would be observed in normotensive older adults is not known.
In terms of mechanism, studies show that while peripheral LDL does not cross the BBB, it may affect the brain indirectly, and that much of the negative impact of elevated peripheral LDL may be related to increased byproducts of excess oxidation. These byproducts may in turn contribute to neural dysfunction (Lesser et al., 2011). Furthermore, a high fat and cholesterol diet was shown to deteriorate the central regulatory capacity by disrupting the BBB in rabbits (Hui et al., 2012). While this latter study focused on indicators of Alzheimer’s pathology as the outcome measure, disruption to the BBB could adversely impact neural functioning via a number of mechanisms. All these factors may explain why higher LDL associates with lower accuracy, discrimination, and slower RTs in a working memory task, as observed in our study.

Our study is the first to explore the relationship between brain activations and LDL levels within a relatively normal clinical range in older adults. Past studies show that middle aged adults (mean age = 51±6) with clinically elevated total cholesterol levels, compared to individuals with cholesterol levels within a normal range, had lower mean activation in task associated regions from baseline during a 2-back task (Pase et al., 2013). Though the finding may not directly contribute to the interpretation of our results because of differences in the activation contrast, age group, and cholesterol measures, this finding does provide evidence that blood cholesterol levels associate with alterations in brain activation. In our study, we found that higher LDL levels were associated lower T-NT % BOLD activation in the left cingulate. However, the left cingulate activation was not associated with any of our performance measures. Hence, LDL’s association with brain activity in the left cingulate did not explain the poorer performance measures predicted by LDL solely.

In conclusion, glucose did not benefit performance or alter functional task-associated activations. Nevertheless, this study uniquely observed that IR and LDL, even within a relatively clinically healthy range, were predictive of 2-back performance (accuracy, discrimination, RTs) and task associated neural activation in older adults with hypertension. Higher IR (perhaps indicative of higher fasting glucose) and lower LDL predicted better performance in a 2-back task. Furthermore, IR and LDL were also associated with activations in the right precuneus and left cingulate, respectively, suggesting that these
regions may be sensitive to these biological variables. Since the right precuneus activations also associated with RTs, this region may act as a mediator between IR and performance. However, there are also other brain regions such as the left precuneus where its neural activity predicted performance without being associated with either IR or LDL. These findings suggest that there are brain regions that are sensitive to biological measures and those that are not, yet they nevertheless predict performance perhaps through a different mechanism. Understanding the complex inter-relationships amongst glucose ingestion, metabolic variables (IR and LDL), brain activation, and cognitive task performance requires further exploration.
### 4.6 Tables

Table 4.6.1: Scanning session protocol and timeline. Steps 1-7 were completed prior to entering scanner. Steps 8-12 were completed in the scanner.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Blood Glucose Measure I</td>
<td>0-3</td>
</tr>
<tr>
<td>2 Drink consumed (Glucose or Placebo)</td>
<td>3-5</td>
</tr>
<tr>
<td>3 Practice 1-back</td>
<td>5-8</td>
</tr>
<tr>
<td>4 Practice 2-back</td>
<td>8-11</td>
</tr>
<tr>
<td>5 Practice Word List Learning and Recall</td>
<td>11-30</td>
</tr>
<tr>
<td>6 Blood Glucose Measure II</td>
<td>30-33</td>
</tr>
<tr>
<td>7 Change into gown</td>
<td>33-43</td>
</tr>
<tr>
<td>8 Enter fMRI scanner and set up</td>
<td>43-58</td>
</tr>
<tr>
<td>9 Brain localizer and reference scans</td>
<td>58-63</td>
</tr>
<tr>
<td>10 Test 2-back</td>
<td>63-68</td>
</tr>
<tr>
<td>11 Test Wordlist Learning and Recall</td>
<td>68-98</td>
</tr>
<tr>
<td>12 TI Structural and FLAIR scans</td>
<td>98-118</td>
</tr>
<tr>
<td>13 Out of Scanner</td>
<td>118-125</td>
</tr>
<tr>
<td>14 Word List delayed recall</td>
<td>125-127</td>
</tr>
<tr>
<td>15 Blood Glucose Measure III</td>
<td>127-130</td>
</tr>
<tr>
<td>16 Change back to regular clothes</td>
<td>130-140</td>
</tr>
</tbody>
</table>
Table 4.6.2: Participant characteristics

<table>
<thead>
<tr>
<th>N=12</th>
<th>Participant averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Yrs)</td>
<td>75±6</td>
</tr>
<tr>
<td>Education (Yrs)</td>
<td>15±3</td>
</tr>
<tr>
<td>BMI</td>
<td>26±3</td>
</tr>
<tr>
<td>Systole (mmHg)</td>
<td>141±18</td>
</tr>
<tr>
<td>Diastole (mmHg)</td>
<td>76±12</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>5/7</td>
</tr>
<tr>
<td>Circumference (cm)</td>
<td>92±9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167±8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72±11</td>
</tr>
</tbody>
</table>

Data are mean ± SD for the 12 study participants.

Table 4.6.3 Blood Measures, cerebrovascular reactivity (CVR) and white matter hyperintensity (WMH)

<table>
<thead>
<tr>
<th>N=12</th>
<th>Participant average</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>5.3±0.3</td>
<td>3.9-5.6</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>0.057±0.002</td>
<td>&lt;0.057</td>
</tr>
<tr>
<td>Fasting Insulin (pmol/l)</td>
<td>53±19</td>
<td>37.7to 173</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.83±0.73</td>
<td>&lt; 2.6</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.26±1.1</td>
<td>&lt; 5.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.46±0.5</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.75±0.4</td>
<td>&gt;1.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.08±0.9</td>
<td>&lt;3.4</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.2±1.5</td>
<td>1-3</td>
</tr>
<tr>
<td>CVR (% BOLD change)</td>
<td>1.6±0.26</td>
<td>Not applicable</td>
</tr>
<tr>
<td>WMH (cc)</td>
<td>1.03±1.1</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

Data are mean ± SD for the 12 study participants. Values for normal ranges were obtained from: American Heart Association for LDL, HDL, total cholesterol, CRP, Triglycerides; American Diabetes Association for fasting insulin and HbA1C, World Health Organization for fasting glucose, and Qu et al for HOMA-IR (2011).
Table 4.6.4 Neuropsychological Measures

<table>
<thead>
<tr>
<th>Declarative memory:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT-II Learning Trials 1 - 5</td>
<td>10.92±4.1</td>
</tr>
<tr>
<td>CVLT-II Short Delay Free Recall</td>
<td>10.5±4.4</td>
</tr>
<tr>
<td>CVLT-II Short Delay Cued Recall</td>
<td>10.25±3.5</td>
</tr>
<tr>
<td>CVLT-II Long Delay Free Recall</td>
<td>10.92±3.1</td>
</tr>
<tr>
<td>CVLT-II Long Delay Cued Recall</td>
<td>10.58±3.6</td>
</tr>
<tr>
<td>WMS-III Logical Memory I, Immediate Recall</td>
<td>13.17±2.4</td>
</tr>
<tr>
<td>WMS-III Logical Memory II, Delayed Recall</td>
<td>13.3±2.5</td>
</tr>
<tr>
<td>WMS-III Faces I Recognition</td>
<td>11.42±0.85</td>
</tr>
<tr>
<td>WMS-R Verbal Paired Associates I, Immediate Recall</td>
<td>10.75±3.0</td>
</tr>
<tr>
<td>WMS-R Verbal Paired Associates II, Delayed Recall</td>
<td>12.25 ± 2.0</td>
</tr>
<tr>
<td>WMS-R Visual Paired Associates I, Immediate Recall</td>
<td>12.67 ± 3</td>
</tr>
<tr>
<td>WMS-R Visual Paired Associates II, Delayed Recall</td>
<td>12.42 ± 1.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Executive Functioning</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phonemic Fluency (FAS)</td>
<td>10.83±3.4</td>
</tr>
<tr>
<td>Semantic Fluency (Animals)</td>
<td>11.67±2.7</td>
</tr>
<tr>
<td>Trails Making Test A</td>
<td>11.9±0.6</td>
</tr>
<tr>
<td>Trails Making Test B</td>
<td>11.0±0.4</td>
</tr>
<tr>
<td>Wisconsin Card Sorting Test – modified Categories</td>
<td>12.17 ± 1.3</td>
</tr>
</tbody>
</table>
### Intellectual Functioning

| Test                                      | Score  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WASI Matrix Reasoning</td>
<td>13.17 ±2.5</td>
</tr>
<tr>
<td>SILS Vocabulary</td>
<td>11.42 ±2.1</td>
</tr>
<tr>
<td>SILS Vocabulary Full Scaled IQ equivalent</td>
<td>110 ±11</td>
</tr>
</tbody>
</table>

### General Cognitive Functioning

| Test                                      | Score  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WMS-III Mental Control</td>
<td>14.17 ±0.9</td>
</tr>
<tr>
<td>WAIS-R Arithmetic</td>
<td>12.08 ±0.8</td>
</tr>
<tr>
<td>WAIS-III Digit Span Total</td>
<td>10.92 ±0.9</td>
</tr>
</tbody>
</table>

Values are the scaled score ± SD

### 4.7 Figures

**Figure 4.7.1:** Participant’s blood glucose concentrations during the glucose and placebo sessions. Shaded blue area represents the time where participants were in the MR. * represents significant difference from time zero for the glucose drink only. (Error Bars = SD)
Figure 4.7.2: Impact of drink ingestion on 2-back A) accuracy, B) discrimination, and C) RT to targets. Data are mean ±SD for 12 participants. There was no significant difference between drinks on any of the 2-back performance measures.

Figure 4.7.3: A main effect of task (target < nontarget) was apparent in the A) right precuneus B) left precuneus C) left cingulate and D) left insula. There was no drink*task effect. *R and L stands for Right and Left; A and P stands for Anterior and Posterior.
Figure 4.7.4: Activation in the right inferior parietal lobule was greater following glucose ingestion, relative to placebo. *R and L stands for Right and Left; A and P stands for Anterior and Posterior.

Figure 4.7.5: (A) T-NT %BOLD activations within the left (p<0.01) and right (p=0.04) precuneus vs. RTs to target. (B) IR vs. T-NT %BOLD activation within the r precuneus (p=0.02) (C) LDL vs. T-NT %BOLD activations within the left cingulate (p=0.03) (D) LDL (p=0.04) and IR (p=0.05) vs. RTs to target.
Figure 4.7.6: (A) left precuneus T-NT % BOLD activation vs. Accuracy ($p<0.01$) (B) left precuneus T-NT % BOLD activation vs. Discrimination ($p<0.01$) (C) IR ($p=0.02$) and LDL ($p<0.01$) vs Accuracy (D) IR ($p=0.04$) and LDL ($p<0.01$) and vs Discrimination
Chapter 5
General Discussion

5 General Discussion

5.1 Major findings
This thesis focussed on how to improve cognition in older adults with hypertension. Using fMRI monitoring a 2-back working memory paradigm, we found that 60g of glucose did not alter task associated brain activity or improve performance in accuracy, discrimination, or RT in a hypertensive elderly population. We defined our task associated activations to be T-NT %BOLD contrast in our event related design of 2-back, which is reflective of an ability to switch between targets and nontargets. Hence, our task contrast may not capture all the regions involved in the 2-back task, but only the ones that have significant T-NT % BOLD activations. Nontarget activations were greater than target activations indicative of greater sustained attentional demands likely due to the relatively higher proportions of nontargets, relative to targets.

Task associated regions identified, which were free from WMH pathologies and effects of CVR, include the left and right precuneus, the left cingulate and the left insula. However, only the left and right precuneus activations were associated with performance. Greater nontarget activations, relative to target, were associated with faster RTs. Similarly, but only in the left precuneus, greater nontarget activations were associated with higher accuracy and discrimination. In sum, greater nontarget activations relative to target activations in the left and right precuneus predicted better performance. This is consistent with the notion that persistent activation (ie. during nontarget events) was associated with better n-back performance likely due to increased sustained attention and working memory (Ball et al, 2011). The left cingulate and left insula did not associate with performance and whether they contributed to task performance through a more indirect route, not identified in the current analyses, is unknown.
Unexpectedly, higher IR, within a relatively healthy range, was associated with better accuracy, discrimination, and faster RTs. Higher IR was associated with a lower T-NT % BOLD change (or greater nontarget activations relative to target) in the right precuneus, and faster RTs. Our finding suggests that sensitivity of the right precuneus to IR (or fasting glucose) may play a contributory role to explain the relationship between IR and RTs. No brain region was found to explain the relation of IR with accuracy or discrimination.

Higher LDL predicted slower RTs and poorer accuracy and discrimination. Interestingly, though our study sample had a relatively normal range of LDL, we found that higher LDL within this range predicted poorer performance in our hypertensive elderly population. In terms of brain activation, higher LDL levels were associated lower T-NT % BOLD in the left cingulate activation. However, since the left cingulate activation was not associated with any performance measures, LDL’s association with brain activity in the left cingulate did not explain the poorer performance measures predicted by LDL solely.

5.2 Relation of major findings to current knowledge

Hypertension is known to exacerbate the effects of healthy aging leading to even greater structural and cognitive decline beyond healthy aging (Freitag et al., 2006; Kilander et al., 1998; Kivipelto et al., 2001). Imaging studies demonstrate that hypertensive individuals have reduced regional cerebral blood flow (rCBF) and cerebrovascular dilation resulting from fundamental disruptions to CVR (Molina et al., 1999; Ficzere et al., 1997; Girouard and Iadecola, 2006). However, there is only a modest number of studies on hypertension which used imaging, and most are confined to anatomical imaging and brain volume measures rather than functional imaging. Results from structural analyses suggest that people with hypertension have lower regional grey matter volume within the anterior cingulate and middle temporal gyrus and greater WMH burden (Raz et al., 2003; Gianaros et al., 2006), both of which can contribute to poorer working memory task performance. However, what happens in terms of neural response free of these hypertension associated structural deficits during task performance are unknown. This study is the first to explore neural function in hypertensive older adults using fMRI in a working memory task and to account for the
potential contributions of CVR and WMH to make inferences about neural activity during task performance.

Nutrients acting as cognitive boosters for elderly have been popularized for the past two decades, and glucose is one of the most cited nutrients and most robust in its effect. In older adults, the extent of glucose’s benefit is primarily observed on tasks associated with episodic memory, where the effect is relatively robust (Kaplan et al., 2000; Kaplan et al., 2001). The effect on working memory has been inconsistent. We are the first to examine the effect of glucose ingestion on cognitive function and neural activation in older adults using fMRI. The dose (60g glucose) and timing of task administration relative to drink ingestion (60 minutes) was based on several studies, including our own, to maximize the likelihood of observing an effect of glucose in the elderly (Meikle et al., 2005; Scholey et al., 2001). However, glucose intake did not improve 2-back working memory performance or alter task associated activations compared to placebo. This observation is consistent with studies reporting no effect of glucose intake on working memory in older adults (Greenwood et al., 2003).

Despite the lack of effect of glucose intake on task relevant neural activity or 2-back performance, glucose intake did increase neural activation in the right inferior parietal lobule, relative to that observed under the placebo condition. These data clearly demonstrate that specific brain regions are sensitive to glucose ingestion. Yet, understanding the biologic and cognitive relevance of increased right inferior parietal lobule activation requires further investigation.

An n-back paradigm is a popular and reproducible method to explore working memory function. Several regions, including those identified in our analyses (left and right precuneus, left cingulate, and left insula), have been consistently identified in the literature review (Owen et al., 2005). The use of an event related design allowed us to identify regions specifically associated with differentiating between target and nontarget events. During 2-back task performance, nontarget activations were greater compared to targets, perhaps indicative of higher sustained attentional demands (ie persistent activation) of nontargets since there was a greater proportion of nontargets relative to targets. Uniquely, our study
explored the relationship between these functional activation patterns, task performance and relevant chronic disorders such as elevated LDL and IR common in older adults.

Our study is the first to associate IR with functional activation. Previous structural imaging studies showed that people with poorer gluco-regulation (either in IFG or T2DM) have greater atrophy in structures relevant to aging and neurodegenerative processes, particularly within the hippocampus and amygdala (Cherbuin et al., 2012; Bruehl et al., 2009; Abbatecola et al., 2006; Raz et al., 2005; Arvanitakis et al., 2006; Jongen et al., 2007). These structures are known to be involved in episodic memory, declarative memory, and emotional processing (Cherbuin et al., 2012). However, little is known about working memory related regions. We attempted to link individual differences in task associated neural activation, measures of task performance, and IR through a series of regression analysis. Even within a relatively healthy clinical range (<2.6), higher IR (perhaps indicative of higher fasting glucose) predicted better performance in a 2-back task (accuracy, discrimination, RTs) and was associated with task T-NT % BOLD activations in the right precuneus. Collectively, these results suggest that the sensitivity of the right precuneus to IR may play a mediatory role in explaining the relationships of IR, neural activations, and RTs.

There is a wealth of literature supporting the notion that LDL contributes to cognitive decline and is associated with increased risk of dementia (Gonzales et al., 2011; Lesser et al., 2011; Oliveira et al., 2011; Vikarunnessa et al., 2013; Hui et al., 2012). Clinically elevated LDL (>3.4 mmol) contributes to cardiovascular risks and oxidation. This in turn may disrupt signalling in the brain either through alternations in vascular integrity or enhancing levels of oxidative byproducts and inflammatory cytokines. In an fMRI study of a 2-back working memory task, associations were observed between serum total cholesterol and task associated activation. Specifically, mean activation intensity in regions associated with this working memory task (ie. inferior parietal lobe, superior frontal gyrus, and right frontal gyrus) were lower in people with higher total cholesterol, relative to those with cholesterol levels within a normal range. Furthermore, lower activation was associated with poorer task performance and slower RT (Gonzales et al., 2011). In our study, we found even within a relatively healthy clinical range, lower LDL predicted better performance in a 2-
back task (i.e. better accuracy, better discrimination, and faster RTs to target). However, we did not identify any brain region that was could explain the association of LDL and performance.

In sum, our findings bring a new perspective to the literature as most studies exploring the effects of chronic disorders, including IR and dyslipidemia, on brain function are limited to structural imaging and do not include functional activation measures. Similarly, studies with a focus on measures of cognitive performance either lack functional neuroimaging or fail to account for the contribution of physiologic measures, such as IR and LDL. We are the first to explore all three factors together - functional activity, performance, and IR/LDL during a 2-back working memory performance in a hypertensive healthy population. Our findings provide new insight into the effect of IR and LDL on brain activity and cognitive performance.

5.3 Implication

The aging Canadian population is estimated to place great demands on our health care system (Whitmer, 2007; Barrett-Connor, 2007; Gorospe and Dave, 2007; Vanhanen et al., 2006; Razay et al., 2006; Craft, 2005). More than fifty percent of elderly Canadians are hypertensive (Wilkins, 2010), and hypertension is known to further exacerbate cognitive decline beyond the effects of normal aging. However, most healthcare planning and budgets do not account for the fact that within each generation of aging Canadians, there is a significant growth in disability associated with cognitive decline and this may reflect the growing prevalence of obesity and its associated disorders in the Canadian population. It is important to find useful yet cost-effective strategies to ameliorate these cognitive deficits.

This study is the first fMRI study of hypertensive elderly to explore differences in neuronal activations associated with a working memory task performance following glucose drink and its association with blood measures of LDL and IR, to make inferences on how they contribute to performance and brain activity. We chose a prevalent patient population in North America to study. These results are important as they argue that more attention to monitoring blood lipid levels may be essential for hypertensive older adults to help minimize their risk of cognitive decline.
The aging population is going to have a large health burden in North America. This may be even higher than expected, given the rise in obesity and obesity-associated disorders such as higher dyslipidemia and IR. Thus, we need to better understand the influences of lifestyle associated disorders to develop preventative strategies. Adverse ranges of LDL and IR blood measures are already under the radar of clinicians. However, the fact that these blood measures even within a healthy range seem to predict changes in brain response and cognitive performance above and beyond mere deficits associated with vascular health related to hypertension may require clinicians to pay more attention and perhaps vigilance to control for these measures in this patient population. Lifestyle and diet changes can be implemented to improve IR and lipid status, hence it is important understand how they relate to cognition and brain function. By identifying the neural foundation and predictors of performance, it may lead to better practices in late life and reduce the cost burden to society. Eventually by targeting decrements in various cognitive processes, it will allow for the development of pharmacologic, nutritional, and other (e.g., exercise) interventions to benefit neuronal health and function in late life. Deeper understanding of the mechanism of neural and cognitive difficulties experienced by older patients with hypertension will help therapists on rehabilitation strategies to improve cognitive functioning in these adults.

5.4 Strengths and Weaknesses

The strengths of this study include being able to monitor brain activity during task performance using fMRI, and investigating its association with a nutrition intervention through influence of baseline IR and LDL. We also used state-of-the-art procedures to account for underlying changes to CVR and presence of WMH – factors which could confound our interpretation of changes in the BOLD signal. This allowed us to focus on our original research question of how neural activity changes during task performance.

To explore the effect of glucose intake, compared to placebo, on neural activity and performance, we pursued the gold standard protocol which is a within subject cross-over design where participants act as their own control. Because participants were well characterized, we are also the first to make inferences on how blood measures of IR and
LDL are related to functional activation and performance. Most studies in the past exploring cognitive performance or brain atrophy at best controlled for these factors and in most instances did not take them into account at all. By correlating IR and LDL to activity in task specific regions, we were able to identify those regions that are sensitive to IR or LDL and infer that they may act as a mediatory step to predict performance.

A limitation of the study is that we only recruited hypertensive older adults, with no healthier older or younger controls with which to compare. As such, we were unable to address the specific effect of hypertension. Also, we chose to explore a working memory task to shed light on the inconsistent literature; however, our findings are confined one specific cognitive domain and cannot be generalized to other cognitive functions. This will be the focus of future work.

A common limitation of most clinical studies is whether the sample is representative of patient populations. Also, because of our rigorous selection and exclusion criteria to recruit a relatively homogenous population, the study ended up being slightly under powered (N=12). Multi-session fMRI studies need to be well powered to accommodate relatively large intra-session subject variation in the fMRI signal. To achieve more significant findings after the stringent multiple comparisons, it has been recommended that a-priori regions of interest should be considered rather than whole brain voxel-wise analysis (Zanbelt et al., 2008). However, we chose to analyze our data as a whole brain analysis since we did not want to make a-priori hypothesis about regions involved in the specific T-NT %BOLD contrast explored using an event related design.

One design limitation that needs to be altered in future experiments is that the left and right hand button pressing (to identify targets versus nontargets) was counterbalanced across sessions, and within individuals. This made it impossible to account for any potential interactions between drinks, sessions and ‘hand’. While this may have influenced the outcome of analyses specifically exploring the effects of glucose versus placebo intakes, it was not an issue in our analysis where we combined the two drink sessions.
Finally, while this study failed to see an effect of glucose intake on 2-back task performance, it is possible that the 2-back task itself lacked the sensitivity to show a glucose effect. Indeed, participants were already performing well under the placebo condition, leaving little room to show an enhancement. To further explore glucose facilitation effects, a more difficult 2-back memory task could be designed to make certain that participants do not reach ceiling performance during the placebo condition. One approach would be to make the events shorter and change the letters to something less identifiable like fractals. Alternatively, a block, rather than event related, design could be used to enable a broader understanding of task performance, beyond the switching from nontarget to target; something which would likely allow for a greater understanding of the contribution of the PFC to task performance and its sensitivity to either glucose intake or physiological factors such as IR and LDL.

5.5 Future Directions

A primary objective of this study was to develop a greater understanding of the effect of glucose intake on brain activity and its association with cognitive performance. As a first step, this study was confined to a single working memory task. Nevertheless, it would be interesting to explore other cognitive domains, such as episodic memory by using a wordlist learning task, to capture hippocampal function. Episodic memory has been consistently shown to benefit from glucose intake. Indeed, participants were also scanned during a word list learning task during this study and the data are awaiting analyses. Furthermore, the hippocampi of each individual have already been traced, allowing for a region of interest analytic approach.

The use of fMRI to understand the effect of diet and nutrient ingestion on brain activity is clearly in its infancy. Results from the current study can provide an early building block to foster second generation studies exploring the role of nutrition in supporting cognitive function with aging. Indeed, there is a large body of epidemiologic literature arguing that a higher quality diet is an important contributor to retention of cognitive function with aging.
Yet, there is currently no understanding of whether diet is playing its role through changes in neural activity.

Second, while the current study was confined to older adults with hypertension, there is a host of other clinical populations at risk for cognitive decline and dementia. Of specific interest are those with mild cognitive impairment and type 2 diabetes. Both groups are at high risk for dementia and understanding the contribution of metabolic disorders to further cognitive decline would allow for the development of new treatment strategies.
Chapter 6
Conclusions

6 Conclusion
The primary objective of this thesis was to explore, using fMRI, the effect of glucose ingestion on 2-back performance (accuracy, discrimination, and RTs to target), its relationship to neural activation, and potential modulation by physiologic factors, specifically IR and LDL, in hypertensive otherwise healthy older adults.

The conclusions that can be drawn from this study include:

A 60 g glucose drink did not benefit 2-back working memory performance or alter T-NT % BOLD task associated activations, relative to a placebo drink.

The left and right precuneus, left cingulate, and left insula were identified as task associated regions. In all 4 regions, nontarget activations were greater compared to target activations. This may be indicative of sustained attentional requirement for continuously identifying nontarget events (since there were more nontarget events than target events).

Greater nontarget activations in the left and right precuneus predicted faster RTs, while greater nontarget activations in the left precuneus predicted better accuracy and discrimination, relative to targets.

Even within a relatively healthy clinical range, higher IR and lower LDL predicted better performance in all 3 measures of 2-back task performance.

IR and LDL were associated with task associated T-NT % BOLD activations in the right precuneus and left cingulate, respectively. This association was interpreted to suggest that these regions may be sensitive to those biological variables. Importantly, right precuneus also associated with RTs, suggesting that this region may act as a mediator between IR and performance.

Collectively, our results suggest that even within a relatively healthy clinical range, IR and LDL may affect performance and brain activity in an elderly hypertensive population.
7 References


