FRONTAL AND TEMPORAL LOBE STRUCTURAL AND FUNCTIONAL COMPLICATIONS IN ADULTS WITH TYPE 2 DIABETES MELLITUS

by

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Abstract

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Type 2 diabetes mellitus (T2DM) has been associated with cognitive impairment, with verbal memory reported as being the most frequently affected, but the underlying cerebral mechanisms remain unclear. There is increasing evidence suggesting that the frontal and temporal lobes may be preferentially affected in T2DM. To comprehensively characterize cognitive functioning and the extent of frontal and temporal lobe involvement in T2DM, the current study compared 46 late middle-aged and elderly patients with T2DM to 50 age- and education-matched controls with no evidence of insulin resistance or T2DM utilizing a comprehensive battery of neuropsychological tests and magnetic resonance (MR) based brain volumetric and diffusion tensor imaging assessment methods.

As expected, individuals with T2DM exhibited clear declarative memory impairment, with verbal memory being more prominently affected than visual memory, along with evidence of compromised verbal learning. Supporting these results, brain assessment revealed volume reductions restricted to the hippocampus bilaterally and extensive microstructural abnormalities in the temporal lobe. The observed verbal memory impairments were associated with compromised microstructural integrity of the left parahippocampal gyrus but not atrophy of the hippocampus. Importantly, this study demonstrated for the first time that systolic blood pressure may partially explain the association between body mass index and hippocampal volume reduction among individuals with T2DM who had normal blood pressure or untreated hypertension. In contrary, the frontal lobe was less extensively affected, with only attention being impaired and evidence of non-specific microstructural abnormalities without obvious tissue loss.

As hypothesized, among individuals with T2DM, glycosylated hemoglobin (HbA_{1C}) was inversely correlated with memory performance but was not the most significant metabolic factor after accounting for age and gender. Having hypertension also did not account for the observed attention deficits. A larger sample is necessary to better understand the independent contributions of metabolic factors and their possible interactions. To clarify the mechanisms underlying the observed impairments, future studies should better characterize the nature and extent of brain involvement, utilizing MR spectroscopy to understand neuronal metabolism and resting-state functional MRI to understand network connectivity. In addition, possible involvement of other potential factors, such as inflammation and impairment in vascular reactivity, should be explored.

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I would like to dedicate this dissertation to my beloved mother.

Having been an IT professional for many years, the decision to go back to school was not an easy one, but it was made with no regrets. I am heartily thankful to my loved ones and dear friends for being forever supportive and for never doubting my decision to embark on this new adventure.

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Table of Contents

List of Tables

List of Figures

Introduction

Research Question and Rationale

Type 2 diabetes (T2DM), in addition to a multitude of secondary health complications, including stroke, retinopathy, and neuropathy, has been linked to impairment in cognitive domains, including declarative memory, attention, executive function, and psychomotor efficiency (Allen et al., 2004; Strachan et al., 1997). Of note, verbal learning and memory, as well as psychomotor efficiency, are among the most consistently reported cognitive functions to be affected among individuals with T2DM. Given that cognitive decline is more prominent and more frequently reported in older than in younger patients with T2DM, it has been proposed that T2DM is associated with accelerated cognitive aging (Hassing et al., 2004a). Although these cognitive implications may seem secondary to the widely known peripheral complications, daily functioning may be compromised (Bruce et al., 2003), particularly in the older T2DM patient population, an issue that has not been systematically explored.

The vast literature on cognitive impairment in T2DM has not provided sufficient clues to its underlying pathological mechanisms. There is a small but growing literature demonstrating cerebral complications in adults with T2DM, which are mostly non-specific brain abnormalities, including cortical and subcortical atrophy (Ciammola et al., 2007; Manschot et al., 2006), enlarged ventricles, increased white matter lesions (WMLs; Manschot et al., 2006), and an elevated risk for lacunar infarction (Jongen et al., 2007). Furthermore, only a handful of these studies have evaluated the relationship between

cerebral complications and cognitive decline (e.g., Manschot et al., 2006; Yau et al., 2009); therefore, the extent and nature of brain involvement in the cognitive decline reported in patients with T2DM remains unclear.

Given that the frequently affected cognitive domains, including declarative memory, executive function, psychomotor efficiency, rely more heavily on the frontal and temporal lobes, it is possible that these brain regions are particularly vulnerable to damage in the presence of T2DM and possibly the common co-morbid conditions (e.g., Gold et al., 2007; Sahin et al., 2007). For example, volume reductions of medial temporal lobe (MTL) structures, including the hippocampus and amygdala, and white matter (WM) microstructural abnormalities have been described in patients with T2DM, and these brain anomalies have also been linked to reduced cognitive performance (Gold et al., 2007; Yau et al., 2009). Vulnerability of the hippocampus to poor glycemic control is also supported by evidence from animal models (Kamal et al., 1999). Other reports of frontal lobe complications, including gray matter (GM) volume reductions (Kumar et al., 2008), diffuse WM microstructural abnormalities (Yau et al., 2009), and altered cerebral metabolism (Modi et al., 2008; Sahin et al., 2007), also highlight the frontal lobe as another frequently affected region. Despite these data, the involvement of frontal and temporal lobe pathology in the cognitive impairments frequently observed in T2DM remains to be better elucidated.

To date, the mechanisms underlying the frequently observed cognitive impairments remain largely unknown. Cognitive impairment has also been reported in type 1 diabetes

mellitus (T1DM) and is often thought to be related to frequent episodes of hypoglycemia, but in T2DM this is rarely the case since severe hypoglycemia is distinctly less frequent among individuals with T2DM (UKPDS 33, 1998). The pathological underpinning of the less frequently reported brain structural abnormalities in T2DM is even less explored. The present study will comprehensively evaluate frontal and temporal lobe structure and function in late middle-aged and elderly individuals with T2DM and their associations with potential explanatory factors, including poor glycemic control (i.e., hyperglycemia and insulin resistance [IR]) and common co-morbid conditions, including obesity, hypertension, and dyslipidemia.

Literature Review

About Type 2 Diabetes Mellitus. Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycemia, with T1DM and T2DM being the more common forms. Unlike T1DM, which is primarily caused by decreased insulin production owing to the destruction of pancreatic β cells, T2DM is characterized by chronic hyperglycemia and increased peripheral resistance to insulin. Of the estimated 24 million people reported to have been affected by diabetes in the United States in 2007, T2DM accounted for 90% - 95% of all diagnosed cases (Center for Disease Control and Prevention [CDC], 2008). It is estimated that over 200 million people worldwide will be diagnosed with diabetes by the year 2010 (Zimmet, Alberti, & Shaw, 2001) and in a 2007 report by the CDC, the prevalence rate of T2DM has risen to approximately 25% in individuals aged 60 and above (CDC, 2008). Due to the chronic nature of the disease and

the multitude of co-morbid health conditions, the mortality rate is high among individuals with T2DM relative to age-matched peers (Desai et al., 2003).

History. The earliest case of diabetes was recorded on the 3rd Dynasty Egyptian papyrus by physician Hesy-Ra. The term diabetes was derived from the Greek verb diabaínein by a physician called Araetus of Cappodocia in the first century (81-133AD). Its derivative "siphon" alludes to the discharge of excessive urine in diabetes. In 1675, a physician named Thomas Willis added the word mellitus (Molnár, 2004), which means honey sweet, referring to the sweetness in the urine of diabetic patients. In 1776, English physician Matthew Dobson discovered that the sweetness was due to excessive amounts of urinary sugar (Dobson, 1776). With the exact nature and cause of the disease being largely unclear, mortality rate was high among individuals with diabetes at the time.

In 1921, inspired by a paper by Barron (1920) titled "The Relation of the Islets of Langerhans to Diabetes with Special Reference to Cases of Pancreatic", a young surgeon Frederick Banting with the help of his assistant Charles Best kept a severely diabetic dog alive for 70 days with injections of canine pancreatic extracts. Dr. Banting summarized his work in a published paper titled "The Beneficial Influences of Certain Pancreatic Extracts on Pancreatic Diabetes." (Banting, Best, Collip, Campbell, & Fletcher, 1922). They later furthered their work in humans by saving a 14-year-old young boy from dying of diabetes with more refined pancreatic extracts from healthy humans. This pancreatic extract, a hormone for glucose regulation, was later termed insulin, the discovery of which had earned Dr. Banting a Nobel prize in Medicine.

Although it was known that both juvenile- and adult-onset diabetes were marked with excess urinary sugar, only those with juvenile-onset diabetes were found to have elevated levels of ketone, a byproduct of fat breakdown, which often accompanies high blood sugar. The mechanisms underlying the two types of diabetes remained puzzling. It was not until the early 1980's that autoimmune destruction of insulin-producing pancreatic β-cells was discovered to be the primary cause of juvenile-onset diabetes, which is now known as insulin-dependent diabetes or T1DM. The adult-onset diabetes, characterized by reduced insulin sensitivity (or increased tissue IR), is termed non-insulin-dependent diabetes or T2DM.

Insulin resistance and glucose dysregulation in T2DM. IR, a key component of T2DM, occurs when there is reduced sensitivity to insulin in target tissues (Kahn, 1995), resulting in attenuation of glucose uptake. When food is digested, it is broken down into glucose, a source of fuel for the body and the brain. Glucose is distributed throughout the body via the bloodstream, and in order for the cells to utilize this fuel, glucose needs to be transported into cells via the insulin receptor-mediated uptake system. At target cells, insulin binds with insulin receptors, triggering a host of intracellular activities that mobilize the insulin-sensitive glucose transporters to the cell membrane such that glucose is brought into the cell (Czech, 1995). Although the underlying mechanism remains elusive, in IR, when insulin binds to those receptors it does not activate the shuttling of the transporters as effectively and higher glucose levels thus remain in the blood.

One possible pathogenic mechanism is the down-regulation of insulin receptors at target cells, resulting in attenuation of glucose uptake and thus reductions of intracellular glucose storage or availability (glycolysis; Review in Kahn, 1995). There may also be a relative reduction of insulin production in T2DM, which is likely due to glucose desensitization at pancreatic β-cells (Chan, Lowe, & Debertin, 1996) or reduced β-cell mass secondary to increased apoptosis (Butler et al., 2003).

Reduction in insulin availability triggers cellular events including glucagon-induced lipolysis (breakdown of fat inside cells) in adipose tissues and ketogenesis (conversion of fatty acids into ketones) in the liver, and ketones in turn inhibit insulin production (Veech, 2004). In T2DM, impaired glucose uptake results in an excessive accumulation of glucose in the bloodstream. In order to maintain normal blood glucose concentrations, there is a compensatory increase in insulin secretion from pancreatic β-cells (in response to excess blood glucose level), a condition called hyperinsulinemia, to offset the reduced insulin sensitivity at target tissues. When the increase in insulin secretion can no longer be maintained due to pancreatic failure, the individual develops hyperglycemia, which is necessary for a diagnosis of diabetes to be made.

T2DM-associated complications. T2DM is associated with macrovascular (e.g., stroke and myocardial infarction), and microvascular complications (e.g., retinopathy, nephropathy and neuropathy). These vascular complications, when coupled with hypertension, create the right environment for stroke (Aronow, 2008). Diabetic retinopathy is one of the earliest complications seen in T2DM and can lead to blindness,

and macular edema may also occur as a result of retinal thickening (Fong, Aiello, Ferris, III, & Klein, 2004). Nephropathy is known to be related to hypertension and poor glycemic control and may progress to kidney failure for which diabetes is the prime risk factor (Zelmanovitz et al., 2009). In peripheral neuropathy, a combination of impaired vascular supply and oxidative damage from too much metabolic substrate can lead to nerve damage, particularly in the lower extremities. When this altered sensation of extremities is coupled with poor circulation, the individual can develop small sores, which can become infected when unattended due to the lack of sensation. Given the poor circulation, these tissues are difficult to repair and in the worst case, can result in a need for amputation.

There is a host of co-morbid conditions in T2DM, which include obesity, hypertension, dyslipidemia and increased inflammation. Obesity is a prime risk factor for developing IR and worse, T2DM (Bloomgarden, 2002). Obesity is linked to IR by impairing insulin signaling via various mechanisms that involve increased release of free fatty acids and adipokine dysfunction (Schmidt et al., 2004). Hypertension, associated with metabolic dysregulation present in T2DM, has serious long-term consequences, including stroke and cardiovascular disease (Bronner, Kanter, & Manson, 1995). Dyslipidemia, also a known risk factor for cardiovascular disease (Miller, 2009), occurs frequently in patients with T2DM (Gadi & Samaha, 2007).

Cognitive impairment has been extensively reported in patients with T2DM, with more prominent cognitive decline among older patients, which suggests that T2DM may

accelerate cognitive aging. The connection between T2DM and the development of Alzheimer's disease (AD) remains controversial (Strachan, Reynolds, Frier, Mitchell, & Price, 2008). With the literature on T2DM-associated brain pathology being so limited, the cerebral mechanisms underlying the cognitive impairment observed in T2DM remain unclear. Since many of the co-morbid conditions also independently relate to cognitive decline and brain abnormalities, it remains a challenge to elucidate the mechanisms underlying the cognitive impairment reported in T2DM.

Cognitive impairment in T2DM. Evidence from both cross-sectional and longitudinal studies has established a link between T2DM and cognitive impairment (Allen, Frier, & Strachan, 2004; Strachan, Deary, Ewing, & Frier, 1997) in addition to increased risks of mild cognitive impairment (MCI; Luchsinger et al., 2007) and dementia (Hassing et al., 2004a). Relative to age- and education-matched non-diabetic controls, patients with T2DM exhibit poorer general cognitive functioning on crude clinical assessment tests such as the Mini Mental State Exam (MMSE; Cockrell & Folstein, 1988) and the Telephone Interview for Cognitive Status (TICS; Brandt, Spencer, & Folstein, 1988), both of which are comprised of questions addressing attention, orientation, immediate verbal memory, and language. Overall intelligence functioning has been reported to be unaffected (e.g., Gold et al., 2007). In general, the cognitive impairment reported in T2DM ranges from mild to moderate, with most consistent findings in verbal memory and learning (Soininen, Puranen, Helkala, Laakso, & Riekkinen, 1992), followed by psychomotor efficiency (van den Berg et al., 2008), executive function (de Wet, Levitt,

& Tipping, 2007; van den Berg et al., 2008), attention (Fontbonne, Berr, Ducimetiere, & Alperovitch, 2001), and working memory (Tun, Perlmuter, Russo, & Nathan, 1987).

Affected cognitive domains. In reviews of earlier (Strachan et al., 1997) and more recent publications (Awad, Gagnon, & Messier, 2004), verbal memory stands out as the most consistently affected cognitive domain in patients with T2DM. Cross sectional studies have shown that individuals with T2DM perform more poorly than age-matched non-diabetic controls on immediate and delayed recall of short paragraphs (e.g., the Logical Memory subtest of the Wechsler Memory Scale - Revised [WMS-R]; Wechsler, 1987; Asimakopoulou, Hampson, & Morrish, 2002), learning of word lists (e.g., the California Verbal Learning Test [CVLT]; Delis, Kramer, Kaplan, & Ober, 1987; Gold et al., 2007), and learning of novel word pairs that are semantically and non-semantically related (i.e. the Verbal Paired Associates subtest of the WMS-R; Cosway, Strachan, Dougall, Frier, & Deary, 2001). Also affected, though less consistently reported, is semantic memory (Arvanitakis, Wilson, Li, Aggarwal, & Bennett, 2006; Yeung, Fischer, & Dixon, 2009). Non-verbal memory such as visual and visuospatial memory, on the other hand, appears to be mostly intact (Cosway et al., 2001; Elias et al., 1997).

Impairment in non-memory domains including attention, executive function, and psychomotor efficiency have also been reported though findings are less consistent than those in memory. Psychomotor slowing seen as slower performance on tests such as the Digit Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale (WAIS; Wechsler, 1981) is commonly reported in T2DM (Gregg et al., 2000). Slower reaction

time is also common among older patients with T2DM (Messier, 2005). Measures of executive function are not uniformly affected. For example, patients with T2DM have also been shown to perform more poorly in mental flexibility as measured by the Trails B test, independent of age, education, hypertension, depression, and cardiovascular disease (Gregg et al., 2000). Frontal inhibitory response, as measured by the Stroop Color Word Interference Test (SCWI; Golden, 1978), has generally been found to be intact (Gold et al., 2007). There are also limited findings of poor performance for abstract reasoning on the Wisconsin Card Sort Test (Heaton, 1980; Reaven, Thompson, Nahum, & Haskins, 1990) and for verbal fluency on tests such as the Controlled Oral Word Association Test (COWAT; Strachan et al., 1997).

Cognitive impairments among elderly patients with T2DM. Most of the literature to date has focused on older adults with T2DM (age > 65), who exhibit more pronounced cognitive decline (Awad et al., 2004) than relatively younger patients (age < 60), with more extensive findings in verbal memory and learning. Effect sizes tend to be moderate to large in elderly patients with T2DM (Stewart & Liolitsa, 1999). Asimakopoulou et al. (2002) showed that after controlling for age, pre-morbid intelligence quotient (IQ), body mass index (BMI), and depression, cognitive impairments among older individuals with T2DM are restricted to verbal memory and mental flexibility (Asimakopoulou et al., 2002). Reductions in verbal learning are also common among older T2DM patients (Helkala, Niskanen, Viinamaki, Partanen, & Uusitupa, 1995). The affected domains encompassing verbal memory and learning, processing speed, and mental flexibility are known to decline with age (Grady, 2008). As aging is accompanied by neurodegenerative

brain changes, it has therefore been suggested that T2DM may accelerate cognitive aging, which has been supported by findings from large prospective studies.

Fontbonne, Berr, Ducimetiere, and Alperovitch (2001) reported that not only did diabetics perform worse on cognitive tests than individuals with normal glucose control and those with impaired fasting glucose, they also exhibited progressive performance decline on tests of verbal learning (i.e. list learning), psychomotor efficiency, and visual attention over a 4-year period. Hassing et al. (2004a) reported that despite comparable baseline cognitive profiles, elderly patients with T2DM showed accelerated decline in episodic memory and processing speed over a 6-year period, relative to age- and education-matched non-diabetic controls. More importantly, the proportion of the diabetic participants who were diagnosed with dementia six years later was double that of the control participants. Similarly, in a prospective cohort study conducted by Okereke et al. (2008), not only did older patients with T2DM have lower baseline scores on all cognitive measures assessed, including global cognitive functioning, verbal memory, and category fluency, they also exhibited greater cognitive decline over a 2-year period.

In a 12-year follow-up study, Robertson-Tchabo, Arenberg, Tobin, and Plotz (1986) evaluated 52 diabetic men and 610 age-matched non-diabetic controls with a limited cognitive battery assessing visuospatial memory with the Benton Visual Retention Test (Benton Sivan, 1992) and semantic memory with the Vocabulary subtest of the WAIS (Uiterwijk, 2001). They found no evidence of impairment in the tested domains at baseline or accelerated cognitive decline among diabetics at 6 and 12 years, which is not surprising

since other cross-sectional studies have shown that patients with T2DM have more difficulties with recent memory (learning new information) than with retrospective memory (remembering things from the past) and that their visuospatial memory may be unaffected. In the Hoorn Study, a population-based cohort study on glucose metabolism, van den Berg et al. (2008) reported poorer performance among elderly individuals with T2DM on processing speed, executive function, and attention, independent of depressive symptoms, relative to those with metabolic syndrome and healthy controls. Their finding of intact memory functioning contradict most of the current literature, which the authors speculated may have been due to the relatively short disease duration and the subjects being metabolically well-controlled.

Cognitive impairments among middle-aged patients with T2DM. Younger and middle-aged adults with T2DM have not been studied as extensively, and in the limited literature, the exact nature of the associations between T2DM and cognitive impairment remains controversial. Consistent with the findings in elderly T2DM patients, Robertson-Tchabo et al. (1986) reported intact visuospatial memory and vocabulary in a group of predominantly middle-aged adults with T2DM both cross-sectionally and longitudinally. Unlike in older patients with T2DM, evidence for verbal learning and memory impairment among middle-aged patients is limited whereas non-memory domains appear to be more affected. A more recent study by Ryan & Geckle (2000) reported psychomotor slowing but intact learning, memory, and problem solving skills in middle-aged adults with T2DM. In the Whitehall II Study, diabetics had significantly lower inductive reasoning but again intact verbal memory relative to non-diabetic controls independent of age, social

economic status, hypertension, or other vascular abnormalities but effect sizes were rather small (Kumari & Marmot, 2005).

There are yet other studies that report no association between T2DM and cognitive dysfunction among middle-aged patients (e.g., Lowe, Tranel, Wallace, & Welty, 1994; Mattlar, Falck, Rönnemaa, & Hyyppä, 1985). In a brief assessment with the Neurobehavioral Cognitive Status Examination (NCSE), Dey, Misra, Desai, Mahapatra, and Padma (1997) found performance decrement in attention, repetition, and memory despite no group difference in MMSE scores. Their cognitive findings were substantiated by detection of delayed electroencephalographic P300 latencies among diabetics, which were inversely correlated with fasting blood glucose, suggesting that metabolic dysregulation present in T2DM may be related to slower neuronal events during cognitive processing. Although memory was reported to be affected, their use of crude assessment tools limits the interpretation of their results.

Some have proposed the threshold theory of cognitive impairment (Satz, 1993) to explain the relatively intact memory function in middle-aged patients (Kumari et al., 2005; Ryan & Geckle, 2000). Since age-related brain degeneration in memory-relevant structures, such as the hippocampus, is broadly estimated to begin at around age 60 (Golomb et al., 1993), T2DM in old age may interact with the aging process to accelerate hippocampal degeneration, with decline in verbal memory and learning as a secondary consequence. Since age-related brain degeneration has not yet commenced in middle age, the magnitude of the T2DM-age interaction effect may not sufficiently impair verbal memory. In a

recent study by Yau et al. (2009), the authors reported performance decrements on immediate and delayed recall of short paragraphs in a mixed group of late middle-aged and elderly patients with T2DM. Given that longitudinal studies have so far focused only on older subjects, the pattern of progressive cognitive decline in T2DM from middle to old age remains to be clarified with a longitudinal design.

Neural correlates of cognitive functions affected in T2DM. The cognitive domains that are more prominently affected in T2DM, including verbal memory, executive function, and psychomotor function, collectively involve both the frontal and temporal lobes. To delineate the cerebral mechanisms underlying these functional abnormalities, it is essential to examine the neural substrates for the affected domains.

The MTL plays an important role in declarative memory (Squire, Stark, & Clark, 2004), which is an organized repository of general knowledge, such as words, concepts and objects, and is acquired through conscious awareness. In particular, verbal memory is highly dependent on temporal lobe structures such as the hippocampus (Squire, 1992). There is an extensive literature also demonstrating frontal lobe involvement during memory formation (Review in Buckner, Kelley, & Petersen, 1999). In addition to its role in memory, the frontal lobe, particularly the dorsolateral prefrontal cortex (DLPFC) is highly involved in executive processes, attention, psychomotor functions, and working memory (Bailey & Mair, 2004; Petrides, 2000; Smith & Jonides, 1999). Tasks that involve inhibitory control, such as the SCWI, have been shown to activate the orbitofrontal cortex (Goldstein et al., 2007).

Additionally, fiber connectivity between the frontal and temporal lobes may also be compromised in T2DM, which contribute to cognitive difficulties. For instance, the temporal stem, a dense fiber bundle that relays information between the frontal and temporal lobes, plays an important role in memory processing (Kier, Staib, Davis, & Bronen, 2004). The internal capsule communicates sensory signals between the thalamus and the cortical areas, and thus psychomotor efficiency is highly dependent on the integrity of this critical pathway (Barnea-Goraly et al., 2005; Madden et al., 2004). Dense corpus callosal fibers are crucial to interhemispheric processing efficiency, with genu of the corpus callosum predominantly involved in frontal interhemispheric signal transmission and splenium in occipital and parietal transmission (Schulte, Sullivan, Muller-Oehring, Adalsteinsson, & Pfefferbaum, 2005). Forceps minor, which extends to the prefrontal regions from the corpus callosum, would be largely responsible for frontal signal transmission and interhemispheric signal transfer. Taken together, reduced structural integrity and fiber connectivity in the frontal and temporal lobes may in part contribute to the cognitive impairments reported in T2DM.

In sum, apart from the general consensus that verbal memory is most affected in T2DM, findings in other cognitive domains vary largely across studies owing to large discrepancies in age, levels of glycemic control, disease duration, and treatment history within and across studies. More importantly, the presence and varying degrees of T2DM-associated complications, which are not always accounted for, may obscure the unique contribution of the disease itself. The inconsistent results from large population- and

community-based studies may be due to crude assessment instruments, which are unstandardized and less sensitive. Furthermore, in large population-based studies, diabetes diagnosis is sometimes based on subject's medical records or self-report (Okereke et al., 2008; van den Berg et al., 2008), which may not accurately reflect their current medical status. In a review of the link between T2DM and cognitive dysfunction in ten longitudinal studies, Allen et al. (2004) pointed out that none of those studies distinguished between T1DM and T2DM. Since T1DM and T2DM only share hyperglycemia in common and are etiologically distinct, it would not be surprising if their patterns of cognitive impairment are also somewhat disparate, despite significant overlap in affected cognitive domains (Kodl & Seaquist, 2008). Despite these methodological limitations, there is compelling evidence supporting cognitive impairment in T2DM and the association between T2DM and accelerated cognitive decline as well as increased incidence of dementia.

Brain abnormalities in T2DM. Diabetes has been shown to be associated with enlarged ventricles and increased rate of brain atrophy (Carmichael et al., 2007; Enzinger et al., 2005; Knopman, Mosley, Catellier, & Sharrett, 2005). A comparative study suggests that the rate of developing brain complications, both structural and functional, is higher in T2DM than in T1DM (Brands et al., 2007). Despite extensive evidence supporting cognitive impairment in T2DM, the existing literature documenting cerebral abnormalities, though expanding, remains limited.

Global volume changes. Cerebral complications documented in T2DM are primarily non-specific gross brain pathologies. Some studies have described cortical and subcortical atrophy utilizing not particularly sensitive measures such as the frontal interhemispheric fissure and bifrontal ratios (e.g., Manschot et al., 2006; Manschot et al., 2007). Others such as Manschot et al. (2008) and van Harten et al. (2006) evaluated cortical atrophy in elderly patients with T2DM with visual rating scales (e.g., the Schelten's Scale; Scheltens et al.,1993) that are commonly used for distinguishing those with AD from non-AD controls, but only the former study identified significantly more atrophies in diabetic patients.

Using more sensitive, automated segmentation methods, which separate an anatomical image into GM, WM, and cerebrospinal fluid (CSF) partitions, other studies have reported reduction in global GM volume in late middle-aged and elderly patients with T2DM (Jongen et al., 2007; Kumar et al., 2008; Last et al., 2007). Some studies also reported enlarged CSF space in addition to global GM volume reduction (Jongen et al., 2007; Last et al., 2007). Using an automated voxel-based morphometry (VBM) approach, Gold et al. (2007) did not find increased global atrophy, which may be explained by the fact that those patients had moderately well-controlled diabetes and that most of the patients were middle-aged. Global WM volume reduction is less frequently reported and findings are even less consistent (Kumar et al., 2008; Last et al., 2007).

White matter lesions. Whereas reports of global WM volume change are limited, there have been many more studies focusing specifically on WML seen as periventricular

and deep white matter hyperintensities (PWMHs and DWMHs) on the fast fluid-attenuated inversion recovery (FLAIR) image. White matter hyperintensities (WMHs) are possible indications of neuronal loss, demyelination or gliosis (Bastin et al., 2009), but the pathological underpinning of the WMH observed in T2DM patients remains largely unclear. A meta-analysis of imaging studies revealed no consistent relationship between T2DM and WMH (Review in van Harten et al., 2006), with some showing an increased occurrence of WML relative to non-diabetic counterparts (Manschot et al., 2008) but others not (Knopman et al., 2005; Schmidt et al., 2004). Overall, the WMHs reported in T2DM patients tend to be diffuse rather than localized and DWMHs tend to occur more frequently and more extensively than PWMHs (Umegaki et al., 2008; van Harten et al., 2006).

A common limitation of many of these reports is the use of semi-quantitative assessment methods, which assign ratings of WML on a categorical scale (e.g., Schelten's Scale) either by visual inspection (van Harten et al., 2007) or by the longest dimension of the lesion (Manschot et al., 2006). Such methods fall short in discerning subtle pathological changes that may not be visible to the naked eye. With a more sophisticated segmentation technique, Jongen et al. (2007) yielded increased WML volume in the absence of WM volume reduction in a relatively small sample of T2DM patients. Using seeds and region-growing techniques, Novak et al. (2006) found more extensive WMHs in the frontal lobe rather than globally.

Specific frontal and temporal lobe abnormalities in T2DM. Studies that assess regional brain integrity in T2DM have identified varying degrees of abnormalities in the frontal (Modi et al., 2008), parietal (Sahin et al., 2007), occipital (Last et al., 2007), and temporal lobes (Gold et al., 2007), with increasing evidence suggesting that the frontal and temporal lobes may be particularly vulnerable to damage in T2DM (e.g., Gold et al., 2007; Sahin et al., 2007).

Evidence of frontal lobe abnormalities in T2DM. There are a handful of reports that characterize frontal lobe abnormalities, including reduced volume and impaired metabolism in patients with T2DM. Using an automated segmentation method, Kumar et al. (2008) identified specific GM volume reductions in prefrontal regions, including the anterior cingulate and orbitofrontal cortex after controlling for total GM volume. Increased CSF volume and reduced cerebral blood flow (CBF) have been reported in the frontal lobe of patients with T2DM (Last et al., 2007). Gold et al. (2007), however, did not find frontal volume reduction or atrophy in a group of late middle-aged and elderly T2DM patients with moderately well-controlled diabetes.

Findings of gross frontal WM abnormalities, however, have been rather inconsistent, which may be attributed to the less sensitive semi-quantitative assessment methods employed in a majority of the studies (e.g., Manschot et al., 2006; van Harten et al., 2006). Many yielded a measure of global rather than regional WML (Manschot et al., 2006). Others such as Last et al. (2007) reported relatively more extensive WMHs in both the frontal and temporal lobe regions in T2DM patients. Using diffusion tensor imaging

(DTI), a sensitive magnetic resonance imaging (MRI) technique for assessment of brain microstructure, Yau et al. (2009) also found more extensive WM microstructural abnormalities in both the frontal and temporal lobes in a mixed group of late middle-aged and elderly patients with T2DM.

Proton magnetic resonance (MR) spectroscopy studies have provided further evidence of frontal lobe abnormalities in T2DM in the form of altered cellular metabolism. Increased myo-inositol (MI) concentrations and myo-inositol-creatine (MI/Cr) ratios in frontal WM have been demonstrated in T2DM patients with or without depression (Ajilore et al., 2006). Another study also found significantly higher MI/Cr ratio in the frontal cortex of late middle-aged patients with T2DM, with more extensive frontal metabolic alterations characterized by lower N-acetylaspartate/creatine (NAA/Cr) and choline/creatine (Cho/Cr) ratios found in those patients with poorer glycemic control (Sahin et al., 2007). These results suggest that T2DM patients may have neuronal dysfunction or structural damage in the frontal lobe. Conversely, a more recent study reported lower Cho/Cr ratio in the occipital but not the frontal lobe among T2DM patients with or without hypothyroidism relative to healthy controls (Modi et al., 2008). However, their highly heterogeneous sample (i.e. age ranging from mid-20's to mid-60's) renders it difficult to generalize the results.

Evidence of temporal lobe abnormalities in T2DM. Evidence of T2DM-related gross temporal lobe atrophy is mixed. For instance, van Harten et al. (2006) failed to detect MTL atrophy in elderly patients with T2DM using a less sensitive visual rating

scale. With a more sensitive method, Last et al. (2007) found increased CSF volume in the temporal lobe in T2DM patients. A small number of reports point to specific MTL structures, including the hippocampus and amygdala, being more susceptible to damage in T2DM. For instance, den Heiji et al. (2007) described volume reductions in the hippocampus as well as the amygdala among elderly patients with T2DM. The hippocampus, in particular, has been shown to be vulnerable to the effect of poor glucose metabolism even in healthy individuals (Convit, Wolf, Tarshish, & de Leon, 2003). In particular, neuronal loss in the hippocampus (i.e. CA1 and CA3 subfields) has been associated with memory deficits (Ramsden et al., 2005). Consistent with these data, animal models of T2DM have demonstrated neuronal loss in the CAl field of the hippocampus (Li, Zhang, & Sima, 2005).

Data from the Honolulu-Asia Aging Study suggest moderately increased risks for hippocampal atrophy among elderly Japanese Americans with T2DM (Korf, White, Scheltens, & Launer, 2006). Similarly, Gold et al. (2007) identified volume reductions restricted to the hippocampus in middle-aged and elderly patients with T2DM using a quantitative manual tracing method. Although the observed volume reduction correlated only modestly with declarative memory impairment, the correlation with elevated HbA_{1C} (a robust indicator of glucose regulation for the previous three months) was moderately strong, which was independent of atherosclerosis, hypertension, and dyslipidemia. Also consistent with these reports, Last et al. (2007) demonstrated more WMHs and enlarged CSF space in the temporal lobe of T2DM patients.

In a whole-brain voxelwise analysis of DTI data, Yau et al. (2009) reported more extensive frontal and temporal WM microstructural abnormalities in late middle-aged and elderly patients with T2DM, with most extensive abnormalities in the temporal stem, even after accounting for overt WM pathology presented as WMHs on the FLAIR image. MR spectroscopy based assessment of hippocampal metabolites in T2DM have so far been done in animal models only. For instance, van der Graaf et al. (2004) found elevated hippocampal tissue concentrations of both glucose and MI in Zucker diabetic rats despite the absence of alterations in other metabolites such as N-acetylaspartate + N-acetylaspartylglutamate (NAA + NAAG) and choline. Impaired insulin receptor signaling has also been reported in the hippocampus of Zucker diabetic rats (Winocur et al., 2005).

Associations between brain abnormalities and cognitive decline in T2DM. Only a few cross-sectional studies have examined the relationship between brain abnormalities and cognitive decline in T2DM. Manschot et al. (2006) found that after controlling for age, gender, and IQ, cortical atrophy, DWMHs, and infarcts were associated with slower processing, whereas subcortical atrophy was associated with worse attention and executive function. Their brain measures were only modestly associated with degree of glycemic control (HbA_{1C}) and disease duration. In van Harten et al. (2007), the authors found that PWMH, which did not distinguish the groups, was the only brain measure that was associated with slower motor speed in elderly patients with T2DM, after controlling for age, gender, level of education, and hypertension. These studies are not without limitations. First of all, their MRI assessment methods were semi-quantitative, which are therefore, less sensitive to subtle structural alterations. Secondly, the MR measures were

primarily identifying non-specific brain abnormalities, which do not target the role of specific brain structures in cognitive functions that are known to be affected in T2DM.

The extent of deep WM lesions has been shown to be associated with psychomotor slowing (Umegaki et al., 2008). In an analysis of 95 non-demented elderly Japanese with T2DM, impairment in memory and mental efficiency was found to be associated with WMHs and subcortical atrophy in structures such as the hippocampus but not with glycemic control, lipid metabolism, or blood pressure (BP; Akisaki et al., 2006).

Gold et al. (2007) found that hippocampal volume reduction but not glycemic control was associated with verbal memory decline though the association was marginally significant. In a more recent study done by the same group, the authors found that fractional anisotropy (FA; a key DTI measure of WM microstructural integrity) values in the left temporal stem explained 10% of the variance in immediate emotional memory after accounting for age, the quantitative insulin sensitivity check index (QUICKI) score as a glycemic measure, and BP (Yau et al., 2009). Their findings suggest that subtle WM abnormalities observed in T2DM may sufficiently impair declarative memory.

In sum, there is evidence suggesting that T2DM patients have gross and microstructural abnormalities and impaired cellular metabolism in the brain. Limited evidence is available to establish the nature of the brain involvement in explaining the cognitive decline observed in T2DM patients. The frontal and temporal lobes may be more vulnerable to the effect of T2DM and possibly other confounding factors. More

comprehensive assessment is necessary to better elucidate the cerebral mechanisms underlying cognitive dysfunction.

Possible explanatory factors. The mechanisms underlying the extensively reported cognitive impairment in T2DM remain under debate. The pathogenesis of the less frequently investigated brain structural abnormalities is even less clear. The link between T2DM and associated cognitive and brain impairments is often obscured by a host of co-morbid conditions, including hypertension, obesity, dyslipidemia, small vessel disease, increased inflammation, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and depression, each of which has also been linked to brain structural and functional abnormalities (Asimakopoulou et al., 2002). These co-morbidities or potential confounding factors may independently exert their effects or mediate the effects of T2DM on cognition. To clarify the underlying pathological mechanisms, in addition to evaluating the association between brain and cognition, we should also characterize the relative contributions of endocrine changes in T2DM and T2DM-associated co-morbidities. As the list of these potential explanatory factors is rather extensive, the present review is restricted to those more common factors, which include the metabolic dysregulation present in T2DM, obesity, hypertension, and dyslipidemia.

Metabolic dysregulation. Our brain relies on glucose, its primary fuel, for its functioning. Since the brain has limited glucose storage, it relies on a continuous glucose supply from the blood, which is supported primarily by the liver. Glucose is transported

via insulin-independent glucose transporters (GLUT1) across the blood brain barrier (BBB) into the interstitial space and brain cells, where glucose metabolism generates the adenosine triphosphate (ATP) needed for cellular function (Maher, Vannucci, & Simpson, 1994). Peripheral insulin may also enter the brain via BBB transporters (Park, 2001). Insulin receptors are found in various brain regions, including the hypothalamus (Unger & Betz, 1998) and the dentate gyrus of the hippocampus (Marks, Porte, Jr., Stahl, & Baskin, 1990). Insulin is known to play an important role in synaptic transmission and glucose utilization during memory processing (Gispen & Biessels, 2000; Hoyer, 2003). Given their direct brain effects as well as their influence on the glucose that is available for the brain to function, metabolic factors may play an important role in the frequently reported brain dysfunction in T2DM.

Hyperinsulinemia and cognition. There is evidence linking elevated blood insulin levels to cognitive decline even in non-diabetic individuals. In middle-aged adults without T2DM, dementia, or stroke, elevated fasting insulin levels have been associated with worse baseline and follow-up performance on delayed word recall, psychomotor efficiency, and word fluency (Young, Mainous, & Carnemolla, 2006). Epidemiological data from the Nurses' Health Study demonstrated an association between elevated fasting insulin levels and accelerated decline in general cognitive functioning and verbal memory among older women without diabetes (van Oijen et al., 2008).

Elderly individuals with normal glycemic control but at high risk for developing T2DM have been shown to perform similarly as individuals with T2DM but worse than

normoglycemic individuals with low risks for T2DM (Bruehl, Sweat, Hassenstab, Polyakov, & Convit, 2010). In a double-blind study by Ryan et al. (2006), patients with T2DM showed performance improvement on working memory but not verbal learning or memory after a 24-week treatment either for improving sensitivity or for stimulating insulin generation from pancreatic β-cells (Ryan et al., 2006). Overall, the relationship between insulin elevation and cognitive impairment in T2DM is, nevertheless, not as well documented.

Hyperinsulinemia and brain. Manschot et al. (2007) was the first study to demonstrate an association between elevated fasting insulin levels and more extensive DWMHs among patients with T2DM (Manschot et al., 2007). In a MR spectroscopy based study, insulin levels were found to inversely relate to frontal NAA/Cr ratio in a group of middle-aged T2DM patients, suggesting possible adverse effect of insulin elevation on neuronal health (Sahin et al., 2007). Microalbuminuria, which often co-occurs with hypertension and T2DM, is strongly associated with IR (Nosadini et al., 1992), and both hypertension and microalbuminuria have been linked with higher risks for WMLs among patients with T2DM (Anan et al., 2008; Manschot et al., 2007).

In a Zucker rat model of T2DM, lean and obese rats received a training of variable-interval delayed alternation test in which they received either a reward or no reward for lever pressing (Winocur et al., 2005). Their results showed that in addition to worse performance relative to their lean counterparts, particularly during long delay, the obese Zucker rats had no increase in hippocampal plasma membrane concentration of insulin

sensitive glucose transporter GLUT4, in the absence of changes in expressions of GLUT4 and insulin receptors. These findings suggest that in T2DM, impaired insulin receptor signaling in the hippocampus may reduce glucose uptake and thus contribute to memory deficits.

Pancreatic β-cells are known to progressively degenerate in T2DM; therefore, blood insulin levels may not directly reflect the degree of IR. Although insulin dysregulation may play a potentially important role in T2DM-associated cognitive and brain impairments, these impairments may be more mediated by the impact this dysregulation has on glucose levels.

Hyperglycemia and cognition. Impaired fasting glucose (IFG) and poor glucose tolerance (the ability to clear glucose from the blood when it increases after feeding) have been linked to cognitive decline in domains such as verbal memory, attention, visuomotor speed, and verbal fluency (Vanhanen et al., 1997; Yaffe et al., 2004) and may be associated with a higher risk for cognitive impairment (Yaffe et al., 2004). Rolandsson et al. (2008) demonstrated that among non-diabetic women, elevations in fasting glucose and glucose levels 2 hours after glucose administration were both associated with poorer episodic memory but not semantic memory. These findings suggest that even minor fluctuations in glucose homeostasis may interfere with normal cognitive processing, particularly in old age, when abnormalities in glucose regulation are highly prevalent.

HbA_{1C} is a marker of long-term glucose regulation, with higher values indicating higher blood sugar levels. Among non-diabetics, HbA_{1C} elevation has been linked to higher incidence of all-cause cardiovascular and ischemic heart disease (Khaw et al., 2001) as well as performance decline in verbal memory and learning (MacLullich, Deary, Starr, Walker, & Secki, 2004). In T2DM patients, particularly those in poor diabetes control (Munshi et al., 2006), elevated HbA_{1C} has been associated with worse performance in learning (Reaven et al., 1990), working memory (Munshi et al., 2006), executive function (de Wet et al., 2007; Perlmuter et al., 1984), psychomotor efficiency (Cukierman-Yaffe et al., 2009; Mogi et al., 2004), and serial learning tasks (Perlmuter et al., 1984).

Manschot et al. (2007) found that among late middle-aged and elderly patients with T2DM, elevated HbA_{1C} was related to lower composite cognitive scores but not brain abnormalities. The QUICKI score, a common metabolic index derived from fasting blood glucose and insulin levels, has also been shown to correlate positively with delayed neutral and emotional memory performance in a group of middle-aged and elderly patients with T2DM (Yau et al., 2009). In addition, elevated HbA_{1C} has been found to be inversely associated with the MMSE score but not performance in delayed word recall (Worrall, Chaulk, & Moulton, 1996). In Dey et al. (1997), blood glucose level but not HbA_{1C} was inversely associated with cognitive performance in middle-aged patients with T2DM.

Hyperglycemia and brain. Elevated HbA_{1C} has been associated with accelerated brain atrophy in healthy middle-aged and elderly individuals (Enzinger et al., 2005). In

two recent imaging studies by Manschott et al. (2006 & 2007), no associations were found between HbA_{1C} and subcortical and cortical atrophy or WMLs among T2DM patients. However, the validity of these findings is limited given that the investigator used somewhat insensitive MR assessment methods.

There is some evidence linking hyperglycemia to frontal lobe abnormalities in patients with T2DM. Utilizing a published reliable frontal lobe parcellation method (Convit et al., 2001), Bruehl et al. (2009a) described an association between HbA_{1C} elevation and volume reduction in the frontal pole among T2DM patients, which was independent of age. The limited findings from MR spectroscopy based brain assessment are rather inconsistent. One study found that in a small group of middle-aged T2DM patients, both HbA_{1C} and insulin levels were inversely associated with frontal cortical NAA/Cr ratio and HbA_{1C} was also inversely associated with Cho/Cr ratio, independent of age, gender, and disease duration (Sahin et al., 2007). Conversely, another study found in a relatively larger group of T2DM patients (age 30-80) without depression, frontal WM MI concentrations were associated with cardiovascular risk factor but not with HbA_{1C} (Ajilore et al., 2006). The inconsistencies may be due to the heterogeneity in age and degree of co-morbid complications. It is also possible that differential mechanisms underlie frontal GM and WM pathologies.

There is stronger evidence supporting the link between hyperglycemia and temporal lobe abnormalities. Convit et al. (2003) described associations between reduced glucose tolerance and poor memory performance as well as more hippocampal atrophies in normal

elderly. In an early study of T2DM utilizing computerized tomography (CT), Soininen, Puranen, Helkala, Laakso, and Riekkinen (1992) found a positive association between fasting glucose levels and right central temporal lobe atrophy in elderly patients with T2DM. In a more recent study that used a manual tracing method, the hippocampal volume reductions observed in T2DM patients were explained by elevated HbA_{1C} but not BMI, hypertension or dyslipidemia (Gold et al., 2007).

Animal studies provide further evidence of hyperglycemia-associated hippocampal damage. The dentate gyrus of the hippocampus is one of the few brain regions where neurogenesis occurs and is thought to be involved in memory formation (Lee & Son, 2009). Using a Goto–Kakizaki rat model of T2DM, Lang et al. (2008) demonstrated hyperglycemia induced proliferation of adult neural progenitors in the dentate gyrus, resulting in impaired neurogenesis in the hippocampus (Lang, Yan, Dempsey, & Vemuganti, 2009). Chronic hyperglycemia may induce changes in neuronal gene transcription and impair long-term potentiation (Kamal, Biessels, Urban, & Gispen, 1999). Impaired hippocampal-based learning in T2DM may be related to compromised glucose metabolism secondary to impairment in insulin signaling at the hippocampus (Winocur et al., 2005).

Hyperglycemia-induced oxidative stress is a potential mechanism underlying cognitive and brain impairments in T2DM. For instance, excess glucose is converted to sorbitol and subsequently to fructose via activation of the sorbitol-aldose reductase pathway (Ahmad, Singh, & Saleemuddin, 2001), and the resulting excessive accumulation of fructose and sorbitol is known to damage nerve cells (Reviews in Brownlee et al., 2001). Sorbitol may

react with proteins and lipids to increase intracellular formation of advanced glycation end products (AGEs; Brownlee, 1992) or increased expression of AGE receptors (Toth et al., 2006), which may in turn damage endothelial cells of vessel walls and contribute to myelin degeneration (Brownlee, 1992; Misur et al., 2004). Moreover, AGEs may upregulate nuclear factor κB, a proinflammatory gene marker, which contributes to neuronal damage (Aragno et al., 2005) in brain structures such as the hippocampus.

Obesity. Obesity in adults is defined as a BMI (generalized obesity) of 30 kg/m^2 or above, or a waist-height ratio (WHR; central obesity) over 0.5. With its escalating rate and a wide array of associated medical complications, such as hypertension, dyslipidemia, heart failure, and cardiovascular disease (Field et al., 2001), obesity represents a major health concern in developed countries (Malnick & Knobler, 2006). Obesity is also a major risk factor for developing IR and T2DM not only in adults (McTernan et al., 2002) but in children and adolescents (Hannon, Rao, & Arslanian, 2005) as well. The obesity effect on brain structure and function has been reported in non-diabetics but has not been extensively investigated in patients with T2DM, particularly because the two conditions are so tightly linked that it is not possible to dissociate them.

Obesity and cognition. There is emerging evidence supporting a link between midlife obesity and accelerated cognitive decline in late life. The Whitehall II Cohort Study demonstrated that among British civil servants, both long-term obesity and underweight were associated with lower MMSE scores and worse memory and executive function in late life, independent of age, sex, and education (Sabia, Kivimaki, Shipley,

Marmot, & Singh-Manoux, 2009). In a 27-year longitudinal cognitive evaluation of 10,276 middle-aged individuals, Whitmer, Gunderson, Barrett-Connor, Quesenberry, and Yaffe (2005) found that relative to normal weight counterparts, obese individuals had the highest rate (74%) of developing dementia, followed by those who were overweight (35%), after controlling for age, gender, race, education, smoking, alcohol use, marital status, and other major cardiovascular risk factors. In five cognitive evaluations of 1351 elderly Latinos conducted over an 8-year period, waist circumference in the middle and high tertiles was found to be associated with elevated risks of dementia/cognitively impaired but not demented (CIND; West & Haan, 2009).

It has been shown that among non-demented individuals, the associations between diabetes and worse semantic memory performance as well as slower perceptual speed become less strong after accounting for BMI (Arvanitakis et al., 2006). Obesity may contribute to the development of hypertension via vasoconstriction or increased renal sodium reabsorption induced by hyperactivation of the rennin-angiotensin system and elevation of aldosterone (Francischetti & Genelhu, 2007). Co-morbid obesity and hypertension may interact to impact cognition independent of T2DM. In the Framingham Heart Study, a prospective analysis of 551 men and 872 females revealed that men with co-morbid hypertension and obesity had worse mental status, memory, and learning than those with either condition 4-6 years later, which was independent of age, level of education, occupation, alcohol use, smoking, cholesterol, and diagnosis of T2DM (Elias, Elias, Sullivan, Wolf, & D'Agostino, 2003); however, no effect was observed among women. In a group of elderly Koreans with T2DM, not only was waist circumference, a

measure of central obesity, associated with lower performance in working memory and tests of reaction time, independent of age, gender, education, and HbA_{1C}, it also interacted significantly with hypertension to impact working memory and verbal memory independent of diagnosis of T2DM (Rivera et al., 2005).

Frontal lobe based functions such as inhibition and attention have been reported to be affected in obese individuals (Golden, 1978). Obesity is known to promote inflammation, which may in turn contribute to the development of atherosclerosis (Rocha & Libby, 2009). In a cross-sectional evaluation of 20 lean and 42 overweight/obese adults, Sweat et al. (2008) found that among females only, there was an inverse relationship between C-reactive protein (CRP), an inflammatory marker, and performance for frontal lobe functions, including IQ, working memory, planning, visual memory. The observed associations were particularly strong in the overweight/obese group. Although the existing literature remains limited and rather inconsistent, it is possible that the increased inflammation that usually accompanies obesity is one of the mechanisms underlying cognitive decline in obese T2DM patients.

Obesity and brain. A few imaging studies were geared towards understanding brain involvement in obesity. For instance, elevated apparent diffusion coefficient (ADC) in GM on the DTI image, a possible indication of increased diffusivity, have been reported in hunger and satiety centers, including the thalamus, hippocampal gyrus, amygdala, orbitofrontal, occipital, dorsolateral and middle temporal cortex, insula, midbrain, suggesting obesity-related alteration in fluid distribution and/or vasogenic edema (Alkan

et al., 2008). Reduced GM density has also been reported in brain regions regulating taste and reward, including the post-central gyrus, frontal operculum, putamen, and middle frontal gyrus (Pannacciulli et al., 2006). The same group demonstrated in another study hypoactivation of the left DLPFC in response to a meal among obese men (Le et al., 2006), which was likely related to a disinhibitory response to food.

There is growing evidence supporting the role of obesity in brain pathology. Global brain volume adjusted for intracranial vault has been reported to be inversely associated with BMI and age in middle-aged adults, after controlling for other cardiovascular risk factors (Ward, Carlsson, Trivedi, Sager, & Johnson, 2005). Striatal WM volume increase has been observed in a Statistical Parametric Mapping (SPM) and VBM based analysis of severely obese individuals (Pannacciulli et al., 2006). Using a similar method, Haltia et al. (2007) also found that relative to lean counterparts, obese individuals had increased cerebral WM volumes, a possible indication of WM expansion, but no GM volume changes. With associations found between increased serum free fatty acid concentration and WM volumes in the left temporal and occipital lobes in obese subjects, the authors speculated that the observed WM expansion may be explained by increased accumulation of fat in central myelin. Furthermore, a subsequent 6-week very low calorie diet was found to partially reverse the observed WM expansion globally and regionally in the left temporal lobe. More sensitive imaging methods such as DTI and MR spectroscopy would be essential to better clarify the nature of the reported WM volume changes.

With CT scans of 290 elderly Swedish women collected in 1992, Gustafson, Lissner, Bengtsson, Bjorkelund, and Skoog (2004) yielded a significant association between BMI and temporal lobe atrophy. However, the poor resolution of CT scans and the semi-quantitative assessment limit the interpretation of their results. Another study also reported MTL atrophies among obese individuals without diabetes. Jagust, Harvey, Mungas, and Haan (2005) also reported hippocampal volume reduction related to central obesity but the authors did not account for the effect of T2DM. The role of obesity in T2DM-associated brain abnormalities has not been extensively examined. Last et al. (2007) found that BMI and CBF were inversely correlated in both T2DM patients and non-diabetic controls. HbA_{1C} but not BMI has been reported to independently predict hippocampal volume among middle-aged and elderly patients with T2DM (Gold et al., 2007). However, in another study examining T2DM patients and matched non-IR controls together, BMI but not HbA_{1C} was inversely associated with hippocampal volume even after controlling for T2DM diagnosis (Bruehl et al., 2009a).

Hypertension. Hypertension is defined as either a sitting systolic BP $\geq$ 130 mmHg or a diastolic BP $\geq$ 85 mmHg according to the National Cholesterol Education Program (NCEP) guidelines (Expert Panel, 2001). Prospective population-based studies have established diabetes as a risk factor for hypertension (Gregg et al., 2000), and both pathologies are prime risk factors for vascular disease (Grossman & Messerli, 2008). Individuals with both T2DM and hypertension have been shown to have higher risk of stroke relative to those with either condition (Junga et al., 2006). Hypertension, like diabetes, is associated with elevated risk of MCI (Elias et al., 1997) and dementia (Bruce,

Harrington, Davis, & Davis, 2001) in old age. Co-morbid hypertension may exacerbate cognitive and brain impairments in T2DM.

Hypertension and cognition. In a review of cross-sectional and longitudinal studies published prior to 1991, Waldstein et al. (1991) summarized that individuals with hypertension generally perform cognitively worse than normotensive individuals, with more consistent findings in memory, attention, and abstract reasoning and less so in perception, constructional ability, mental flexibility, and psychomotor speed (Waldstein, Manuck, Ryan, & Muldoon, 1991).

There is accumulating evidence supporting an association between BP elevation and cognitive decline in both mid- and late life (van den Berg, Kloppenborg, Kessels, Kappelle, & Biessels, 2009). Accelerated cognitive decline has been described in late middle-aged hypertensive diabetics over a 6-year period (Knopman et al., 2001). In a comprehensive cognitive evaluation of healthy, non-demented community-dwelling individuals ranging from middle-age to elderly, Knecht et al. (2008) described an association between elevated BP within the normal range and lower composite score of multiple cognitive domains. Harrington et al. (2000) found that relative to normotensive counterparts, hypertensive elderly with no evidence of dementia or vascular disease had slower reaction time and worse performance in working memory, immediate and delayed word recognition, spatial memory, and attention (van Beek, Claassen, Rikkert, & Jansen, 2008).

The adverse effect of elevated systolic BP in midlife on cognition in late life has been extensively reported in large prospective population-based studies (Knopman et al., 2001; Whitmer et al., 2005). In a 25-30 year longitudinal study, individuals with sustained elevation in systolic BP from mid- to late life performed worse on verbal memory and learning whereas those with decreased systolic BP had psychomotor slowing (Swan, Carmelli, & LaRue, 1998). A 20-year longitudinal evaluation showed that in both young (ages 18 – 46) and old (ages 47 – 83) age groups, higher BP was associated with worse performance on tests of visualization and fluid abilities (Elias, Elias, Robbins, & Budge, 2004). In an analysis of a large combined sample from the Rotterdam Study and the Leiden 85-Plus Study, Euser et al. (2009) reported that baseline systolic and diastolic BP elevations predicted lower cognitive performance at the 11-year follow-up among those between ages 65 and 74 but not those below age 65, suggesting that hypertension-associated cognitive decline may be more prevalent in the elderly.

Hypertension in midlife may predispose one to higher risk of dementia in late life, particularly among those with diabetes (Posner, Luchsinger, Langigua, Stern, & Mayeux, 2002). Hypertension may account for cognitive dysfunction independent of diabetes (Bruce et al., 2001). Yau et al. (2009) found that elevated systolic BP was linked to worse immediate emotional memory recall in late middle-aged and elderly individuals with or without T2DM, even after controlling for age. Hypertension may, as suggested by results from the Framingham study, interact with T2DM to exacerbate cognitive impairment in elderly individuals (Elias et al., 1997). Hassing et al. (2004b) showed that although elderly individuals with both T2DM and hypertension had the greatest decline over a 6-

year period in overall cognitive functioning as indexed by MMSE scores, T2DM but not hypertension was associated with accelerated cognitive decline.

In a longitudinal evaluation of middle-aged adults, psychomotor slowing at follow-up was associated with both diabetes and hypertension at baseline whereas verbal fluency was predicted by diabetes but not hypertension (Knopman et al., 2001). Both poorer inductive reasoning (Kumari et al., 2005) and impaired verbal memory (Gold et al., 2007) have been reported in patients with T2DM independent of age, hypertension, vascular complications. In a comprehensive evaluation of cognitive functioning of elderly patients with T2DM, however, no associations were found between hypertension and any of the cognitive measures (van Harten et al., 2007).

Hypertension and brain. Hypertension is known to independently affect brain integrity, with increased occurrences of WMLs being the more frequently reported anomalies. For example, Wiseman et al. (2004) described increased occurrences of subcortical and total periventricular WMH, in addition to smaller whole brain volumes, among older mildly to moderately hypertensive individuals relative to their normotensive counterparts (Wiseman et al., 2004). There is also evidence demonstrating an association between hypertension during midlife and more extensive brain morphological damage in late life. In a prospective analysis, Swan et al. (1998) found that those with high BP during midlife had larger WMH volume in addition to greater decline in cognitive performance in late life (Swan et al., 1998). Similarly, in the NHLBI (National Heart, Lung and Blood Institute) Twin Study, de Carli et al. (1999) described an association

between high BP in midlife and increased WML volumes in late-life in a group of 414 men.

Hypertension and T2DM may synergistically increase the risk for macrovascular and microvascular abnormalities (UK Prospective Diabetes Study Group, 1998) by contributing to increased BBB permeability (Starr et al., 2003); however, the link between hypertension and T2DM-assocaited brain pathology has been inconsistently reported. More silent multiple cerebral infarcts, reduced cerebral *N*-acetyl aspartate concentrations, and lower cerebrovascular reserve have been described in elderly hypertensives with T2DM relative to those without (Kario et al., 2005). Last et al. (2007) also reported more extensive atrophy globally and regionally in the frontal and temporal lobes in addition to finding an association between hypertension and lower CBF in the temporal lobe among T2DM patients with hypertension (mean age in the early 60's). Conversely, Knopman et al. (2005) found an association between enlarged ventricular size and diabetes but not hypertension in a prospective analysis of a middle-aged cohort. Jongen et al. (2007) also failed to find an association between hypertension and cerebral atrophy or increased incidences of WMH among T2DM patients (mean age of mid-60's).

There is emerging evidence suggesting that the frontal lobe may be particularly vulnerable to the adverse effect of hypertension. Specific prefrontal volume reduction and increased frontal WMHs as well as more perseverative errors on the WCST have been reported even in individuals with controlled hypertension (Raz, Rodrigue, & Acker, 2003). Gold et al. (2005) also reported significantly smaller frontal lobe volumes and a non-significant trend

of prefrontal volume reduction, in addition to poorer scores on the Tower of London test (Davis, Bajszar, & Squire, 1994), in hypertensive individuals independent of age, gender, and BMI. Moreover, association with hypertension was restricted to the prefrontal volume. Hypertension-associated frontal hypoperfusion has also been described in T2DM patients (Last et al., 2007).

Hypertension appears to have less impact on the temporal than the frontal lobe. Wiseman et al. (2004) reported non-significantly increased hippocampal atrophy, which was unrelated to BP elevation. Findings from the LADIS (Leukoaraiosis and Disability) study demonstrated an association between MTL atrophy and diabetes but not hypertension in elderly individuals (Korf et al., 2007). In line with these reports, Gold et al. (2005) described volume reductions in the frontal but not temporal lobe among hypertensive individuals. Another report showed that men with untreated midlife high BP had increased risk for hippocampal atrophy (Korf, White, Scheltens, & Launer, 2004).

Dyslipidemia. T2DM and dyslipidemia are both major risk factors for atherosclerosis and cardiovascular disease. Dyslipidemia may be either characterized by reductions in high density lipoprotein (HDL) cholesterol, an elevation in total cholesterol and low density lipoprotein (LDL), and/or triglyceride levels. Cholesterol is an integral part of cell membranes and also plays an important role in protein signaling (Green et al., 1999). Excessive LDL can be accumulated in arterial walls and oxidation of LDL can stimulate endothelial protein expression, which in turn triggers an inflammatory response leading to atherosclerosis (Farmer, 2008). Elevated blood triglyceride levels can impair

endothelial expansion (Hadi, Carr, & Al Suwaidi, 2005). HDL, the so-called "good" cholesterol, protects against atherosclerosis through various mechanisms, including inhibition of LDL oxidation and clearance of cholesterol from foam cells (Barter, 2005). Reduced HDL concentrations are common in patients with metabolic syndrome or T2DM (Brunzell & Ayyobi, 2003).

Dyslipidemia is thought to be related to IR, a key feature in T2DM (Adiels, Taskinen, & Boren, 2008). While the role of lipids in cognition is not well understood, the association between impaired lipid metabolism and cognitive and brain impairments reported in T2DM is even less certain.

Dyslipidemia and cognition. Cholesterol plays an important role in cognitive function. In an 8-year prospective evaluation of a population-based cohort of healthy middle-aged women, better memory performance was associated with higher concentrations of total cholesterol and LDL but not with HDL or triglyceride concentrations measured 3 years earlier (Henderson, Guthrie, & Dennerstein, 2003). In line with these findings, a cross-sectional study also found associations between slower visuomotor speed and lower serum total cholesterol and LDL concentrations, respectively, among young and middle-aged men (Zhang, Muldoon, & McKeown, 2004). These findings suggest that higher lipid levels within the normal range may better support normal cognitive functioning.

HDL may have a protective effect against dementia, particularly in old age, and reductions in HDL have been more consistently shown to relate to lower cognitive performance or higher risk for cognitive decline. For instance, a population-based study of 85-year-old individuals demonstrated that low HDL levels were related to higher risk for dementia as measured by the MMSE independent of level of education and concentrations of LDL and triglycerides (van Exel E et al., 2002). Cross-sectional and post-mortem data also support a higher risk for AD in individuals with reduced HDL levels (Kuo et al., 1998; Merched, Xia, Visvikis, Serot, & Siest, 2000).

In a population-based study of elderly women, those with metabolic syndrome at baseline as well as those with lower baseline levels of HDL had an elevated risk of memory decline at the 12-year follow-up (Komulainen et al., 2007). Also among older adults, higher baseline triglyceride levels have been shown to be associated with worse semantic memory at the 10-year follow-up (de Frias et al., 2007). In the same study, the authors also reported that total cholesterol levels moderate the effect of apolipoprotein E (APOE), a plasma protein for transporting cholesterol and other lipids, on episodic memory. Although these findings suggest that lipid dysregulation may adversely affect memory, there is no clear evidence that memory is preferentially affected.

In a cross-sectional analysis of 246 middle-aged and elderly outpatients with T2DM, Perlmuter et al. (1988) found that T2DM patients with higher triglyceride levels had worse memory and attention and slower reaction time than patients with lower triglyceride levels. Jagusch, Cramon, Renner, and Hepp (1992) reported associations between higher

fasting triglyceride levels and slower reaction time, and between higher fasting cholesterol and worse auditory and visual attention in elderly patients with T2DM. In addition, increased triglyceride levels have been associated with worse performance on verbal fluency among elderly patients with T2DM (Helkala et al., 1995). Among middle-aged individuals with T2DM, the observed psychomotor slowing may alternatively be explained by metabolic dysregulation rather than high BP or triglyceride levels (Ryan et al., 2000). In a comprehensive cognitive evaluation of elderly patients with T2DM, however, neither cholesterol nor HDL level was independently associated with cognitive performance (van Harten et al., 2007).

Dyslipidemia and brain. Cholesterol constitutes approximately 2% of the weight of the brain. As the brain can synthesize most of its required cholesterol, only relatively limited peripheral cholesterol crosses the BBB. The association between lipid levels and brain integrity remains unclear. In a cross-sectional study of elderly individuals, cerebral atrophy as measured by ventricular size was not associated with cholesterol or triglyceride levels (Knopman et al., 2005). In a 6-year follow-up of neurologically asymptomatic elderly individuals, neither elevated cholesterol nor triglyceride level was associated with the baseline brain parenchymal fraction (Enzinger et al., 2005).

The AD Cholesterol-Lowering Treatment trial reported hippocampal volume reductions, particularly on the right side, which were statistically significant, among elderly AD patients who had received a 1-year cholesterol-lowering treatment (Sparks et al., 2008). The authors speculated that hippocampal neuronal loss in AD may be accompanied by

increased intracellular fluid resulting in expanded volumes and therefore, the post-treatment volume reductions may reflect improved fluid balance and thus restored volumes, which in essence mean a healthier hippocampus. It is unclear why the authors did not validate their results with a VBM-based assessment of hippocampal tissue density. Alternatively, DTI-based assessment of water diffusivity in brain tissues can help verify whether fluid balance was indeed improved.

The effect of dyslipidemia on brain integrity in T2DM is not well established. In diabetic mice, not only does a high cholesterol diet impair short term memory retention, it induces increased lipid peroxidation, likely as a consequence of increased oxidative stress, in the frontal cortex and hippocampus (Xie & Du, 2005). This suggests that lipid dysregulation may trigger a chain of cellular events leading to nerve cell damage that may subsequently impact memory and learning. Conversely, high cholesterol diets have been associated with enhanced memory in rabbits (Schreurs, Smith-Bell, Darwish, Stankovic, & Sparks, 2007). Among T2DM patients, the use of statin, a lipid lowering drug, is associated with better cognitive performance and less severe cortical atrophy in T2DM patients (Manschot et al., 2007). The literature remains inconsistent, as there have been two cross-sectional studies of T2DM patients that showed no association between elevated triglyceride levels and global atrophy (Last et al., 2007) or hippocampal volume reduction (Bruehl et al., 2009a).

In summary, several co-morbid conditions, including obesity, hypertension, and dyslipidemia, complicate the interpretation of the literature on the effects of T2DM on

cognition and brain integrity. There is substantial evidence suggesting that the temporal lobe, in particular memory-mediating MTL structures such as the hippocampus, may be differentially affected by hyperglycemia in a cascade of cellular events leading to neuronal damage. Although there is some preliminary evidence of obesity-related temporal lobe atrophy, obesity is likely to play a more crucial role in frontal lobe structural and functional abnormalities, which are more extensively reported. The frontal lobe is highlighted as the brain region more susceptible to the adverse effect of hypertension. The interactive effect of obesity and hypertension may also exacerbate frontal lobe abnormalities. Given the association between obesity and hypertension during midlife and accelerated cognitive decline in late life, it is possible that those who in addition have T2DM in middle age may be predisposed to an even higher risk of accelerated cognitive aging. Given that dyslipidemia is a major risk factor for atherosclerosis, which is known to contribute to cognitive impairment, the role of dyslipidemia in cognition and brain integrity in T2DM is in need of better clarification.

Purpose

The present study seeks to comprehensively evaluate cognitive performance in late middle-aged and elderly individuals with T2DM, while trying to minimize the confounding effects of neurological disease, psychiatric disorder, alcohol and substance abuse relative to a group of age- and education-matched control subjects without IR or T2DM. Although cognitive decline may not be as pronounced among late middle-aged

participants with T2DM, our selection of a comprehensive and sensitive neuropsychological test battery would enable us to detect subtle cognitive decrements.

We would comprehensively evaluate brain integrity, while emphasizing the frontal and temporal lobes. Sensitive imaging analysis methods, including manual and automated volumetric assessment and DTI-based microstructural assessment, were used to characterize gross and microstructural frontal and temporal lobe alterations, respectively. To better delineate the mechanisms underlying the hypothesized frontal and temporal lobe abnormalities**,** both structural and functional, we would ascertain whether the anticipated brain complications could be attributed to poor glycemic control, per se, or by the multitude of common co-morbid conditions.

Hypotheses

Specific aims. To ascertain whether adults with T2DM have both structural and functional complications in the frontal and temporal lobes, the following hypotheses will be tested:

Hypothesis 1 Participants with T2DM perform worse than controls on verbal learning and memory measures but not on visual memory.

Hypothesis 2 Participants with T2DM score lower than controls in non-memory domains including psychomotor efficiency, attention, and executive function, but the effect sizes are anticipated to be smaller than those observed in verbal learning and memory.

Hypothesis 3 Participants with T2DM exhibit lower WM (FA reduction) and GM (ADC elevation) microstructural integrity as measured on DTI images, particularly in the frontal and temporal lobes.

Hypothesis 4 Participants with T2DM exhibit gross volume reductions and cortical thinning specifically in the frontal and temporal regions, and in particular, based on prior work in our lab (Gold et al., 2007) and that of others (den Heijer et al., 2003), hippocampal volumes are hypothesized to be reduced among participants with T2DM relative to controls.

Secondary hypotheses. Exploratory analyses will be conducted to ascertain the influence of quality of glycemic control, hypertension, obesity, and lipid profile on the anticipated brain structural and functional abnormalities among adults with T2DM. The following hypotheses will be tested:

Hypothesis 5 Glycemic parameter, HbA_{1C}, contributes significantly to the anticipated group differences in temporal lobe structure and function (i.e., verbal memory).

Hypothesis 6 Hypertension contributes significantly to the anticipated group differences in frontal lobe structure and function.

Methods

Participants

Participants were consecutively recruited community-residing middle-aged and elderly individuals participating in an ongoing diabetes study at the Brain, Obesity, and Diabetes Laboratory (BODyLab) in the Department of Psychiatry, New York University School of Medicine (NYUSOM). The sample was typical of a research clinical population and was not a randomly drawn sample from the general populaton. Participants were referred by collaborating endocrinologists or individuals responding to advertisements on the internet or in local periodicals, participating in longitudinal aging studies, or were friends, relatives, or associates of other participants.

Forty six middle-aged and elderly patients with T2DM (ages 42.52 – 73.26 years; 24 F / 22 M; education 12 - 20 years) and 50 non-insulin resistant controls (ages 43.09 - 75.75 years; 29 F / 21 M; education 12 - 21 years), well matched for age, gender, and education (see Table 1) were evaluated. All participants were cognitively within the non-demented range, medically within the normal range (other than having diabetes, hypertension, or dyslipidemia) and had a minimum of a high-school education in order to reduce the potential variance in the cognitive assessment caused by including participants with lower education levels (Stern et al., 1994).

Procedures

The protocol was approved by the Internal Review Boards (IRB) at the NYUSOM and Queens College of the City University of New York. Once having passed the initial phone or questionnaire screening and having met the study entry criteria, participants received medical, endocrine, psychiatric, neuropsychological, and brain MRI evaluations/assessments during a comprehensive 8-hour evaluation completed over 2 visits on separate days within a one-month period. The neuropsychological assessment was conducted in the post-prandial state, split over two sessions with a total time of approximately 2.5 hours.

First visit - medical and psychiatric evaluations. On the first visit, the participant received medical and psychiatric evaluations by qualified medical professionals at the BODyLab of NYUSOM. An overnight fast of 10 to 12 hours was required for the blood test. Free breakfast or lunch was provided after some of the medical evaluations as needed. Comprehensive clinical evaluations included: (i) Laboratory tests consisting of blood analysis (a baseline blood draw of 35cc) for determining the status of metabolic regulation, for example, fasting glucose level, fasting insulin level, lipid profile, thyroid functions, etc., (ii) Electrocardiogram (EKG) for assessment of coronary disease, (iii) Measures of diastolic and systolic BP for assessing the status of hypertension, (iv) Psychiatric assessments of depression and alcohol and substance abuse using the National Institute of Mental Health Quick Diagnostic Interview Schedule III-Revised (Q-DIS; Marcus, Robins, & Bucholz, 1988) and the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960), (v) Assessment of global functioning

and screening of dementia using the Global Deterioration Scale (GDS; Bergmann, 1975) and the MMSE (Folstein, Folstein, & McHugh, 1975), (vi) Modified Hachinski Ischemia Scale for detection of significant cerebrovascular disease (Rosen, Terry, Fuld, Katzman, & Peck, 1980; see below for details). Part 1 of the neuropsychological battery was also administered.

Second visit - neuropsychological and neuroimaging assessment. Part 2 of the neuropsychological assessment was conducted. MR sequences of the participants were acquired for brain gross and microstructural assessment. All procedures were conducted by trained MRI technicians with the principal investigator present to ensure adherence to our predefined procedures. Total scanning time per participant was estimated to be 30 - 45 minutes. MR images were downloaded with dummy-coded filenames and subsequently transported to our local secured SUN servers for storage and analysis. All written documents were kept in locked cabinets at our center.

Informed consent. All participants signed an informed consent (see Appendix B), which detailed the purpose, procedures, possible risks, and benefits of their participation, and were informed that their participation was voluntary and that they could withdraw at any time and for any reason. The consent form was drafted in accordance with the policies of the IRB of Queens College and of the IRB of NYUSOM.

Medical and Psychiatric Evaluations

Medical and psychiatric evaluations and brief cognitive screening were conducted by qualified medical professionals at the BODyLab, NYUSOM.

Physical and neurological exams. A qualified medical staff member completed a physical examination including waist, hip, and height measurement and evaluation of peripheral vascular disease. See Appendix C for a copy of the demographic questionnaire. The Quantitative Medical Evaluation Rating Scale was administered. Patient and family medical history, current medical conditions, medications, and complaints were documented. A brief neurological exam was conducted to rule out neurologic disease. All participants were screened for significant cerebrovascular disease using the Rosen modification of the Hachinski Ischemia scale. The scale includes items such as history of hypertension, history of stroke, focal neurological symptoms, and focal neurological signs, with the maximum score being 12.

Subject classification. A participant was classified as having T2DM if their fasting glucose level was above 126 mg/dL, casual plasma glucose concentration above 200 mg/dL, or 2-hour post-load glucose concentration above 200 mg/dL during an oral glucose tolerance test (OGTT; Stumvoli et al., 2004; CDC, 2006), which is known to estimate insulin release and sensitivity with reasonable accuracy. The QUICKI score is a reliable measure of insulin sensitivity calculated from fasting glucose and insulin levels,

which has been validated against the "gold standard" glucose clamp and minimal model analysis (Katz et al., 2000).

Participants with a fasting glucose of less than 110 mg/dl but with a QUICKI score less than 0.35 were given an OGTT, which entails receiving 75 grams of glucose orally after a 12-hour overnight fast. Participants with a 2-hour glucose value post-load above 200 mg/dl were considered diabetics. Those with values between 140 mg/dl and 199 mg/dl were considered to have impaired glucose tolerance were classified as having non-diabetic insulin resistance and were not excluded from the present study. Those with 2-hour values of less than 140 mg/dl were classified as non-insulin resistant. Participants were classified as non-diabetic controls with no evidence of insulin resistance if they had no current diagnosis of diabetes and had no significant IR, as demonstrated by a QUICKI score of 0.35 or above (Katz et al., 2000).

BMI was calculated by dividing weight in kg by height in meters squared. Sitting BP was determined by averaging two readings obtained during the second visit: 30 minutes after arrival and at the end of that evaluation. Participants were classified as hypertensive if they received anti-hypertensive treatment or had a sitting BP above the NCEP cut-off (a systolic BP $\geq$ 130 mmHg or a diastolic BP $\geq$ 85 mmHg; Expert Panel, 2001).

A participant was classified as having dyslipidemia if any of the following conditions was met:

- Prior or current statin treatment

- HDL ≤ 41 for men or HDL ≤ 50 for women
- Triglyceride level ≥ 200
- LDL ≥ 130

Psychiatric and behavioral assessments.

National Institute of Mental Health Quick Diagnostic Interview Schedule III-Revised (Q-DIS). The Q-DIS is a brief computerized version of the highly structured DIS-Revised used to screen individuals for alcohol or substance abuse and potential psychiatric disorders, such as depression, as defined by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria (Marcus, Robins, & Bucholz, 1988), which, if needed, was followed-up with the appropriate National Institute of Mental Health Diagnostic Interview Schedule (DIS; Robins, Helzer, Croughan, & Ratcliff, 1981). The test consists of 25 diagnostic sections presented as questions on a computer monitor and the subject keys in the responses. As opposed to the hierarchical diagnostic determination used in the DIS-Revised, the Q-DIS employs a categorical diagnostic system by which a diagnostic decision is made when minimum diagnostic criteria are met or not. The original validation study showed that with the exception of obsessive-compulsive disorder and generalized anxiety disorder, the Q-DIS shows high sensitivity and specificity (kappa coefficient ranging from 0.47 to 1.00 as well as excellent diagnostic agreement with the DIS-Revised (Marcus et al., 1988).

Hamilton Depression Rating Scale (HAM-D). In addition to the Q-DIS, the HAM-D, a clinician-administered assessment scale, was used to quantify sub-clinical depressive symptoms (Hamilton, 1960). The HAM-D is a clinician-administered assessment scale that measures the severity of symptoms, such as depressive mood, anhedonia, insomnia, hypochondriasis, and obsessive and compulsive symptoms. It was originally designed to assess severity of depression in previously diagnosed depressed in-patients but is widely used in both clinical and research settings. The HAM-D has high discriminatory power for separating depressed from non-depressed (Fava, Kellner, Munari, & Pavan, 1982) and for other emotional disorders (Stukenberg, Dura, & Kiecolt-Glaser, 1990). The clinician rates the severity of symptoms for each of the 21 items by the subject's responses and by the clinician's observations. Scores 0 – 15 are considered within normal range; scores 15 – 25 are classified as moderate depression, and scores 25 and above indicate severe depression.

Brief cognitive assessment.

Global Deterioration Scale (GDS). The GDS is 7-stage rating scale of subjective evaluation of global functioning and is widely used as a brief assessment of dementia (Bergmann, 1975) as well as an AD staging instrument (Eisdorfer et al., 1992). The scale, chosen for the exclusion of individuals with dementia in the present study, has excellent validity (Paul et al., 2002), reliability and correlates well with other dementia severity instruments. It is scored based upon subjective complaints of memory difficulty and objective observation of deficits based on careful interview by a trained clinician. Scores

range from 1 - 7: scores 1 - 2 are classified as being functionally normal, a score of 3 is indicative of possible MCI, and scores above 3 are classified as indicating possible dementia.

Mini-Mental State Exam (MMSE). The MMSE was originally designed to be a brief cognitive assessment tool for psychiatric Patients (Folstein, Folstein, & McHugh, 1975) but has since been used as a screening tool for dementia. The test has proven validity (Cockrell & Folstein, 1988), good test-retest reliability (Mitrushina & Satz, 1991), minimal practice effects (Tombaugh, 2005), and has been shown to be sensitive to health-related cognitive decline (Eslinger, Swan, & Carmelli, 2003). The MMSE score also correlates well with other verbal memory measures (Mitrushina et al., 1991). It is a 30-point questionnaire that assesses orientation, registration, attention, calculation abilities, recall and copying ability. Scores of less than 23 are considered indicative of possible dementia.

Subject Exclusion

Based on the outcome of clinical and psychiatric evaluations, a participant was excluded from the study if he/she had:

Medical Evaluation:

- A history of current insulin treatment to avoid the possible confounding effects of hypoglycemic episodes.
- Fasting glucose < 70 or > 126 mg/dL.
- Uncontrolled hypertension (BP > 150/90 mm/Hg).

- Significant coronary ischemic disease detected on the EKG or a Modified Hachinski Ischemia Scale score >4.
- Any focal neurological signs.
- Current diagnosis or past history of stroke, or a significant head trauma.
- Evidence of tumor on the structural MR scan

Psychiatric Evaluation:

- A history of significant psychiatric disorders such as schizophrenia, mania, and bipolar diseases; seizures and/or other serious neurological disorders.
- Indication of significant depression, or alcohol or substance abuse/dependence on the Q-DIS, and/or a HAM-D rating above 16.

Global Functioning:

- A family history of early-onset AD
- Indication of dementia as reflected by a GDS score of 3 or above, or a MMSE score below 27.

Neuropsychological Assessment

Each participant received a comprehensive battery of neuropsychological tests that have been described in detail by Lezak et al. (2004), targeting cognitive domains, including verbal memory, visual memory, working memory, attention, executive function, and psychomotor functions. These standardized tests have known validity and reliability and that have been used extensively to assess frontal and temporal lobe functions. Participants

were also evaluated for their overall intellectual functioning. Given the large number of cognitive variables generated by this comprehensive test battery, only selective key measures that have been shown to statistically differentiate between non-diabetics and diabetics in prior reports from our lab were evaluated (e.g., Bruehl et al., 2009a; Gold et al., 2007).

General intellectual functioning.

Shipley Institute of Living Scale (SILS). The SILS was originally designed to detect mild intellectual impairment in individuals with normal pre-morbid intelligence but has since been used as a brief and reliable estimate of intellectual functioning (Shipley, 1940). The SILS remains one of the most popular brief intelligence scales used to-date and correlates strongly with IQ scores obtained from the Wechsler Adult Intelligence Scale - Revised (WAIS-R; Weiss & Schell, 1991) and from the Kaufman Brief Intelligence Test (Bowers & Pantle, 1998). It consists of vocabulary and logic subtests, the scores of which are combined to yield a WAIS-R estimated Full Scale IQ score. The Vocabulary subtest consists of 40 items in multiple-choice format where the participant chooses one of four possible words that has the same meaning as the target word. The Abstraction subtest consists of 20 items in a completion format where the individual is presented with a logical sequence and is asked to fill in the numbers or letters that best complete the sequence. The combined score of the two subtests is converted to a conceptual quotient, which then yields a WAIS-R Full Scale score estimate stratified by age.

Learning and memory.

California Verbal Learning Test (CVLT). The CVLT is designed to assess learning, short- and long-term verbal memory, verbal learning strategies, and conceptualization (Delis, Kramer, Kaplan, & Ober, 1987). The test has been extensively used to detect learning and memory difficulties in normal aging and in a wide range of patient populations, including those with MCI (Petrella et al., 2007), AD (Delis et al., 2010), dementia (Lekeu et al., 2010), Parkinson's disease (O'Brien et al., 2009), Huntington's disease (Lafosse, Corboy, Leehey, Seeberger, & Filley, 2007), depression (Elderkin-Thompson, Mintz, Haroon, Lavretsky, & Kumar, 2007), and epilepsy (Loring et al., 2008). The participant is given five trials to learn and recall a shopping list of 16 items (the Monday List), four in each of the semantic categories (clothing, fruit, tools, and herbs and spices). After an intercepting new list of 16 items (the Tuesday List), short delay free and cued recall of the first shopping list is tested, followed 20 minutes later by free and cued recall and recognition.

The CVLT generates a large number of measures targeting different aspects of learning and memory, and only selective measures were utilized in the present study. The total number of words recalled over the five learning trials of the Monday List was used to assess learning, with scores ranging from 0 to 80. Raw scores for the immediate and delayed free as well as cued recall were reported, with values ranging from 0 to 16 (number of words recalled). Recognition hits is the number of words recalled for the Monday list.

Wechsler Memory Scale Revised (WMS-R). The WMS-R is a comprehensive learning and memory assessment tool targeting verbal memory, auditory and visuospatial working memory, visual memory, attention, and concentration, covering ages 16 - 90 (Wechsler, 1987). The WMS-R is one of the most widely used memory assessment instrument used in clinical research (Shadmehr, Brandt, & Corkin, 1998). With the exception of the subtests of attention/concentration and working memory, each test has both immediate and delayed conditions. Only raw scores are reported in the present study.

The *Logical Memory subtest* assesses one's ability to learn and recall contextual verbal information in two brief paragraphs, and it demonstrates high discriminating power in differentiating healthy older adults from those with very mild dementia (Storandt & Hill, 1989). The participant is tested for recall, word for word, immediately after each paragraph is read and, again, 20 - 25 minutes later. The maximum score for the two paragraphs combined is 50 for each recall condition.

In the *Verbal Paired-Associates subtest,* the participant learns eight novel word pairs, half of which are related semantically and half non-semantically. The list of word pairs is read to the participant in different orders over six learning trials. During the learning trials, the first word in the pair is read and the participant is asked to produce the paired word. If the response is incorrect, the examiner provides the correct response. If all the items are answered correctly in trial 3, no further learning trials are administered. The total number of correct responses over the first three trials yields the score for immediate recall, with 24

being the maximum score. The participant is tested for delayed recall 20 - 25 minutes later, with the maximum score being 8.

In the *Visual Paired-Associates subtest,* the participant learns six novel color-figure pairs. In each of the six trials, the participant is first shown the six color-figure pairs, and each figure is then presented to the participant, who is asked to identify the paired color on the key card. Any incorrect response is corrected. Learning discontinues if the participant answers all the items correct in trial 3. The number of correct responses over the first three trials yields the total score for the immediate condition, with 18 being the maximum score. Delayed recall is tested 20 - 25 minutes later, with the maximum score being 6.

The *Digit Span subtest* has two components, one targeting short-term auditory memory (Digit Span Forward) and the other phonological working memory (Digit Span Backward). The forward condition consists of six items with two trials each, with the number of digits increasing from 3 to 8. The participant is read sequences of digits and is asked to repeat the digits in the same order. In the backward condition, the participant is asked to repeat sequences of 2 to 7 digits in the reverse order. The test is discontinued after failure on both trials of any item. The maximum score is 12 for each condition.

Similar to the Digit Span subtest, the *Visual Memory Span subtest* is designed to assess visuospatial working memory. The test administer taps sequences of boxes printed on a cardboard and asks the participant to repeat the tapping sequences in the same (Visual Span Forward; sequences of 2 - 8 boxes; seven items of two trials each) or reverse order

(Visual Span Backward; sequences of 2 - 7 boxes; six items of two trials each). The test is discontinued after failure on both trials of any item. The maximum scores are 14 and 12 for the forward and backward conditions, respectively.

The *Visual Reproduction subtest* is a test of non-verbal learning and memory. The participant is shown four cards of different geometric designs of increasing complexity, one at a time, for 10 seconds and is asked to reproduce the design from memory on a piece of a paper immediately after the design is presented. The participant is asked to reproduce the designs from memory again after 20 - 25 minutes later. The accuracy of the design reproduction is judged by the shape, angle, length, proportions of parts, and symmetry. The maximum score is 41 for each recall condition.

Guild Memory Test (GMT). The GMT is a test of verbal learning and memory, which has been shown to be sensitive to both aging (Ferris, Crook, Clark, McCarthy, & Rae, 1980) and dementia (Catalano, Levee, & Catalano, 1968) and to be associated with hippocampal atrophy (Convit et al., 1997). The test consists of immediate and delayed recall of two paragraphs with interference tasks. The participant is read the first paragraph and then asked to recall, verbatim, as many components as possible. The paragraph is read to the participant a second time before an interference task is performed. Delayed recall of the first paragraph is then tested 15 minutes later followed by the administration of the second paragraph in the same format. The second paragraph is read a second time after immediate recall, and delayed recall is tested after a second interference task. The

scores for the two paragraphs are averaged to yield the overall score for immediate and delayed recall, respectively. The maximum score is 21.

Attention.

Digit Vigilance (DVT). The DVT is a paper-and-pencil test of sustained attention (Lewis & Rennick, 1979) with established validity and reliability (Kelland & Lewis, 1996). The test consists of lines of randomly organized digits printed on two pages. The participant is asked to cross out the number "9" as quickly as possible. The total time taken to complete the two pages is the key measure.

Psychomotor efficiency.

WAIS Digit Symbol Substitution Test (DSST). The DSST is a non-verbal test designed to assess complex attention in coordination with visuomotor skills (Wechsler, 1981). The test has been shown to be a useful tool for detection of psychiatric disorders (e.g., Dickinson, Ramsey, & Gold, 2007) and neurological diseases (Maddocks & Saling, 1996) and is known to be sensitive to age-related frontal lobe dysfunction (Parkin & Java, 1999). Using 10 symbol-digit pairs as a guide, the participant is asked to write out as quickly as possible the associated symbol for each digit listed in random repetitions within a 90-sec time limit. Scoring is based on the number of correct matches.

Perceptual Speed Test (PST). The PST is a cancellation test that measures attention (Moran & Mefferd, 1959). The test consists of lines of randomly organized digits printed on two pages. In each row, the participant is asked to cross out digits that are the same as the first circled digit of that row. The test yields the total number of correct cancellations within 150 min.

Executive function.

Tower of London (TOL). The TOL is a computerized test that is extensively used to assess planning and strategy formation (Shallice, 1982). The TOL is known to be sensitive to frontal lobe damage (Shallice & Burgess, 1991) as it has been shown to activate the fronto-parieto-thalamic network (Wagner, Koch, Reichenbach, Sauer, & Schlosser, 2006); in particular, the prefrontal cortex (PFC) plays an important role. The participant is instructed to re-arrange a set of colored beads on pegs, one at a time, to match the goal arrangement. The goal is to complete each arrangement with the least number of moves. Each trial is followed by a break of a few seconds. The key measure used is the total number of excess moves.

Wisconsin Card Sort Test (WCST). The WCST assesses one's abstract reasoning and ability to change problem solving strategies as shifts in the sorting principle occur. The WCST has been used extensively to assess frontal dysfunction in both aging (Bryan & Luszcz, 2000) and clinical neuropsychological research (review in Nyhus & Barcelo, 2009). The present study uses a computerized version of the WCST, which consists of a

deck of 128 response cards with symbols that vary in color (red, green, yellow, and blue), number (1 - 4), and shape (triangle, star, cross, and circle). Four reference cards are presented on the screen, each having a different combination of those parameters (four red crosses, two yellow circles, and so forth). The participant is asked to match the response card to one of the four reference cards presented on the screen. The participant is asked to figure out how the cards should be matched without being told how to do so. The only feedback the participant receives is whether a response is "right" or "wrong". Once the participant successfully matches the cards in a specific number of consecutive trials, an unannounced shift in rule occurs. The WCST continues until all cards are sorted or when six correct sorting criteria have been reached. The test takes about 12-20 minutes to complete. The number of perseverative errors was used as the key measure.

Stroop Color Word Interference Test (SCWI). The SCWI measures frontal inhibitory response as well as processing speed in three subtests (Golden, 1978). The goal to assess one's flexibility to switch perceptual set by suppressing an automatic response in favor of an incompatible response. The participant is given 45 seconds to complete each of the three subtests, and the number of errors is subtracted from the number of correct responses to yield the total score for that subtest. On the first Word subtest, the participant is asked to read out words of color (i.e. "GREEN", "BLUE", and "RED") printed in black ink as quickly as possible. The second Color subtest requires the participant to name the color in which each symbol is printed in. In the last Color-Word subtest, participant is presented with words of color printed in incompatible colors (e.g., the word 'GREEN' printed in red ink) and is asked to name the color of the ink each word is printed in. The

goal is to measure how well one can override the *automatic response* to the semantic meaning of the word by identifying the color of the ink (an *incompatible response*). An interference score is computed by subtracting the predicted number of correct responses from the actual score for the Color-Word subtest. The predicted Color-Word score is calculated by multiplying the scores from the Word and Color subtest and then divided by the sum of the two scores. A higher interference score indicates high resistance to interference. Key measures used were the score of Color-Word subtest and interference scores.

Controlled Oral Word Association Test (COWAT). The COWAT is a sensitive test of phonetic verbal fluency (Benton & Hamsher, 1976). Verbal fluency is known to be affected in neurodegenerative disease such as dementia (Pasquier, Lebert, Grymonprez, & Petit, 1995) and AD (Pachana, Boone, Miller, Cummings, & Berman, 1996) and has been shown to be sensitive to lesions in the frontal and temporal lobes (Coslett, Bowers, Verfaellie, & Heilman, 1991). The participant is asked to produce quickly and spontaneously as many common words as possible that begin with a given letter of the alphabet ("F", "S", or "A") within 60 seconds. The total number of words generated in the three trials yields the total score.

Magnetic Resonance Imaging (MRI) Assessment

The goal of the MR-based assessment was to assess brain morphological changes using manual-based volumetric assessment, automated cortical thickness measurement, and

voxelwise assessment of brain microstructural integrity. One participant with T2DM was missing all MR scans. A total of 95 participants received structural MR scans either on the 1.5 T Siemens Avanto machine (59 participants: 32 participants with T2DM and 27 controls) or the 1.5 T General Electric Vision machine (36 participants: 13 participants with T2DM and 23 controls) using equivalent sequences. Of the participants scanned on the Siemens machine, DTI and other MR sequences for supporting DTI analysis were also acquired. The DTI scan of one participant with T2DM was excluded from the analysis due to extensive movement artifact, leaving a total of 58 DTI scans (31 participants with T2DM and 27 controls) for analysis.

Magnetic resonance image acquisition.

Sequences acquired on the Siemens 1.5T Avanto machine. A T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (TR 1300 ms; TE 4.38 ms; TI 800 ms; FOV 250 x 250; 196 coronal slices; slice thickness 1.2 mm; no gap; NEX 1; Flip angle 15°) was used for volumetric assessment. A DTI echo planar sequence (TR 6100 ms; TE 75 ms; delay in TR = 0; b values 0, 1000 s/mm^2; 6 diffusion directions; FOV 210 × 210; 4 averages and 1 concatenation; 50 axial slices; no gap; voxel size 1.64 x 1.64 x 3 mm^3) was acquired to assess brain microstructural integrity. Two additional MR sequences, a T2-weighted and a FLAIR sequence were acquired to support DTI analysis. The T2-weighted sequence (TR 9000 ms, TE 94 ms; TI 2000 ms; FOV 210 x 210; 50 axial slices; slice thickness 3 mm) was used along with the MRPAGE image as anatomical guide to process the DTI images. The FLAIR sequence (TR 9000 ms; TE 97

ms; FOV 210 x 210; 1 average and 2 concatenations; 50 axial slices; no gap; Flip angle 145°) was used so as to assess group differences in DTI metrics after accounting for gross WM abnormalities. The FLAIR sequence was also used together with the MPRAGE to rule out primary neurological disease.

To optimize image registration, the DTI, T2-weighted, and FLAIR sequences were acquired in the same orientation, number of slice, and thickness and were standardized at a scan angle parallel to a line drawn between the anterior and posterior commissure (AC-PC line).

Sequences acquired on the General Electric 1.5T Vision machine. The T1-weighted spoiled-gradient inversion recovery (SPGIR) sequence (TR 30 ms; TE 2 ms; FOV 250×250 mm; 124 coronal slices; slice thickness 1.5 mm; no gap; NEX 1; Flip angle 60°), equivalent to the T1-weighted MPRAGE sequence acquired on the Siemens scanner, was also used for volumetric assessment. Validation analysis that was previously conducted on MR structural scans acquired on both scanners for 10 individuals confirmed no inter-machine volume differences.

MRI-derived brain analysis.

Brain volumetric assessment – manual tracing method. Volumetric assessment was done using an in-house developed software called Multimodal Image Data Analysis System (MIDAS) v.1.11 (Tsui, 1995). The structural MPRAGE and SPGIR images were

re-formatted into the standardized "pathological angle" coronal and sagittal planes. The signal intensity was first normalized to correct for signal inhomogeneity so as to ensure adequate gray–white matter contrast for accurate volume assessment. The regions of interest (ROIs) were manually drawn, blind to participants' identity and diagnosis, for the intracranial vault (ICV) in the sagittal plane and for the frontal and temporal regions in the coronal plane, which included the prefrontal region (PFR), dorsolateral prefrontal region (DLPFR), hippocampus, and superior temporal gyrus (STG). The ROIs were manually traced on threefold enlarged images in MIDAS and were re-checked for accuracy and corrected when necessary in the orthogonal planes. Volume for an ROI was estimated from the voxel counts times the voxel volume over the slices chosen for that region.

ICV volume and global atrophy. To account for inter-subject variability in brain size, in the volumetric measures of regional structures, we obtained an estimate of 'premorbid' brain size by manually tracing the ICV of the supratentorial compartment in the sagittal plane, following the margins of the dura and tentorium (see Figure 1). The manual tracing was done on every 5^{th} slice, and the overall ICV volume was estimated by multiplying the obtained volume by 5. Given that CSF has higher signal intensity than brain tissues, an operator-based intensity thresholding procedure was used to identify voxels above a threshold for the CSF portion of the ICV, which yields an estimate of the global atrophy (see Figure 1).

Hippocampus. The hippocampal volume was manually traced on the coronal plane in MIDAS using a published reliable method described in Convit et al. (1997). This

method has been validated against post-mortem MRI evaluations (Bobinski et al., 2000). The structural image was simultaneously displayed in orthogonal views (see Figure 2), which allowed us to better define the hippocampus-amygdalar boundaries using a published reliable method (Convit et al., 1999). The sampling of slices for the structure encompassed the neck of the hippocampus, where the anterior margin of the lateral geniculate body is, and the tail of the hippocampus, corresponding to the level of the posterior pulvinar. The hippocampus is bound laterally by the medial wall of the temporal horn and the hippocampal body is bound medially by the CSF of the choridal, hippocampal, and transverse fissures. The inferior boundary is the parahippocampal gyrus (PHG), which is not included in the hippocampal volume estimation. For further methodological details, please refer to Convit et al. (1997).

STG. As a medial border, the STG has a line joining a reference point in the middle of the temporal horn to the most medial and inferior extension of the Sylvian fissure (Convit et al., 1997; see Figure 3). The STG is bound superiorly by the Sylvian fissure and inferiorly by the superior temporal sulcus.

DLPFR and PFR. The DLPFR and PFR were chosen as the frontal regions of interest given their specific involvement reported in studies of varied frontal lobe-based functions, including working memory, attention, and executive function. Using a frontal lobe parcellation method (Convit et al., 2001), which has been validated with all inter-rater intra-class correlation coefficients > 0.94, the DLPFR was defined on the MPRAGE in the coronal orientation. The DLPFR is bounded anteriorly by the cingulated sulcus and

posteriorly by the anterior margin of the supplementary motor cortex (See Figure 4). The DLPFR ROI was expanded anteriorly to include the frontal pole, which together with the DLPFR, form the overall PFR ROI. Please refer to Convit et al. (2001) for details.

Regional atrophy estimation and ICV adjusted volumes. Using the same intensity thresholding procedure mentioned above, the CSF portion for each ROI is estimated and subtracted from the total volume of that ROI to yield the actual regional volume. To account for "pre-morbid" brain size in regional volume estimation, a common method is to express the regional volume as a ratio of the ICV volume, which has however been shown to yield spurious correlations (Van Petten, 2004). Consequently, we obtained an ICV-adjusted volume for each of the ROIs (left and right sides combined) using a linear regression analysis with the regional volume as the dependent variable and the ICV volume as the independent variable. This yielded the unstandardized residual, which represents the ICV adjusted volume for that region.

Brain volumetric assessment – FreeSurfer-based cortical thickness measurement. The cerebral cortex is a highly convoluted sheet of neurons with extensive folds. Measurement of cortical thickness provides valuable information that is buried in the cortical folds inaccessible with conventional volumetric assessment methods. Normal aging is often accompanied by macroscopic changes including global and regional volume reduction (Anderton, 2002) and cortical thinning (Magnotta et al., 1999; Salat et al., 2004). Cortical thinning has also been characterized in conditions such as MCI (Julkunen et al., 2009), dementia (Du et al., 2007), Huntington's disease (Selemon, Rajkowska, &

Goldman-Rakic, 2004), and schizophrenia (Schultz et al., 2010), and similar to brain volumetric measures, higher cortical thickness is associated with higher IQ (Narr et al., 2007) and better performance in memory (Dickerson et al., 2008) and executive function (Andersson, Ystad, Lundervold, & Lundervold, 2009).

Manual tracing of the cortical ribbon is rather labor-extensive, even for an experienced neuroanatomist. More recent advancement has made it possible to automatically and accurately measure the thickness of the cortical ribbon. The FreeSurfer (FS) toolkit is a common Linux-based image analysis software package freely available on the internet (http://surfer.nmr.mgh.harvard.edu) that employs an automatic computational approach to accurately measure cortical thickness. The FS surface-based cortical thickness approach has gained increasing popularity and provided valuable information regarding cortical thickness in normal development (Westlye et al., 2010), aging (Fjell, Amlien, Westlye, & Walhovd, 2009) and pathological conditions (Gutierrez-Galve et al., 2009; Lehmann et al., 2010; Lyoo, Ryu, & Lee, 2010) with high inter-machine reliability (Han et al., 2006) and within-subject reliability for association with cognition (Dickerson et al., 2008).

The structural MPRAGE or SPGIR scans are processed using standard options in FS taking approximately 24 hours of processing time per scan. The structural image is converted to FS image format followed by a series of preprocessing steps. Intensity normalization (Sled, Zijdenbos, & Evans, 1998) is first performed to correct for intensity variations due to common artifacts in MR acquisition and to match with the FS atlas image intensity histogram so as to standardize and optimize tissue classification. Using a

watershed/surface deforming algorithm, the brain is then skull-stripped to remove non-brain tissue (Segonne et al., 2004). The intensity normalized and skull-stripped brain is then subjected to an affine transformation to Talairach space.

Thickness at any given point on the cortex is defined as the distance between the gray-white boundary and the pial surface. To define these boundaries, a WM segmentation procedure is used to preliminarily label voxels as either WM or non-WM tissue based on intensity and geometric information (Fischl et al., 2002; see Figure 5a), which yields an initial WM volume that may have mislabeled voxels (CSF contamination or missing WM voxels). To refine the segmentation results, WM is filled and a tessellation process is used to connect the surfaces of the filled WM voxels into a continuous surface using patterns of small triangles. The reconstructed surface, which becomes somewhat jagged after tessellation, is subjected to topological correction and smoothing (Fischl, Liu, & Dale, 2001; Segonne et al., 2004)

The processed brain is automatically separated into two hemispheres and surface deformation is done by hemisphere from this point on (Fischl, Sereno, & Dale, 1999). The cortex is first inflated and the resulting ballooned figure is the pial surface or the GM/CSF boundary. The inflated cortex is then flattened and morphed into a two-dimensional cortex surface per hemisphere. Inter-subject registration of each hemispheric cortical surface is performed by aligning the cortical surface to a standard spherical cortical surface-based atlas (coordinate system) by the folding patterns of the cortex point to point, which yields the spherical left and right hemispheres. FS uses an automatic

cortical parceling algorithm to assign an anatomical label to each triangular vertex on the entire cortex using probabilistic information estimated from manually labeled data with stored prior statistics about cortical structures (Fischl et al., 2004).

The average cortical thickness for each anatomical label is represented by the distance between the gray/white boundary to the gray/CSF (pial) boundary at each vertex (Fischl & Dale, 2000; see Figure5b). FS also provides estimates of the surface area for each region examined. Given the extensive number of brain regions generated by FS, only the frontal and temporal regions that corresponded to the results from the automated DTI-based analysis described below were selected for the statistical analysis. The average cortical thickness of each selected region was determined by the average of the cortical thickness, weighted by the surface areas of both the left and right sides.

DTI-based cerebral white and gray matter microstructural assessment. DTI is a non-invasive, powerful MR technique that makes use of the Brownian motion of water molecules in tissues to capture microstructural and physiological information in skeletal tissues, muscular structures, and brain tissues, which cannot be detected using conventional MRI techniques (Rugg-Gunn, Symms, Barker, Greenwood, & Duncan, 2001). "Tensor" is a mathematical construct used to describe tension forces, which can be conceptualized as a set of three-dimensional vectors. The diffusion tensor can be visualized as an ellipsoid with three mutually perpendicular eigenvectors, each of which has a corresponding eigenvalue representing the principal diffusivity along the axis. The

two most commonly used DTI indices are FA and ADC, for white and gray matter assessment, respectively.

Diffusion is said to be anisotropic, namely directionally dependent, in tissues such as nerve fibers where there is a strong resistance to water diffusion orthogonal to the direction of the axon. In cerebral WM fiber, water diffusion is greatest along the long axis where water resistance is the weakest. In contrast, diffusion is isotropic, namely directionally independent, in CSF. The most commonly used DTI index for assessing WM integrity is FA, which measures the degree of diffusion anisotropy and thus provides an estimate of the degree of fiber organization, directional coherence, or integrity. FA values range from 0 (fully isotropic diffusion) to 1 (fully anisotropic diffusion; Basser & Pierpaoli, 1996).

Cerebral WM FA has been shown to decline with age (Pfefferbaum et al., 2000). In conditions such as depression (Alexopoulos, Kiosses, Choi, Murphy, & Lim, 2002), schizophrenia (Ardekani et al., 2005a), fronto-temporal dementia (Olesen, Nagy, Westerberg, & Klingberg, 2003), and drug abuse (Ma et al., 2009), FA decline has been observed in brain regions where abnormalities are known to be prevalent. FA values of regional WM structures have also been associated with cognitive performance (Kubicki et al., 2003; Nestor et al., 2004; Schulte, Sullivan, Muller-Oehring, Adalsteinsson, & Pfefferbaum, 2005); for example, our lab has previously reported using the same methodology described below an association between FA reduction in the temporal stem

and memory performance (Yau et al., 2009), suggesting that subtle fiber disruptions may hinder the signal transduction necessary for supporting normal cognitive functioning.

ADC, which represents the mean magnitude of water diffusivity, provides an assessment of tissue density (Gupta et al., 2000) and is commonly used to assess GM integrity. Elevated ADC values represent high diffusivity, which may reflect increased extracellular space or increased edema, a possible indication of neuronal damage. This is supported by findings of inverse relationships between ADC and levels of neuronal metabolites such as choline and NAA levels, both of which are indices of neuronal structures (Irwan, Sijens, Potze, & Oudkerk, 2005). ADC is known to be elevated in normal aging (Ardekani, Kumar, Bartzokis, & Sinha, 2007) and has been found to be useful in classification of brain disorders (Sener, 2001). Association between ADC elevation and cognitive impairment has been described in conditions such as MCI (Ray et al., 2006) and multiple sclerosis (Benedict et al., 2007). Consistent with data from volumetric studies, ADC values of the frontal and temporal lobe structures have been implicated in memory processing (Ray et al., 2006; Sasson, Doniger, Pasternak, & Assaf, 2010).

Normalization of FA & ADC maps to standard space. The goal of the DTI processing was to correct for spatial distortion artifacts on the DTI images, register to a target MPRAGE image (voxel size 1 x 1 x 1 mm) reformatted in the axial plane in Talairach space, and create group FA and ADC maps for voxelwise comparisons. Intra-subject and inter-subject registrations were performed using MIDAS and the Automated Registration Toolkit 2 (ART2) software developed at the Nathan KIine Institute (NKI;

Ardekani et al., 2005b). In an evaluation of 14 non-linear deformation algorithms for image registration used worldwide, ART2 was ranked among top four yielding the best registration results and was among top two producing consistently high accuracy (Klein et al., 2009).

First, the structural native MPRAGE image was manually skull-stripped to remove non-brain tissue, which was then spatially normalized to the target image using a 3D non-linear warping algorithm (Ardekani et al., 2005b). This step yielded a 3D warp field containing transformation parameters necessary for spatial normalization of the DTI maps.

Second, a rigid-body linear transformation was used to optimize the registration of the T2 to the MPRAGE image (Ardekani, Braun, Hutton, Kanno, & Iida, 1995) by first re-slicing the native MPRAGE image to match the T2 image in orientation, number of slices, FOV, and voxel size, which yielded the first 4 x 4 transformation matrix. A second transformation matrix was produced by iteratively correcting for registration errors due to subject motion (Ardekani et al., 1995). The two matrices were multiplied and the product was inverted to produce the final T2-to-MPRAGE transformation matrix.

Third, with a non-linear 2D warping algorithm developed by Ardekani (Ardekani et al., 2005b), the *b0* non-diffusion-weighted image was iteratively warped to correct for spatial distortions using the skull-stripped T2 image as a guide, which produced a 2D warp field containing distortion correction and spatial transformation information.

Fourth, the FA and ADC values were computed and overlay maps were generated from the native DTI images using algorithms described in Basser and Pierpaoli (1998) and Basser (1995). *Finally,* to reduce interpolation errors, we combined the three transformations from the previous steps into one single transformation, which was applied to spatially correct and normalize the FA and ADC maps to standard space.

To ascertain whether there were significant group differences in WM microstructural integrity even after controlling for overt WM abnormalities, the FLAIR image was used as a covariate in the voxelwise analysis. The FLAIR was therefore also normalized to the target image by applying the same transformations used for the DTI maps.

Statistical Analysis

Power analyses were conducted using GPOWER (Faul & Erdfelder, 1992) to determine the appropriate sample size necessary to attain statistical power of 0.80 and a medium effect size at an alpha level of 0.05. For *t*-tests, power analysis estimated a minimum of 45 participants in each group needed for a medium-large effect size of 0.6 and adequate power. For multivariate analysis of variance (MANOVA) with 5 dependent variables and two independent samples, the analysis yielded a minimum of 92 participants. Our selection of 46 participants with T2DM and 50 controls totaling 96 participants exceeded the minimum requirements for those analyses.

Data normality and homogeneity of variances. The Kolmogorov-Smirnova (for sample size >=50) and Shapiro-Wilk (for sample size < 50) tests were used to evaluate normality. Variables that have non-normal distributions were transformed using natural logarithm before group comparisons were conducted. If data transformation could not adequately normalize these variables, data analysis was done on raw values instead. The assumption of homogeneity of variance was evaluated using the Levene's test of equality of error variance.

Demographics, endocrine and psychiatric data. Group differences in demographic, endocrine, and psychiatric data were evaluated using the independent samples *t*-test for continuous variables and the chi-square test for independence for categorical variables (gender, ethnicity, hypertension, and dyslipidemia). Variables that were not normally distributed in at least one of the groups were also tested with non-parametric statistics (Mann-Whitney *U* test). For ease of interpretation, if the results from the Mann-Whitney *U* tests and *t*-tests were confirmed to be identical, the results from the *t*-tests were presented.

Defining cognition and brain structural domains. Given the large number of cognitive and imaging variables, data reduction was first conducted to organize similar measures into functional domains.

The cognitive variables were organized into eight key domains:

- *Verbal Learning* – the scores from CVLT trials 1 - 5

- *Verbal Memory Immediate Recall* – CVLT Short Delay Free Recall, CVLT Short Delay Cued Recall, WMS-R Logical Memory Immediate Recall, GUILD Paragraph Immediate Recall, and WMS-R Verbal Paired Associates Immediate Recall
- *Verbal Memory Delayed Recall* – CVLT Long Delay Free Recall, CVLT Long Delay Cued Recall, WMS-R Logical Memory Delayed Recall, GUILD Paragraph Delayed Recall, and WMS-R Verbal Paired Associates Delayed Recall
- *Visual Memory Immediate Recall* - WMS-R Visual Paired Associates Immediate Recall, and Visual Reproduction Immediate Recall
- *Visual Memory Delayed Recall* – WMS-R Visual Paired Associates Delayed Recall, and Visual Reproduction Delayed Recall
- *Attention and Working Memory* – DVT total time, WMS-R Digit Span Forward, WMS-R Digit Span Backward, WMS-R Visual Span Forward, and WMS-R Visual Span Backward
- *Executive Function* – WCST number of perseverative errors, TOL number of excess moves, Stroop interference score, and COWAT total score
- *Psychomotor Efficiency* –DSST total score and PST total correct score

The imaging variables for brain volumetric and cortical thickness assessment were organized into frontal and temporal lobe domains:

- *Frontal Lobe Domain* – Volumes for the PFR and DLPFR (both residualized to head size) and cortical thickness for the regions that corresponded to those clusters identified in the voxelwise DTI analysis described below.

- *Temporal Lobe Domain* – ICV-adjusted volumes for the hippocampus and STG and cortical thickness for the regions that corresponded to those clusters identified in the voxelwise DTI analysis described below.

To minimize the number of comparisons, the volumes of the left and right sides of each manually drawn ROI were summed to yield an overall volume for that region. Since FS provides cortical thickness information for each region by hemisphere, the average cortical thickness for a selected brain region was determined by averaging the cortical thickness of both sides, weighted by their surface areas.

Cognition and brain structural analysis. IQ and global atrophy are general measures and do not fall under any of the functional domains; therefore, they were evaluated using independent samples *t*-tests. Verbal learning measured by the CVLT trials 1 - 5 was evaluated using ANOVA, with diagnosis (0 for controls and 1 for participants with T2DM) as the between-subjects factor and the CVLT trials as the repeated-measures factor, and group differences in the learning curves were evaluated by examining the interaction between diagnosis and CVLT trials. Since the PST was replaced by the DVT at a later stage in this study, data were available for only 46% of the participants (26 participants with T2DM and 18 controls). The psychomotor efficiency domain was therefore evaluated using *t*-tests.

For the remaining 6 cognitive and 2 imaging domains, if at least 80% of the variables within a domain were normally distributed or became normally distributed after a natural

log transformation, MANOVA was conducted, with diagnosis (0 for controls and 1 for participants with T2DM) as the between-subjects factor; otherwise, the variables were evaluated with *t*-tests and the results were confirmed with the Mann-Whitney *U* test. For MANOVA, the Wilks' Lambda statistics were reported, and follow-up univariate ANOVAs were conducted when significant main effect for diagnosis was present in order to identify the individual measures within the domain that showed a significant group difference. As the WCST was incorporated in the neuropsychological battery at the midpoint of the study, data were available for 50% of the participants (22 participants with T2DM and 26 controls), and the number of perseverative errors of the WCST was analyzed separately using the *t*-test. Similarly, data were available for only 45% of the participants (16 participants with T2DM and 27 controls) for the DVT, which was therefore also evaluated with the *t*-test.

By domain, the Bonferroni-Holm step-down test (Holm, 1979) was used to control for Type 1 error across the follow-up univariate ANOVAs in MANOVAs and in *t*-tests. This method sorts the *p*-values from the ANOVAs or *t*-tests in ascending order and uses a significance level of α/k for the most significant ANOVA (smallest *p* value), α/k-1 for the next most significant ANOVA (the next smallest *p* value) and so on, where α equals 0.05 and k is the number of dependent variables for that domain. For effect size estimates, Cohen's *d* (0.2 small; 0.5 medium; 0.8 large) was used for *t*-tests and partial eta squares (η_p^2, 0.01 small; 0.06 medium; 0.14 large; Cohen, 1973) for multivariate analyses.

Voxelwise DTI analysis. Of the 58 cases that had DTI scans, two scans of participants with T2DM were excluded due to major distortions from image normalization, leaving 56 cases (29 participants with T2DM and 27 controls) for group comparisons. Group images created from the spatially corrected and normalized FA maps and were subjected to a two-tailed voxelwise analysis of covariance (VANCOVA), with age and the FLAIR image as covariates. Utilizing the FLAIR image as a covariate ensured that areas of WM hyperintensity were accounted for in the voxelwise analysis. For group comparison of ADC maps, the VANCOVA was conducted with age as the only covariate. WM and GM masks, created from the average MPRAGE image of the participants, were used to confine FA and ADC analyses within those regions, respectively. To minimize the chance of Type I errors, we restricted the accepted cluster size to those having at least 100 contiguous voxels (each voxel is 1 mm^3, thus, a minimum cluster size of 0.1 cc in volume) and by then choosing a false discovery rate (FDR) less than 0.01. A *p*-value threshold of 0.01 was chosen to ensure that the FDR would be kept below 0.01 (Benjamini & Hochberg, 1995).

Correlation/regression analyses. Correlation and regression analyses were conducted to assess whether the cognitive or brain measures were associated with any of the four possible dimensions of metabolic dysregulation, namely hyperglycemia, obesity, hypertension, and dyslipidemia.

Construction of the composite score for each cognitive domain that was significantly affected among participants with T2DM. Within any cognitive or

imaging domain that differentiated the groups, if only one variable yielded statistical significance, that variable was used as the dependent variable for that domain in the correlation/regression analyses; otherwise, a composite score was calculated for that domain by first converting each variable that yielded at least a statistical trend, upon controlling for multiple comparisons, to a *z*-score on the participant level using the mean and standard deviation of the control group. The *z*-scores were then averaged by domain to derive a composite score per participant to be entered as the dependent variable in the stepwise regression analysis.

Variable selection for each type of metabolic factor. In the regression analyses predicting cognitive performance or brain integrity by metabolic factors, one variable was selected to represent each type of metabolic factor. Indices of poor glycemic control, namely, HbA_{1C}, fasting glucose, and fasting insulin, are available from the blood tests. Neither fasting glucose nor insulin levels reflect the degree of long-term glucose control as they are snapshots of glucose regulation at the moment of blood collection. Furthermore, individuals with failing pancreatic function would have diminished insulin production and thus exhibit lower fasting insulin levels, which may be misinterpreted as an indication of better insulin status. More importantly, fasting insulin level does not accurately reflect the degree of insulin sensitivity. HbA_{1C}, on the other hand, is a robust measure of glucose regulation for the previous three months and thus a better measure of long-term diabetes control; therefore, it was chosen as the key measure of hyperglycemia in the correlation and regression analyses predicting cognition and brain integrity.

Although there are many obesity measures, such as BMI, waist-height ratio, and waist circumference, BMI, which is a measure of generalized obesity, was chosen as the obesity measure given that associations have been established between elevated BMI and brain and cognitive abnormalities in both obesity (Gonzales et al., 2010; Ward, Carlsson, Trivedi, Sager, & Johnson, 2005) and T2DM (Bruehl et al., 2009a) studies, and it would therefore be of interest to explore whether BMI would also help explain the anticipated abnormalities in the current study.

With advancing age, diastolic BP stabilizes or declines whereas systolic BP rises and is independently associated with increased cardiovascular risk (Kannel, 2000) and mortality (Perry, Miller, Baty, Carmody, & Sambhi, 2000). In fact, only two participants, both individuals with T2DM, had elevated diastolic BP in the hypertensive range. Systolic BP was thus chosen as the variable of interest for hypertension.

Triglycerides, HDL, LDL, and total cholesterol levels are among the lipid measures available in the present study. Although each of these lipid measures has been independently linked to cognitive functioning and brain integrity, HDL was selected as the variable of interest for lipid metabolism given that it is the least affected by anti-dyslipidemia treatment and would thus provide a better estimate of lipid metabolism.

Bivariate correlation explaining cognitive and brain measures using metabolic factors. Exploratory analyses with Spearman's correlation examined associations amongst cognitive and imaging measures that distinguished between the groups and

amongst possible explanatory factors, including glycemic parameters (HbA_{1C}), hypertension (systolic BP), obesity (BMI), and lipid factors (HDL). Associations between diabetes duration and cognitive and brain measures were explored, with two participants with T2DM who had diabetes duration beyond 2 standard deviations from the mean being excluded.

Exploratory regression analyses explaining cognition and brain volumes using metabolic factors among participants with T2DM. Stepwise regression models were constructed to determine among participants with T2DM whether glycemic parameter (HbA_{1C}) was the strongest metabolic parameter associated with cognitive performance or brain volume after accounting for age and gender. Please note that in the regression models, dichotomous variables were used for hypertension and dyslipidemia. To explain the rationale for this, it is important to note that brain complications likely accompanying hypertension or dyslipidemia may not be reversible with treatment even when the conditions are significantly ameliorated. To avoid the potential masking effects of treatment of either hypertension or dyslipidemia, dichotomous variables indicating whether a participant was classified as having hypertension or dyslipidemia, respectively, were used in the regression models instead of the continuous variables (systolic BP and HDL, respectively). Please refer to the Subject Classification section for the classification of hypertension and dyslipidemia.

In each regression model, demographic variables, age and gender (dichotomous variable with values 0 [female] and 1[male]), were entered as covariates in Step 1. In Step 2, a

stepwise method was used to determine whether glycemic parameter (HbA_{1C}), obesity (BMI), hypertension (dichotomous variable with values 0 [no hypertension] and 1 [hypertension]), or dyslipidemia (dichotomous variable with values 0 [no dyslipidemia] and 1 [having dyslipidemia]) could explain the anticipated cognitive or brain volume differences.

Exploratory analyses explaining cognition using imaging measures among participants with T2DM. Correlation and stepwise regression analyses were conducted to examine among participants with T2DM whether the anticipated temporal lobe-based cognitive domains that differentiated group performances were associated with regional temporal lobe volumes or microstructural integrity of a selection of temporal lobe (FA or ADC) group difference clusters generated in the VANCOVA DTI analysis. To derive the FA or ADC value for each chosen significant cluster at the case level, the cluster was mapped onto the individual DTI maps from which the mean FA or ADC value was derived.

Bivariate correlation analyses evaluated the associations of the composite or raw score representative of the cognitive domain with regional temporal lobe volumes and the FA or ADC values of the chosen clusters. For each cognitive domain that differentiated the groups, a stepwise regression model was conducted including only those individuals with T2DM, with age and gender entered as covariates in Step 1. Step 2 used a stepwise approach to evaluate which of the chosen imaging measures was the most significant factor(s) explaining the observed cognitive performance.

Results

Comprehensive analyses were conducted to test the study hypotheses that addressed between-group differences in cognitive functioning and brain structural integrity. Descriptive statistics for demographic, endocrine, and psychiatric data are presented first, followed by the inferential statistics addressing the primary hypotheses. To test the secondary hypotheses, exploratory data are presented to highlight potential factors that may help elucidate the mechanisms underlying the anticipated brain functional and structural complications in T2DM.

Demographic, Endocrine and Psychiatric Evaluations

Demographic, endocrine and psychiatric data are first evaluated for normality and then tested for group differences.

Of the variables evaluated for normality, a number of measures had significantly skewed distributions in at least one of the groups, which included years of education, BMI, the QUICKI score, HbA_{1C}, fasting glucose, fasting insulin, cholesterol level, triglyceride concentration, LDL, fibrinogen, CRP, HAM-D score, MMSE score, and GDS score (see Table 1-2).

Tables 3-5 summarize the group comparison results for demographic, endocrine, and psychiatric data. The groups were comparable in age (participants with T2DM, *M* = 58.79, *SD* = 8.20; controls, *M* = *58*.79, *SD* = 7.91) and years of education (participants with T2DM, *M* = 15.43, *SD* = 2.49; controls, *M* = 15.84, *SD* = 2.06). A majority of the

participants were in their late middle age, with 76% participants with T2DM and 74% controls below age 65. Chi-square tests revealed no significant group difference in gender distribution ($\chi^2[1] = 0.33$, *n.s.*) but a non-significant trend in ethnicity distribution (participants with T2DM, 27 Caucasians, 15 Hispanic/African Americans, and 4 Asians; controls, 40 Caucasians, 9 Hispanic/African Americans, and 1 Asian; $\chi^2[2] = 5.67$, $p = 0.059$).

As expected, participants with T2DM had significantly worse glycemic control than non-diabetic controls, as reflected by a lower QUICKI score and elevated levels of HbA_{1C}, fasting glucose, and fasting insulin. Diabetes duration was available for 34 of the participants with T2DM, with duration ranging from 0.07 year to 29.88 years ($M = 7.48$, $SD = 6.69$). Women with T2DM ($M = 7.28$, $SD = 1.18$) had significantly better diabetes control, as reflected by higher HbA_{1C} levels, than men with T2DM ($M = 8.43$, $SD = 2.26$; $t[43] = 2.12$, $p = 0.042$, adjusted for unequal variances).

Participants with T2DM had significantly higher BMI than controls ($t[92] = 6.72$, $p < 0.0001$), where 57% of the participants with T2DM and 12% of the control participants were obese. More participants with T2DM were classified as hypertensive (70% participants with T2DM vs. 34% controls; $\chi^2[1] = 12.13$, $p = 0.0005$). Of those who were hypertensive, 63% participants with T2DM and 10% controls had a history of hypertension treatment. Given that a larger number of the participants with T2DM received anti-hypertensive medication and thus their BP was likely to be better controlled, this may explain the non-significant group differences in systolic and diastolic BP.

There was also a significantly larger proportion of the participants with T2DM than controls with dyslipidemia (85% participants with T2DM and 44% controls; $\chi^2[1] = 17.20$, $p < 0.0001$), with participants with T2DM having significantly lower HDL levels and elevated triglyceride concentrations. Contrary to expectation, participants with T2DM had significantly lower levels of total cholesterol and LDL than controls, also likely due to the fact that more participants with T2DM received statin treatment (57% participants with T2DM vs. 12% controls). The groups also did not differ significantly in their scores on the HAM-D, GDS, or MMSE.

The variables that were not normally distributed were also evaluated with the Mann-Whitney *U* test (see Table 6), and the results remained unchanged. For ease of interpretation, the results of *t*-tests for those variables were presented and were marked with a '§' in Tables 3 - 5.

Neuropsychological Results

To address primary Hypotheses 1 - 2, group differences were evaluated in eight cognitive domains including verbal learning, verbal memory immediate recall, verbal memory delayed recall, visual memory immediate recall, visual memory delayed recall, attention and working memory, executive function, and psychomotor efficiency.

Tables 7 - 12 summarize the descriptive and normality statistics for the cognitive variables. Tests of normality showed that 23 of the 31 cognitive variables were non-normally distributed in at least one of the groups. The remaining eight variables that were normally distributed were CVLT trial 2 score, WMS-R Logical Memory Immediate Recall, DVT total time, WMS-R Visual Span Backward, Stroop interference score, COWAT total score, DSST total score, and PST correct score. Natural log transformation did not improve the skewness in any of those variables that were not normally distributed; therefore, after the raw values were evaluated using *t*-tests, the results were confirmed with the Mann-Whitney *U* tests.

Relative to controls, participants with T2DM had a significantly lower estimated IQ score (see Table 13). Results addressing primary Hypotheses 1 and 2 are presented below.

Hypothesis 1. Participants with T2DM were hypothesized to perform worse than controls on verbal learning and memory but not visual memory.

Verbal learning. Results of the mixed ANOVA revealed a non-significant between-subjects main effect of diagnosis ($F[1, 91] = 2.52$, *n.s.*), despite consistently lower scores in the diabetic group relative to controls across all five learning trials. Since the Mauchly's test of sphericity showed that the assumption of sphericity in CVLT learning trials was not met (Mauchly's $W = 0.37$, $p < 0.0001$), the Greenhouse-Geisser correction was used in the test of the within-subjects effect (CVLT learning trials). Statistical significance was obtained ($F[1, 91] = 151.68$, $p < 0.0001$). The interaction

between CVLT trials and diagnosis, however, did not reach significance ($F[1,91] = 0.63$, *n.s.*), indicating that participants with T2DM and controls did not differ in their verbal learning rates, most likely due to the indiscernible group differences for trials 1 - 3. The descriptive and normality statistics are presented in Table 7.

Figure 6 illustrates the learning curves of the subject groups. Both groups showed a steady increase in the number of words learned from one trial to the next, with the learning rates becoming progressively slower after trial 3, but this was much more prominent among participants with T2DM. Follow-up analysis revealed no significant group differences across trials 1 – 3, respectively, but relative to controls, participants with T2DM learned significantly fewer words than controls in both trials 4 ($t = 2.05$, $p = 0.044$) and 5 ($t = 2.13$, $p = 0.036$).

Verbal memory immediate recall. Overall, participants with T2DM performed worse than controls on all 5 measures of verbal memory immediate recall, with statistical significance attained in all but the WMS-R Verbal Paired Associates Immediate Recall, which showed a statistical trend (see Table 13). The effect sizes ranged from small-medium to medium-large. After controlling for multiple comparisons with the Bonferonni-Holm step-down test (p-value threshold at each step is also stated in parentheses), the Guild Paragraph Immediate Recall ($p = 0.001$; p-value threshold = 0.010), CVLT Short Delay Free Recall ($p = 0.006$; p-value threshold = 0.013), and WMS-R Logical Memory Immediate Recall measures ($p = 0.013$; p-value threshold = 0.017) remained significant whereas the CVLT Short Delay Cued Recall ($p = 0.039$; p-value

threshold = 0.025) and WMS-R Verbal Paired Associates Immediate Recall (p = 0.073; p-value threshold = 0.050) measures trended towards significance.

Verbal memory delayed recall. Participants with T2DM also scored lower across all 5 measures of delayed recall of verbal materials, with statistical significance attained for 3 measures, that is, the WMS-R Logical Memory Delayed Recall (p = 0.005), Guild Paragraph Delayed Recall measures (p = 0.020), and CVLT Long Delay Free Recall (p = 0.048), and statistical trend in WMS-R Verbal Paired Associates Delayed Recall (p = 0.093), all with small-medium to medium-large effect sizes (see Table 13). The group difference for the CVLT Long Delay Cued Recall was, however, non-significant. After controlling for multiple comparisons, the WMS-R Logical Memory Delayed Recall score remained significant (p-value threshold = 0.010) and the Guild Paragraph Delayed Recall score became a statistical trend (p-value threshold = 0.013), but the group difference for the CVLT Long Delay Free Recall score was no longer significant (p-value threshold = 0.017).

Visual memory immediate and delayed recall. Although participants with T2DM scored lower than controls on all immediate and delayed recall measures of visual memory, only the WMS-R Visual Reproduction Delayed Recall yielded significant finding (p = 0.044; p-value threshold = 0.025), which became a statistical trend after controlling for multiple comparisons. The statistical trend observed for the WMS-R Visual Paired Associates Immediate Recall (p = 0.071; p-value threshold = 0.025), however, was not retained after controlling for multiple comparisons (see Table 14).

Of those verbal and visual memory measures that were not normally distributed (marked with a § in the respective *t*-test results tables), the *t*-test results were consistent with those from the Mann-Whitney *U* test (see Table 18).

In sum, participants with T2DM scored lower than controls across all measures of verbal learning and all measures of verbal and visual memory. Despite the non-significant group difference in verbal learning, the results strongly support the hypothesized verbal memory impairment in both immediate and delayed recall among participants with T2DM. Contrary to expectation, visual memory was also affected among participants with T2DM, though less prominently than verbal memory. Overall, these results provide overwhelmingly strong support for Hypothesis 1.

Hypothesis 2. Participants with T2DM were hypothesized to score lower than controls in non-memory domains, including attention/working memory, executive function, and psychomotor efficiency, but that the effect sizes were anticipated to be smaller than those observed for verbal learning and memory.

Attention and working memory. Table 15 summarizes the *t*-test results for the attention and working memory domains. A *t*-test was conducted for a subset of the participants who were administered the DVT (22 participants with T2DM, age, $M = 56.94$, $SD = 7.91$, 14 F / 12 M; 18 controls, age, $M = 56.89$, $SD = 7.30$, 9 F / 9 M). The results indicated that participants with T2DM took a longer time to complete the DVT ($p = 0.015$;

p-value threshold = 0.010), which was a statistical trend after controlling for multiple comparisons.

Relative to controls, participants with T2DM performed about the same or lower on the Digit Span and Visual Span subtests of the WMS-R. However, the group differences did not achieve statistical significance.

Executive function. Table 16 summarizes the results of the between-group comparisons of the executive function measures. Although participants with T2DM performed worse than controls on the TOL, SCWI, and COWAT, *t*-tests revealed no significant group differences and the effect sizes were small. In a subset of the participants who were also administered the WCST (22 participants with T2DM, age *M* = 58.32, *SD* = 7.84, 11 F /11 M; 26 controls, age *M* = 59.06, *SD* = 7.88, 14 F / 12 M), participants with T2DM made non-significantly more perservative errors than controls, with a medium effect size (Cohen's *d* = 0.47).

Psychomotor efficiency. The *t*-test revealed no significant group difference on the DSST (see Table 17). Sixteen participants with T2DM (age *M* = 59.83, *SD* = 8.19, 8 F / 8 M) and 27 controls (age M = 58.61, *SD* = 8.68, 17 F / 10 M) were also administered the PST, which also did not attain statistical significance.

Of those measures that were not normally distributed (marked with a § in the respective *t*-test results tables), the *t*-test results were consistent with those found using the Mann-Whitney *U* test (see Table 18).

Overall, the findings are consistent with Hypothesis 2 in that individuals with T2DM performed consistently worse than controls on all the non-memory domains, with effect sizes considerably smaller than those reported in the memory domains. Among the non-memory measures examined, attention was the only non-memory measure that significantly differentiated the groups with a large effect size.

DTI Voxelwsie Results

Hypothesis 3. Participants with T2DM were hypothesized to exhibit lower WM (FA reduction) and GM (ADC elevation) microstructural integrity as measured on the DTI images, particularly in the frontal and temporal lobes.

To address Hypothesis 3, VANCOVA analyses for FA and ADC data were conducted on a total of 56 participants who had DTI scans (29 participants with T2DM, age, $M = 57.87$, $SD = 7.14$, 15 F / 14 M; 27 controls, age, $M = 58.22$, $SD = 7.42$, 15 F / 12 M). Consistent with the hypothesis, the results revealed both extensive WM and GM microstructural abnormalities among participants with T2DM, independent of age at $p < 0.01$ (FDR below 0.01).

White matter FA results. The VANCOVA analysis identified a total of 15 significant WM clusters ($p < 0.01$), 13 of which demonstrated significant FA reductions among participants with T2DM, with a total volume of 3228 voxels or 3.23 cc. These results were independent of age and overt WM abnormalities seen on the FLAIR image. Given the extensive number of clusters of abnormality, we chose to only itemize the largest 10 clusters on Table 19. This same set of selected clusters is displayed on an average brain structural image in Figure 7. Of those 13 clusters, six remained significant at a more restrictive significance level of 0.005 (marked with a "*" on Table 19).

The clusters were found in all four lobes, with more clusters identified in the left hemisphere. More clusters were found in the temporal regions, including bilateral arcuate fasciculi, left superior temporal WM, and right middle temporal WM, which may be involved in auditory and memory processing. The finding of significant FA reductions in the left temporal stem among individuals with T2DM is consistent with our prior reports on adults (Yau et al., 2009) and obese adolescents with T2DM (Yau et al., 2010). Three clusters were found in the parietal lobe, where the largest WM FA cluster was identified; three were in the frontal lobe and two in the occipital lobe.

The two clusters (132 and 111 voxels in size, respectively) demonstrating significant WM FA elevations among participants with T2DM were among the smallest 4 clusters identified and neither remained significant at $p < 0.005$.

Gray matter ADC results. The VANCOVA analysis revealed extensive GM ADC elevations among participants with T2DM relative to controls. A total of 26 clusters demonstrating significant group differences in GM ADC were identified at $p < 0.01$ (FDR < 0.01); three of those clusters demonstrating GM ADC elevations among participants with T2DM were discarded due to significant CSF contamination. The largest cluster also had CSF contamination that was substantial but proportionally small relative to its overall size of 1034 voxels; it was therefore retained. All but one of the 23 remaining clusters, totaling 5694 voxels or 5.69 cc in volume, demonstrated significant ADC elevations among participants with T2DM relative to controls.

Of the 22 clusters of significant ADC elevations among participants with T2DM, 10 were located in the temporal regions, including bilateral Heschl's gyri, bilateral PHG, bilateral fusiform areas, and right insular cortex. Five clusters were located in the occipital lobe, mostly along the calcarine fissure bilaterally, and five clusters were also identified in the frontal lobe, primarily in the PFC. There were only two clusters of ADC elevations found in the parietal lobe. Table 20 displays the largest 10 clusters in order of size. In Figure 8, the same clusters are illustrated in orthogonal views on an average brain structural image. Thirteen of those clusters with ADC elevations remained significant at $p < 0.005$ (marked with a "*" in Table 20). The only cluster (113 voxels or 0.113 cc in volume) demonstrating significant ADC reduction among participants with T2DM was in the occipital lobe, with a size among the 3 smallest clusters.

Please also note that the cluster of abnormality on the left Heschl's gyrus was in close proximity to the WM cluster found in the left arcuate fasciculus. Further investigation is needed to confirm whether they are functionally connected and whether they have memory involvement.

In sum, the extensive findings of WM FA reductions and GM ADC elevations are more highly concentrated in but not restricted to the temporal lobe. Although clusters of abnormality were also identified in the frontal lobe, the damage was less extensive. Overall, these findings strongly support Hypothesis 3.

Group Comparisons on MR-based Assessment of Brain Volume and Cortical Thickness

Hypothesis 4. Participants with T2DM were hypothesized to exhibit volume reductions and cortical thinning specifically in the frontal and temporal regions. In particular, hippocampal volumes were hypothesized to be reduced among participants with T2DM relative to controls.

Participants with T2DM (M = 109.06 cc, *SD* = 41.97) exhibited non-significantly more ICV-adjusted global atrophy than controls (M = 98.15 cc, SD = 47.32; $t[92] = 1.54$, *n.s.*). Please note that the data presented in parentheses are volumes in cc though the group comparison was done on the ICV-adjusted values. Regional assessments are presented

below. To address Hypothesis 4, operator-based brain volumes and FS-based cortical thickness assessments were used to evaluate frontal and temporal lobe integrity.

Frontal lobe findings. The DTI analysis identified a number of clusters of GM ADC elevation in the frontal lobe. To minimize the number of comparisons, an average cortical thickness of the frontal lobe was calculated by taking a weighted average of the cortical thickness of the frontal regions generated by FS, which included the frontal pole, superior frontal cortex, caudal middle frontal cortex, rostral middle frontal cortex, and lateral and medial orbitofrontal cortex. The average cortical thickness of the frontal lobe, as well as the manually derived DLPFR and PFR volumes, were evaluated for group differences.

Table 21 summarizes the descriptive and normality statistics for the three frontal lobe variables. Test of normality showed that PFR volume was non-normally distributed in the diabetic group. Natural log transformation did not improve normality; therefore, the raw values were subjected to group comparisons using *t*-tests and the result confirmed with Mann-Whitney *U* tests. The other two frontal lobe measures were also evaluated with the *t*-test.

The results showed that participants with T2DM had non-significantly smaller PFR volumes than controls, and the results were confirmed with the Mann-Whitney *U* test (z = -1.04, *n.s.*; see Table 22). No discernible group differences were found for DLPFR volume or frontal lobe cortical thickness.

Temporal lobe findings. Volumetric assessment evaluated volumes of the hippocampus and STG. To minimize the number of comparisons, the average volume of the left and right sides of each structure was utilized in the analyses. Cortical thickness was also assessed in selective regions that are relevant to memory function or corresponded to those identified to have microstructural alterations in the voxelwise ADC analyses. Those regions included the STG, fusiform area, MTL, PHG, and entorhinal cortex. For each cortical region, a weighted average of both the left and right sides was calculated and utilized in the group comparisons.

Of the seven temporal lobe measures evaluated, only the average PHG and entorhinal cortical thickness measures were non-normally distributed in at least one of the groups (see Table 23). Since natural log transformation did not improve the skewness, the raw values were tested using *t*-tests and followed up by Mann-Whitney *U* tests. The remaining five temporal lobe measures were subjected to MANOVA analysis.

Results from the *t*-tests revealed no discernible group difference in the average PHG ($t[92] = 0.14$, *n.s.*) or entorhinal cortical thickness ($t[92] = 0.39$, *n.s.*); it was therefore unnecessary to confirm the result with the Mann-Whitney *U* test. The overall results from the MANOVA on the 5 temporal lobe measures revealed a significant main effect of diagnosis ($F[5, 88] = 2.69$, $p = 0.026$; $\eta_p^2 = 0.133$), with an overall large effect size, indicating that a diagnosis of T2DM accounted for 13.3% of the overall variance (see Table 24). Tests of between-subjects effects yielded significantly smaller average ICV-

adjusted average hippocampal volume in participants with T2DM relative to controls, with a medium effect size η_p^2 of 0.091, and the result remained significant even after accounting multiple comparisons (p-value threshold = 0.01). The group difference in average MTL cortical thickness achieved a statistical trend, which did not remain so after controlling for multiple comparisons comparisons (p-value threshold = 0.013).

Given the prominent finding of average hippocampal volume reduction in participants with T2DM, follow-up analyses were conducted with t-tests to evaluate the laterality of hippocampal damage. The test results showed that the ICV-adjusted volumes of both the left (participants with T2DM, M = 2.72 cc, SD = 0.32; controls, M = 2.90 cc, SD = 0.33; t[92] = -2.78, p = 0.007, Cohen's d = 0.54) and right hippocampi (participants with T2DM, M = 2.78 cc, SD = 0.35, controls, M = 2.96 cc, SD = 0.30; t[92] = -2.94, p = 0.005, Cohen's d = -0.53) were also significantly reduced in participants with T2DM relative to controls, with effect sizes being moderate.

Although neither frontal lobe volume nor cortical thickness was found to be affected in participants with T2DM, the finding of hippocampal volume reduction in participants with T2DM was prominent and consistent with prior reports (den Heijer et al., 2003; Gold et al., 2007). Overall, the results support Hypothesis 4.

Explaining Temporal lobe Structural and Functional Abnormalities

Hypothesis 5. The glycemic parameter, HbA_{1C}, was hypothesized to contribute

significantly to the anticipated group differences in temporal lobe structures and functions (i.e. verbal memory).

Explaining temporal lobe function. Correlation and regression analyses were conducted to determine whether the metabolic factors (i.e. hyperglycemia, obesity, hypertension, and dyslipidemia) explained cognitive performance for variables that remained significant or trended towards significance after accounting for multiple comparisons in the between-group analyses. Those variables included all five measures of verbal memory immediate recall, two measures of verbal memory delayed recall (WMS-R Logical Memory Delayed Recall and Guild Paragraph Delayed Recall), and WMS-R Visual Reproduction Delayed Recall in the visual memory delayed recall domain. For each verbal memory domain, the raw scores of each measure were z-score transformed using the mean and standard deviation of the control group and the resulting z-scores were then averaged to yield a composite score for each subject. For WMS-R Visual Reproduction Delayed Recall, one diabetic outlier, who was beyond 3 standard deviations below the mean, was excluded from the analyses. Since controls had a restricted range of normal HbA_{1C} values (4.00 - 6.20%), analyses with HbA_{1C} were conducted for participants with T2DM only (5.00 - 12.4%).

All subjects. Among all subjects, BMI was inversely associated with verbal memory immediate recall composite score ($rs[87] = -0.23$, $p = 0.032$), verbal memory delayed recall composite score ($rs[89] = -0.19$, $p = 0.078$), and WMS-R Visual Reproduction Delayed Recall ($rs[90] = -0.24$, $p = 0.019$). Higher systolic BP was also

weakly associated with worse performance on all three measures, with the strongest association found for WMS-R Visual Reproduction Delayed Recall ($rs[92] = -0.21$, $p = 0.039$). The associations did not change when excluding those with treated hypertension. Higher HDL was modestly associated with verbal memory immediate ($rs[89] = 0.30$, $p = 0.004$) and delayed ($rs[91] = 0.28$, $p = 0.007$) recall. The positive association between HDL and WMS-R Visual Reproduction Delayed Recall was rather weak ($rs[93] = 0.17$, $p = 0.093$). Further analysis confirmed that the associations became weaker when excluding those with a history of statin treatment.

Participants with T2DM only. Table 25 summarizes the bivariate correlations of memory performance with metabolic factors and diabetes duration for participants with T2DM only. Diabetes duration was not associated with verbal memory immediate ($rs[28] = -0.08$, *n.s.*) or delayed recall ($rs[29] = -0.002$, *n.s.*) and was only weakly correlated with WMS-R Visual Reproduction Delayed Recall ($rs[28] = -0.16$, *n.s.*). Verbal memory immediate recall composite score was correlated moderately with HbA_{1C} ($rs[41] = -0.30$, $p = 0.053$) but weakly with the other metabolic factors (BMI, systolic BP, and HDL). Associations with systolic BP or HDL remained weak even after excluding those with a history of hypertension treatment or statin treatment, respectively.

The verbal memory delayed recall composite score was also moderately correlated with HbA_{1C} ($rs[42] = -0.29$, $p = 0.060$) but not with BMI or systolic BP regardless of a history of hypertension medication. Higher HDL was, however, weakly correlated with better performance in verbal memory delayed recall ($rs[43] = 0.15$, *n.s.*) but the correlation

became stronger but remained non-significant among those participants with T2DM who had no history of statin treatment (*rs*[18] = 0.29, *n.s.*).

Better performance on WMS-R Visual Reproduction Delayed Recall was strongly correlated with lower levels of HbA_{1C} (*rs*[42] = -0.29, *p* = 0.063), BMI (*rs*[43] = -0.34, *p* = 0.025), and systolic BP (*rs*[42] = -0.36, *p*=0.017) but was weakly correlated with higher HDL (*rs*[42] = 0.14, *n.s.*). When excluding those participants with T2DM with treated hypertension, the association with systolic BP became stronger (*rs*[15] = -0.40, *n.s.*), but when excluding those with a history of statin treatment, the association with HDL remained weak (*rs*[18] = 0.16, *n.s.*).

To address the question whether HbA_{1C} would contribute significantly to the temporal lobe functions, stepwise regression analyses were conducted to examine the effects of the metabolic factors on performance in each of the 3 memory domains among participants with T2DM only. In each regression model, age and gender (values 0 [female] and 1[male]) were entered in Step 1. In Step 2, stepwise analysis was used to identify the most significant factors among which included HbA_{1C}, BMI, hypertension (dichotomous variable with values = 0 [non-hypertensive] or 1 [hypertensive], dyslipidemia (dicohotomous variable with values = 0 [no dyslipidemia] or 1 [having dyslipidemia]). Refer to Table 26 for a summary of the regression models.

For verbal memory immediate recall, the composite score was entered as the dependent variable. The overall model was statistically significant (*F*[2,40] = 3.18, *p* = 0.052), with

age and gender together accounting for 13.7% of the variance, where lower performance was moderately associated with advancing age but a weak gender effect was observed. In Step 2, the stepwise analysis, however, found that none of the factors significantly explained performance in verbal memory immediate recall.

For verbal memory delayed recall, the composite score was entered as the dependent variable. The overall model was statistically significant ($F[3,40] = 2.82$, $p = 0.051$), accounting for a total of 17.4% of the variance. In Step1, age and gender together accounted for 8.2% of the variance ($\Delta F[2, 41] = 1.83$, *n.s.*), where lower performance was modestly associated with advancing age and a weak gender effect was observed. Contrary to expectation, stepwise analysis identified dyslipidemia as the only significant metabolic factor, accounting for an additional 9.2% of the variance ($\Delta F[1, 40] = 4.48$, $\Delta p = 0.041$), which was statistically significant. This suggests that having dyslipidemia is associated with worse performance in delayed verbal memory.

For visual memory delayed recall, WMS-R Visual Reproduction Delayed Recall was entered as the dependent variable. The overall model was statistically significant ($F[3,39] = 4.25$, $p = 0.011$), accounting for a total of 24.7% of the variance. In Step 1, age and gender together accounted for 7.6% of the variance ($\Delta F[2,40] = 1.64$, *n.s.*), where lower performance was more modestly associated with advancing age and no discernible gender effect was observed. In Step 2, stepwise analysis identified BMI as the only significant metabolic factor, accounting for an additional and significant 17.1% of the variance

independent of age and gender ($\Delta F[1, 39] = 8.83$, $\Delta p = 0.005$), where higher BMI accounted for lower performance on WMS-R Visual Reproduction Delayed Recall.

Explaining hippocampal volume. Of the temporal lobe volume and cortical thickness measures, only hippocampal volumes (average, left, and right) significantly differentiated the groups from each other after controlling for multiple comparisons. Although the left and right hippocampal volumes also differentiated the groups, to minimize the number of analyses, correlation and regression analyses involving the hippocampus were conducted on the average volume of the left and right sides, adjusted to ICV.

All subjects. A strong inverse relationship was found between BMI and average hippocampal volume among all subjects ($rs[90] = -0.45$, $p < 0.0001$). Average hippocampal volume was weakly associated with both systolic BP ($rs[92] = -0.12$, *n.s.*) and HDL ($rs[92] = 0.20$, $p = 0.06$). The correlations remained weak with systolic BP ($rs[56] = -0.16$, *n.s.*) when excluding those with treated hypertension and also with HDL when excluding those with a history of statin treatment ($rs[62] = 0.22$, $p = 0.077$).

Pariticipants with T2DM only. Average hippocampal volume was not associated with diabetes duration ($rs[30] = 0.067$, *n.s.*). Contrary to expectation, HbA_{1C} was not associated with average hippocampal volume ($rs[41] = -0.008$, *n.s.*) among participants with T2DM. Higher BMI remained strongly associated with smaller average hippocampal volume ($rs[42] = -0.51$, $p < 0.0005$) when examining participants with T2DM alone. No

association was found with HDL (*rs*[42] = 0.06, *n.s.*) even when excluding those with a history of statin treatment (*rs*[18] = 0.11, *n.s.*). Although higher systolic BP was only weakly associated with average hippocampal volume among all participants with T2DM (*rs*[42] = -0.20, *n.s.*), an exploratory analysis revealed a remarkably strong inverse relationship in a diabetic subgroup of 17 after excluding those with treated hypertension (nine normotensive and eight pre-hypertensive or untreated hypertensive; *rs*[15] = -0.77, $p < 0.0005$; see Figure 9). As obesity and hypertension are common co-morbid conditions, BMI (one lean, 8 overweight, and 8 obese) was found to correlate positively with systolic BP (*rs*[15] = 0.35, *n.s.*) and also inversely with average hippocampal volume (*rs*[15] =

-0.54, $p = 0.014$; see Figure 10) for this diabetic subgroup. However, neither HDL nor HbA_{1C} was associated with average hippocampal volume for those participants with T2DM.

A hierarchical regression model was thus constructed to explore whether systolic BP partially explained the association between BMI and hippocampal volume among this diabetic subgroup with no hypertension or with untreated hypertension. In the first model, the overall model was statistically significant, accounting for 67.6% of the variance in the ICV-adjusted average hippocampal volume ($F[4,12] = 6.24$, $p = 0.006$). In Step 1, age and gender, together, accounted for 8.4% of the variance (β[age] = 0, β[gender] = 0.11, $\Delta F[2,14] = 6.42$, $p = 0.025$), where no age effect was found and men with T2DM had slightly larger hippocampal volume than women with T2DM. In Step 2, BMI accounted for an additional 30.3% of the variance ($\beta = -0.014$, $\Delta F[1,13] = 6.42$, $\Delta p = 0.025$), with

higher BMI accounting for smaller hippocampi. In Step 3, systolic BP accounted for an additional 28.9% of the variance (β = -0.018, ΔF[1,12] = 10.68, Δp = 0.007) independent of age, gender, and BMI, where higher systolic BP was also associated with smaller hippocampi.

In the second model, inverting the order of entry of BMI and systolic BP, after controlling for age and gender, systolic BP explained an additional 55.0% of the variance in the ICV-adjusted average hippocampal volume (β = -0.018, ΔF[1,13] = 19.51, Δp = 0.001). In Step 3, BMI, however, accounted for only 4.2% of the variance (β = -0.014, ΔF[1,12] = 1.55, *n.s.*). The results suggest that systolic BP may partially explain some of the variance in the association between BMI and the ICV-adjusted average hippocampal volume among participants with T2DM who are non-hypertensive or hypertensive with no history of anti-hypertensive medication.

Explaining Frontal Lobe Structural and Functional Abnormalities

Hypothesis 6. It was hypothesized that hypertension would explain the anticipated group differences in frontal lobe structure and function.

Explaining frontal lobe function. Among the frontal lobe based functions, only DVT total time yielded a statistical trend. Correlation and regression analyses were thus conducted to assess whether DVT total time was associated with any of the metabolic factors.

All subjects. Contrary to the hypothesis, when examining controls and participants with T2DM together, no association was found between DVT total time and systolic BP (*rs*[42] = 0.03, *n.s.*). Significant associations were, however, found between longer DVT total time *and* higher BMI (*rs*[41] = 0.30, $p = 0.052$) and lower HDL (*rs*[24] = -0.31, $p = 0.041$).

Participants with T2DM only. A weak inverse relationship was found between diabetes duration and DVT total time (*rs*[13] = -0.21, *n.s.*), which may represent spurious effects since diabetes duration was available for only 14 of the participants with T2DM who were administered the DVT. Even when examining participants with T2DM alone, DVT total time correlated only weakly with systolic BP (*rs*[24] = 0.19, *n.s.*). BMI correlated modestly with DVT total time (*rs*[24] = 0.25, *n.s.*). The positive association between DVT total time and HbA_{1C} (*rs*[24] = 0.41, $p = 0.037$) was significant whereas the negative association with HDL was a statistical trend (*rs*[24] = -0.33, $p = 0.100$). These results are also summarized in Table 25.

A regression analysis was then conducted to evaluate, among the 26 participants with T2DM who were administered the test, which of the four metabolic factors (HbA_{1C}, BMI, hypertension, and dyslipidemia) significantly predicted performance on the DVT after accounting for age and gender. The result showed that the overall model was non-significant (*F*[2, 23] = 1.57, *n.s.*; see Table 26), with age and gender together accounting for 12.0% of the variance in Step 1, where increasing age was weakly associated with

longer completion time and men took substantially longer to complete the test than women. In Step 2, the stepwise analysis revealed that none of the metabolic parameters significantly explained performance on the DVT after accounting for age and gender.

Explaining frontal lobe structural integrity. Since none of the frontal lobe volume or cortical thickness measures significantly distinguished the groups, no correlation analysis was conducted.

Exploratory Analysis to Associate Cognition and Brain Measures among Participants with T2DM

Given that the most prominent findings were restricted to temporal lobe structure and function, exploratory analyses were conducted to examine, among participants with T2DM only, possible associations between verbal memory performance, both immediate and delayed recall, and temporal lobe measures including ICV-adjusted average hippocampal volume and DTI measures that differentiated the groups.

Associations between verbal memory and hippocampal volume. Bivariate correlations were conducted to evaluate associations between average hippocampal volume and the composite scores of verbal memory immediate and delayed recall, respectively, among participants with T2DM. Average hippocampal volume was not associated with the composite scores for verbal memory immediate ($rs[37] = 0.063$, *n.s.*) or delayed ($rs[37] = 0.002$, *n.s.*) recall although stronger correlations were found among

controls (immediate recall, *rs*[44] = 0.28, *p* = 0.063, and delayed recall, *rs*[44] = 0.13, *n.s.*). No correlation was found among participants with T2DM even when examining left or right hippocampal volume separately.

Association between verbal memory and DTI-based microstructural changes. Given the extensive DTI findings from the voxelwise analysis, only selective temporal lobe clusters demonstrating significant FA reduction or ADC elevation in those subjects with DTI scans were included in the analyses. To ensure that the selected FA and ADC clusters were located in functionally cohesive temporal lobe regions, those clusters spanning across multiple regions/structures were excluded. The seven selected clusters included the left and right arcuate fasciculi FA clusters (368 and 286 voxels, respectively), left superior temporal WM FA cluster (223 voxels), left temporal stem FA cluster (161 voxels), left PHG ADC cluster (442 voxels), and left (118 voxels) and right (227 voxels) Heschl's gyri ADC clusters.

The preliminary correlation analysis revealed that among controls, the FA or ADC values of those clusters did not correlate or correlated weakly with the composite scores for both verbal memory immediate and delayed recall. For example, among controls, left PHG ADC correlated positively and weakly with verbal memory immediate (*rs*[22] = 0.09, *n.s.*) and delayed recall (*rs*[23] = 0.00, *n.s.*). Conversely, among the 28 participants with T2DM with DTI scans, the correlations were more substantial, particularly for the ADC value of the left PHG and the FA value of the left Superior temporal WM (details described below). Therefore, the associations between DTI-based microstructural

integrity and verbal memory performance were further evaluated only for the diabetic group.

For each verbal memory composite score, a regression model was constructed with age and gender accounted for in Step 1. In Step 2, a stepwise analysis identified the most significant among the selected clusters, which were associated with verbal memory performance. A summary of the regression models are presented in Table 26.

Verbal memory immediate recall. The verbal memory immediate recall composite score was found to correlate strongly with the ADC value of the left PHG ADC cluster ($rs[25] = -0.54$, $p = 0.004$; see Figure 11 and Table 27 for a summary of the correlation results) and moderately with the FA value of the left superior temporal WM. The cluster in the left PHG was the 3rd largest among those identified in the voxelwise GM ADC analysis and is illustrated in Figure 8.

The overall regression model significantly accounted for a total of 29.6% of the variance ($F[3, 23] = 3.23$, $p = 0.041$). In Step 1, age and gender, together, accounted for 13.4% of the variance (β[age] = -0.01, β[gender] = -0.24, $\Delta F[2, 24] = 1.86$, *n.s.*), where in this small subset of the participants with T2DM, the age effect was indiscernible and men performed only slightly worse than women. In Step 2, stepwise analysis identified the ADC value of the left PHG as the only significant factor accounting for 16.2% of the variance in verbal memory immediate recall independent of age and gender ($\beta = -3.5$, $\Delta F[1, 23] = 5.30$, $\Delta p =$

0.031), demonstrating that lower microstructural integrity of the left PHG was associated with lower performance for immediate recall of verbal memory.

There were two diabetic outliers with verbal memory immediate composite scores over 2 standard deviations below the mean (see Figure 11). When they were excluded, the results remained largely unchanged, with the ADC value of the left PHG cluster being the only significant DTI measure, accounting for a significant percent of the variance (i.e. 16%) independent of age and gender.

Verbal memory delayed recall. The verbal memory delayed recall composite score was also found to correlate strongly with the ADC value of the left PHG cluster only ($rs[26] = 0.44$, $p = 0.020$; see Figure 12, and Table 27 for a summary of the correlation results). The overall regression model accounted for a total of 24.4% of the variance ($F[3, 24] = 2.58$, $p = 0.077$), which was a statistical trend. In Step 1, age and gender together accounted for 9.6% of the variance (β[age] = 0.02, β[gender] = -0.51, $\Delta F[2, 25] = 1.33$, *n.s.*), where age effect was indiscernible whereas men performed worse than women. In Step 2, stepwise analysis identified the ADC value of the left PHG as the only significant factor accounting for an additional 14.8% of the variance ($\beta = -4.10$, $\Delta F[1, 24] = 4.70$, $\Delta p = 0.040$), independent of age and gender, again demonstrating that lower microstructural integrity of the left entorhinal cortex was associated with lower performance on delayed recall of verbal memory.

The results demonstrated that GM microstructural abnormalities in the left PHG, which were the most extensive in the temporal lobe, rather than volume reduction in the hippocampus, may help explain the verbal memory impairment observed in participants with T2DM. Notably, these preliminary findings are the first to illustrate a possible link between temporal lobe structural and functional abnormalities reported among individuals with T2DM.

Summary of Results

Consistent with the study hypotheses, late middle-aged and elderly adults with T2DM present predominantly temporal lobe structural and functional abnormalities, which are characterized by verbal memory impairment, hippocampal volume reduction, and extensive microstructural abnormalities of temporal lobe GM and WM. To further substantiate these findings, the present study established an association between the observed verbal memory impairment and microstructural abnormalities in the left PHG, which is a key structure in memory process, among participants with T2DM. Although not anticipated, visual memory was also affected in participants with T2DM. Consistent with expectation, frontal lobe functions were found to be intact among participants with T2DM, with the exception of attention. In line with the cognitive results, participants with T2DM also did not exhibit gross structural changes in the frontal lobe but non-specific microstructural changes were evident.

Discussion

T2DM and the many health complications and factors associated with it represent major health risks worldwide. Although it is well established that individuals with T2DM have impairment in declarative memory, our knowledge of the extent or nature of T2DM-related brain structural complications remains limited. To comprehensively evaluate cognitive functioning and the extent of brain involvement in T2DM, the current study contrasted 46 late middle-aged and elderly patients with T2DM with 50 age- and education-matched controls with no evidence of IR or T2DM. Overall, the results present clear evidence of both temporal lobe structural and functional complications in a group of T2DM patients free of significant psychiatric or neurodegenerative disorders, which strongly support the primary study hypotheses.

As hypothesized, patients with T2DM exhibited declarative memory impairment, with verbal memory being more prominently affected than visual memory, along with evidence of compromised verbal learning. Supporting these cognitive results, both gross and microstructural abnormalities of memory-associated temporal lobe structures were also identified in patients with T2DM. In particular, brain volume reductions were restricted to the hippocampus bilaterally whereas microstructural abnormalities were found extensively in temporal lobe regions relevant to memory processing. Of great importance, this is the first report demonstrating that systolic BP explains some of the variance in the association between BMI and hippocampal volume reduction in T2DM as well as the first preliminary evidence linking verbal memory impairment in patients with T2DM and compromised

microstructural integrity of the left PHG, another key memory relevant region. In contrast to our extensive temporal lobe findings, as hypothesized psychomotor efficiency and frontal lobe based functions were largely intact in patients with T2DM, with the exception of attention, while there were non-specific frontal lobe microstructural abnormalities in the absence of obvious tissue loss.

Cognitive Findings

Impairments in temporal lobe based functions. Consistent with the existing literature, the current findings demonstrated verbal learning difficulties and specific declarative memory decline among late middle-aged and elderly patients with T2DM whereas verbal and visual working memory were largely preserved. Participants with T2DM also had lower estimated intellectual functioning.

On the CVLT, the group differences were negligible initially (Trials 1 - 3) but diverged substantially as participants learned more words (Trials 4 - 5). It is possible that participants with T2DM had a reduced short-term memory span; however, no significant group differences were found on the WMS-R Digit Span subtest, which measures short-term and working auditory memory. It is possible that repeating sequences of 2 - 8 digits in serial order requires less cognitive resources than learning 16 items in random order on the CVLT. Alternatively, it may be due to reduced vigilance observed among participants with T2DM. Since only a subset of the sample was given the DVT and no significant memory differences were found for those participants, it was not possible to ascertain

whether the observed learning differences were partly explained by differences in sustained attention. Exploratory correlation analyses also found inconsistent associations of completion time on the DVT for the scores on trial 4 (*rs*[23] = -0.33, *n.s.*) and trial 5 (*rs*[23] = -0.19, *n.s.*) of the CVLT. Future studies with a larger sample are needed to better clarify the effect of reduced vigilance on the attenuated verbal learning observed here.

Overall, participants with T2DM scored consistently lower than controls across all measures of verbal memory, and more importantly, more significant differences were found on tests that require learning of contextual materials than those involving word lists or associated word pairs. Of the verbal memory measures used in the present study, the GMT is the most challenging since it employs verbatim scoring, which is more sensitive to memory problems in aging than gist recall (Abikoff et al., 1987). Similarly, participants with T2DM performed worse than controls on the less stringent WMS-R Logical Memory subtest, another paragraph recall test, which assigns partial credits for gist and full credits for verbatim recall.

Overall, the verbal memory tests yielded more significant results for immediate than for delayed recall. For instance, participants with T2DM performed significantly worse than controls for both short and long delay free recall on the CVLT, but for cued recall, the groups differed significantly only on the short delay condition. It is possible that participants with T2DM benefited more from the categorical cues than controls, particularly in the short delay condition where participants with T2DM scored almost one

point higher than in free recall but almost no difference was found for controls. Although not reported here, the groups also did not score differently on the number of hit or false positive responses during recognition, likely reflecting a retrieval rather than an encoding problem, which is typical of MCI (Clement, Belleville, & Mellah, 2010) but not AD, which shows clear encoding problems (Pillon, Deweer, Agid, & Dubois, 1993). Interestingly on the CVLT, participants with T2DM showed some improvements for long versus short delay recall, particularly in the free recall condition, which may be due to participants with T2DM being more vulnerable to interference by the preceding Tuesday list in the short delay recall.

As hypothesized, of the two visual memory tests administered, participants with T2DM had the most difficulty memorizing complex geometric figures than associations between color and less complex geometric figures. The results clearly confirm that visual memory is less affected than verbal memory in individuals with T2DM. It is likely that verbal and visual memory processes involve overlapping yet divergent networks, which may be differentially affected in T2DM.

Impairments in frontal lobe based functions. Also consistent with the study hypothesis, psychomotor efficiency and frontal lobe based functions were largely preserved in participants with T2DM with the exception of attention, which showed the largest effect size. Reports of impairments in frontal lobe based functions have been rather inconsistent in T2DM. It is possible that our diabetic sample (predominantly of age under 65) is relatively younger than those (predominantly of age 65 or above) in studies that

found impairments of frontal lobe functions (van den Berg et al., 2008; Verdelho et al., 2007; Yeung, Fischer, & Dixon, 2009).

Imaging Findings

The comprehensive brain evaluations have generated a number of important findings, which support regional rather than global atrophy in addition to extensive subclinical microstructural abnormality among individuals with T2DM. The finding that participants with T2DM did not exhibit more global atrophy than controls is consistent with some reports (Bruehl et al., 2009a; van Harten, Oosterman, van Loon, Scheltens, & Weinstein, 2006) but not others (Kumar et al., 2008). Notably, there is no clear lateralization of either gross or microstructural GM abnormalities in participants with T2DM whereas there are more extensive WM microstructural abnormalities in the left hemisphere where 70% of the WM clusters of FA reductions were found.

Temporal lobe structural abnormalities. Congruent with data from both manual (den Heijer et al., 2003; Gold et al., 2007) and automated (Brundel, van den Heuvel, de Bresser, Kappelle, & Biessels, 2010) brain assessments in T2DM, the current report represents one of the very few documenting bilateral hippocampal atrophy in addition to clear evidence of diminished hippocampal-based declarative memory performance in a group of T2DM patients who are mostly late middle-aged. Together with prior reports from our lab (Bruehl, Wolf, & Convit, 2009b; Gold et al., 2007), the current data provide solid evidence highlighting the hippocampus as the brain structure particularly vulnerable

to damage not only in adults but in adolescents with T2DM as well (Bruehl, Sweat, Tirsi, Shah, & Convit, Under Submission).

In the absence of obvious structural changes, the DTI analysis identified widespread GM and WM microstructural abnormalities across all four lobes, with more clusters of abnormality identified bilaterally in the temporal lobe. Utilizing the same analysis techniques, our lab has previously demonstrated similar microstructural abnormalities in both adults (Yau et al., 2009) and adolescents with T2DM (Yau et al., 2010); in particular, the robust finding of microstructural abnormality in the left temporal stem replicated those reported previously. The temporal stem, which consists of fiber projections from various sources, is known to be involved in memory processing (Kier, Staib, Davis, & Bronen, 2004). It is unclear whether the cluster represents fronto-temporal connections relevant to memory as there is so far limited evidence linking temporal stem damage to memory decrements in T2DM (Yau et al., 2009). Nonetheless, these findings suggest that this dense fiber bundle, like the hippocampus, may also be particularly vulnerable to damage in T2DM.

The PHG was another key memory-associated temporal region affected bilaterally in participants with T2DM and the cluster found in the left hemisphere was the most extensive found in the temporal lobe. Additional temporal regions affected include bilateral arcuate fasciculi and Heschl's gyri; in particular, those clusters in the left hemisphere were located in close proximity to each other. Whether those affected fibers represent projections to the Heschl's gyrus, known for its involvement in phonological

processing, remains to be further explored. More interestingly, as seen on the panel displaying the left superior longitudinal fasciculus FA cluster in sagittal view in Figure 7, there were a few other clusters of WM abnormalities visible in plane, namely the PFR WM, superior longitudinal fasciculus, arcuate fasciculus, and temporal stem, which may represent parts of a functional network related to language, auditory processing, and memory (Breier, Hasan, Zhang, Men, & Papanicolaou, 2007; Glasser & Rilling, 2008; Kier et al., 2004; Warrier et al., 2009). In order to examine possible interconnections, techniques such as DTI tractography can be used to visualize the fiber projections based on directionality information. The fusiform gyri, which play a key role in face recognition (Kanwisher, McDermott, & Chun, 1997), also exhibited microstructural abnormalities bilaterally in the absence of cortical thinning, a possible indication of reduced GM density. However, no clear evidence of functional manifestations of the observed fusiform atrophies was detected here.

Of the temporal lobe regions that exhibited microstructural abnormalities among participants with T2DM, only the MTL also showed non-significantly reduced cortical thickness among participants with T2DM. There has been so far one study that demonstrated cortical thinning in T2DM (Brundel et al., 2010). Also using FS-based analysis, the authors found, in a group of elderly T2DM patients, bilateral reductions of volume and cortical thickness of the hippocampal region and of the surface area and volume of the middle temporal gyrus in addition to global cortical volume and surface area reductions in the right hemisphere. It is unclear whether the hippocampal region examined was strictly the hippocampus itself. The lack of cortical thickness changes

among our diabetic participants may be due to our sample being considerably younger (10 years in average) and having much shorter diabetes duration than those evaluated in the Brundel et al. (2010) study.

Frontal lobe structural abnormalities. Congruent with the cognitive findings, evidence of frontal lobe structural damage is also less extensive than in the temporal lobe. Neither volumetric nor cortical thickness assessment revealed widespread gross structural damage within the frontal lobe. In order to minimize the number of comparisons, the cortical thickness of frontal lobe subregions was combined to yield an overall frontal lobe measure, which might have masked subregional effects within the frontal lobe. Though not reported here, the preliminary data revealed no significant group differences in those individual subregions. Instead, the DTI-based microstructural assessment provided limited evidence of subtle frontal GM and WM damage among participants with T2DM, primarily in the PFR, which is implicated in memory encoding (Golby et al., 2001). Whether these frontal microstructural changes are related to the reported memory problems in participants with T2DM remains to be clarified.

The limited evidence of frontal lobe involvement, both structural and functional, is somewhat unexpected, considering that disproportionately more participants with T2DM had hypertension, which is often linked to frontal lobe-specific complications, such as reduced frontal lobe volume (Raz, Rodrigue, & Acker, 2003a) and increased occurrences of WML (Gold et al., 2005; Raz, Rodrigue, & Acker, 2003b). The present study also did not explore specific frontal lobe involvement or possible interactions of fronto-temporal

microstructural abnormalities in relation to the declarative memory impairment observed. WM volume reductions coupled with hypoperfusion in the frontal lobe have been described in T2DM patients of a similar age range but with considerably longer disease durations (an average of 13 years; Last et al., 2007). Future work should employ other MR techniques to fully assess frontal lobe involvement in T2DM. As an example, MR spectroscopy can be used to characterize abnormalities in frontal cellular metabolism in T2DM patients (Ajilore et al., 2006; Sahin et al., 2007). The use of automated brain segmentation methods would help tease out possible differential effects of T2DM on frontal WM and GM. In light of the findings of extensive yet subtle GM and WM abnormalities, future work should explore possible fronto-temporal disconnections in the memory networks using techniques such as DTI tractography and resting-state functional MRI.

Structural abnormalities in other brain regions. The calcarine fissure, which is the location of primary visual cortex, was another region affected in participants with T2DM. Given that none of the participants with T2DM had evidence of visual disorders, such as retinopathy, it is unclear what functional involvement those clusters represent. Notably, the calcarine fissure has large inter-subject variability in patterns of gyral folding and is thus prone to registration errors. Moreover, since those GM clusters were also located in close proximity to neighboring WM, the results must be interpreted with caution. The most extensive WM microstructural abnormality was found in the left parietal lobe. Although visuospatial functions tend to be right lateralized (Stephan, Fink, & Marshall, 2007), a close examination revealed that the cluster contained partly fiber

extensions from the forceps major, which consists of fiber projections from the splenium of the corpus callosum to the occipital lobe. Although interhemispheric transfer mediated the callosal fibers is crucial to visuospatial representation (Saenz & Fine, 2010; Schulte, Sullivan, Muller-Oehring, Adalsteinsson, & Pfefferbaum, 2005), given that this cluster of WM tracts is rather extensive in size and thus may represent great functional diversity, its involvement in the observed visual memory decline is ambiguous and therefore, the current study did not explore those associations. Given that WM microstructure is more extensively affected in the left than in the right hemisphere, whether this helps explain why visual memory (right lateralized) is less affected than verbal memory (left lateralized) in T2DM remains to be clarified.

Explaining Brain Structural and Functional Abnormalities in T2DM

Bivariate correlation and regression analyses were conducted to ascertain whether the metabolic parameters, particularly HbA_{1C}, an indicator of how well diabetes has been controlled during the preceding 3 months (the mean length of survival of hemoglobin-carrying red cells), were associated with the memory measures that differentiated the groups, namely immediate and delayed recall of verbal memory (composite scores) and delayed recall of visual memory (WMS-R Visual Reproduction). In addition, analyses were conducted to evaluate whether having hypertension was the strongest factor associated with deficits in sustained attention (DVT total time).

Associations between cognitive impairments and metabolic factors.

Effects of age and gender. Overall, the regression analysis results revealed modest to moderate associations between advancing age and poorer performance for immediate and delayed recall of verbal memory and for delayed recall of visual memory among participants with T2DM, but no age effect was found for sustained attention. The observed age effects for memory are consistent with normal age-related memory decline (Deary et al., 2009). Given that sustained attention is generally well preserved into old age (Filley & Cullum, 1994), the lack of age associations among participants with T2DM suggest that the observed deficits in sustained attention are likely related to T2DM or co-morbid conditions. Conversely, the gender effect was weak for both verbal and visual memory but was rather substantial for sustained attention, where women with T2DM completed the test considerably faster than men with T2DM. Gender effects in cognitive functioning have not been studied extensively in T2DM. The stronger gender effect for sustained attention observed in the small subset of the participants with T2DM (14 women and 12 men) needs to be clarified with a larger sample. Diabetes duration, on the other hand, did not reliably predict cognitive performance.

Effects of metabolic factors. Consistent with Hypothesis 5, HbA_{1C} was the only metabolic parameter independently correlated with all four cognitive domains that differentiated the groups. However, after accounting for age and gender, HbA_{1C} was no longer a significant factor. This was likely due to the moderate gender effect on HbA_{1C},

showing significantly better glucose control in women than in men with T2DM, and the lack of power owing to the relatively small sample size, particularly for attention, which was measured on only 26 of the participants with T2DM. The link between gender and HbA_{1C} suggests possibly differential gender effects in the associations between cognitive performance and metabolic factors. Exploratory regression analysis showed that the results were mainly driven by women with T2DM. In addition to having better glucose control as reflected by lower HbA_{1C}, women with T2DM also had higher HDL levels than men with T2DM. Moreover, women with T2DM scored descriptively higher on the verbal memory tests than men with T2DM. This may be due to that the facts that women are known to have higher HDL than men (Johnson et al., 2004) and that higher HDL may have neuroprotective effect (Barzilai, Atzmon, Derby, Bauman, & Lipton, 2006). Nevertheless, limited by the relatively small sample size, we are not presenting those preliminary data here as it warrants future exploration with a larger sample.

On the other hand, having dyslipidemia was independently associated with both immediate and delayed recall of verbal memory, but the effect was stronger for delayed recall, which showed a marginally significant difference between those diabetic participants with dyslipidemia and those without even after accounting for age and gender. Poor lipid metabolism has been associated with memory decline in individuals with (Helkala et al., 1995; Perlmuter et al., 1988) or without diabetes (e.g., de Frias et al., 2007; Komulainen et al., 2007). Our lab has also previously reported associations between dyslipidemia and declarative memory deficits among middle-aged individuals with T2DM (Bruehl et al., 2009a). Though the underlying mechanisms remain elusive, dyslipidemia

may exert its effect on memory via atherosclerosis or increased inflammation (van den Kommer, Dik, Comijs, Jonker, & Deeg, in press).

Among participants with T2DM, poor visual memory of complex geometric figures was moderately associated with poor glycemic control (elevated HbA_{1C}), obesity (high BMI), and having hypertension. However, after accounting for age and gender, BMI was the only significant metabolic factor. Obesity has been associated with memory decline (Sabia et al., 2009) and increased risk for dementia (Gunderson et al., 2005), and it may exert an effect on memory independent of T2DM (e.g., Elias et al., 2003; Rivera et al., 2005). Although BMI also correlated modestly with completion time on the DVT, it did not help explain the observed attention deficits after accounting for age and gender. Contrary to expectation, having hypertension also did not explain the attention deficits observed among participants with T2DM after accounting for age and gender.

Associations between hippocampal volume reduction and metabolic factors. The associations between hippocampal volume and metabolic parameters were also evaluated. Contrary to expectation, no age effect was observed. Men with T2DM had larger ICV-adjusted hippocampal volumes than women with T2DM. The temporal lobe is known to be particularly sensitive to dysregulation in blood glucose levels among individuals with large dysregulation (Lamport, Lawton, Mansfield, & Dye, 2009) and even mostly normal glucose control (Convit, Wolf, Tarshish, & de Leon, 2003). As such, one of the primary goals was to establish a link between the anticipated hippocampal volume and the degree of glucose regulation as indexed by HbA_{1C}. Contrary to

Hypothesis 5, the findings support no association with HbA_{1C} but rather a strong inverse relationship with BMI. These data are consistent with a handful of reports demonstrating temporal lobe atrophies, such as hippocampal volume reduction and microstructural abnormalities, in obese individuals (e.g., Alkan et al., 2008; Gustafson et al., 2004; Jagust et al., 2005). Our lab has previously reported inconsistent findings among adults with T2DM, where hippocampal volume reductions were associated with elevated HbA_{1C} in one study (Gold et al., 2007) but with BMI in another (Bruehl et al., 2009a). This is likely due to a combination of factors, such as the degree of diabetes control and the severity and extent of the co-morbid conditions.

The most intriguing finding was that when examining all participants with T2DM together, no association was found between systolic BP and hippocampal volume, but when restricting the analysis to those diabetics who were non-hypertensive and those with untreated hypertension, a strong inverse relationship was found. There is, so far, only one report linking hypertension to reduced CBF in the temporal lobe among patients with T2DM (Last et al., 2007) but none exist pointing specifically to associations with hippocampal atrophy. The current finding is consistent with increased risk for hippocampal atrophy previously reported among individuals with untreated midlife BP elevation (Korf et al., 2004). There is also evidence associating elevated BP with memory decline and reductions in concentrations of hippocampal glutamate, an essential component of long-term potentiation (LTP; Westhoff et al., 2010). More importantly, the data demonstrated, for the first time, that systolic BP partially explains the effect of obesity on hippocampal structural integrity among individuals with T2DM. Interactions

between obesity and hypertension have been reported to affect cognition (e.g., Elias et al., 2003) but none exists in relation to brain abnormality. The current findings emphasize that the adverse effect of elevated BP on brain integrity must be interpreted with caution in the presence or a history of anti-hypertensive treatment.

Other potential explanatory factors for the temporal lobe structural and functional abnormalities. Due to the extensive scope of the present study, we did not comprehensively explore other possible explanatory factors. Though no direct associations were found for the observed brain abnormalities, hyperglycemia is known to have many detrimental side effects. Animal data show that chronic hyperglycemia may impair LTP in brain structures such as the hippocampus (Biessels, van der Heide, Kamal, Bleys, & Gispen, 2002; Kamal, Biessels, Urban, & Gispen, 1999). Hyperglycemia is known to be accompanied by increased oxidant byproducts of intracellular glucose metabolism coupled with decreased levels of antioxidants (reviews in Brownlee et al., 2001). Increased oxidative stress that accompanies hyperglycemia may also induce selective oligodendrocyte death, resulting in demyelination (Smith, Kapoor, & Felts, 2009), which may help explain the observed WM microstructural abnormalities. Increased intracellular formation of AGE may damage endothelial cells of vessel walls and thus contribute to myelin degeneration (Brownlee et al., 1992; Mišur et al., 2004). Increased AGE accumulation may enhance vascular inflammation that in turn affects cognitive function (Takeuchi & Yamagishi, 2008).

Chronic mild inflammation, which often accompanies obesity, may also contribute to the brain functional and structural complications reported here. Inflammation, like oxidative stress, is known to promote neuronal death and impaired neurogenesis (Das & Basu, 2008; Ghosal, Stathopoulos, & Pimplikar, 2010). There is evidence linking elevated CRP, a pro-inflammatory marker, and lower cognitive performance, particularly in overweight or obese women (Sweat et al., 2008). Our lab has preliminary data supporting an inverse relationship between elevated CRP concentrations and ICV-adjusted hippocampal volumes among individuals with T2DM (data not reported here), which is consistent with a recent report demonstrating more hippocampal atrophy among elderly non-demented patients with T2DM with high CRP levels relative to those with low CRP levels (Anan et al., 2010). Elevated lipid levels, such as LDL and triglycerides, coupled with high inflammation has been associated with lower cognitive performance in the elderly (van den Kommer et al., in press). Therefore, interactions between inflammation and metabolic dysregulation are among other plausible mechanisms that may underlie cognitive and brain dysfunction in T2DM.

With a high co-localization of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR; McEwen, De Kloet, & Rostene, 1986), the hippocampus is the prime target for the actions of glucocorticoid (GC) and it is also known to play a pivotal role regulating the HPA axis by inhibiting the secretion of GC via a negative feedback mechanism (Heuser & Lammers, 2003). Whereas activation of MR appears to have neuroprotective effects (Woolley, Gould, Sakai, Spencer, & McEwen, 1991), hyperactivation of GR increases vulnerability to excitoxicity in the hippocampus, resulting

in increased neuronal death via apoptosis (Sapolsky, 2000). In Cushing's disease, an increase in GC levels is known to increase blood glucose levels (Newell-Price, Bertagna, Grossman, & Nieman, 2006). In T2DM, there is evidence of elevated basal cortisol levels as well as a reduction in suppression to Dexamethasone during the Dexamethasone-suppressed corticotropin-releasing hormone stimulation test, an indication of impairment in the HPA axis feedback mechanism, which is associated with poor glycemic control (Bruehl et al., 2007). Hippocampal volume reduction has also been associated with a blunted cortisol awakening response in T2DM patients (Bruehl et al., 2009b). It is possible that in T2DM, hippocampal damage induces HPA axis dysregulation, resulting in persistently elevated GC, which in turn causes more damage to the hippocampus, via GR-mediated apoptosis, and subsequent memory deficits (Stranahan et al., 2008).

Evidence Linking Temporal Lobe Structural and Functional Abnormalities in T2DM.

Association between verbal memory impairment and hippocampal damage.

The existing literature provides limited evidence linking memory impairment and abnormalities in memory-mediating brain structures in T2DM. For instance, van Harten et al. (2007) failed to establish an association between MTL atrophy and cognitive dysfunction among elderly patients with T2DM (van Harten et al., 2007), likely due to the less sensitive, qualitative brain assessment method employed. The current data, despite the reliable quantitative hippocampal volume assessment, do not support an association between the observed hippocampal volume reduction and declarative memory impairment

in patients with T2DM. This is not surprising as a meta-analysis of results from 33 studies found little evidence of a relationship between hippocampal volume and memory ability (Van Petten, 2004). There is emerging evidence suggesting that different subregions in the hippocampus may have distinct cognitive roles (Brickman, Stern, & Small, in press), which may help explain why hippocampus-memory associations have been inconsistent so far.

Other dimensions of hippocampal abnormalities may help explain the memory decline observed in patients with T2DM. For example, there is evidence of altered cellular metabolism in T2DM from MR spectroscopy studies (Ajilore et al., 2006; Sahin et al., 2007). Abnormal levels of metabolites in the hippocampus, such as reductions in NAA/Cr ratio, have been linked to lower cognitive performance in non-demented individuals (Zimmerman et al., 2008) and patients with MCI or AD (Kantarci et al., 2002). Although there is preliminary MR spectroscopy data for a subset of the participants with T2DM included in this study, no meaningful results were generated owing to the small sample size. Future work should more comprehensively evaluate cellular metabolism in the hippocampus and its associations with cognitive function.

The formation of new memories involves a constellation of neuronal events, including LTP, synaptic plasticity, and neurogenesis (Neves, Cooke, & Bliss, 2008), and the hippocampus is one of the few brain sites where neurogenesis occurs (Eriksson et al., 1998). Chronic hyperglycemia is known to induce changes in neuronal gene transcription and impair LTP in brain structures such as the hippocampus (Biessels et al., 2002; Kamal

et al., 1999). In animal models of T2DM, hyperglycemia has been shown to increase proliferation of neural progenitors but due to blunted responsiveness of neural progenitors to growth factors such as fibroblast growth factor 2 (FGF2) and insulin-like growth factor 1 (IGF1), the survival of newly formed neurons is diminished (Lang, Yan, Dempsey, & Vemuganti, 2009). Normally, these growth factors, known to promote cell division and survival of new neurons (Johnson-Farley, Patel, Kim, & Cowen, 2007) and regulate neurogenesis after injury (Yoshimura et al., 2001), act through insulin receptors, but in T2DM, it is speculated that disruptions in insulin signaling pathway may, in part, account for the reduced responsiveness of progenitors to growth factors, resulting in impaired neurogenesis.

Evidence of involvement of other temporal lobe structures. Since memory involves highly interconnected neural networks, the hippocampus, thought to be a major contributor to memory processing, is merely one of the many brain components involved. Of the temporal cortical clusters shown to be affected on the DTI images, only the ADC values of the left PHG, which represented the most extensive GM microstructural abnormalities found in the temporal lobe, correlated significantly with both immediate and delayed recall of verbal memory, independent of age and gender. This is the first evidence of an association between verbal memory decline and microstructural abnormality of the temporal cortex in T2DM. Conversely, the results did not show any involvement of the left or right Heschl's gyri in the memory decline reported in participants with T2DM.

It is also possible that the connectivity between the hippocampus and other memory-relevant structures is impaired in individuals with T2DM. In a group of elderly patients with T2DM, a recent report demonstrated reduced resting-state functional connectivity of the hippocampus with brain regions in the "default mode network", including the frontal gyrus, fusiform gyrus, temporal gyrus and anterior cingulate (Zhou et al., 2010). As discussed earlier, participants with T2DM also had reductions in microstructural integrity in WM tracts in the left hemisphere possibly involved in language, auditory processing, and verbal memory, namely PFR WM, superior longitudinal fasciculus, and arcuate fasciculus. Although information regarding brain functional connectivity is unavailable in the current study, extrapolating from the existing literature documenting gross WM abnormalities (Manschot et al., 2007; Novak et al., 2006; Sahin et al., 2007) and the previous (Yau et al., 2009) and current findings of extensive reductions of microstructural WM integrity among adults with T2DM, it is possible that subtle disruptions in fiber connectivity within the memory network is another contributing factor.

Of the extensive temporal WM abnormalities identified, the FA values of the superior temporal WM cluster (the 6th largest FA cluster listed in Table 19), which was in close proximity to the left Heschl's gyrus where ADC elevation was found (not shown in Table 20), also correlated positively but less strongly with immediate recall of verbal memory (see Table 27). Whether these WM and GM abnormalities have an impact on phonological processing that is relevant to memory processing cannot be established without further investigation. The lack of association between the FA values of the left temporal stem cluster and verbal memory performance is consistent with a prior report of

an association with immediate emotional but not neutral memory (Yau et al., 2009). Notably, the fact that the temporal lobe GM and WM clusters correlated with verbal memory were all in the left hemisphere is in line with the well established view of left-lateralization of verbal memory (Jansen et al., 2009).

Impaired vascular reactivity as another possible underlying mechanism. Lastly, there is evidence of peripheral and cerebral vascular reactivity impairment in IR (Tooke & Hannemann, 2000) and T2DM (Last et al., 2007; Stansberry et al., 1996). When performing a cognitive task, there is an increase in synaptic activity in the brain region involved during brain activation. In the normal brain, this results in regional vasodilation and thus an increase in glucose availability to that region to support the increased cognitive demand (Benton, Parker, & Donohoe, 1996). Therefore, vascular reactivity, which is integral to well-regulated cerebral blood flow, is key to maintaining optimal neuronal environment during brain activation (Drake & Iadecola, 2007). Although the underlying mechanisms remain elusive, it is proposed that impairments in vascular reactivity, such as in the hippocampus, may contribute to the reductions in memory performance reported in individuals with T2DM.

Conclusions and Future Directions

The current study has many strengths. Unlike many studies that use brief cognitive assessment tools, this study utilized a comprehensive battery of standardized neuropsychological tests with known validity and reliability. Additionally, robust and

state-of-the-art image analysis techniques were utilized so as to maximize the quality and reproducibility of the results. The operator-derived hippocampal volumes were derived using a manual tracing method that has been validated against post-mortem MRI evaluations (Bobinski et al., 2000). In anticipation of subtle brain abnormalities in addition to hippocampal atrophy, the current study used an automated analysis method for an unbiased, sensitive, and comprehensive assessment of brain microstructure on the DTI images. The chosen software package has been demonstrated to be among the best currently available in a recent comparison study (Klein et al., 2009). FS is one of the most common and sophisticated image analysis packages available for unbiased assessment of cortical thickness, which is otherwise done using manual methods that are labor-intensive and that require expert neuroanatomists.

Future work should incorporate other imaging techniques to further characterize the brain network involved in the memory impairment in T2DM. As mentioned previously, MR spectroscopy can be useful for assessing changes in metabolite levels in the frontal and temporal lobes. The DTI data have revealed novel information that may help decipher possible impairment in network connectivity in individuals with T2DM. The relatively poor spatial resolution and spatial distortions inherent in echo-planar acquisitions, such as DTI, limit the ability to ascertain the integrity of relatively small fiber tracts relevant to memory functions, such as the angular bundle and in brain regions that are adjoining CSF (e.g., the fornix and cingulum). Moreover, these fibers are also highly susceptible to registration errors. DTI tractography may help improve the accuracy of sampling and perhaps lead to improved comparability across subjects. DTI tractography may also prove

to be useful for isolating fronto-temporal connections within the temporal stem (Kier, Staib, Davis, & Bronen, 2004) that are more relevant to declarative memory, particularly verbal memory. Arterial spin labeling is another MR technique that can help address whether there are vasomotor reactivity impairments in the hippocampus, which may explain the observed declarative memory impairments in patients with T2DM.

The current study has a few limitations. The overall sample size was reasonably large, but for analyses concerning participants with T2DM alone, the sample size was more limited and did not yield sufficient power. The sample size also limited the ability to explore possible gender effects on metabolic contributions to the observed cognitive and brain abnormalities. The relatively broad range of age (early 40's to mid 70's) and of diabetes duration (0.07 years up to 29.88 years) add further variability to the data. In spite of these concerns, the solid findings obtained in study participants who were relatively younger than those studied previously allowed us to show that brain and cognitive complications are not limited to T2DM patients in old age.

The present report presents strong evidence associating systolic BP and obesity with hippocampal integrity among participants with T2DM who are either non-hypertensive or have untreated hypertension. Future work should confirm these relationships with a larger sample. Nonetheless, this will remain a limitation for future investigations given that T2DM patients who are free of hypertension or have untreated hypertension represent a very small patient subset indeed. Please also note that the same applies to dyslipidemia. Prior studies have demonstrated improvement of cognitive performance with

improvements in glucose control (Ryan et al., 2006). Also to be addressed is whether the brain abnormalities present in T2DM patients are reversible with lipid- or BP-lowering treatments, or weight loss. Future studies should also explore possible interaction effects of the metabolic parameters and T2DM on cognition and brain structures, such as those previously reported (Elias, Elias, Sullivan, Wolf, & D'Agostino, 2003; Elias et al., 1997; Rivera et al., 2005).

To comprehensively evaluate insulin sensitivity, future research should employ techniques such as the hyperinsulinemic–euglycemic clamp test (DeFronzo, Tobin, & Andres, 1979). A better understanding of the intricate relationships among hippocampal integrity, hyperglycemia/IR, and dysregulation of the HPA axis may provide further insight into the mechanisms underlying the hippocampal atrophy in T2DM. The role of pro-inflammatory markers on cognition and brain integrity should also be explored further. Interestingly, the temporal cortices that showed microstructural abnormalities among diabetics, namely the middle and inferior temporal gyri and fusiform gyri, are the same cortices that have been demonstrated to be the first to show volume decline in the progression from MCI to dementia of the Alzheimer's type (Convit et al., 2000). Given that T2DM is associated with an increased risk for dementia, it would be important to track the progression of those microstructural cortical changes and their relationships with the cognitive status in individuals with T2DM over time so as to better assess the possible link between T2DM and dementia.

The cross-sectional design employed here limits the interpretation of causality. To better delineate the mechanisms underlying the T2DM-related brain complications, future studies employing longitudinal designs should track pathological changes from the pre-clinical stages of insulin resistance into T2DM. In particular, studying children and adolescents would give us the advantage of tracking brain structural and functional changes from pre-diabetes stages to T2DM without the confounding effects of clinically significant vascular complications in older adults, which are unlikely to have developed in such young ages.

Clinical Implications

T2DM is a serious health condition with a constellation of known health complications, with prevalence rates continuing to escalate worldwide not only in adults but also in children. The escalating prevalence of T2DM puts increasing burden on the individual as well as society given the cost and health care resources necessary for the management of the disease itself and the myriad of associated health complications. While the existing literature documenting cognitive and brain complications in T2DM is primarily on elderly patient populations, the current report emphasizes that diabetes related cognitive and brain complications are not limited to those in old age. The current report demonstrates that although adults with T2DM with well controlled diabetes and relatively short disease duration may function cognitively within the normal range, they may manifest measurable memory decline coupled with brain structural complications. What deserves even greater

emphasis is that the existing literature has also provided evidence for similar complications in adolescents with T2DM (Yau et al., 2010).

The myriad of common health complications along with the evidence of accelerated brain aging for older adults and possibly delayed brain maturation in children with T2DM, raise concerns regarding the potential negative impact on patients' daily functioning and the increased likelihood of progression to neurological diseases such as dementia. Future work should better characterize the T2DM-associated brain structural and functional complications and the underlying mechanisms, which will help foster new strategies for the treatment of T2DM and possible prevention of its associated complications.

Tables

Tables 1 – 27 are presented in the subsequent pages.

Table 1. Demographic, Endocrine, and Psychiatric Data - Descriptive and Normality Statistics

	T2DM			Control		
	n	Mean ± *SD* *(Min - Max)*	Normality Statistics	n	Mean ± *SD* *(Min - Max)*	Normality Statistics
Age (years)	46	58.79 ± 8.20 *(42.52 - 73.26)*	0.96	50	58.79 ± 7.91 *(43.09 - 75.75)*	0.11 [K]
Education (years)	46	15.43 ± 2.49 *(12.00 - 21.00)*	0.92 *	50	15.84 ± 2.06 *(12.00 - 20.00)*	0.17 [K]*
BMI (kg/m^2)	46	32.80 ± 6.97 *(21.04 - 51.10)*	0.93 *	48	24.81 ± 4.14 *(18.31 - 38.06)*	0.96
Waist-Height Ratio	44	0.65 ± 0.09 *(0.47 - 0.88)*	0.99	44	0.52 ± 0.07 *(0.38 - 0.65)*	0.98
QUICKI Score	46	0.32 ± 0.04 *(0.26 - 0.49)*	0.86 *	50	0.39 ± 0.03 *(0.35 - 0.48)*	0.12 [K]
HbA_{1C} (%)	45	7.84 ± 1.86 *(5.00 - 12.40)*	0.85 *	49	5.32 ± 0.43 *(4.00 - 6.20)*	0.94 *
Fstg Gluc (mg/dL)	46	142.28 ± 54.57 *(84.00 - 310.00)*	0.76 *	50	77.74 ± 8.92 *(59.00 - 100.00)*	0.10 [K]
Fstg Ins (µIU/mL)	46	14.16 ± 10.69 *(1.00 - 47.00)*	0.82 *	50	5.57 ± 2.21 *(2.00 - 9.00)*	0.13 [K]*
Syst BP (mmHg)	46	122.39 ± 11.77 *(98.00 - 147.00)*	0.98	50	118.53 ± 13.75 *(90.00 - 153.00)*	0.98 [K]
Diast BP (mmHg)	46	72.11 ± 7.22 *(58.00 - 90.00)*	0.98	49	71.64 ± 7.32 *(54.00 - 84.00)*	0.97
Chol (mg/dL)	46	171.70 ± 34.87 *(119.00 - 284.00)*	0.93 *	50	191.24 ± 36.36 *(141.00 - 302.00)*	0.15 [K]*
HDL (mg/dL)	46	45.13 ± 12.15 *(24.00 - 72.00)*	0.95	50	56.66 ± 13.98 *(36.00 - 98.00)*	0.10 [K]
Triglyc (mg/dL)	46	131.54 ± 91.08 *(45.00 - 591.00)*	0.71 *	50	90.00 ± 34.78 *(43.00 - 192.00)*	0.16 [K]*
LDL (mg/dL)	45	102.11 ± 30.77 *(49.00 - 205.00)*	0.95 *	50	116.58 ± 34.31 *(62.00 - 218.00)*	0.16 [K]*
Fibrinogen (mg/dL)	45	369.16 ± 97.73 *(235.00 - 700.00)*	0.86 *	49	326.08 ± 90.41 *(198.00 - 697.00)*	0.87 *
CRP (mg/dL)	38	3.56 ± 3.87 *(0.10 - 20.10)*	0.74 *	48	2.15 ± 2.92 *(0.10 - 14.70)*	0.27 *

Fstg Gluc = Fasting Glucose; Fstg Ins = Fasting Insulin; Syst BP = Systolic Blood Pressure; Diast BP = Diastolic Blood Pressure; Chol = Cholesterol; Triglyc = Triglycerides

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

* $p < 0.05$ (Distribution is non-normally distributed)

Table 2. Demographic, Endocrine, and Psychiatric Data - Descriptive and Normality Results

	T2DM					Control				
	n	**Mean *(Min***	**±**	***SD* *Max)***	**Normality Statistics**	**n**	**Mean *(Min***	**±**	***SD* *Max)***	**Normality Statistics**
HAM-D Score	45	2.80 *(0.00*	± -	2.94 *12.00)*	0.83 *	50	2.72 *(0.00*	± -	3.56 *16.00)*	0.22 [K]*
MMSE Score	46	29.15 *(27.00*	± -	0.97 *30.00)*	0.80 *	50	29.44 *(26.00*	± -	0.91 *30.00)*	0.35 [K]*
GDS Rating	46	1.87 *(1.00*	± -	0.50 *3.00)*	0.67 *	49	1.71 *(1.00*	± -	0.61 *3.00)*	0.31 *

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

* $p < 0.05$ (Distribution is non-normally distributed)

Table 3. Demographics – *t*-test Results

	T2DM	Control			Cohen's
	Mean ± SD	**Mean ± SD**	***t***	***p***	***d***
Age (years)	58.79 ± 8.20	58.80 ± 7.91	-0.01	0.996	0.00
[§] **Education (years)**	15.43 ± 2.49	15.84 ± 2.06	-0.87	0.386	-0.18
Gender			χ^2=0.33	1.000	0.57
Female	52%	58%			
Male	48%	42%			
Ethnicity			χ^2 =5.67	0.060	0.55
Caucasian	27 (59%)	40 (80%)			
Hispanic/African American	15 (33%)	9 (18%)			
Asian	4 (9%)	1 (2%)			

[§] These variables were non-normally distributed in at least one of the groups and the *t*-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* tests).

Table 4. Metabolic Parameters – *t*-test Results

	T2DM Mean ± SD	Control Mean ± SD	*t*	*p*	Cohen's *d*
[§] **BMI (kg/m^2)**	32.80 ± 6.97	24.81 ± 4.14	6.72[a]	< 0.0001	1.40
[§] **Waist-Height Ratio**	0.66 ± 0.10	0.53 ± 0.08	6.82[a]	< 0.0001	1.43
[§] **QUICKI Score**	0.32 ± 0.04	0.39 ± 0.03	-9.17	< 0.0001	-1.87
HbA1C (%)	7.84 ± 1.86	5.32 ± 0.43	8.87[a]	< 0.0001	1.90
[§] **Fstg Gluc (mg/dL)**	142.28 ± 54.57	77.74 ± 8.92	7.93[a]	< 0.0001	1.69
[§] **Fstg Ins (µIU/mL)**	14.16 ± 10.69	5.57 ± 2.21	5.35[a]	< 0.0001	1.14
Hypertension (Y/N)	70% / 30%	34% / 66%	χ^2=12.13	0.0005	
Syst BP (mmHg)	122.39 ± 11.77	118.53 ± 13.75	1.47	0.144	0.30
Diast BP (mmHg)	72.11 ± 7.22	71.64 ± 7.32	0.31	0.756	0.06
Dyslipidemia (Y/N)	85% / 15%	44% / 56%	χ^2=17.20	< 0.0001	
[§] **Chol (mg/dL)**	171.70 ± 34.87	191.24 ± 36.36	-2.68[a]	0.009	-0.55
HDL (mg/dL)	45.13 ± 12.15	56.66 ± 13.98	-4.30	< 0.0001	-0.88
[§] **Triglyc (mg/dL)**	131.54 ± 91.08	90.00 ± 34.78	2.91[a]	0.005	0.61
[§] **LDL (mg/dL)**	102.11 ± 30.77	116.58 ± 34.31	-2.16	0.034	-0.44
[§] **Fibrinogen (mg/dL)**	369.16 ± 97.73	326.08 ± 90.41	2.22	0.029	0.46
[§] **CRP (mg/dL)**	3.56 ± 3.87	2.15 ± 2.92	1.93	0.057	0.42

Fstg Glu = Fasting Glucose; Fstg Ins = Fasting Insulin; Syst BP = Systolic Blood Pressure; Diast BP = Diastolic Blood Pressure; Chol = Cholesterol; Triglyc = Triglycerides

[a] Adjusting for unequal variances

[§] These variables were non-normally distributed in at least one of the groups and the *t*-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* tests).

Table 5. Psychiatric Evaluation – *t*-test Results

	T2DM			Control					Cohen's
	Mean	±	SD	Mean	±	SD	*t*	*p*	*d*
§ **HAM-D Score**	2.80	±	2.94	2.72	±	3.56	0.12	0.906	0.02
§ **MMSE Score**	29.15	±	0.97	29.44	±	0.91	-1.51	0.135	-0.31
GDS Rating	1.87	±	0.50	1.71	±	0.61	1.36[a]	0.178	0.28

§ These variables were non-normally distributed in at least one of the groups and the t-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* tests).

a Adjusted for unequal variances

Table 6. Demographic, Endocrine, and Psychiatric Data - Non-Parametric Mann-Whitney *U* Test Results for Non-Normally Distributed Variables

		T2DM			Control			
		n	Mean Rank	Sum of Ranks	n	Mean Rank	Sum of Ranks	z	*p*
	Education	46	45.32	2084.50	50	51.43	2571.50	-1.10	0.273
*	**BMI**	46	64.63	2973.00	48	31.08	1492.00	-5.96	<0.0001
*	**Waist-Height Ratio**	45	62.13	2796.00	46	30.22	1390.00	-5.76	<0.0001
*	**QUICKI Score**	46	26.89	1237.00	50	68.38	3419.00	-7.29	<0.0001
*	**HbA_{1C}**	45	70.78	3185.00	49	26.12	1280.00	-7.94	<0.0001
*	**Glucose**	46	72.96	3356.00	50	26.00	1300.00	-8.25	<0.0001
*	**Insulin**	46	65.62	3018.50	50	32.75	1637.50	-5.78	<0.0001
*	**Cholesterol**	46	40.49	1862.50	50	55.87	2793.50	-2.70	0.007
*	**Triglycerides**	46	56.71	2608.50	50	40.95	2047.50	-2.77	0.006
*	**LDL**	45	41.66	1874.50	50	53.71	2685.50	-2.13	0.033
*	**Fibrinogen**	45	55.18	2483.00	49	40.45	1982.00	-2.62	0.009
	CRP	38	51.87	1971.00	48	36.88	1770.00	-2.77	0.006
	HAM-D Score	45	50.53	2274.00	50	45.72	2286.00	-0.87	0.387
	MMSE Score	46	44.04	2026.00	50	52.60	2630.00	-1.67	0.095
	GDS	46	51.61	2374.00	49	44.61	2186.00	-1.47	0.143

* Remained significant after controlling for multiple comparisons

Table 7. Verbal Learning Domain - Descriptive and Normality Results

	T2DM			Control		
	n	**Mean ± *SD* *(Min - Max)***	**Normality Statistics**	**n**	**Mean ± *SD* *(Min - Max)***	**Normality Statistics**
CVLT Trial 1	45	8.22 ± 2.50 *(4.00 - 15.00)*	0.96	48	8.65 ± 2.50 *(5.00 - 14.00)*	0.95 *
CVLT Trial 2	45	11.04 ± 2.24 *(5.00 - 16.00)*	0.97	48	11.65 ± 2.41 *(6.00 - 16.00)*	0.97
CVLT Trial 3	45	12.53 ± 2.13 *(8.00 - 16.00)*	0.96	48	12.83 ± 2.60 *(6.00 - 16.00)*	0.91 *
CVLT Trial 4	45	12.87 ± 2.00 *(7.00 - 16.00)*	0.92 *	48	13.83 ± 2.51 *(6.00 - 16.00)*	0.82 *
CVLT Trial 5	45	13.22 ± 2.39 *(7.00 - 16.00)*	0.90 *	48	14.25 ± 2.26 *(7.00 - 16.00)*	0.78 *

The Shapiro-Wilk test was used to evaluate normality for $n < 50$

* $p < 0.05$ (Distribution is non-normally distributed)

Table 8. Estimated FSIQ and Verbal Memory Immediate and Delayed Recall Domains - Descriptive and Normality Results

	T2DM			Control		
	n	Mean ± *SD* *(Min - Max)*	Normality Statistics	n	Mean ± *SD* *(Min - Max)*	Normality Statistics
WAIS-R FSIQ	46	106.48 ± 11.81 *(84.02 - 131.86)*	0.98	50	112.16 ± 11.39 *(76.25 - 139.13)*	0.13 [K]*
Verbal Memory Immediate Recall						
CVLT SDFR	46	11.00 ± 3.72 *(2.00 - 16.00)*	0.92 *	49	12.90 ± 2.74 *(5.00 - 16.00)*	0.90 *
CVLT SDCR	46	11.91 ± 2.70 *(6.00 - 16.00)*	0.95 *	50	13.04 ± 2.56 *(6.00 - 16.00)*	0.14 [K]*
WMS-R LMIR	45	27.56 ± 6.32 *(15.00 - 39.00)*	0.97	50	31.04 ± 6.99 *(15.00 - 44.00)*	0.11 [K]
Guild PAIR	46	5.51 ± 2.23 *(1.00 - 10.00)*	0.95	48	7.26 ± 2.51 *(3.50 - 16.00)*	0.91*
WMS-R VERPI	44	19.84 ± 4.01 *(7.00 - 24.00)*	0.87 *	50	21.26 ± 3.58 *(6.00 - 24.00)*	0.24 [K]*
Verbal Memory Delayed Recall						
CVLT LDFR	46	11.70 ± 3.25 *(4.00 - 16.00)*	0.94 *	50	12.96 ± 2.94 *(4.00 - 16.00)*	0.15 [K]*
CVLT LDCR	46	12.46 ± 2.79 *(6.00 - 16.00)*	0.93 *	50	13.24 ± 2.45 *(6.00 - 16.00)*	0.14 [K]*
WMS-R LMDR	45	22.38 ± 7.81 *(12.00 - 42.00)*	0.95 *	50	27.02 ± 7.89 *(9.00 - 42.00)*	0.98 [K]*
Guild PADR	46	7.32 ± 3.18 *(1.50 - 15.00)*	0.98	48	8.95 ± 3.48 *(3.50 - 19.50)*	0.95 *
WMS-R VERPD	44	7.41 ± 0.92 *(5.00 - 8.00)*	0.67 *	50	7.70 ± 0.71 *(4.00 - 8.00)*	0.44 [K]*

WAIS FSIQ = WAIS Estimated Full-Scale IQ; CVLT SDFR = CVLT Short Delay Free Recall; CVLT SDCR = CVLT Short Delay Cued Recall; WMS-R LMIR = WMS-R Logical Memory Immediate Recall; GUILD PAIR = GUILD Paragraph Immediate Recall; WMS-R VERPI = WMS-R Verbal Paired Associates Immediate Recall; CVLT LDFR = CVLT Long Delay Free Recall; CVLT LDCR = CVLT Long Delay Cued Recall; WMS-R LMDR = WMS-R Logical Memory Delayed Recall; GUILD PADR = GUILD Paragraph Delayed Recall; WMS-R VERPD = WMS-R Verbal Paired Associates Delayed Recall

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

* $p < 0.05$ (Distribution is non-normally distributed)

Table 9. Visual Memory Immediate and Delayed Recall Domains - Descriptive and Normality Results

	T2DM			Control		
	n	Mean ± *SD* (*Min - Max*)	Normality Statistics	n	Mean ± *SD* (*Min - Max*)	Normality Statistics
Visual Memory Immediate Recall						
WMS-R VISPI	43	14.47 ± 3.71 *(1.00 - 18.00)*	0.84 *	47	15.81 ± 3.25 *(5.00 - 18.00)*	0.70 *
WMS-R VISRI	45	32.96 ± 4.87 *(16.00 - 41.00)*	0.91 *	50	34.48 ± 4.63 *(19.00 - 41.00)*	0.13 [K]*
Visual Memory Delayed Recall						
WMS-R VISPD	43	5.42 ± 1.18 *(1.00 - 6.00)*	0.57 *	47	5.74 ± 0.82 *(2.00 - 6.00)*	0.36 *
WMS-R VISRD	45	29.04 ± 7.29 *(4.00 - 40.00)*	0.94 *	50	31.86 ± 6.16 *(13.00 - 40.00)*	0.93 [K]*

WMS-R VISPI = WMS-R Visual Paired Associates Immediate Recall; WMS-R VISRI = WMS-R Visual Reproduction Immediate Recall; WMS-R VISPD = WMS-R Visual Paired Associates Delayed Recall; WMS-R VISRD = WMS-R Visual Reproduction Delayed Recall

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n >= 50); otherwise, the Shapiro-Wilk Test was used (n < 50)

* $p < 0.05$ (Distribution is non-normally distributed)

Table 10. Attention/Working Memory Domain - Descriptive and Normality Results

	T2DM					Control				
	n	Mean (Min - Max)	±	SD	Normality Statistics	n	Mean (Min - Max)	±	SD	Normality Statistics
DVT Tot Time	26	429.35 (311.00 - 584.00)	±	59.37	0.96	18	383.50 (254.00 - 470.00)	±	58.68	0.95
WMS-R DSF	44	8.50 (4.00 - 12.00)	±	2.35	0.95 *	50	8.84 (5.00 - 12.00)	±	1.98	0.16 [K]*
WMS-R DSB	44	7.32 (3.00 - 12.00)	±	2.59	0.95	50	7.86 (3.00 - 12.00)	±	2.56	0.13 [K]*
WMS-R VSF	43	8.07 (5.00 - 11.00)	±	1.74	0.94 *	47	8.45 (4.00 - 14.00)	±	1.83	0.92 *
WMS-R VSB	43	7.28 (2.00 - 11.00)	±	2.07	0.96	47	7.32 (4.00 - 12.00)	±	1.66	0.96

WMS-R DSF = WMS-R Digit Span Forward; WMS-R DSB = WMS-R Digit Span Backward; WMS-R VSF = WMS-R Visual Span Forward; WMS-R VSB = WMS-R Visual Span Backward

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

Please note that only 26 participants with T2DM and 18 controls were administered the DVT

* $p < 0.05$ (Distribution is non-normally distributed)

Table 11. Executive Function Domain - Descriptive and Normality Results

	T2DM					Control				
	n	Mean (*Min*	± -	*SD* *Max)*	Normality Statistics	n	Mean (*Min*	± -	*SD* *Max)*	Normality Statistics
TOL Excess Moves	40	14.58 *(0.00*	± -	25.83 *165.00)*	0.42 *	43	11.44 *(0.00*	± -	18.93 *121.00)*	0.48 *
WCST Persev. Error	22	16.23 *(3.00*	± -	11.63 *45.00)*	0.89 *	26	10.88 *(3.00*	± -	11.29 *59.00)*	0.60 *
Stroop Interf. Score	46	-2.09 *-(26.01*	± -	8.86 *13.50)*	0.98	50	-1.20 *-(12.19*	± -	6.49 *17.46)*	0.11 [K]
COWAT Tot. Score	41	46.17 *(25.00*	± -	12.57 *72.00)*	0.96	42	48.31 *(22.00*	± -	14.99 *83.00)*	0.98

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

* $p < 0.05$ (Distribution is non-normally distributed)

Table 12. Psychomotor Efficiency - Descriptive and Normality Results

	T2DM					Control				
	n	Mean (*Min*	±	*SD* *Max)*	Normality Statistics	n	Mean (*Min*	±	*SD* *Max)*	Normality Statistics
DSST Tot Score	43	54.14 *(28.00*	±	12.18 *82.00)*	0.97	50	56.78 *(36.00*	±	10.12 *86.00)*	0.07 [K]
PST Correct Score	16	71.13 *(44.00*	±	15.22 *98.00)*	0.97	27	71.59 *(49.00*	±	12.85 *96.00)*	0.95

[K] Indicates that normality was tested using the Kolmogorov-Smirnov Test (for n>=50); otherwise, the Shapiro-Wilk Test was used (n < 50)

Please note that only 16 participants with T2DM and 27 controls were administered the PST

Table 13. Estimated FSIQ and Verbal Memory Immediate and Delayed Recall Domains - *t*-test Results

		T2DM	Control			
		Mean ± SD	Mean ± SD	*t*	*p*	Cohen's *d*
§	**WAIS-R FSIQ**	106.48 ± 11.81	112.16 ± 11.39	-2.40	0.018	-0.49
	Verbal Memory Immediate Recall					
* §	**CVLT SDFR**	11.00 ± 3.72	12.90 ± 2.74	-2.82	0.006	-0.58
† §	**CVLT SDCR**	11.91 ± 2.70	13.04 ± 2.56	-2.10	0.039	-0.43
*	**WMS-R LMIR**	27.56 ± 6.32	31.04 ± 6.99	-2.54	0.013	-0.52
* §	**Guild PAIR**	5.51 ± 2.23	7.26 ± 2.51	-3.57	0.001	-0.74
† §	**WMS-R VERPI**	19.84 ± 4.01	21.26 ± 3.59	-1.81	0.073	-0.37
	Verbal Memory Delayed Recall					
§	**CVLT LDFR**	11.70 ± 3.25	12.96 ± 2.94	-2.00	0.048	-0.41
§	**CVLT LDCR**	12.46 ± 2.79	13.24 ± 2.45	-1.47	0.146	-0.30
* §	**WMS-R LMDR**	22.38 ± 7.81	27.02 ± 7.89	-2.88	0.005	-0.59
† §	**Guild PADR**	7.32 ± 3.18	8.95 ± 3.48	-2.37	0.020	-0.49
§	**WMS-R VERPD**	7.41 ± 0.92	7.70 ± 0.71	-1.70	0.093	-0.36

WAIS FSIQ = WAIS Estimated Full-Scale IQ; CVLT SDFR = CVLT Short Delay Free Recall; CVLT SDCR = CVLT Short Delay Cued Recall; WMS-R LMIR = WMS-R Logical Memory Immediate Recall; GUILD PAIR = GUILD Paragraph Immediate Recall; WMS-R VERPI = WMS-R Verbal Paired Associates Immediate Recall; CVLT LDFR = CVLT Long Delay Free Recall; CVLT LDCR = CVLT Long Delay Cued Recall; WMS-R LMDR = WMS-R Logical Memory Delayed Recall; GUILD PADR = GUILD Paragraph Delayed Recall; WMS-R VERPD = WMS-R Verbal Paired Associates Delayed Recall

* Remained significant after controlling for multiple comparisons

† Statistical trend after controlling for multiple comparisons

§ These variables were non-normally distributed in at least one of the groups and the t-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* test).

Table 14. Visual Memory Immediate and Delayed Recall Domains - *t*-test Results

	T2DM	Control			
	Mean ± SD	Mean ± SD	*t*	*p*	Cohen's *d*
Visual Memory Immediate Recall					
[§] **WMS-R VISPI**	14.47 ± 3.71	15.81 ± 3.25	-1.83	0.071	-0.39
[§] **WMS-R VISRI**	32.96 ± 4.87	34.48 ± 4.63	-1.57	0.121	-0.32
Visual Memory Delayed Recall					
[§] **WMS-R VISPD**	5.42 ± 1.18	5.74 ± 0.82	-1.51	0.136	-0.32
[†§] **WMS-R VISRD**	29.04 ± 7.29	31.86 ± 6.16	-2.04	0.044	-0.42

WMS-R VISPI = WMS-R Visual Paired Associates Immediate Recall; WMS-R VISRI = WMS-R Visual Reproduction Immediate Recall; WMS-R VISPD = WMS-R Visual Paired Associates Delayed Recall; WMS-R VISRD = WMS-R Visual Reproduction Delayed Recall

[†] Statistical trend after controlling for multiple comparisons

[§] These variables were non-normally distributed in at least one of the groups and the t-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* test).

Table 15. Attention / Working Memory Domain - *t*-test Results

		T2DM		Control				
		Mean	± SD	Mean	± SD	*t*	*p*	Cohen's *d*
†	**DVT Total Time**	429.35	± 59.37	383.50	± 58.68	2.53	0.015	0.78
§	**WMS-R DSF**	8.50	± 2.35	8.84	± 1.98	-0.76	0.449	-0.16
§	**WMS-R DSB**	7.32	± 2.60	7.86	± 2.56	-1.02	0.311	-0.21
§	**WMS-R VSF**	8.07	± 1.74	8.45	± 1.83	-1.00	0.320	-0.21
	WMS-R VSB	7.28	± 2.07	7.32	± 1.66	-0.10	0.919	-0.02

WMS-R DSF = WMS-R Digit Span Forward; WMS-R DSB = WMS-R Digit Span Backward; WMS-R VSF = WMS-R Visual Span Forward; WMS-R VSB = WMS-R Visual Span Backward

† Statistical trend after controlling for multiple comparisons

§ These variables were non-normally distributed in at least one of the groups and the t-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* test).

Please note that only 26 participants with T2DM and 18 controls were administered the DVT.

Table 16. Executive Function Domain - *t*-test Results

	T2DM		Control				
	Mean	± SD	Mean	± SD	*t*	*p*	Cohen's *d*
[§] **TOL Excess Moves**	14.58	± 25.83	11.44	± 18.93	0.63	0.528	0.14
[§] **WCST Persev. Error**	16.23	± 11.63	10.88	± 11.29	1.61	0.114	0.47
Stroop Interf. Score	-2.09	± 8.86	-1.20	± 6.49	-0.56	0.574	-0.12
COWAT Tot. Score	46.17	± 12.57	48.31	± 14.99	-0.70	0.484	-0.15

[§] These variables were non-normally distributed in at least one of the groups and the t-test results were confirmed to be consistent with those obtained using non-parametric tests (Mann-Whitney *U* test).

Table 17. Psychomotor Efficiency Domain - *t*-test Results

	T2DM	Control			
	Mean ± SD	**Mean ± SD**	***t***	***p***	**Cohen's *d***
DSST Tot Score	54.14 ± 12.18	56.78 ± 10.12	-1.14	0.257	-0.24
PST Correct Score	71.13 ± 15.22	71.59 ± 12.85	-0.11	0.915	-0.03

Please note that only 16 participants with T2DM and 27 controls were administered the PST

Table 18. Neuropsychological Data - Non-Parametric Mann-Whitney *U* Test Results for Non-Normally Distributed Variables

	T2DM			Control				
	n	Mean Rank	Sum of Ranks	n	Mean Rank	Sum of Ranks	z	*p*
*WAIS-R FSIQ	46	40.87	1880.00	50	55.52	2776.00	-2.57	0.010
Verbal Memory Immediate Recall								
† CVLT SDFR	46	40.85	1879.00	49	54.71	2681.00	-2.47	0.014
† CVLT SDCR	46	42.23	1942.50	50	54.27	2713.50	-2.13	0.033
* Guild PAIR	46	37.62	1730.50	48	56.97	2734.50	-3.45	0.001
† WMS-R VERPI	44	41.28	1816.50	50	52.97	2648.50	-2.10	0.036
Verbal Memory Delayed Recall								
CVLT LDFR	46	42.47	1953.50	50	54.05	2702.50	-2.05	0.040
CVLT LDCR	46	44.51	2047.50	50	52.17	2608.50	-1.36	0.174
* WMS-R LMDR	45	39.61	1782.50	50	55.55	2777.50	-2.82	0.005
Guild PADR	46	41.57	1912.00	48	53.19	2553.00	-2.07	0.039
WMS-R VERPD	44	43.77	1926.00	50	50.78	2539.00	-1.58	0.114
Visual Memory Immediate Recall								
† WMS-R VISPI	43	38.81	1669.00	47	51.62	2426.00	-2.37	0.018
WMS-R VISRI	45	43.22	1945.00	50	52.30	2615.00	-1.61	0.108
Visual Memory Delayed Recall								
† WMSR - VISPD	43	42.06	1808.50	47	48.65	2286.50	-1.79	0.073
† WMSR - VISRD	45	41.97	1888.50	50	53.43	2671.50	-2.03	0.043
Attention and Working Memory								
WMS-R DSF	44	45.33	1994.50	50	49.41	2470.50	-0.73	0.465
WMS-R DSB	44	44.47	1956.50	50	50.17	2508.50	-1.02	0.309
WMS-R VSF	43	42.59	1831.50	47	48.16	2263.50	-1.03	0.303
Executive Function								
TOL Excess Moves	40	45.01	1800.50	43	39.20	1685.50	-1.10	0.271
WCST Persev. Error	22	29.20	642.50	26	20.52	533.50	-2.15	0.032

Table 18 continued…

WAIS FSIQ = WAIS Estimated Full-Scale IQ; CVLT SDFR = CVLT Short Delay Free Recall; CVLT SDCR = CVLT Short Delay Cued Recall; GUILD PAIR = GUILD Paragraph Immediate Recall; WMS-R VERPI = WMS-R Verbal Paired Associates Immediate Recall; CVLT LDFR = CVLT Long Delay Free Recall; CVLT LDCR = CVLT Long Delay Cued Recall; WMS-R LMDR = WMS-R Logical Memory Delayed Recall; GUILD PADR = GUILD Paragraph Delayed Recall; WMS-R VERPD = WMS-R Verbal Paired Associates Delayed Recall; WMS-R VISPI = WMS-R Visual Paired Associates Immediate Recall; WMS-R VISRI = WMS-R Visual Reproduction Immediate Recall; WMS-R VISPD = WMS-R Visual Paired Associates Delayed Recall; WMS-R VISRD = WMS-R Visual Reproduction Delayed Recall; WMS-R DSF = WMS-R Digit Span Forward; WMS-R DSB = WMS-R Digit Span Backward; WMS-R VSF = WMS-R Visual Span Forward

* Remained significant after controlling for multiple comparisons

† Statistical trend after controlling for multiple comparisons

Table 19. Ten Largest Clusters Demonstrating Significant White Matter FA Reductions among Participants with T2DM ($p < 0.01$)

			Talairach Coordinates		
Clusters	**Size (Voxels)**	**Mean *t* value**	***x***	***y***	***z***
* **Left Parietal WM**	814	-3.02	25.4	61.9	11.4
* **Right Arcuate Fasciculus**	368	-3.07	-42.2	50.3	-10.3
* **Left Arcuate Fasciculus**	286	-2.93	45.2	52.7	-5.4
* **Right Medial Occipito-Temporal WM**	256	-3.24	-18.3	83	-15.4
* **Right Parietal WM**	251	-3.08	-27	50.8	26.4
Left Superior Temporal WM	223	-2.88	37.3	43.8	-14.1
Left Superior Longitudinal Fasciculus	200	-3.03	44.9	18.1	4.7
Left Temporal Stem	161	-2.86	45.9	28.7	-34.2
Left Occipital WM	158	-2.99	14.1	85.1	-2.5
* **Left Parietal WM**	144	-3.54	49.9	57.6	13.1

Voxel size = 1 mm^3; a *p*-value threshold of 0.01 was chosen to keep FDR below 1%

∗ Remained significant at $p < 0.005$

Table 20. Ten Largest Clusters Demonstrating Significant Gray Matter ADC Elevations among Participants with T2DM ($p < 0.01$)

	Clusters	Size (Voxels)	Mean *t* value	*Talairach Coordinates* *x*	*y*	*z*
[1] *	**Calcarine Fissure**	1034	3.08	0.2	103.7	-6.2
*	**Right PHG / Cerebellum**	741	2.86	-18.4	55.6	-37.8
*	**Left PHG**	442	2.98	16.6	46.8	-29.6
*	**Right Calcarine Fissure**	275	2.92	-12.1	80.7	-10.8
*	**Left Posterior Fusiform**	272	3.09	23	65.3	-31.2
*	**Right PFC**	241	3.08	-30.5	23.5	41.4
*	**Left Parietal Cortex**	234	3.22	65.7	54.9	9.6
*	**Right Heschl's Gyrus**	227	3.05	-46.2	30.6	-13.6
*	**Right PFC**	226	2.99	-2.2	-0.8	27.5
*	**Right PFC**	216	3.09	-3.3	6.1	39.9

Voxel size = 1 mm^3; a *p*-value threshold of 0.01 was chosen to keep FDR below 1%

* Remained significant at $p < 0.005$

[1] Please note that this cluster had substantial CSF contamination, which was proportionally small relative to its overall size; therefore, the cluster was retained.

Table 21. Frontal Lobe Volumes and Cortical Thickness - Descriptives and Normality Test

	T2DM			Control		
	n	Mean ± *SD* *(Min - Max)*	Shapiro-Wilk	n	Mean ± *SD* *(Min - Max)*	Kolmogorov-Smirnov
ICV-Adj. PFR Vol. (cc)	44	271.82 ± 36.38 190.68 - 350.09	0.95 *	50	279.72 ± 36.03 204.28 - 373.14	0.08
ICV-Adj. DLPFR Vol. (cc)	44	218.83 ± 32.62 154.82 - 299.32	0.95	50	218.85 ± 28.16 168.14 - 284.03	0.08
Tot Frontal Cort. Thick. (mm)	44	2.43 ± 0.15 2.00 - 2.72	0.98	50	2.46 ± 0.14 2.14 - 2.75	0.08

* $p < 0.05$ (Distribution is non-normally distributed)

Table 22. Frontal Lobe Volumes and Cortical Thickness – *t*-test Results

Measures	T2DM M ± SD	Control M ± SD	*t*	*p*	Cohen's *d*
ICV-Adj. PFR Vol. (cc)	271.82 ± 36.38	279.72 ± 36.03	-1.20	0.234	-0.25
ICV-Adj. DLPFR Vol. (cc)	218.83 ± 32.62	218.85 ± 28.16	0.38	0.705	0.08
Tot Frontal Cort. Thick. (mm)	2.43 ± 0.15	2.46 ± 0.14	-1.03	0.307	-0.21

ICV-adjusted brain volumes were being analyzed; for ease of interpretation, raw values for the descriptive data are presented.

Table 23. Temporal Lobe Volumes and Cortical Thickness - Descriptives and Normality Test

	T2DM			Control		
	n	Mean ± *SD* *(Min - Max)*	Shapiro-Wilk	n	Mean ± *SD* *(Min - Max)*	Kolmogorov-Smirnov
ICV-Adj. Hipp. Vol. (cc)	44	2.75 ± 0.33 2.24 - 3.38	0.96	50	2.93 ± 0.30 2.48 - 3.55	0.09
ICV-Adj. STG Vol. (cc)	44	23.15 ± 3.44 17.40 - 34.71	0.98	50	23.02 ± 3.23 18.03 - 34.61	0.11
Avg STG Cort. Thick. (mm)	44	2.58 ± 0.16 2.23 - 2.88	0.97	50	2.62 ± 0.16 2.25 - 2.95	0.08
Avg Fusiform Cort. Thick. (mm)	44	2.60 ± 0.15 2.25 - 2.93	0.98	50	2.63 ± 0.13 2.35 - 2.88	0.06
Avg MTL Cort. Thick. (mm)	44	2.75 ± 0.19 2.31 - 3.12	0.98	50	2.82 ± 0.18 2.43 - 3.09	0.09
Avg PHG Cort. Thick. (mm)	44	2.59 ± 0.27 1.97 - 3.13	0.98	50	2.58 ± 0.26 2.13 - 3.18	0.13 *
Avg Entorhinal Cort. Thick. (mm)	44	3.39 ± 0.35 2.65 - 4.04	0.98	50	3.36 ± 0.33 2.58 - 3.84	0.19 *

ICV-adjusted brain volumes were being analyzed; for ease of interpretation, raw values for the descriptive data are presented.

* $p < 0.05$ (Distribution is non-normally distributed)

Table 24. Temporal Lobe Volumes and Cortical Thickness – MANOVA & *t*-test Results

Measures	T2DM M ± SD	Control M ± SD	*F*	*p*	η_p^2
Overall Results (Wilks' Lambda)			2.69	0.026	0.133
* **ICV-Adj. Hipp. Vol. (cc)**	2.75 ± 0.33	2.93 ± 0.30	9.19	0.003	0.091
ICV-Adj. STG Vol. (cc)	23.15 ± 3.44	23.02 ± 3.23	0.24	0.622	0.003
Avg STG Cort. Thick. (mm)	2.58 ± 0.16	2.62 ± 0.16	1.60	0.209	0.017
Avg Fusiform Cort. Thick. (mm)	2.60 ± 0.15	2.63 ± 0.13	1.54	0.218	0.016
Avg MTL Cort. Thick.(mm)	2.75 ± 0.18	2.82 ± 0.18	3.38	0.069	0.035
			t	*p*	**Cohen's *d***
Avg PHG Cort. Thick. (mm)	2.59 ± 0.27	2.58 ± 0.26	0.14	0.893	0.03
Avg Entorhinal Cort. Thick. (mm)	3.39 ± 0.35	3.36 ± 0.33	0.39	0.700	0.08

ICV-adjusted brain volumes were being analyzed; for ease of interpretation, raw values for the descriptive data are presented.

* Remained significant after controlling for multiple comparisons

Table 25. Correlation Matrix for Cognitive Measures and Metabolic Factors among Participants with T2DM

	Verbal Memory Imm. Recall Composite Score	Verbal Memory Del. Recall Composite Score	WMS-R Visual Reproduction Delayed Recall	DVT Total Time
Diabetes Duration	-0.08	-0.002	-0.16	-0.21
HbA_{1C}	-0.30*	-0.29 †	-0.29 †	0.41*
BMI	0.05	0.05	-0.34*	0.25
Systolic BP	-0.06	-0.11	-0.36*	0.19
HDL	0.14	0.15	0.14	-0.33 †
Hypertension (Yes/No)	-0.19	-0.07	-0.24	0.03
Dyslipidemia (Yes/No)	-0.28†	-0.30*	-0.18	0.13

† $p < 0.10$ * $p < 0.05$

Table 26. Summary of Regression Models for Predicting Cognitive Measures among Participants with T2DM by (A) Metabolic Factors (B) Left PHG ADC After Controlling for Age and Gender

(A)

	Age and Gender				Metabolic Factors		
	β (Age)	*β (Gender)*	*ΔR²*	*ΔP*	*β*	*ΔR²*	*ΔP*
Verbal Memory Imm. Recall - Comp Score	-0.04	0.02	0.137	0.052	--		
					Dyslipidemia		
Verbal Memory Del. Recall – Comp Score	-0.03	0.21	0.082	0.173	-0.78	0.920	0.041
					BMI		
WMS-R Visual Reprod. Del. Recall	-0.30	-0.22	0.076	0.207	-0.44	0.171	0.005
DVT Total Time	-1.15	42.39	0.120	0.229	--		

(B)

	Age and Gender				Left PHG ADC		
	β (Age)	*β (Gender)*	*ΔR²*	*ΔP*	*β*	*ΔR²*	*ΔP*
Verbal Memory Imm. Recall - Comp Score	-0.01	-0.24	0.134	0.177	-3.50	0.162	0.310
Verbal Memory Del. Recall - Comp Score	0.02	-0.51	0.096	0.282	-4.101	0.148	0.040

Note: Gender (0 for women and 1 for men) and dyslipidemia (0 for NO and 1 for YES) are dichotomous variables.

Table 27. Correlation between Verbal Memory and Temporal Lobe Structures among Participants with T2DM

	Verbal Memory Imm Recall Comp. Score		Verbal Memory Del Recall Comp. Score	
	Spearman's rho		Spearman's rho	
ICV-Adj. Average Hipp. Volume	0.02		-0.01	
ADC - Left PHG	-0.54	*	-0.44	*
ADC - Left Heschl's Gyrus	-0.12		-0.04	
ADC - Right Heschl's Gyrus	-0.10		0.10	
FA - Left Superior Temporal WM	0.28		0.11	
FA - Left Arcuate Fasciculus	0.16		0.11	
FA - Right Arcuate Fasciculus	-0.01		-0.14	
FA - Left Temporal Stem	0.03		-0.19	

* $p < 0.05$

Figures

Figures 1 – 12 are presented in the subsequent pages.

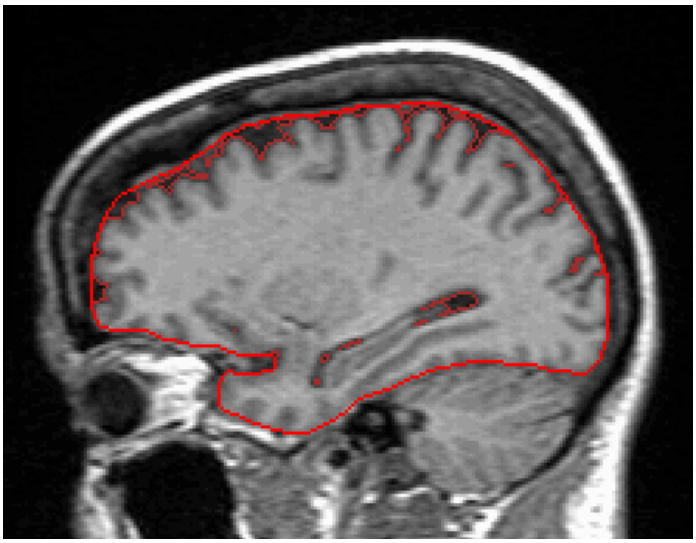

Figure 1. Intracranial Vault and Atrophy
Manual tracing of the intracranial vault (bright red) and overall atrophy estimation (dark red) by a thresholding method.

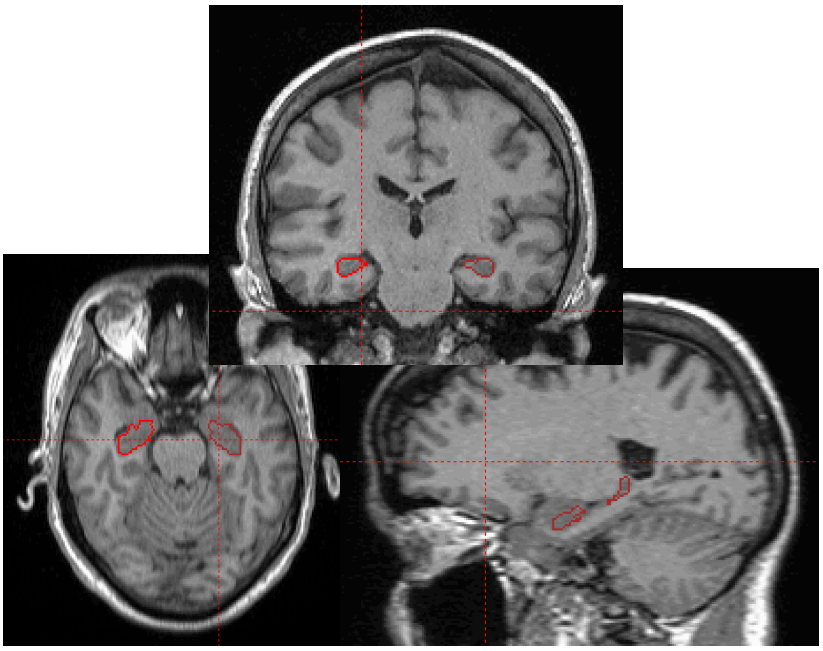

Figure 2. Manual Tracing of the Hippocampus
The drawing of the left and right hippocampi is done in the coronal plane (top), with the image also displayed in axial (left) and sagittal (right) views.

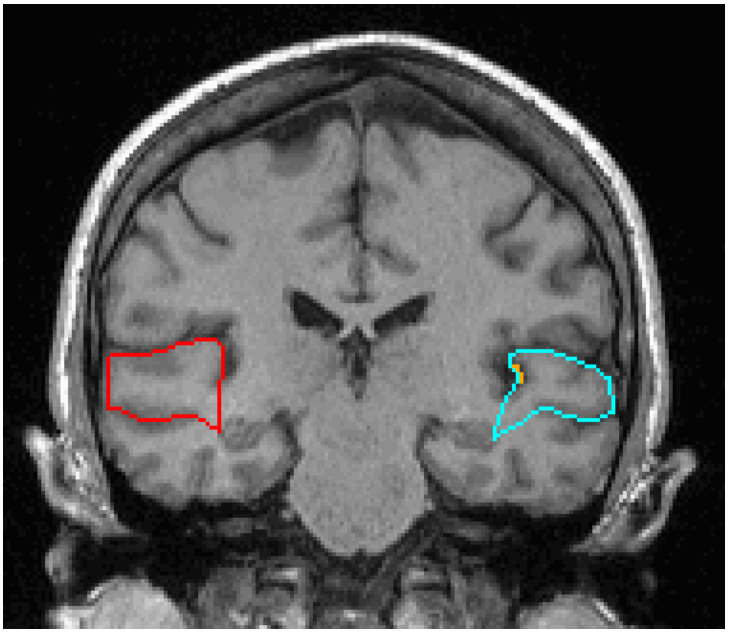

Figure 3. Manual Tracing of the STG with CSF Windowing
The drawing of the left (blue) and right STG (red) is done in the coronal plane. CSF windowing is shown in yellow in the left hemisphere.

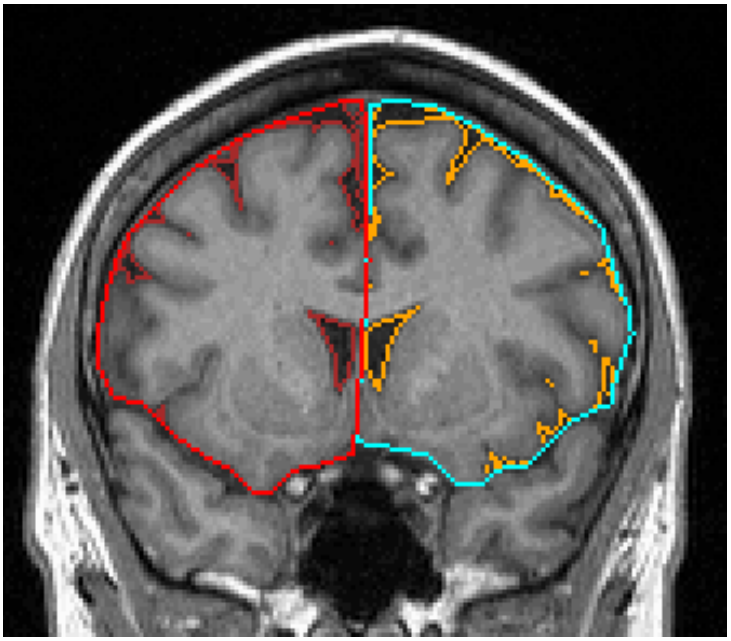

Figure 4. Manual Tracing of the DLPFR with CSF Windowing
The drawing of the left (blue) and right (bright red) DLPFR is done in the coronal plane. CSF windowing is shown in yellow and dark red in the left and right hemispheres, respectively.

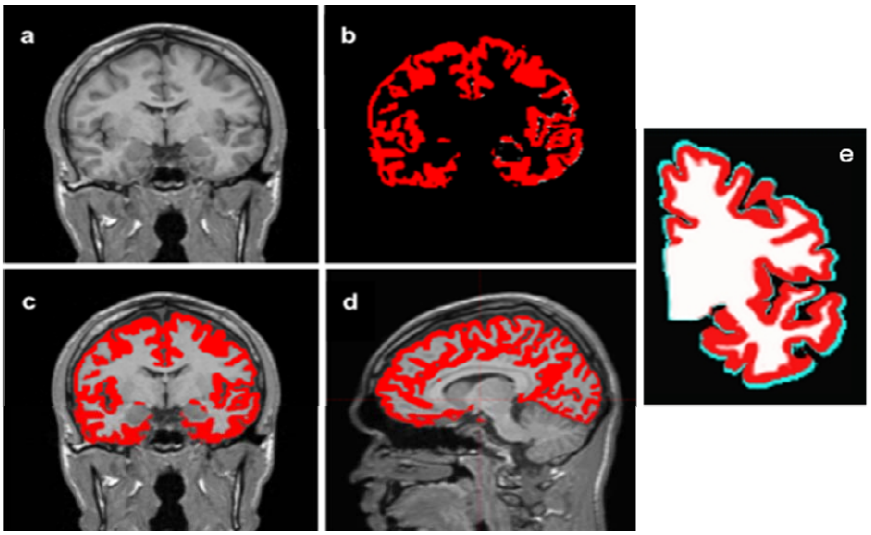

Figure 5. FreeSurfer-based Cortical Thickness Assessment
In the raw structural image (a), the cortical layer (b) is illustrated in the coronal (c) and sagittal (d) views. In panel (e), cortical thickness represents the distance between the pial surface (shown in blue) and the gray/white boundary, which is shown as the boundary between red (*gray matter*) and white (*white matter*) regions.

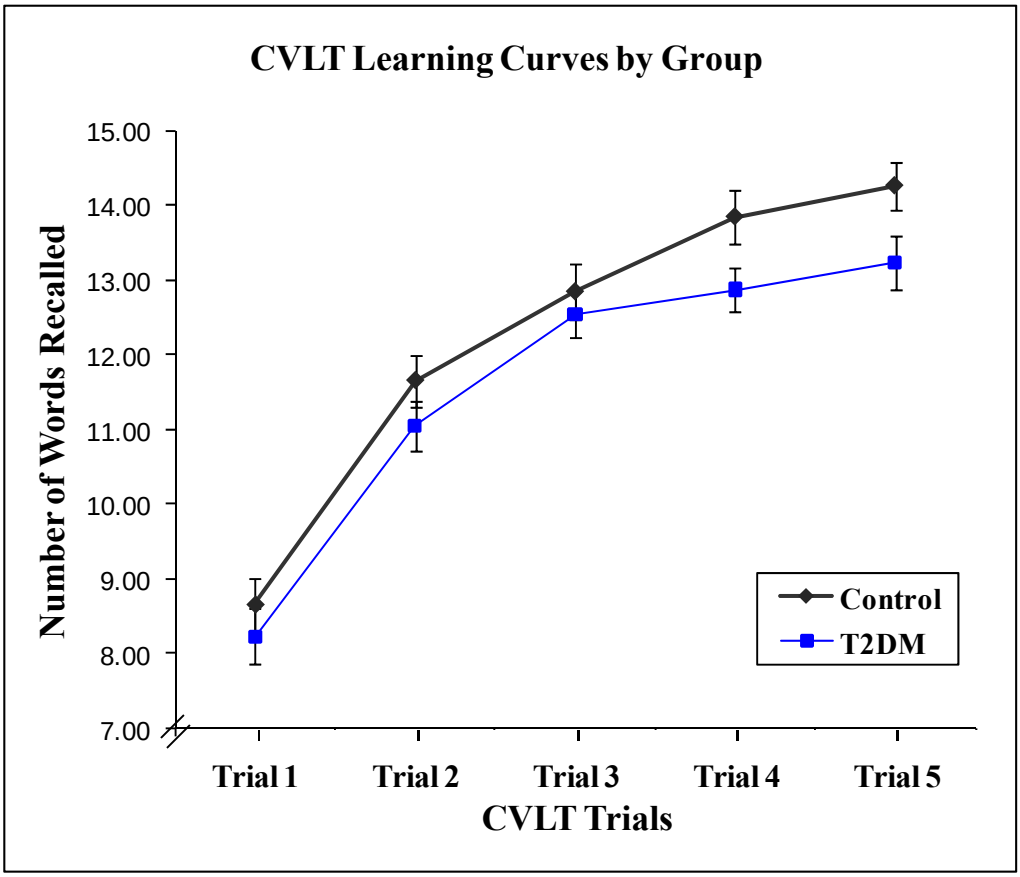

Figure 6. CVLT Learning Curves by Group

The learning curves across the 5 CVLT trials are illustrated for the groups, with the diabetic group performing consistently worse than the control group. Both groups showed a steady increase in the number of words learned from one trial to the next, with the learning rates becoming progressively slower particularly after trial 3, which was somewhat more prominent among participants with T2DM. The displayed values are mean ± SEM.

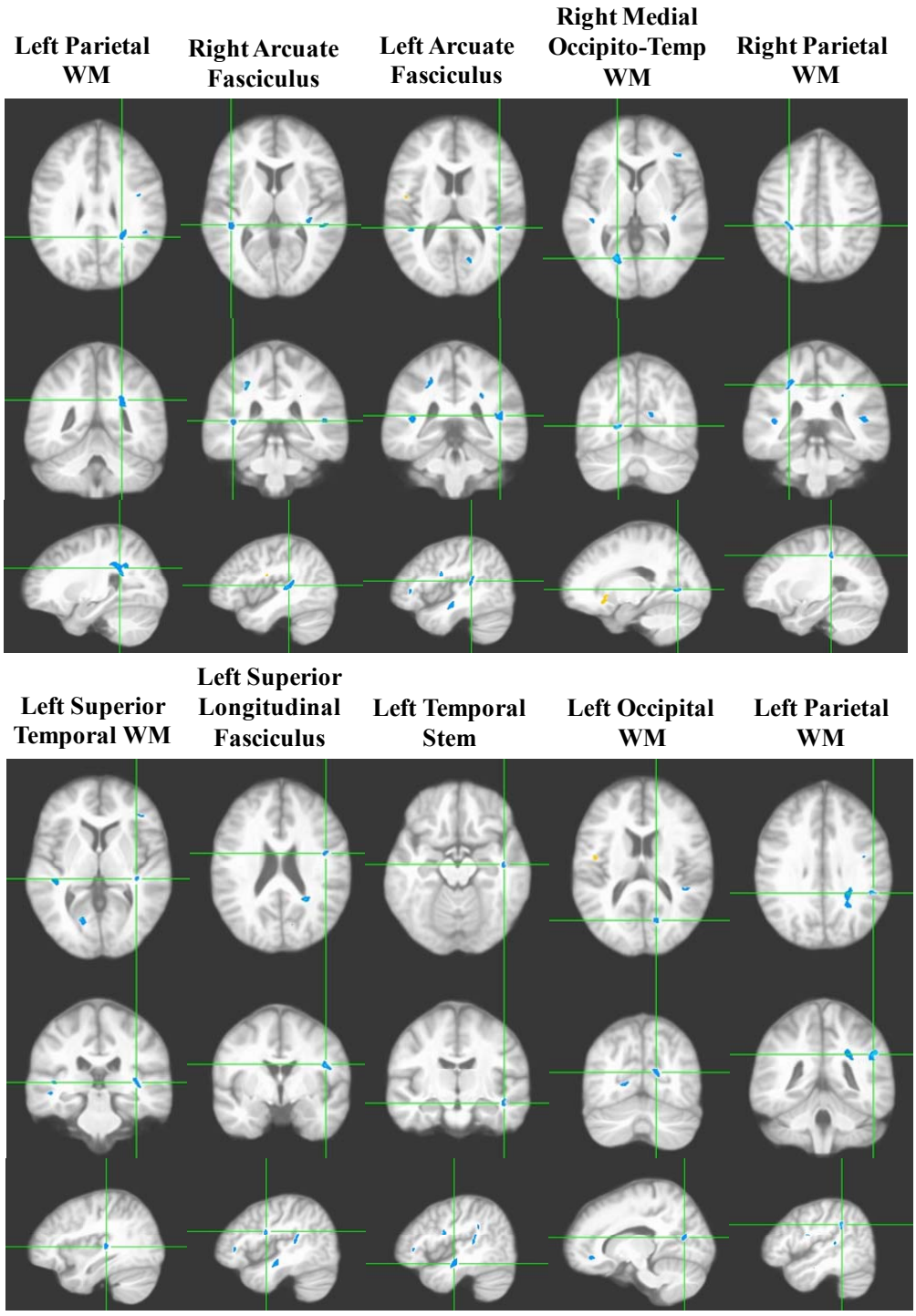

Figure 7. Ten Largest Clusters Demonstrating Significant White Matter FA Reduction among Participants with T2DM

The significant clusters of FA reduction (blue) are displayed in order of size, from left to right. In each of the two panels, the three rows of images, each showing an orthogonal orientation axial, coronal, and sagittal), display the clusters with the axes going through the centroid (coordinates in Table 19).

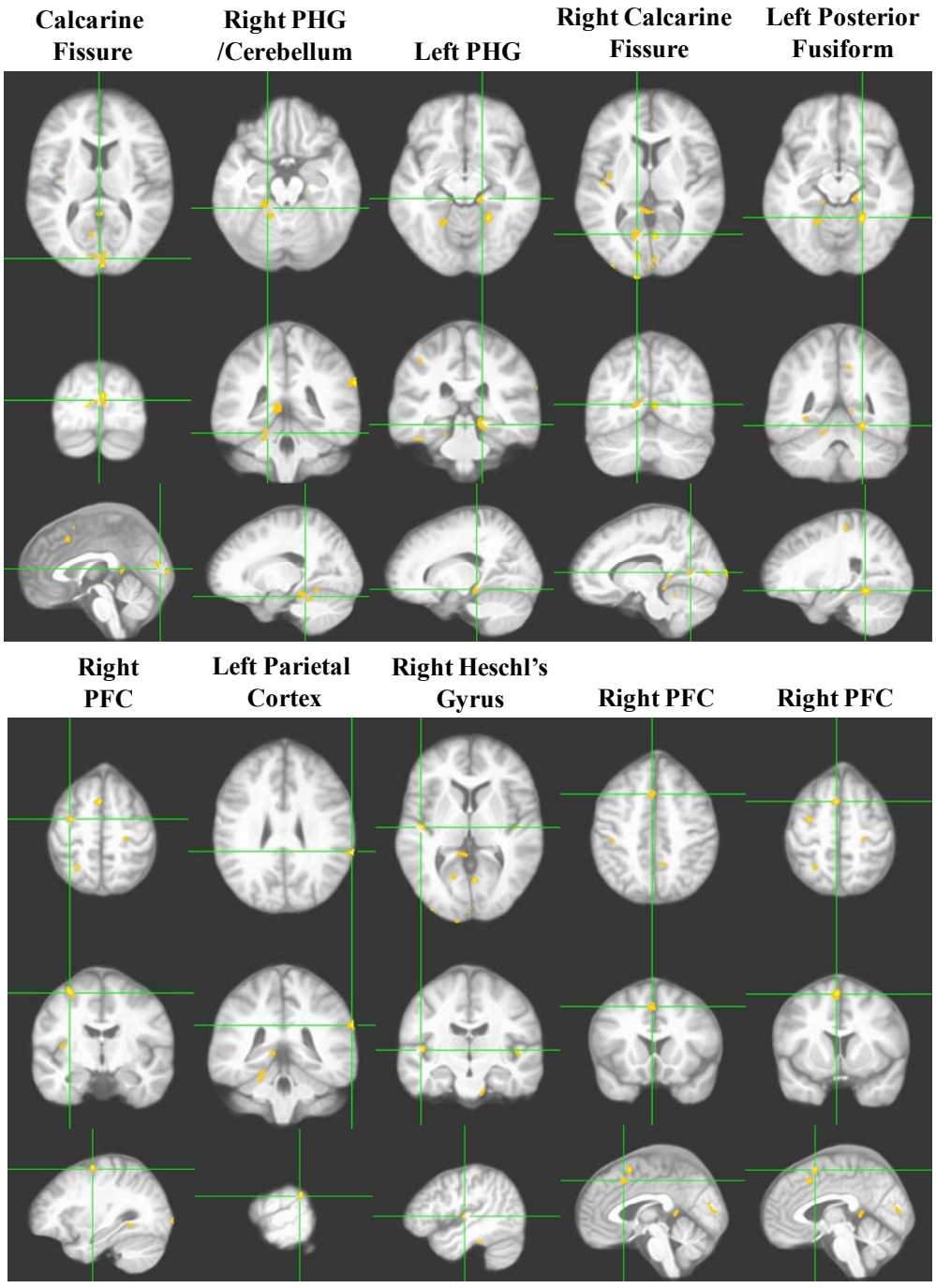

Figure 8. Ten Largest Clusters Demonstrating Significant Gray Matter ADC Elevation among Participants with T2DM

The significant clusters of ADC elevation (yellow/orange) are displayed in order of size, from left to right. In each of the two panels, the three rows of images, each showing an orthogonal orientation (axial, coronal, and sagittal), display the clusters with the axes going through the centroid (coordinates in Table 20).

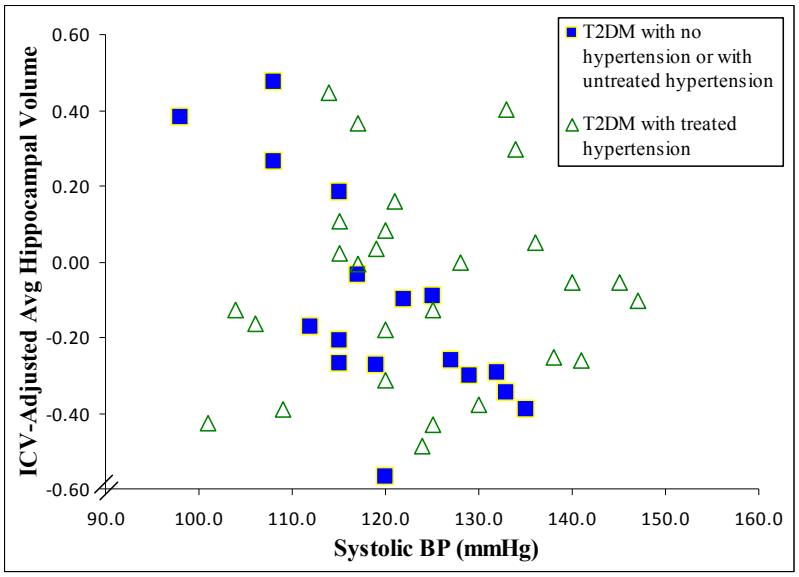

Figure 9. Association between Systolic BP and ICV-Adjusted Average Hippocampal Volume among Participants with T2DM

Systolic BP correlated inversely with ICV-adjusted average hippocampal volume among participants with T2DM without hypertension or with untreated hypertension together (blue squares) but no correlation was found for those with a history of anti-hypertensive medication (green triangles).

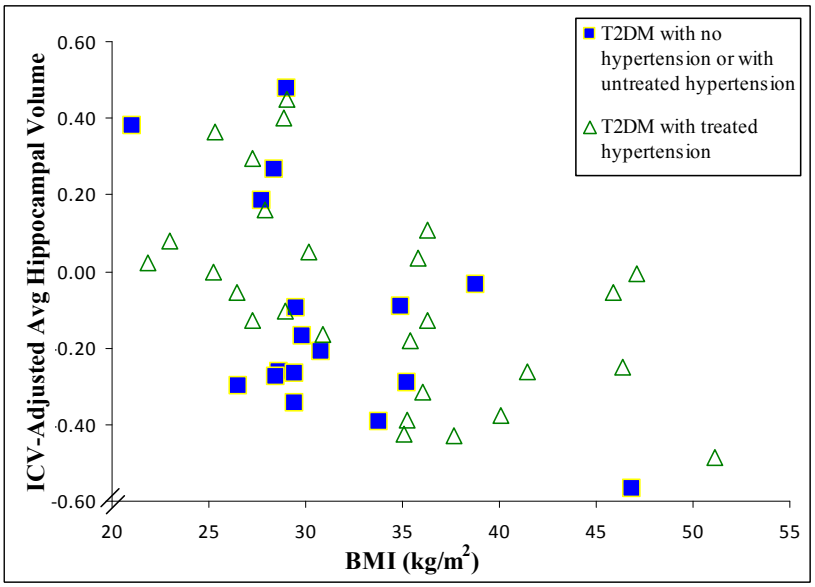

Figure 10. Association between BMI and ICV-Adjusted Average Hippocampal Volume among Participants with T2DM

BMI correlated inversely with ICV-adjusted average hippocampal volume among participants with T2DM with no hypertension or with untreated hypertension together (blue squares) and for those with a history of anti-hypertension medication (green triangles).

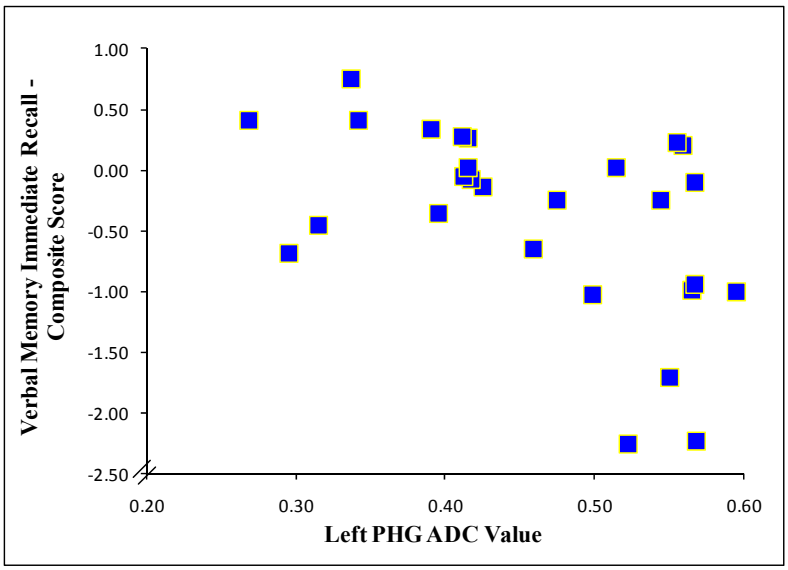

Figure 11. Inverse Relationship between ADC Values in the Left PHG Cluster and Verbal Memory Immediate Recall Composite Score among Participants with 2DM

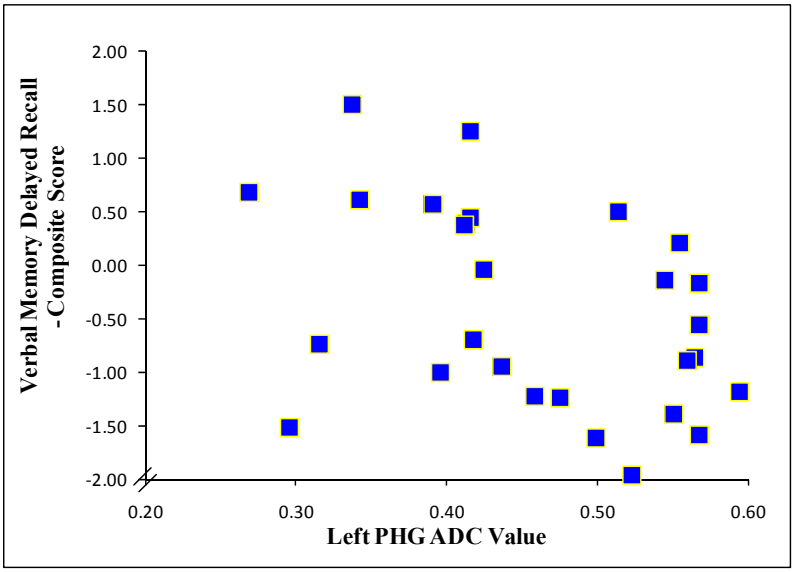

Figure 12. Inverse Relationship between ADC Values in the Left PHG Cluster and Verbal Memory Delayed Recall Composite Score among Participants with T2DM

Appendix A. Abbreviations

AD	Alzheimer's disease
ADC	Apparent diffusion coefficient
AGE	Advanced glycation end products
APOE	Apolipoprotein E
ART2	Automated registration toolkit 2
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BMI	Body mass index
BODyLab	Brain, Obesity, and Diabetes Laboratory
BP	Blood pressure
CBF	Cerebral blood flow
CDC	Center for Disease Control and Prevention
Cho	Choline
CIND	Cognitively impaired but not demented
COWAT	Controlled Oral Word Association Test
Cr	Creatine
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computerized tomography
CVLT	California Verbal Learning Test
DLPFC	Dorsolateral prefrontal cortex
DLPFR	Dorsolateral prefrontal region
DSB	Digit span backward
DSF	Digit span forward
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSST	Digit Symbol Substitution Test
DTI	Diffusion tensor imaging
DVT	Digit Vigilance Test
DWMH	Deep white matter hyperintensity
EKG	Electrocardiogram
FA	Fractional anisotropy
FDR	False discovery rate
FGF2	fibroblast growth factor-2
FLAIR	Fast fluid-attenuated inversion recovery
FOV	Field of view
FS	FreeSurfer

GDS	Global Deterioration Scale
GLUT	Glucose transporter
GM	Gray matter
GMT	Guild memory test
HAM-D	Hamilton Depression Scale
HbA_{1C}	Hemoglobin A1C
HDL	High density lipoprotein
HPA	Hypothalamic-pituitary-adrenal
ICV	Intracranial vault
IFG	Impaired fasting glucose
IGF-1	Insulin-like growth factor 2
IQ	Intelligence quotient
IR	Insulin resistance
IRB	Internal Review Board
LADIS	Leukoaraiosis and Disability
LDCR	Long delayed cued recall
LDFR	Long delayed free recall
LDL	Low density lipoprotein
LMDR	Logical memory delayed recall
LMIR	Logical memory immediate recall
LTP	Long term potentiation
MANOVA	Multivariate analysis of variance
MCI	Mild cognitive impairment
MI	Myo-inositol
MIDAS	Multimodal image data analysis system
MMSE	Mini Mental State Exam
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTL	Medial temporal lobe
n.s.	non-significant
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NCEP	National Cholesterol Education Program
NCSE	Neurobehavioral Cognitive Status Examination
NEX	Number of averages
NHLBI	National Heart, Lung and Blood Institute
NKI	Nathan Kline Institute

NYUSOM	NYU School of Medicine
OGTT	Oral glucose tolerance test
PAIR	Paragraph immediate recall
PADR	Paragraph delayed recall
PFC	Prefrontal cortex
PFR	Prefrontal region
PHG	Parahippocampal Gyrus
PST	Perceptual speed test
PWMH	Periventricular white matter hyperintensity
Q-DIS	Quick Diagnostic Interview Schedule III-Revised
QUICKI	Quantitative insulin sensitivity check index
ROI	Region of interest
SCWI	Stroop color word interference test
SDCR	Short delayed cued recall
SDFR	Short delayed free recall
SES	Socioeconomic status
SILS	Shipley Institute of Living Scale
SPGIR	Spoiled-gradient inversion recovery
SPM	Statistical parametric mapping
STG	Superior temporal gyrus
T1	Spin–lattice relaxation time
T1DM	Type 1 diabetes mellitus
T2	Spin–spin relaxation time
T2DM	Type 2 diabetes mellitus
TE	Echo time
TI	Inversion time
TICS	Telephone Interview for Cognitive Status
TOL	Tower of London
TR	Repetition time
VANCOVA	Voxelwise analysis of covariance
VBM	Voxel-based morphometry
VERPD	Verbal paired associates delayed recall
VERPI	Verbal paired associates immediate recall
VISPD	Visual paired associates delayed recall
VISPI	Visual paired associates immediate recall
VISRD	Visual reproduction delayed recall
VISRI	Visual reproduction immediate recall

VSB	Visual span backward
VSF	Visual span forward
WAIS	Wechsler Adult Intelligence Scale
WAIS-R	Wechsler Adult Intelligence Scale - Revised
WASI	Wechsler Abbreviated Scale of Intelligence
WCST	Wisconsin Card Sorting Test
WHR	Waist-height ratio
WM	White matter
WMH	White matter hyperintensity
WML	White matter lesion
WMS-R	Wechsler Memory Scale - Revised

Appendix B. Informed Consent

Office of Institutional Board of Research Associates
NYU School of Medicine
550 First Ave. Building #VET
10 West
NY, NY 10016
Phone: 212.263.4110
Fax: 212.263.4147

Principal Investigator: Antonio Convit, M.D.

INFORMED CONSENT FORM TO PARTICIPATE AND AUTHORIZATION FOR RESEARCH

TITLE OF RESEARCH: *Diabetes, Cognition, and the Brain*

A. PURPOSE OF THE STUDY:

You are being asked to volunteer in a research study. Sugar or glucose is the only source of fuel for the brain and the glucose the brain uses comes from the blood. The purpose of this study is to assess whether how well your brain is working (memory and other cognitive functions) is related to how your body handles glucose. We are inviting three types of individuals to participate in this study: 1) those having Type 2 (adult onset) diabetes, who have never been treated with insulin or sulfonylureas; 2) those having normal fasting glucose levels and impaired glucose tolerance; and 3) those with normal fasting glucose levels and glucose tolerance. You will qualify for this study if you do not have an active medical (other than Type 2 diabetes), neurological, or psychiatric condition that will preclude your participation. In addition to assessing your glucose tolerance, we will assess how your cortisol (stress hormone) levels interact with your glucose regulation in influencing your memory function. This study may indirectly benefit you in that an oral glucose tolerance test will be performed, which could uncover a metabolic problem needing medical treatment. Your participation may help us understand the factors that may be causing some diabetics to have memory problems, and thus be in a position to prevent them.

B. SUBJECT PARTICIPATION:

We estimate that the following number of subjects will enroll in this study:
At this site: 150 Total at all sites: 150

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SUBJECT PARTICIPATION:
☐ Inpatient
☒ Outpatient
☒ other [healthy subjects, etc.] Please specify: Healthy controls

[The IBRA expects that research will be available, as appropriate, to all persons regardless of race, gender, age, or economic circumstances]

Your participation will involve 3 visits, which will take place over less than 2 months.

Each of these visits will take the following amount of time: 2-6 hours depending on the visit.

C. DESCRIPTION OF THE RESEARCH:

You will have had a medical and neurological examination, and some memory tests as part of your participation in the Center for Brain Health or the Aging and Dementia Research Center prior to this study. As part of that assessment you will have also received an MRI of your brain. You may have already completed parts of this study during earlier research participation.

As part of those previous evaluations you will have blood drawn to determine your apolipoprotein genotype (ApoE genotyping). One variant of this gene may be a risk factor for Alzheimer's Disease (AD). DNA for ApoE genotyping will be prepared, and in addition, your blood will be stored for future research use. ApoE genotyping is not a diagnostic test for AD and is not part of the recommended diagnostic evaluation for AD. Consequently, the results will not be made available to you. However, if you wish to learn of the ApoE results we can arrange for your genotype to be determined by an outside clinical laboratory and you will be billed directly for that service

This study will involve three separate visits:

On the first visit we will ask you questions about your medical health and your diet, and will measure your height and weight, as well as administer some cognitive memory tests. During this visit we will give and explain how to complete special food record forms, where you will record what you eat for the three days before you come back for tests during the second visit. On the first visit you will also be given a container to collect your urine on the day before you come for your second visit. We will give you detailed instructions on how to fill out the diet record and collect the urine.

On the second visit you will receive an Oral Glucose Tolerance Test. For the Oral

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Glucose Tolerance Test, you will have an intravenous catheter placed in one arm (small plastic tube in your vein, similar to what is used to give people fluid by vein when they are in the hospital). We will ask you to drink a standardized sweet beverage containing 75 grams of glucose (it will be like a very sweet soda) and at different times we will obtain 6 blood samples to monitor your glucose levels, insulin levels, and cortisol levels for a 4-hour period. Each time we will obtain 6 or 9cc (2 or 3 Tsp) dependent on the sample for a total of about 54 cc or 11/4 oz.

The night before your third visit we will ask you to take 1.5 mg of dexamethasone by mouth at 11:00 pm. We will give you the dexamethasone pills when you come in for your second visit. Dexamethasone is a synthetic steroid that is very similar to cortisol, the stress hormone (see below regarding the safety of taking it). We will ask you to come to our laboratory for the third visit at 1:00 pm. We will first give you a standardized lunch. After lunch we will place an intravenous catheter in one arm. At 3:00 pm we will give you 100 micrograms of Corticotropin Releasing Hormone (CRH). CRH is a hormone that your body naturally produces (see below regarding the safety of taking it). We will be giving it to you to test how your cortisol release is controlled by the dexamethasone. From 2:45 to 4:15 we will draw a total of six blood samples through the intravenous catheter we placed in your arm. Each time we draw your blood we will take 6 cc (2 Tsp.) for a total of 36 cc (about 1 ounce). The blood samples will be used to measure cortisol, as well as ACTH, another hormone involved in the stress response. We will also administer a few brief memory tests both before and after you receive the CRH.

DONATION OF BLOOD: 6 or 9cc, (equivalent to 2 or 3 Tsp), depending on the sample. Number of Blood Withdrawals: 12 (over the 2 sessions). Total amount: 90 cc. The potential risks of donating blood may occasionally include pain, bruising, fainting or a small infection at the puncture site. To reduce risks, the procedures will be performed by experienced medical personnel using sterile methods and materials.

Any questions concerning your participation in the study can be addressed to Antonio Convit, M.D. at (212) 263-7565.

D. COSTS/REIMBURSEMENTS:

This research will be conducted at no cost to you. This study is being sponsored by grants from the National Institutes of Health to the NYU School of Medicine GCRC and Dr. Antonio Convit. Portions of Dr. Convit's and his research team's salaries are being paid by Dr. Convit's grant.

As part of your participation in the study we will compensate you for your time and inconvenience. For the two experimental sessions we will compensate you $100 for the oral glucose tolerance test and $200 for the Dex/CRH test for a total of $300. If for whatever reason

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you do not complete all of the experimental sessions, your compensation will be prorated to those experimental sessions that you participated in.

E. POTENTIAL RISKS AND DISCOMFORTS:

We discuss below the risks or discomforts that may be associated with participation in this research. It is possible that the particular treatment or procedure may involve risks to you (or to the embryo or fetus, if you are or may become pregnant), which are not currently known or foreseeable. In spite of all precautions, you might develop medical complications.

Dexamethasone may cause an increase in blood pressure, fluid retention, and perhaps an exaggerated sense of well being. However, given that you will only receive a low dose (1.5 mg) only once, these effects are very unlikely. Moreover, the careful physical exam you will receive as part of your participation in the Center for Brain Health or Aging and Dementia Research Center has been designed to identify any disease condition, such as uncontrolled high blood pressure, that might be affected by the dexamethasone you will be given.

Corticotropin Releasing Hormone (CRH) may cause a slight, transient increase in blood pressure (10 – 20 mm Hg systolic), mild flushing in the face, or slight increase in heart rate (10 beats per minute). Some individuals describe a sensation like a "rush" in the chest. These reactions are rare and brief, rarely lasting longer than a few minutes. As part of your medical evaluation you will have been screened for any disease condition (such as high blood pressure) that might be affected by giving you CRH; people with uncontrolled high blood pressure will not be given CRH. In addition, we will be monitoring your blood pressure during these procedures.

During the oral glucose tolerance test your blood sugar will rise to levels similar as those after a very large meal. However, the risk from this is only minimal. Study participants with non-insulin dependent diabetes will only be included if their blood sugar is well controlled.

There is a small chance that where we place the intravenous catheters there may be tenderness or bruising afterwards. There is a very slight chance of infection at the site where we place the intravenous catheters, but this is minimized by taking special precautions. We are experienced in conducting these procedures as they are used routinely in some of our other studies.

F. POTENTIAL BENEFITS:

There is no direct benefit to you expected from your participation in this study. It is hoped the knowledge gained will be of benefit to others in the future.

There are several indirect medical benefits to you by participating in this study include the comprehensive medical evaluation (a physical exam, a neurological exam and laboratory measurements) as well as the Glucose Tolerance Test, which evaluates your body's ability to regulate its blood sugar levels. We will give you results of this medical evaluation and let you

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know if there are any problems. On the rare occasion that during our evaluation we uncover a problem that might need medical attention, we will instruct you to follow up with your own doctor. If you do not have a doctor, we will make a referral for you.

There is the possibility (in non-diabetic participants) that you may have some level of glucose intolerance that will be diagnosed by the Glucose Tolerance Test. If any glucose intolerance is diagnosed in you by this test, we will inform you of this and instruct you to follow up with your own doctor, or will give you a referral if needed. Your doctor will want to monitor any glucose intolerance, and perhaps would modify your diet to make sure you do not have any problems with diabetes in the future.

Although you may receive no direct medical benefits from participating in this study, this project may provide information on how glucose tolerance and cortisol affect blood flow in the brain as well as memory. The brain is not normally used as one of the organs that are evaluated to monitor the potential complications of diabetes (currently the retina, kidneys, and nerves are predominantly used). If this study is successful it may highlight the importance of assessing the brain and its function in diabetes.

G. ALTERNATIVES TO PARTICIPATING IN THE STUDY:

You will not be prevented from participating in other research programs. Also, another alternative is not to participate in the study.

H. CONFIDENTIALITY:

Private information about you that identifies you may be used or shared for the purposes of this research project. This section of the consent/authorization form describes how your information will be used and shared in this research, and the ways in which NYU School of Medicine will safeguard your privacy and confidentiality.

If you agree to be in this research program, Dr. Convit and his study team will ask you to have the tests outlined in the procedure section above (pages 2-4). Results of tests and studies are done just for this research study and are not as part of your regular care and will not be included in your medical record. Only a research record will be kept.

Other persons and organizations, including co-investigators, federal and state regulatory agencies, and the IRB(s) overseeing the research may receive your information during the course of this study. Except when required by law, study information shared with persons and organizations outside of New York University School of Medicine (NYUSM) will not identify you by name, social security number, address, telephone number, or any other direct personal identifier.

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When your study information will be disclosed outside of NYUSM as part of the research, the information that can identify you as listed above will be removed and your records will be assigned a unique code number. NYUSM will not disclose the code key, except as required by law.

Confidentiality of Your Study Information
Your study records include information that identifies you and that is kept in research files. We will everything we can to keep this information confidential, but we cannot guarantee it. If data from this study are to be published or presented, we will first take out the information that identifies you.

Retention of Your Study Information
The study results will be kept in your research record for at least six years or until after the study is completed, whichever is longer. At that time either the research information not already in your medical record will be destroyed or information identifying you will be removed from such study results at NYU. Any research information in your medical record will be kept indefinitely.

Your HIPAA Authorization
A new federal regulation, the federal medical Privacy Rule, has taken effect as required by the Health Insurance Portability and Accountability Act (HIPAA). Under the Privacy Rule, in most cases we must seek your written permission to use or disclose identifiable health information about you that we use or create [your "protected health information"] in connection with research involving your treatment or medical records. This permission is called an Authorization.

If you sign this form you are giving your Authorization for the uses and sharing of your protected health information described below. You have a right to refuse to sign this form. If you do not sign the form you may not be in the research program, but refusing to sign will not affect your health care (or payment for your health care) outside the study.

This Authorization will not expire unless you withdraw it in writing. You have the right to withdraw your authorization at any time, except to the extent that NYU has already relied upon it or must continue to use your information to complete data analysis or to report data for this study. The procedure for revoking your authorization is described below in Section K.

By signing this form you authorize the use and disclosure of the following information for this research:

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- Your research record
- Results of laboratory tests
- Clinical and research observations made during your participation in the research.

By signing this form you authorize the following persons and organizations to receive your protected health information for purposes related to this research:

- Every research site for this study, including this hospital, and including each sites' research staff and medical staff
- Every health care provider who provides services to you in connection with this study
- Any laboratories and other individuals and organizations that analyze your health information in connection with this study in accordance with the study's protocol
- The following research sponsors and the people and companies they use to oversee, administer, or conduct the research: NIH, Departmental
- The United States research regulatory agencies and other foreign regulatory agencies
- The members and staff of the hospital's affiliated Institutional Review Board
- Queens College-CUNY Institutional Review Board
- The members and staff of the hospital's affiliated Privacy Board
- Principal Investigator: Antonio Convit, M.D.
- Study Coordinator
- Members of the Research Team
- The Patient Advocate or Research Ombudsman (GCRC)
- Members of the NYU/NYUMC Clinical Trials Office/Office of Research and Sponsored Programs
- Data Safety Monitoring Board/Clinical Events Committee
- Others (as described below): Laboratory at the University of Duesseldorf or Dresden, Germany, where the analyses for glucose, insulin, cortisol, ACTH, CRH, and dexamethasone are conducted. Samples are identified only by a code, known only to NYU researchers.

If any of the companies or institutions listed above merges or is sold during the course of this research, your Authorization will cover uses and disclosures of your protected health information to the new company or institution that assumes responsibility for the research.

Please be aware that once your protected health information is disclosed to a person or organization that is not covered by the federal medical Privacy Rule, the information is no longer protected by the Privacy Rule and may be subject to redisclosure by the recipient.

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I. COMPENSATION/TREATMENT IN THE EVENT OF INJURY:

All forms of medical (or mental health) diagnosis and treatment – whether routine or experimental – involve some risk of injury. In addition, there may be risks associated with this study that we do not know about. In spite of all precautions, you might develop medical complications from being in this study.

If you sustain any injury during the course of the research or experience any side effect to a study drug or procedure, please contact the Principal Investigator Antonio Convit, M.D. at the following telephone number (212) 263-7565. If such complications arise, the study doctor will assist you in obtaining appropriate medical treatment but this study does not provide financial assistance for medical or other injury-related costs. You do not give up any rights to seek payment for personal injury by signing this form.

J. VOLUNTARY PARTICIPATION AND AUTHORIZATION:

Your decision as to whether or not to take part in this study is completely voluntary (of your free will). If you decide not to take part in this study it will not affect the care you receive and will not result in any loss of benefits to which you are otherwise entitled.

You will be told of any significant new findings developed during the course of the research that may influence your willingness to continue to participate in the research.

Your decision as to whether to give your Authorization for the use and disclosure of your protected health information for this study is also completely voluntary; however, if you decline to give your Authorization or if you withdraw your Authorization you may not participate in the study.

K. WITHDRAWAL FROM THE STUDY AND/OR WITHDRAWAL OF AUTHORIZATION:

If you decide to take part in the study, you may withdraw from participation at any time without penalty or loss of benefits to which you would otherwise be entitled. You may also withdraw your Authorization for us to use or disclose your protected health information for the study. If you do decide to withdraw your consent, we ask that you contact Dr. Antonio Convit in writing and let him know that you are withdrawing from the study. His mailing address is Center for Brain Health, NYU School of Medicine, 550 First Ave. HN-400, New York, NY 10016. If you wish to withdraw your Authorization as well as your consent to be in the study, you must contact Dr.

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Convit in writing. Remember that withdrawing your Authorization only affects uses and sharing of information after your written request has been received, and you may not withdraw your Authorization for uses or disclosures that we have previously made or must continue to make to complete analyses or report data from the research.

The Principal Investigator or another member of the study team will discuss with you any considerations involved in discontinuing your participation in the study. You will be told how to withdraw from the study and may be asked to return for a final check-up.
The study doctor may also decide to withdraw you from the study for certain reasons. Some possible reasons for withdrawing a subject from the study would be worsening health or other conditions that might make it harmful for you.
The following circumstance may also lead to you being withdrawn from the study:
(a) failure to keep appointments, follow directions or take medications as instructed
(b) a serious adverse reaction to drug therapy
(c) the need for treatment that is not allowed in the study
(d) termination or cancellation of the study by study sponsor.

L. PERMISSION TO CONTACT YOU ABOUT FUTURE RESEARCH:

I authorize the principal investigator and his or her co-investigators to contact me about future research on Peripheral Glucose Regulation, Brain, and Cognition within the Department of Psychiatry, Center for Brain Health provided that this future research is approved by the original IRB of record and that the principal investigator and co-investigator are affiliated with the research protocol.
If I agree, then someone from Dr. Convit's research staff might contact me in the future and he or she will tell me about a research study. At that time, I can decide whether or not I am interested in participating in a particular study. I will then have the opportunity to contact the researcher to schedule an appointment to be fully informed about the research project.

☐ I agree to be contacted by the Principal Investigator or Co-Investigators of the research study titled: ***Diabetes, Cognition and the Brain***

☐ I **do not** want to be contacted by the Principal Investigator or Co-Investigator of the research study titled: ***Diabetes, Cognition and the Brain***

Signature of participant Date

 Subject's Initials: _____ Date: _____

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This Consent Document is approved for use by the New York University's Institutional Review Board (IRB). Only the IRBA-stamped approved form may be used.

Approved: From: 20-Apr-2009 *To:* 19-Apr-2010
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10/21/09 to 10/20/10

Your permission to allow us to contact you about future research would be greatly appreciated, but it is completely voluntary. If you choose not to allow us to contact you, it will not affect your care at any of the NYUSM facilities. Please understand that giving your permission to do this is only for the purpose of helping us identify subjects who may qualify for one of our future research studies. It does not mean that you must join in any study.

M. CONTACT PERSON(S):

For further information about your rights as a research subject, or if you are not satisfied with the manner in which this study is being conducted and would like to discuss your participation with an institutional representative who is not part of this study, please contact the Administrator, Institutional Board of Research Associates, Telephone No. 212-263-4110.

If you have any questions or sustain any injury during the course of the research or experience any adverse reaction to a study drug or procedure, please contact the Principal Investigator Antonio Convit, M.D. at the following telephone number (212) 263-7565.

AGREEMENT TO PARTICIPATE AND AUTHORIZATION FOR THE USE OR DISCLOSURE OF PROTECTED HEALTH INFORMATION:

Part of the consent process includes your Authorization to use Protected Health Information for the purposes of this study, as described above. If you do not want to authorize the use of this PHI, you should not agree to be in this study.

☐ I have read this consent form
or
☐ it was read to me by: _____.

Any questions I had were answered by: _____.

I ☐ am ☐ am not participating in another research project at this time.
(If yes, you should discuss this with your study doctor.)

I voluntarily agree to participate in this research program at:

☐ NYUSM [Skirball Institute; Nelson Institute of Environmental Medicine; Post Graduate Medical School]

☐ The NYU Hospitals Center (Tisch Hospital; the Rusk Institute of Rehabilitation Medicine);

 Subject's Initials: _____ Date: _____

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☐Bellevue Hospital Center: this form and your study information will be available to Bellevue Hospital administration and their auditors.

☐Hospital for Joint Diseases Orthopedic Institute;

☐NYU College of Dentistry;

☐The New York Campus of the Veteran's Affairs New York Harbor Healthcare System.

☐Other, please specify:

I understand that I am entitled to and will be given a copy of this signed Consent/Authorization Form.

By signing this Consent/Authorization form, I give my Authorization for the uses and disclosures of my protected health information as described above.

WHEN THE SUBJECT IS AN ADULT:

Print Name of Participant	Signature of Participant	Date
Print Name of Person Obtaining Consent	Signature of Person Obtaining Consent	Date

 Subject's Initials: _____ Date: _____

(IRB Official Use Only)

This Consent Document is approved for use by the New York University's Institutional Review Board (IRB). Only the IBRA-stamped approved form may be used.

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Appendix C. Demographic Questionnaire

Please send completed application to:

NYU School of Medicine
Center for Brain Health
ATTN: Victoria Sweat
Dept. of Psychiatry, MHL 400
550 First Avenue
New York, NY 10016

(All information supplied in this questionnaire is strictly confidential)

DATE: _____/_____/_____

APPLICANT'S NAME: _____
First Initial Last

ADDRESS: _____

_____

HOME: ( ) _____- _____ WORK: ( ) _____- _____

E-MAIL: _____

AGE: _____ SEX: _____ HEIGHT: _____ WEIGHT: _____

RELIGION: _____ BIRTHDATE:_____

SS#: _____ BIRTHPLACE: _____

REFERRED BY: _____

PRIMARY CONTACT PERSON:

NAME: _____

RELATIONSHIP TO APPLICANT: _____

ADDRESS: _____

_____

HOME: ( ) _____- _____ WORK: ( ) _____- _____

E-MAIL: _____

RELATIVE OR FRIEND:(in addition to PRIMARY CONTACT PERSON above)

NAME: _____

RELATIONSHIP TO APPLICANT: _____

ADDRESS: _____

_____

HOME: ( ) _____- _____ WORK: ( ) _____- _____

E-MAIL: _____

1.1 PRINCIPAL FAMILY PHYSICIAN

NAME: _____

OFFICE PHONE: ( ) _____ - _____

ADDRESS: _____

_____

_____

While we will never ask you for specific information, we just need to know whether you have health insurance:

_____ Yes, I have health insurance _____ No, I do not have health insurance

1.1.1 APPLICANT INFORMATION

PRIMARY LANGUAGE SPOKEN: _____

SECOND LANGUAGE(S): _____

MARITAL STATUS (Check one): SINGLE __ MARRIED __ WIDOWED ___ DIVORCED __ SEPARATED __

If married, name of spouse: _____

RACE: BLACK ____ WHITE _____ ASIAN _____ OTHER (please specify)_____

ETHNICITY: Hispanic _____ Non-Hispanic _____

OCCUPATION

Please tell us your occupation or the type of work you have done most of your life:

_____

_____

CURRENTLY EMPLOYED: ____ yes ____ no

RETIRED: ____ yes ____ no

YEARS SINCE RETIRING: _____
HIGHEST LEVEL OF EDUCATION: _____ DEGREE: _____

1.2 CHECK ONLY ONE RELEVANT COLUMN FOR EACH SYMPTOM

(from not at all present to severe):

SYMPTOM	NOT AT ALL	MILD	MODERATE	SEVERE
ANXIETY	_____	_____	_____	_____
TENSION	_____	_____	_____	_____
AGITATION	_____	_____	_____	_____
DEPRESSION	_____	_____	_____	_____
CONFUSION	_____	_____	_____	_____
DISORIENTATION	_____	_____	_____	_____
POOR MEMORY	_____	_____	_____	_____
POOR CONCENTRATION	_____	_____	_____	_____
REDUCED ACTIVITIES	_____	_____	_____	_____
POOR MOTIVATION	_____	_____	_____	_____
FATIGUE	_____	_____	_____	_____
INSOMNIA	_____	_____	_____	_____
DISTURBED SLEEP	_____	_____	_____	_____
POOR APPETITE	_____	_____	_____	_____
SEXUAL PROBLEMS	_____	_____	_____	_____
INCONTINENCE	_____	_____	_____	_____
PANIC REACTIONS	_____	_____	_____	_____
IRRATIONAL THOUGHTS	_____	_____	_____	_____
DELUSIONS	_____	_____	_____	_____
HALLUCINATIONS	_____	_____	_____	_____
OTHER(S):_____	_____	_____	_____	_____

1.3 MEDICAL HISTORY

APPLICANT'S MEDICAL HISTORY – OPERATIONS AND OTHER HOSPITALIZATIONS:

DATE	TYPE OF OPERATION	TREATMENT

PLEASE LIST ALL CURRENT MEDICTIONS WITH THEIR TRADE NAMES:

NAME OF MEDICATION	DOSAGE/FREQUENCY	REASON

MEDICAL PROBLEMS:

_____ HEART ATTACK	_____ SPLEEN DISEASE	_____ ASTHMA
_____ ANGINA	_____ LIVER DISEASE	_____ DIABETES
_____ PACEMAKER	_____ GASTRIC DISEASE	_____ THYROID
_____ ARRYTHMIA	_____ BOWEL DISEASE	_____ BLINDNESS
_____ HIGH BLOOD PRESSURE	_____ LUNG DISEASE	_____ DEAFNESS
_____ LOW BLOOD PRESSURE	_____ BRONCHIAL DISEASE	_____ VENEREAL DISEASE
_____ KIDNEY DISEASE	_____ ALLERGIES	_____ PAGET's DISEASE

OTHER PROBLEMS (PLEASE SPECIFY):

_____

_____

_____

PLEASE GIVE DETAILS OF CHECKED ITEMS:

_____

_____

_____

NEUROLOGICAL PROBLEMS:

_____ HEAD INJURY WITH UNCONSCIOUSNESS	_____ BRAIN SURGERY	_____ STROKE
_____ HEAD INJURY WITHOUT DISORDER UNCONSCIOUSNESS	_____ MIGRAINE	_____ SPEECH
_____ MENINGITIS	_____ DIZZY SPELLS	_____ FLACCID OR SPASTIC
_____ ENCEPHALITIS	_____ EPILEPSY/SEIZURES	_____ APPLICANT WAS AN AMATEUR OR PROFESSIONAL BOXER

_____ POLIOMYELITIS _____ LOU GEHRIG's DISEASE

_____ MULTIPLE SCLEROSIS _____ PARKINSON'S DISEASE

OTHER NEUROLOGICAL PROBLEMS (PLEASE SPECIFY):

_____

_____

_____

PLEASE GIVE DETAILS OF CHECKED ITEMS:

_____

_____

_____

PSYCHIATRIC PROBLEMS:

APPLICANT'S HISTORY OF PSYCHIATRIC PROBLEMS:

_____ PSYCHIATRIC HOSPITALIZATIONS _____ DEPRESSION _____BIPOLAR DISORDER

_____ PSYCIATRIC TREATMENT _____ ALCOHOLISM

_____ SCHIZOPHRENIA _____ DRUG ABUSE

PLEASE SPECIFY:_____

_____

_____

APPLICANT'S ***FAMILY HISTORY*** OF PSYCHIATRIC PROBLEMS:

_____ PSYCHIATRIC HOSPITALIZATIONS _____ DEPRESSION _____ BIPOLAR DISORDER

_____ PSYCHIATRIC TREATMENT _____ ALCOHOLISM

_____ SCHIZOPHRENIA _____ DRUG ABUSE

PLEASE SPECIFY:_____

_____

_____

1.4 SUBSTANCE USE

HAS THERE EVER BEEN A PERIOD IN YOUR LIFE WHEN YOU HAD THREE OR MORE DRINKS PER DAY FOR THREE OR MORE DAYS IN A ROW?

___ YES ___ NO IF YES, HOW LONG AGO?_____ WHEN WAS THE LAST TIME? _____

HAVE YOU USED DRUGS LIKE MARIJUANA, COCAINE or HALLUCINGENS?
TYPE?_____

HOW OFTEN? _____

WHEN DID YOU LAST USE? _____

DO YOU TAKE VALIUM, SLEEPING PILLS, TRANQUILIZERS OR PAIN KILLERS?
TYPE? _____

HOW OFTEN? _____

WHEN DID YOU LAST USE? _____

WHY DID YOU CHOOSE TO PARTICIPATE IN THIS RESEARCH STUDY?_____

_____

_____

_____

_____

_____

_____

NAME: _____ ID#: _____ DATE: ___/___/___ PERIOD: _____

2 MEMORY ASSESSMENT CLINICS QUESTIONNAIRE (MAC-Q)

Memory complaint questionnaire: As compared to when you were in high school, how would you describe your ability to perform the following tasks involving your memory?

	Much Better Now (1)	Somewhat Better Now (2)	About the Same Now (3)	Somewhat Poorer Now (4)	Much Poorer Now (5)
1. Remembering the name of a person just introduced to you.					
2. Recalling telephone numbers or zip codes that you use on a daily or weekly basis.					
3. Recalling where you have put objects (such as keys) in your home or office.					
4. Recognizing people who recognize you.					
5. Remembering the item(s) you intended to buy when you arrive at the grocery store or pharmacy.					
	(2)	(4)	(6)	(8)	(10)
6. In general, how would you describe your memory as compared to when you were in high school?					

TOTAL SCORE:_____

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