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# Episodic memory performance and associated grey matter volume in older adults with type 2 diabetes mellitus

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**EPISODIC MEMORY PERFORMANCE AND ASSOCIATED GREY MATTER  
VOLUME IN OLDER ADULTS WITH TYPE 2 DIABETES MELLITUS**

by

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B.S., University of Maryland - College Park, 2013

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

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## **DEDICATION**

I would like to dedicate this work to my father for inspiring me to pursue life with  
passion.

## **ACKNOWLEDGMENTS**

I would like to acknowledge my thesis mentor and friend, Dr. Peter Fried. I would also like to acknowledge Dr. Aaron Boes for helping me with the MRI analysis, Ann Connor for helping me carry out the study procedures, and Dr. Horton for patient recruitment for the research study. Lastly, I would like to acknowledge the Berenson Allen Center for Noninvasive Brain Stimulation staff, as well as the MSCI program faculty, particularly Stacey Hess-Pino for her guidance and support.

# **EPISODIC MEMORY PERFORMANCE AND ASSOCIATED GREY MATTER**

## **VOLUME IN OLDER ADULTS WITH TYPE 2 DIABETES MELLITUS**

**SADHVI SAXENA**

### **ABSTRACT**

Type 2 Diabetes Mellitus (DM2) results in peripheral and central nervous system complications. Recent studies suggest that DM2 accelerates age-related cognitive decline and is specifically linked to Alzheimer Disease (AD). A commonly reported impairment reported in DM2 is in learning and memory, and macroscopic brain changes that could mediate memory impairments can be detected by quantifying grey matter volume with Magnetic Resonance Imaging (MRI). This thesis project predicts that older adults in DM2 have impaired learning and memory compared with older adults without DM2. Additionally, the DM2 group would have decreases in grey matter volume of the hippocampus and associated brain regions, which would mediate memory function. The study found that the DM2 group performed significantly worse on two validated neuropsychological measures of learning, recall and recognition. The difference was highly significant in the learning and memory of face-name pairs, suggesting that assessing higher-level memory functions could be a sensitive marker for subtle memory impairments. However, the two groups did not differ in grey matter volume of the hippocampus, the medial temporal lobe, or the hippocampal network. Additionally, grey matter volume was not associated with learning and memory measures. The findings suggest that memory changes in DM2 may not be mediated by brain atrophy, rather could

be mediated by microscopic brain changes earlier in AD progression such as beta amyloid accumulation, hyperphosphorylated tau protein, or reduced synaptic plasticity.

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## LIST OF ABBREVIATIONS

AD.....	Alzheimer Disease
ADCS-ADL .....	Alzheimer’s Disease Cooperative Study Activities of Daily Living Scale
aMCI.....	Amnesic Mild Cognitive Impairment
CNS.....	Central Nervous System
DM .....	Diabetes Mellitus
DM2 .....	Type 2 Diabetes Mellitus
DMN .....	Default Mode Network
fMRI.....	Functional Magnetic Resonance Imaging
FNAME .....	Face Name Associative Memory Exam
GDS .....	Geriatric Depression Scale
HbA1c.....	Glycosylated hemoglobin A1c
HCCRC.....	Harvard Catalyst Clinical Research Center
MCI.....	Mild Cognitive Impairment
MMSE.....	Mini Mental Status Examination
MTL .....	Medial Temporal Lobe
MRI.....	Magnetic Resonance Imaging
NACC-UDS .....	National Coordinating Center’s Uniform Data Set
OHC .....	Older Healthy Control
PET .....	Positron Emission Topography
RAVLT .....	Rey’s Auditory Verbal Learning Test
WTAR.....	Weschler Test of Adult Reading

## INTRODUCTION

### **Type II Diabetes Mellitus**

Diabetes Mellitus (DM) affects approximately 29 million people in the United States. 8 million of these cases are undiagnosed and therefore untreated.<sup>1</sup> Type II Diabetes Mellitus (DM2) is referred to as latent-onset diabetes and accounts for 90 to 95% of all diagnosed cases of DM.<sup>2</sup> In cases of DM2, beta cells of the pancreas are unable to secrete enough insulin in response to elevated blood glucose levels. In addition, insulin resistance occurs in DM2.<sup>3</sup> Insulin resistance refers to an inadequate response to insulin by its target in muscle, liver and adipose tissues, and therefore these cells do not respond to the physiological effects of insulin.<sup>4,5</sup> DM2 is diagnosed when there are fasting blood glucose levels higher than 126 mg/dL, glycosylated hemoglobin A1c (HbA1c) levels of 6.5% or higher, or glucose levels higher than 200 mg/dL 2 hours after an oral glucose tolerance test.<sup>3</sup>

DM2 is a major cause of disability and death. It is well known that individuals with DM2 develop neurological complications, such as peripheral neuropathy and resultant sensory loss, weakness, gait disturbances, balance problems and pain in the feet or hands. Alterations in autonomic nervous system activity also occur, including slowed digestion or erectile dysfunction.<sup>2</sup> Such complications of the peripheral and autonomic nervous system can be detected early, characterized, and also followed longitudinally using neurophysiological techniques.<sup>2</sup> Central nervous system (CNS) damage is also common in DM2, but the neurobiological substrates and clinical profile of these changes are less clear.<sup>6</sup>

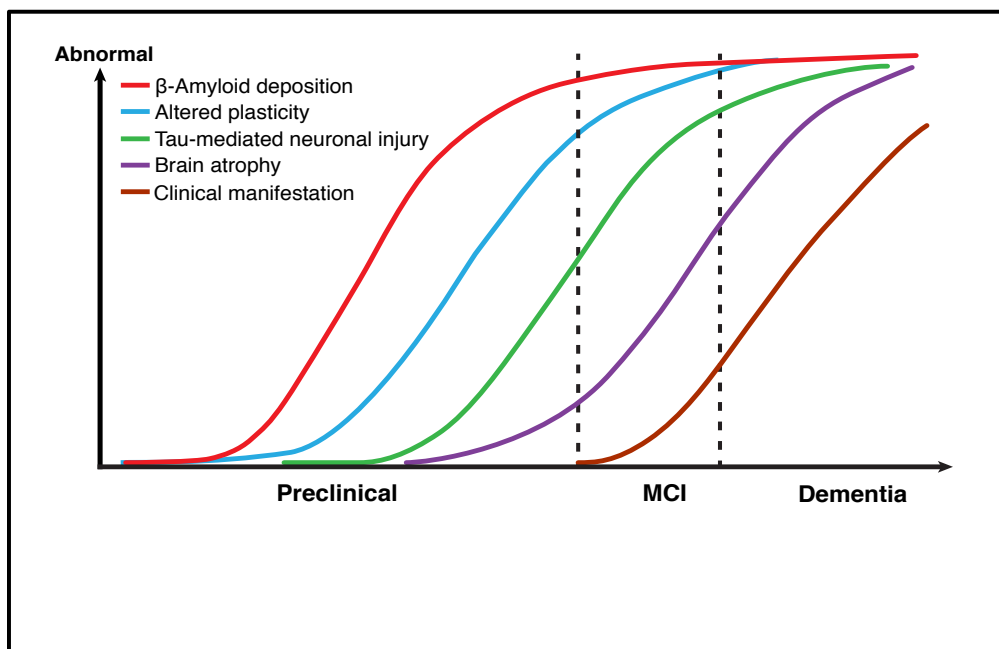
## **Relationship Between DM2 and Alzheimer Disease (AD)**

Several studies have examined the aforementioned CNS changes and found that DM2 accelerates brain aging.<sup>7-12</sup> Likely as a result of DM-related damage to the CNS, older adults with DM2 also show both cognitive and functional impairments compared with controls.<sup>13</sup> DM2-related cognitive impairment, in particular, has been consistently reported in cross-sectional, case-control and longitudinal studies.<sup>6,9,12,14-22</sup> Furthermore, small clinical trials demonstrate that control of DM2 is associated with improvements in cognitive functioning<sup>21,23-25</sup>, and improvements in glycemic control can partially reverse cognitive dysfunction.<sup>26</sup> However, the benefits of glucose management in DM2 remain under debate. Results from a recent, large-scale clinical trial demonstrated that those with DM2 showed no cognitive benefit from glucose management.<sup>27,28</sup> Thus, the causal relationship between DM2 and cognitive functioning remains unresolved.

The most commonly reported changes in DM2 are in learning and memory, mental flexibility and mental speed.<sup>7,8,15</sup> These changes are shown as early as the pre-diabetes phase where individuals have elevated glucose levels below the threshold for DM2 diagnosis.<sup>29</sup> The aforementioned changes in memory have been of great interest in the literature because impairments in memory are a hallmark of Alzheimer Disease (AD). AD is a neurodegenerative disorder that makes up about 50% of all dementia cases.<sup>30</sup> It affects about 5 million Americans,<sup>3</sup> but these numbers are expected to triple by 2050.<sup>31</sup>

AD is a progressive neurodegenerative disease, and its neuropathological changes occur prior to the cognitive changes that are used to guide AD diagnosis.<sup>2</sup> However, by this point, there is irreversible brain damage from beta-amyloid deposition,

hyperphosphorylated tau protein accumulation, reduced synaptic plasticity and resulting atrophy from neuronal loss (Sperling 2011). Mild cognitive impairment (MCI) is the transitional phase before AD where there are neuropsychological changes, but not enough to be diagnosed as dementia.<sup>46,47</sup> Only those with amnesic MCI (aMCI) go on to develop dementia.<sup>33</sup> As a result, much effort has been made to detect risk factors of AD that put individuals in stages of preclinical dementia, prior to the development of cognitive symptoms. These could serve as targets for early intervention and prevention of cognitive decline. The changes that occur and their time course are demonstrated in Figure 1.



**Figure 1: Time course of brain changes in preclinical, MCI, and AD.** AD pathology begins with beta amyloid deposition, which alters synaptic plasticity. There is then neuronal injury and loss from hyperphosphorylation of the tau protein, leading to brain atrophy. These brain changes occur prior to MCI, where only a subset of these individuals proceeds to develop AD. At AD diagnosis, permanent brain damage and cognitive abnormalities have already occurred.

Many studies have found that DM2 is associated with an increased risk of developing AD.<sup>2,34-36</sup> It is also known that patients with AD are at an increased risk of developing reduced insulin resistance,<sup>37</sup> with up to 80% of all cases of AD having a diagnosis of DM2 or elevated fasting glucose levels.<sup>38</sup> The prevalence of both DM2 and AD increase with age, and as their incidence increases rapidly, the complications and underlying mechanisms that link the two diseases must be addressed.

### **Underlying Mechanisms That Link Type 2 Diabetes and Alzheimer Disease**

AD is well characterized by pathological features including beta amyloid deposition, neurofibrillary tangles of hyperphosphorylated tau protein, as well as cerebral inflammation.<sup>39</sup> The mechanisms that underlie brain changes in DM2, on the other hand, are unclear. DM2 is caused by a combination of physiological, genetic and lifestyle factors,<sup>30</sup> making it a complex metabolic disorder with many variations and comorbidities.<sup>40,41</sup> Studies have suggested that increased glucose levels lead to neuronal dysfunction and resulting neuronal loss, which may result in AD-like brain changes. Insulin resistance from DM2 also occurs in the brain, as the insulin receptor signal transduction pathway is perturbed. As a result, there is reduced glucose metabolism in the brain<sup>42-44</sup> and neuronal dysfunction. Rodent models suggest that these insulin receptors are mainly localized in areas of the brain that mediate learning and memory performance, including the hippocampus, prefrontal cortex and the cingulate gyrus.<sup>38,45,46</sup> Therefore, abnormal glucose metabolism as a result of DM2 may have a negative effect on memory performance, increasing the risk of developing AD.

Brain changes as a result of insulin resistance and compensatory hyperinsulinemia

resemble AD pathology. DM2 interferes with amyloid metabolism by facilitating amyloid secretion and interfering with amyloid breakdown,<sup>38,41,47,48</sup> and insulin dysfunction leads to tau hyperphosphorylation.<sup>48,49</sup> Lastly, elevated plasma insulin levels are associated with elevated inflammatory markers such as C-reactive protein and interleukin-6,<sup>50</sup> which would make individuals receiving insulin for DM2 treatment more prone to cerebral inflammation.

Recent interventional studies have shown that diabetes medications, such as insulin, can improve memory performance in patients with AD, which has also been shown in rodent studies as well.<sup>51</sup> A combination of insulin and other diabetes medication has also lowered neuritic plaque density in patients with AD.<sup>52</sup> These studies provide further evidence that brain insulin resistance contributes to cognitive impairment in mild cognitive impairment (MCI), dementia and AD.<sup>53,54</sup>

Nonetheless, many questions need to be answered, as pathological studies have failed to confirm the link between DM2 and AD on a microscopic level.<sup>55,56</sup> While underlying mechanisms are unresolved, there is a critical need for the parallel development of surrogate markers for macroscopic CNS changes that can be localized and prevented, or at least treated early in the disease progression.

### **Shared Memory Impairments DM2 and AD**

Given that DM2 is associated with an increased risk of cognitive decline and AD, and that insulin receptors in the brain are localized in the hippocampus, much focus has been on examining memory changes in individuals with DM2. Episodic memory, the ability to remember concrete and personal experiences,<sup>57</sup> is a common cognitive

impairment in amnesic MCI and AD.<sup>57</sup> It is also shown in cases of DM2.<sup>2</sup> Verbal memory is a type of explicit declarative memory, and neuropsychological measures of verbal learning tests reflect the ability to retain new information.<sup>57</sup> Verbal memory assessments are commonly used tools of memory impairments in dementia and preclinical AD.<sup>10,12,15,20,58,59</sup>

Learning and memory impairments are a signature of early AD. Given that the clinical symptoms of these changes only occur after pathological changes in AD have progressed, they may not be sufficient to detect mild changes in cognition that occur before the onset of irreversible changes. More recently, researchers have been focusing on asymptomatic individuals who are at preclinical AD phases, so that the risk for developing symptoms of AD can be detected and treated prior to disease onset. One such cognitive process is the daily task of learning the name of someone that one meets, and remembering this face-name association. This daily task is especially challenging because the association is inherently unrelated.<sup>60</sup> Additionally, it becomes even more challenging as one's age progresses.<sup>61</sup> Recent Positron Emission Topography (PET) and postmortem pathological studies have shown that "asymptomatic" individuals, free of cognitive complaints but nonetheless perform worse at paired-associate memory tasks, have higher levels of beta amyloid in the brain.<sup>61</sup> Researchers then developed neuropsychological measures that are sensitive to these subtle memory impairments of what investigators have called preclinical AD,<sup>61,62</sup> and that lead to the buildup of beta-amyloid. If these paired-associative memory changes are detected in DM2, then

interventions could be targeted before irreversible brain damage from substantial neuronal loss and neuropathological changes occur that lead to symptoms of AD.

### **Using Magnetic Resonance Imaging to Explore Macroscopic Changes in Type 2 Diabetes Mellitus**

Since several studies have shown that DM2 is associated with impairments in memory that resemble preclinical AD, aMCI or AD, it is important to detect the neural correlates of these changes. One way to localize the macroscopic correlates of these changes is through Magnetic Resonance Imaging (MRI). MRI is a sensitive tool that is used to detect biomarkers of neurological diseases. In particular, structural MRIs can be used to quantify grey matter volume differences<sup>63</sup> and thus identify regions that may be shrinking due to neuronal loss.

Structural MRI studies have shown that AD progression starts in the limbic regions of the brain, and spread through the temporal, parietal and frontal lobes as the disease progresses.<sup>64</sup> Specifically, early stages of AD demonstrate atrophy in the hippocampus and entorhinal cortex in the medial temporal lobe (MTL).<sup>65</sup> aMCI pathology also starts in the entorhinal cortex and hippocampus<sup>66</sup> and can be detected as early sites of abnormalities prior to the development of AD.

Similarly, there is reduced hippocampal volume in DM2,<sup>20,58,67-72</sup> as well as lower volumes of other MTL structures such as the entorhinal cortex.<sup>68,73</sup> This association has also been reported in adolescents with DM2<sup>44</sup> and elderly people with prediabetes.<sup>74</sup> The similarities in MTL structural changes are supported by evidence that DM2 is associated with an accelerated progression from MCI to dementia,<sup>75</sup> and that individuals with DM2

and MCI have greater reduction in hippocampal volume than individuals with only MCI.<sup>33</sup>

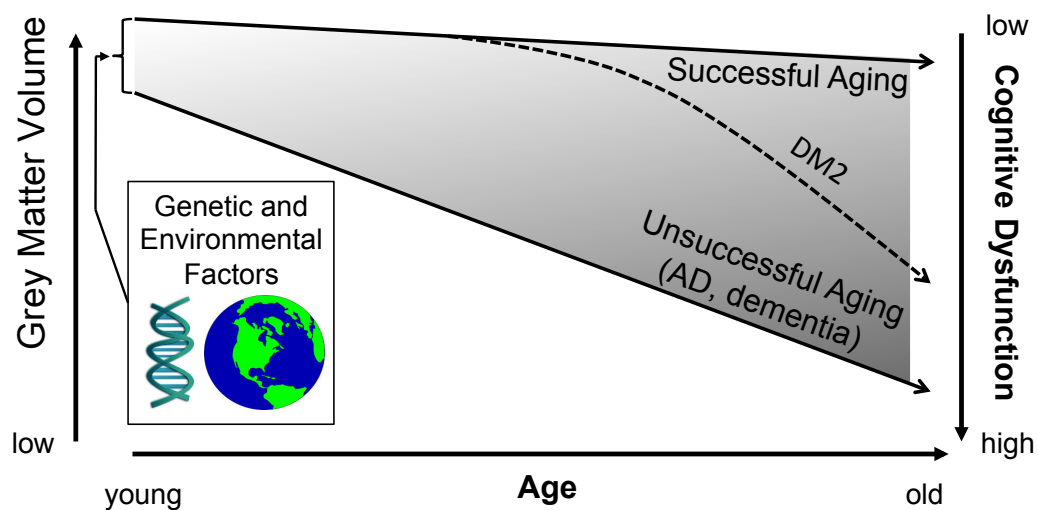
The process of memory formation and retrieval, however, is not limited to the hippocampus and entorhinal cortex. Recently, it has been shown that many cognitive processes are driven by the dynamic communication between large-scale neural networks.<sup>76</sup> Thus, memory depends on more than just the hippocampus and the medial temporal lobe.<sup>77</sup> This network has been identified by functional MRI (fMRI) and PET studies and is known as the default mode network (DMN).<sup>78</sup> The regions that make up the DMN are functionally connected with the hippocampus and are consistently reported in episodic memory and paired-associative memory formation. Disruptions of the DMN and resultant disconnectivity are being explored as neural substrates for cognitive disorders, as they deactivated during cognitive tasks such as hippocampal-dependent memory tasks. The episodic memory network that is impaired in AD includes the medial temporal lobe structures (hippocampus, entorhinal cortex, parahippocampus, amygdala), as well as medial and lateral parietal areas of the default mode network (precuneus/posterior cingulate, angular gyrus)<sup>77,78,79</sup> Atrophy in this network of regions can be detected through structural imaging in very mild stages of AD or even preclinical AD.<sup>77,80,81</sup> Furthermore, DMN alterations also overlap with amyloid deposition in AD.<sup>82,83</sup>

Although there is no concrete evidence that amyloid plaques are accumulated in DMN regions in cases of DM2, there is evidence that poor glycemic control and high insulin resistance are also associated with reduced gray matter volume in the episodic memory network regions including the amygdala, prefrontal cortex and precuneus.<sup>2,34-36</sup>

Studies have also found gray matter atrophy in bilateral hippocampal regions and also temporal, frontal and cingulate cortices.<sup>84</sup> However, there are still substantial gaps to fill as to whether hippocampal and memory changes in DM2 are even related, as several studies have failed to show this association.<sup>20,72</sup>

### Specific Aims and Objectives

Given the limited evidence linking structural brain changes to cognitive performance, there is a need for additional studies that relate grey matter volume reductions to cognitive dysfunction. More studies are needed to assess if structural brain changes play a mediating role in the association between DM2 and memory performance. The hypothesized mediating effect is shown in Figure 2.



**Figure 2: Accelerated brain aging due to DM2.** As age increases, grey matter volume decreases and cognitive dysfunction increases. These changes occur due to normal aging, but DM2 may lead to accelerated brain aging, caused by gray matter volume reductions and resulting cognitive impairments.

The presented analysis focuses on two types of memory with well-known involvement in early or preclinical stages of AD: verbal learning and memory and paired-associative memory. These two measures will be used to investigate the etiology of brain damage in DM2. This analysis aimed to localize differences in cortical volume in regions of the DMN associated with episodic and paired-associative memory in individuals with DM2 compared with healthy controls.

The study investigates whether there are changes in episodic memory performance in older adults with DM2, and if these changes are mediated by reductions in grey matter volume of the hippocampus and associated brain regions. The specific aims of the study include the following:

1. To determine if DM2 is a predictor of impairments in learning and memory compared with controls.
2. To determine if DM2 is a predictor of reductions in grey matter volume in the hippocampus and associated regions.
3. To determine if memory performance is associated with grey matter volume in brain regions associated functionally with learning and memory.

### **Study Rationale**

Ultimately, this analysis aims to contribute to the idea that individuals with DM2 are at high risk of cognitive decline and AD-like pathologies. Additionally, specific structural changes can be targets for early detection and prevention of cognitive decline that results from DM2. Currently there are no ways of reversing cognitive decline. If DM2 is a risk factor for AD, and if the cognitive and neural substrates are well

characterized, then these can be targets of early prevention and intervention to ultimately decrease the public health burden of these diseases on our aging population.

## METHODS

### *Subject Population*

This secondary analysis of a cross-sectional study included individuals DM2 between the ages of 50 and 80 years old, and healthy controls between 50 and 80 years old. DM2 subjects were recruited in part through physician-approved letters sent to patients of the Joslin Diabetes Center. In addition, DM2 and control subjects were recruited through flyers posted around the Beth Israel Deaconess Medical Center. All potential participants were prescreened on the phone for eligibility, and all study sessions took place at the Beth Israel Deaconess Medical Center. The study protocol was approved by the Institutional Review Board at the Beth Israel Deaconess Medical Center (BIDMC).

Inclusion for DM2 subjects included a confirmed diagnosis of DM2, treatment of DM2 through diet and/or medication, and HbA1c levels of 10% or less, indicating diabetes that is well controlled. For the control subjects, inclusion criteria included normal fasting glucose (< 100 mg/dl) and HbA1c levels (< 6.4%), no evidence of dementia, and a minimum completion of 8th grade education. Subjects in both groups were excluded if they had any unstable health condition, as well as standard exclusion criteria for MRI (e.g., implanted devices or ferrous metal in the body). Specific exclusion criteria included: any significant macrovascular disease including cardiovascular disease, peripheral vascular disease or stroke; significant microvascular disease including vision impairing retinopathy, severe neuropathy or renal insufficiency; history of serious neurological disorders affecting cognitive function; past or current history of major

depression or any other major psychiatric condition; history of neurodevelopmental disorders or intellectual disability; any metal in the brain, skull, or elsewhere in the body that are not MRI compatible; or a BMI > 40 due to MRI scanner weight and width limits.

### ***Screening Procedures, Baseline Measurements, and Neuropsychological Assessment***

All participants provided written and verbal consent at the initial screening visit. After obtaining informed consent, a Research Nurse at the Harvard Catalyst Clinical Research Center (HCCRC) performed a venous blood draw for baseline measurements of fasting blood glucose levels, creatinine levels and HbA1c levels.

Medical history was then collected in a structured clinical interview including a review of current medications, history of diabetes, history of high cholesterol, history of hypertension, family history of dementia, and history of other medical conditions that may exclude subjects from the study. All participants underwent a brief neurological examination by a study physician to confirm that the subject was eligible to continue with protocol procedures.

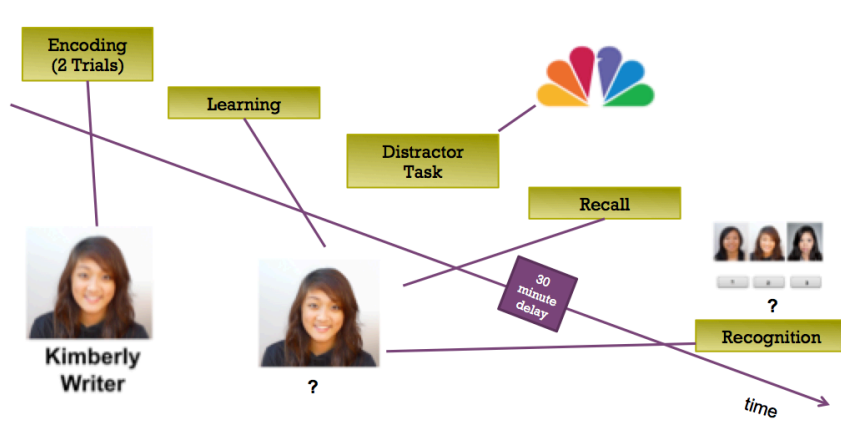
The screening visit also included a standard neuropsychological battery. Tests and inventories for baseline characteristics were drawn from the National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS) and consisted of the Geriatric Depression Scale (GDS), Mini Mental Status Examination (MMSE). In addition, the Alzheimer's Disease Cooperative Study Activities of Daily Living scale (ADCS-ADL) and Weschler Test of Adult Reading (WTAR) were administered to assess functional independence, cognitive impairment and general intelligence, respectively.

### ***Verbal Learning and Paired-Associative Learning Assessment***

To further probe learning and memory function, two tests were administered. Verbal memory performance was measured by a 10-item version of the Rey Auditory Verbal Learning Test (RAVLT). The RAVLT reflects the process of encoding, learning, recalling and recognizing verbally presented words<sup>57</sup> and is a validated predictor of conversion to AD.<sup>85,86</sup> Each participant was read a list of 10 semantically unrelated words in 5 consecutive trials. After each list is given, the participant was asked to immediately recall as many words as possible. After 20 minutes, participants were asked to recall as many words as possible from the same list. There are three outcomes from the RAVLT task. Immediate recall, indicative of verbal learning, was scored as the average words correctly recalled over the 5 trials. Delayed recall was calculated as the proportion of correct words recalled after a 20-minute delay. Delayed recognition was calculated as the proportion of correct words recalled after a verbal cue.

Paired-associative memory was assessed by the Face Name Associative Memory Exam (FNAME). The FNAME is a validated tool to assess paired-associative memory performance of names and occupations at initial retrieval, cued retrieval, and delayed retrieval after a 30 minute delay. The FNAME task is a tool for dissociating memory impairments related to preclinical AD from those associated with normal aging.<sup>87</sup> Participants were presented 12 faces with names and occupations written below, and were given 8 seconds to read aloud and remember the name and occupation that went with each face. At the end of the first trial, the participants were shown each face in the same order, and they were asked to recall the name and occupation. Each participant was given a second learning trial with the faces presented in a different order. The composite

score of the two learning trials was calculated as the participant's learning ability. During short delay of 4 to 5 minutes, participants were administered a distractor task. In this task, they were shown a company logo and were then asked to name the company to which each logo corresponds, as well as the product of the company. After this distractor task, participants were again shown the 12 faces and asked to recall the name and occupation with each face. The total names and occupations correctly recalled after the distractor task comprised of recall scores. After a 30-minute delay, participants were shown 3 faces, and had to determine which face they recognized from before. Then, participants were asked to recall the name and occupation of the face. The total number of names and occupations recalled comprised the recognition score. The composite of each subscale will be used as the primary outcome for paired-associative memory. Face-name retrieval (combined learning, recall, and recognition) and face-occupation retrieval (combined learning, recall, and recognition) scores will be assessed. Lastly, individual subscale scores will be assessed to isolate the memory functions assessed in this task: learning, recall and recognition. A diagram outlining the administration of the FNAME task is presented in Figure 3.



**Figure 3: Face Name Associative Memory Exam (FNAME) Administration Sequence.** The task begins with learning trials of reading and remembering the name and occupation that goes with each face. Then, learning of the names and occupation that belong to each face is assessed. After a distractor task, the participants are shown the faces again and asked to recall the name and occupation, assessing recall. After a 30-minute break, participants were shown the faces again and asked to recall the names and faces to assess recognition, as they were cued by recognizing the face.

### ***ROI selection***

Regions of interest (ROIs) for MRI analysis were selected *a priori* from a review of literature related to imaging memory networks in dementia. ROIs included the hippocampus and surrounding entorhinal cortex, parahippocampus, amygdala, posterior cingulate/precuneus, and angular gyrus. These regions have been associated with learning and memory performance and collectively comprise the default mode network, a resting-state network that is disrupted in early stages of dementia.

### ***MRI acquisition and Image Processing***

Each participant's MRI was performed on BIDMC's 3T Signa MR scanner (LX 15, GE Healthcare, Illinois) using an 8-channel proton head coil. A 3D spoiled gradient echo sequence was used with the following parameters: 162 axial-oriented slices for whole-brain coverage; 240 mm isotropic field-of-view; 0.937 mm x 0.937 mm x 1 mm native resolution; flip angle = 15°; TE/TR ≥ 2.9/6.9 ms; duration ≥ 432 s. Individual images were processed using the recon-all pipeline in Freesurfer (Martinos Center for Biomedical Imaging, Boston, MA, USA).

### ***Statistical Analysis***

Descriptive statistics were used to compare all variables between groups, including education level, MMSE score, GDS score, ADL score, HbA1c levels, fasting

glucose levels, creatinine levels, and BMI. A Student's t-test for independent means was used to assess differences for continuous variables, and a Chi-squared test was used for categorical variables.

### ***Episodic Memory Performance Analysis***

Between-group differences in verbal learning, delayed recall, and delayed recognition on the RAVLT were compared using a Student's t-tests for independent means. Between-group differences in initial name recall, cued name recall, and 30-minuted delayed cued recall were compared using a Student's t-test for independent means.

### ***Grey Matter Volume Analysis***

MRI analysis was performed using FreeSurfer Image Analysis Suite, Version 4.0.2, which was downloaded online at <http://surfer.nmr.mgh.harvard.edu>.<sup>88</sup> FreeSurfer processes and normalizes each individual MRI, and performs grey matter volume calculations after automatic parcellation and segmentation of the regions of interest based on a predefined atlas.<sup>89</sup> For each individual, grey matter volume for each ROI was calculated as the proportion of gray matter volume (cm<sup>3</sup>) in the selected ROI over the estimated Total Intracranial Volume (eTIV). This method has been suggested to account for individual differences in head size that may account for differences in structural volume. Differences in proportions of grey matter volume between the groups were analyzed using a Student's t-test for independent means.

Differences between DM2 and controls in total gray matter volume were also analyzed to assess global hippocampal and associated regional atrophy.

### **DM2 and Potential Confounders as Predictors of Outcome Measures**

A linear regression model was performed on the outcomes that were significantly different between the DM2 and control groups at a  $p < 0.05$  levels. To assess if DM2 was a still significant predictor after adjusting for potential confounds, a linear model was created to control for BMI, MMSE, GDS age, sex, and education. Since HbA1c levels are reliable predictors of DM2-related brain changes,<sup>73</sup> a separate linear regression model looked at the association between HbA1c levels and outcome measures when controlling for the same variables as the previous model. The analysis did not create a separate model for fasting glucose measures, as HbA1c more sufficiently captures the dose-response relationship.

### **Association Between Memory Performance and Structural Brain Changes**

A linear regression analysis was performed to determine if there is an association between cognitive measures and grey matter volume in the targeted areas. An analysis was performed within the two groups. In the DM2 group, this association could be due to age rather than diabetes status. Associations between HbA1c levels and age in the two groups were also assessed as potential predictors that may confound the relationship. For significant associations found, a separate model was created to adjust for HbA1c and/or age.

## RESULTS

### **Baseline Characteristics**

Table 1 highlights demographic and baseline information collected from the 27 individuals with DM2 and 26 controls that were administered the RAVLT. Of these 53 subjects, 15 individuals with DM2 and 15 controls were administered the FNAME task.

On the Geriatric Depression Scale (GDS), the DM2 group reported significantly higher levels of depression than the control group ( $p=0.023$ ). On average, the DM2 group was older, had fewer years of education, had lower global cognitive status (MMSE), reported a lower level of functional independence in daily activities (ADL), and scored lower on the measure of general intelligence (W-TAR). These differences were not significant, suggesting that the two groups were balanced in overall cognitive, functional and independence status. However, it is important to note that true differences may not be detected due to the small sample size. As expected, the DM2 group had significantly elevated HbA1c and fasting glucose levels ( $p<0.001$  for both measures), consistent with diabetes status.

**Table 1: Demographic and Baseline Characteristics.**

	<b>OHC</b>	<b>DM2</b>
<b>Baseline and Demographic Characteristics</b>		
<b>N<sup>^</sup></b>	26	27
<b>Sex (% Male)</b>	53.8%	55.6%
<b>Age (years)</b>		
Mean (S.D.)	61.7 (9.1)	66.0 (7.7)
Range	50-77	60-80
<b>Education (years)</b>		
Mean (S.D.)	15.8 (2.4)	15.6 (2.6)
Range	12-21	10-20
<b>BMI (kg/m<sup>2</sup>)*</b>		
Mean (S.D.)	26.4 (4.8)	29.9 (3.8)
Range	19.4-39.2	22.6-35.9
<b>Neurocognitive Status</b>		
<b>MMSE</b>		
Mean (S.D.)	29.5 (0.8)	29.0 (0.96)
Range	27-30	27-30
<b>ADL</b>		
Mean (S.D.)	76.2 (2.6)	76.0 (2.6)
Range	67-78	69-78
<b>GDS*</b>		
Mean (S.D.)	0.4 (0.8)	1.3 (1.8)
Range	0-3	0-7
<b>W-TAR</b>		
Mean (S.D.)	113.7 (10.1)	112.3 (10.1)
Range	92-126	78-126
<b>Diabetes-status</b>		
<b>Fasting glucose (mg/dL)**</b>		
Mean (S.D.)	89.1 (7.5)	138.4 (37.0)
Range	76-102	90-220
<b>Creatinine</b>		
Mean (S.D.)	0.84 (0.2)	0.89 (0.2)
Range	0.58-1.3	0.62-1.6
<b>HbA1c (%)**</b>		
Mean (S.D.)	5.6 (0.3)	7.3 (1.1)
Range	5.1-6.2	5.9-9.7

\*p<0.05

\*\*p<0.001

<sup>^</sup> = FNAME administered to 15 OHC and 15 DM2 subjects.

- HbA1c collected on 16 HC and 19 DM2, creatinine on 18 HC and 15 DM2, fasting glucose on 17 HC and 18 DM2.
- BMI collected on 21 DM2 and 21 OHC.
- GDS reported on a scale from 1-13, with higher score indicating greater severity of self-reported depressive symptoms.
- ADL has a maximum score of 78, with higher score demonstrating greater functional independence in activities of daily living.

### **Verbal Learning and Memory Results**

The DM2 group scored lower on the RAVLT learning, recall and recognition subsections compared with controls (Table 2). However, the decrease in performance was only significant for immediate recall and delayed recognition measures. Therefore, the DM2 group showed impairments in episodic memory retrieval. It is important to note that the RAVLT consists of 10 words, and the proportion of correctly recalled words DM2 group on average was one word less than the control group.

**Table 2: Performance on RAVLT measures (verbal learning, recall and recognition) in DM2 group compared with control group.**

RAVLT Scores (proportion correct)	OHC (n=26)	DM2 (n=27)	p-value
<b>Learning</b>	0.81 (0.1)	0.70 (0.1)	0.0015*
<b>Recall</b>	0.8 (0.2)	0.7 (0.3)	0.1066
<b>Recognition</b>	0.98 (0.04)	0.89 (0.11)	0.0015*

### **Paired-Associative Memory Results**

The DM2 group had significantly lower scores on the face-name measures of the FNAME task, but only certain face-occupation measures (Table 3). The DM2 group performed significantly worse than the control group on measures of learning face-name and face-occupation pairs ( $p < 0.001$  and  $p = 0.019$ , respectively) after the learning trial.

Composite FNAME scores, but not FOCC scores, were significantly reduced in the DM2 group. These results suggest that the mechanism for remembering face-name and face-occupation associations are different, and that face-name associations are more difficult to learn and retrieve in in cases of DM2 compared with controls.

**Table 3: Performance on FNAME measures in DM2 group compared with control group.**

FNAME Scores (number correct)	OHC (n=15)	DM2 (n=15)	p-value
<b>Face-name learning</b>	14.6 (3.8)	8.9 (4.2)	0.0005*
<b>Face-occupation learning</b>	20.7 (3.0)	17.0 (4.9)	0.019*
<b>Face-name recall</b>	9.7 (1.9)	5.8 (2.98)	0.0002*
<b>Face-occupation recall</b>	11.0 (2.0)	9.4 (2.6)	0.0707
<b>Face-name recognition</b>	9.2 (2.7)	4.8 (2.9)	0.0002*
<b>Face-occupation recognition</b>	11.0 (1.6)	9.5 (2.1)	0.0300*
<b>FNAME Task Total Score</b>	76.3 (13.8)	51.7 (17.1)	0.0002*
<b>Face-name cumulative (learning, recall and recognition)</b>	33.5 (7.9)	19.9 (10.7)	0.0005*
<b>Face-occupation cumulative (learning, recall and recognition)</b>	42.7 (6.6)	38.1 (13.3)	0.24

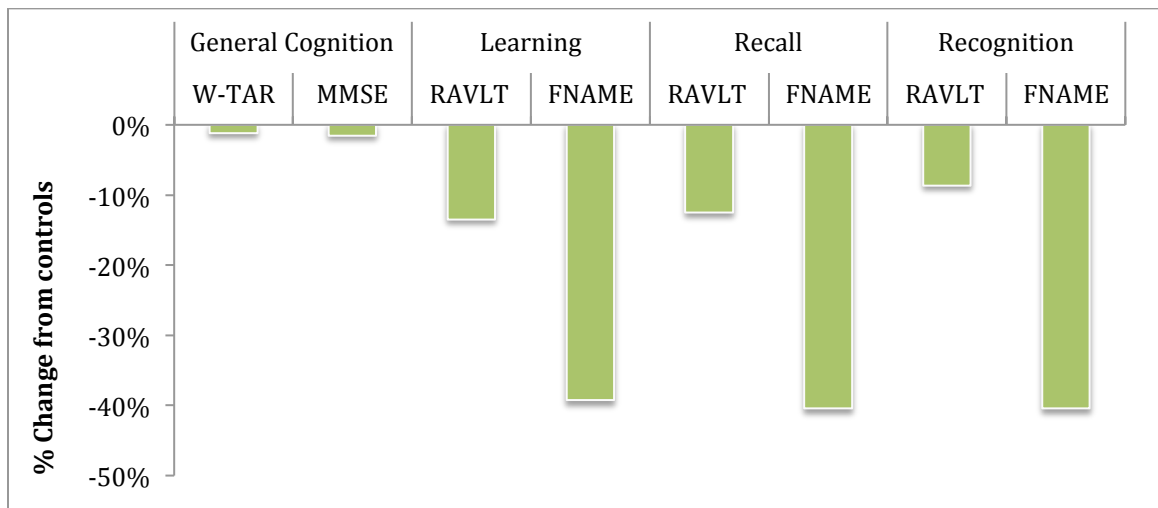
Represented by mean (SD)

\*=p<0.05

### **FNAME and RAVLT Comparison Results**

Figure 4 outlines that the two groups were similar in general cognition and general intelligence, but compared to the control group, performed significantly worse on

both measures of learning and memory. Particularly, the DM2 group was significantly impaired in the learning, recall, and recognition of face-name pairs compared with controls. These results that the FNAME task is a reliable marker of the memory impairments that are shown in older adults with DM2.



**Figure 4: DM2 performance on RAVLT and face-name measures compared to controls.** Despite similarities in general cognition and intelligence, the DM2 group performed significantly worse on the FNAME (face-name pairs only) measures compared to controls. They performed worse on RAVLT measures, but the differences were of higher significant in forming and remembering face-name pairs. These results suggest that the FNAME task is a more sensitive measure of memory impairments as a result of DM2.

### Region-of-Interest Gray Matter Volume Results

Table 4 shows that the DM2 group showed reductions in the left and right posterior cingulate cortex, left and right precuneus, left and right angular gyrus, right and left angular cingulate cortex, left parahippocampus and right entorhinal cortex. None of these reductions were significant, though there was a statistical trend for reductions in right posterior cingulate cortex volume ( $t=1.99$ ,  $p=0.053$ ). Interestingly, the DM2 group

had a higher proportion of grey matter volume in the left entorhinal cortex, but this difference was not significant.

**Table 4: ROI gray matter volume differences (average proportion of gray matter volume of ROI/total gray matter volume of each individual) in DM2 group compared with control group.**

	<b>OHC (n=26)</b>	<b>DM2 (n=27)</b>	<b>p-value</b>
<b>Medial Temporal Lobe</b>			
<b>L-hippocampus</b>	0.0026 (0.0003)	0.0026 (0.0004)	0.98
<b>R-hippocampus</b>	0.0026 (0.0003)	0.0026 (0.0004)	0.55
<b>L-amygdala</b>	0.0011 (0.0001)	0.0011 (0.0002)	0.68
<b>R-amygdala</b>	0.0012 (0.0001)	0.0012 (0.0002)	0.63
<b>L-parahippocampus</b>	0.0013 (0.0003)	0.0012(0.0003)	0.69
<b>R-parahippocampus</b>	0.0011 (0.0002)	0.0011(0.0003)	0.52
<b>L-entorhinal</b>	0.00080 (0.0002)	0.00085 (0.0002)	0.35
<b>R-entorhinal</b>	0.00078 (0.0002)	0.00073 (0.0002)	0.40
<b>Default Mode Network</b>			
<b>L-posterior cingulate cortex</b>	0.0018 (0.0017)	0.0017 (0.0003)	0.14
<b>R-posterior cingulate cortex</b>	0.0019 (0.0002)	0.0017 (0.0003)	0.05
<b>L-precuneus</b>	0.0055 (0.0006)	0.0054 (0.0006)	0.46
<b>R-precuneus</b>	0.0057 (0.0005)	0.0055 (0.0006)	0.31
<b>L-angular gyrus</b>	0.0034 (0.0006)	0.0032 (0.0005)	0.22
<b>R-angular gyrus</b>	0.0040 (0.0007)	0.0037(0.0007)	0.15

\*All values are reported in cm<sup>3</sup>

\*All GMV calculates are the measure of the ROI as a proportion of estimated Total Intracranial Volume to account for differences in head size.

### **Global Gray Matter Volume Results**

Table 5 represents the proportion of total gray matter volume, including both cortical and subcortical structures. The total gray matter volume was compared between the two groups. Overall, the DM2 group had reduced grey matter volume compared to the control group, but this reduction was marginally significant ( $t=2.04$ ,  $p=0.05$ ). This association was no longer marginally significant after adjusting for age, suggesting that it

could be the higher average age of the DM2 group that contributed to the reduction in total gray matter volume.

**Table 5: Global gray matter volume differences (average proportion of gray matter volume of cortical and subcortical regions/total gray matter volume of each individual) in DM2 group compared with controls.**

	<b>OHC</b>	<b>DM2</b>	<b>t-value</b>	<b>p-value</b>
<b>Total gray matter volume</b>	.40 (0.02)	0.38 (0.05)	2.03	0.05

\*All values are reported in cm<sup>3</sup>

\*GMV measured as total cortical and subcortical grey matter volume as a proportion of estimated Total Intracranial Volume to account for differences in head size.

### Association between DM2 Diagnosis and Outcome Measures

After controlling for the effects of BMI, MMSE, GDS, age, sex, and education, DM2 was still associated with lower RAVLT learning (p=0.0115) and recognition scores (p=0.0422). For the FNAME measures, DM2 remained a significant predictor of total FNAME task performance (p=0.0006), face-name learning, recall and recognition (p=0.0015), face-name learning (p=0.0005), face-occupation recall (p=0.0124), face-name recall (p=0.0010), face-name recognition (p=0.0023), and face-occupation recognition (p=0.0345) after the adjustment.

Interestingly, when adjusting for the same variables, higher HbA1c levels were significantly associated with most but not all tasks. Higher HbA1c was still associated with lower recognition (p=0.0086), FNAME task performance (p=0.0177), face-name learning, recall and recognition (p=0.0089), face-name learning (p=0.0080), face-name recall (p=0.0080), and face-name recognition (p=0.0261). No associations were found

between HbA1c levels and RAVLT learning ( $p=0.1402$ ) and face-occupation recognition ( $p=0.1691$ ).

### **Association between episodic memory performance and brain structure**

In the DM2 group, there were no associations found between immediate recall and grey matter volume in the regions-of-interest. However, there were significant associations between higher scores on long-term memory retrieval measures (delayed recall and delayed recognition) and lower right amygdala volume (RAVLT delayed recall:  $p=0.044$ ; RAVLT delayed recognition:  $p=0.044$ ). Therefore, in individuals with DM2, memory consolidation and retrieval may be mediated by the amygdala. Lower right amygdala was also associated with lower face-occupation recognition in the DM2 group ( $p=0.0452$ ). This suggests that face-occupation associative memory may be similarly mediated by the amygdala. In the DM2 group, lower left angular gyrus was also significantly associated with higher total FNAME scores ( $p=0.0131$ ), face-name recall ( $p=0.008$ ), and face-name recognition ( $p=0.0310$ ) scores. Thus, this higher-level episodic memory process of forming and retrieving face-name associations may require input from the DMN network specifically the angular gyrus.

In the control group, lower RAVLT recall scores was associated with lower right posterior cingulate volume ( $p=0.0428$ ), which is another area of the DMN network that plays a role in episodic memory retrieval. Lower RAVLT recognition scores were associated with lower left entorhinal volume ( $p=0.0349$ ). These findings suggest that impairments verbal memory consolidation and retrieval may be due to affected regions other than the hippocampus. Interestingly, only in the control group were there

associations with the cognitive measures and hippocampal volume. Specifically, decreasing face-occupation associative memory scores were associated with decreases in hippocampal volume. This includes total face-occupation scores ( $p=0.0481$ ), face-occupation recall ( $p=0.0411$ ) and face-occupation recognition ( $p=0.0456$ ).

When looking at the association between age and structural measures, increasing age was significantly associated with reductions in left hippocampal volume in the OHC group ( $p=0.004$ ). However, even after controlling for age, decreased face-occupation associative memory performance was still significantly associated with reductions in left hippocampal volume.

## DISCUSSION

Most DM2 research has focused on DM2-related complications of the peripheral and autonomic nervous system. However, DM2 can also affect the CNS by inducing lasting cognitive abnormalities and accelerating the rate of cognitive aging. The impact of DM2 nonetheless contributes to cognitive deficits and exacerbates functional impairments and depressive symptoms that are commonly reported in DM2. CNS-related complications that are undetected and untreated may result in show poor adherence to treatment and poor diabetes control.<sup>90</sup> Moreover, the increasing prevalence and financial burden of DM2 and dementia management will pose a challenge to our already strained health care system. It is therefore critical to identify if DM2 is in fact a risk factor for AD. If so, then the specific brain changes and cognitive profile must be well understood so that these cases can be detected and treated prior to permanent brain damage and irreversible cognitive dysfunction.

The findings of this analysis contribute to the knowledge of episodic memory performance in DM2 and structural changes in associated brain regions. Two types of episodic memory were chosen, one in verbal learning and memory, and the other in paired-associative memory. Impairments in paired-associative memory were more significantly impaired in the DM2 group than impairments in verbal learning and memory. This could be because semantically unrelated words, such as the list of 10 words of the RAVLT, allows one to categorize each word and more easily retrieve them from his or her memory. Thus, the RAVLT captures a more simple episodic memory process. Additionally, similar previous studies, this analysis failed to report significant

reductions in the formation and retrieval face-occupation pairs,<sup>61</sup> aside from a delayed recall after 30 minutes. Perhaps DM2 did not predict impairments retrieving face-occupation associations because one can rely on previously stored semantic knowledge. Yet, long-term retrieval of face-occupation associations is impaired in DM2, which is a more complicated process of long-term memory. Ultimately, the formation of face-name associations is a more complex process compared with the RAVLT measures and the face-occupation measures because there are no contextual properties with which to form the associative link.<sup>61</sup> Therefore, forming this association requires a higher level of cognitive demand. The results suggest that the FNAME task is a sensitive marker for the earliest stages of cognitive decline or accelerated brain aging. Moreover, DM2 may predict accelerating aging and a decline in forming face-name associations. Thus, the FNAME may be a reliable tool for the early detection and prevention of these changes.

The impairments in episodic memory remained significant even after controlling for age, BMI, global cognitive status (MMSE), depression, sex and education. An identical model was created using HbA1c levels, rather than diagnosis, as a predictor of episodic memory performance. This model supports that HbA1c is also a reliable predictor of episodic memory performance. Therefore, the results from the cognitive measures suggest that both DM2 and HbA1c are predictors of episodic memory impairments and are independent of potential confounders.

For the structural outcome measures, the results of this analysis are inconsistent with reports of medial temporal lobe grey matter volume reductions in individuals with DM2 compared with controls. This analysis did not find significant decreases, or

reductions at all, in commonly reported areas of the episodic memory network: the amygdala, the precuneus, the anterior cingulate cortex, and the angular gyrus. A marginally significant reduction was found in the right posterior cingulate cortex. This analysis found reductions in global gray matter volume, including both cortical and subcortical structures. The results were only significant on a  $p < 0.10$  level. However, it is important to note that the lack of statistical significance could be due to the small sample size rather than clinical importance. The results of the structural analysis are consistent with previous studies that did not find hippocampal reductions in DM2, but had instead found global reductions in grey matter volume. However, after controlling for age, the association of this analysis was not as strong. Therefore, the reduction could have been due to the fact that the DM2 group was older on average, rather than the effect of diabetes.

In looking at within-group associations between cognitive and structural measures, interesting associations were found and discussed in the results. However, these differences are not of clinical significance since the DM2 group did not show any reductions in grey matter volume alone in the regions of interest. Additionally, not all regions of the medial temporal lobe and default mode network were associated with episodic memory performance, suggesting that the entire network and its dynamic communication may not be affected in DM2 as it is in preclinical AD or AD.

Several limitations of this analysis must be discussed. First, the small sample size may hinder the power of the analysis. The sample size was enough to detect significant differences in memory, particularly in paired-associative memory, even after controlling

for variables that may confound the relationship, such as BMI, self-reported depression, and age. However, the small sample size may have hidden true difference between the DM2 and control group in structural volume as well as in baseline characteristics such as general cognition or intelligence. Additionally, recent literature reports that effect sizes to detect impairments in cognitive functioning range from 0.4 to 1.0.<sup>91</sup> If a sample size calculation were to be performed before the study, to find a moderate effect size of 0.4 with a standard deviation of 0.5, 80% power, and an alpha of 0.05, each study arm would need to have 26 subjects. Therefore the results found only on the FNAME task would be underpowered. Another statistical limitation is that although significant differences were found, this analysis did not correct for multiple comparisons, which also increases the likelihood of a type I error.

Another limitation is the generalizability of the analysis, as patients from the Joslin Diabetes Center are those who are receiving care for DM2 from a specialist as opposed to a primary care physician. Therefore, the individuals in this analysis may have poorly controlled DM2 and resultant irreversible cognitive damages, and the results would not apply to those with early stages or well-controlled DM2 who are only being seen by a PCP. Additionally, in our population, there are several individuals with undetected DM2, and many who cannot access or afford treatment of DM1. Therefore, the consequences of diabetes on these poorly controlled individuals may be different, as they are not receiving treatment through oral agents or insulin. A similar limitation applies to the control subjects. There were individuals with HbA1c levels in the pre-diabetes range. Since HbA1c is the average glycosylated hemoglobin in the past 3

months, those with elevated HbA1c levels may have had HbA1c levels in the diabetes range (>6.4%) in the past. As a result, they may have already developed DM2-related brain changes. Therefore, this analysis included individuals with pre-diabetes who were not true controls to the DM2 group, and this may have hindered true differences between individuals with and without DM2.

Another limitation is the lack of access to all study charts to control for the potentially confounding effect of hypertension, high cholesterol, and genetic risk on the outcome measures. This lack of information may affect the results of the study. For example, cases of dementia may be mediated by vascular risk factors besides DM2. These risk factors are comorbidities of DM2 and include hypertension, high cholesterol and obesity. In 2009, a systematic review showed that these four risk factors were associated with a decrease in cognitive functioning, but the significance was highest for diabetes and obesity.<sup>92</sup> Another study showed, however, that learning memory were significantly impaired, but when the analysis was adjusted for the presence of hypertension, the difference was not significant.<sup>93</sup> In this analysis, both groups included individuals who were receiving lipid-lowering and blood pressure medications. This analysis controlled for BMI, but the true differences between groups may be masked if control subjects had hypertension and cholesterol. Moreover, the structural and cognitive changes detected may be exacerbated by the presence hypertension and high cholesterol in the DM2 group. Therefore, future studies should examine the independent effect as well as the interactions of these risk factors with DM2 on as predictors of structural and cognitive measures. Lastly, genetic predisposition could also have a modulating effect on

the relationship between DM2 and the outcome measures, specifically the presence of the E4 allele of the Apolipoprotein E (APOE) is a gene on chromosome 9. Individuals who are heterozygous for the E4 allele have a 4-fold risk of developing AD, while homozygous individuals are at a 9-fold increased risk.<sup>9</sup> This analysis did not adjust for the genetic risk of developing AD, which could result in a false positive association between DM2 and the outcome measures.

It is important to note the clinical significance of the cognitive measures reported in this analysis. The study protocol of this analysis used a 10-word list for the RAVLT, which is a modified form of the original 15-word version. There are no norms published for the modified version. Although the DM2 group performed significantly worse on measures of learning and recognition, indicating impaired verbal learning and long-term memory, the decrease in performance of the DM2 group may not be clinically meaningful. Similarly, the FNAME task has published norms for different age groups, but not for the version of the task that the study protocol used.

Future studies are needed to further investigate the link between DM2 and AD, as well as to specify the etiology of brain changes due to DM2 directly. A future study could enroll individuals at the pre-diabetes stage and assess episodic memory performance and structural MRI changes longitudinally. This study design would give insight into changes that may occur as early as the pre-diabetes phase. If there are changes, individuals should be followed and treated for these changes prior to DM2 diagnosis. Secondly, this analysis consisted of several regions of the episodic memory network. There is little evidence of grey matter reductions in these regions. A future study could take a large database of

DM2 subjects (n>100) for whom there are structural MRIs. A whole brain morphometry analysis should be performed to isolate specific that have gray matter volume loss in DM2 compared with controls. Then, a ROI-based morphometry analysis can therefore be performed that associates episodic memory performance to grey matter volume in the more specific ROIs.

One important finding of this analysis is the marginally significant reductions in posterior cingulate cortex volume in lieu of the small sample size. A follow-up study should be performed to focus on posterior cingulate cortex volume in DM2. Posterior cingulate cortex reductions are reported in mild, moderate and severe cases of AD. Furthermore, the functional connectivity of the posterior cingulate cortex to other regions of the DMN is reduced, and this reduction in connectivity is exacerbated with increasing AD severity.<sup>33</sup> A future cross-sectional study should take a larger sample of individuals with and without DM2 and examine both gray matter volume of the posterior cingulate cortex and functional connectivity with the hippocampus and other DMN regions.

This analysis concludes that there are significant impairments in episodic memory individuals with DM2. There is not enough evidence to conclude that grey matter volume of brain regions in the episodic memory network is associated with these impairments in episodic memory formation and retrieval. The results of the analysis nonetheless contribute to the need of validated neuropsychological measures, such as the FNAME task, that measure higher-level cognitive functions in conjunction imaging-based biomarkers of cognitive decline in population-based studies of DM2. Together, these tools can help identify and predict risk factors of cognitive decline prior to AD onset.

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**CURRICULUM VITAE**

