Impaired Macromolecular Protein Pools in Fronto-Striato-Thalamic Circuits in Type 2 Diabetes Revealed by Magnetization Transfer Imaging

Running title: Impaired brain macromolecular protein pools in T2DM

Shaolin Yang, PhD 1,2, *; Olusola Ajilore, MD, PhD 1; Minjie Wu, PhD 1; Melissa Lamar, PhD 1; Anand Kumar, MD 1, *

1 Department of Psychiatry, 2 Department of Radiology, University of Illinois at Chicago, Chicago, Illinois 60612, USA

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* Correspondence to:

Shaolin Yang, Ph.D.
Assistant Professor
Departments of Psychiatry and Radiology
University of Illinois at Chicago
1601 W. Taylor St., Suite 512
Chicago, IL 60612, USA
Phone: 312-413-3818
Fax: 312-966-2344
Email: syang@psych.uic.edu

Anand Kumar, M.D.
Lizzie Gilman Professor, Department Head
Department of Psychiatry
University of Illinois at Chicago
1601 W. Taylor St., Suite 573
Chicago, IL 60612, USA
Phone: 312-996-7383
Fax: 312-966-2344
Email: akumar@psych.uic.edu
Abstract (limit 200 words)

Previous research has shown that type 2 diabetes mellitus (T2DM) is associated with white matter microstructural changes, cognitive impairment, and decreased resting-state functional connectivity and spontaneous brain activity. This study used magnetization transfer (MT) imaging to examine, for the first time, the integrity of macromolecular protein pools in fronto-striato-thalamic circuits and its clinical and cognitive correlates in patients with T2DM. T2DM patients without mood disorders (n=20, age=65.05±11.95 years) and healthy controls (n=26, age=62.92±12.71 years) were recruited. Nodes of fronto-striato-thalamic circuits, including the head of the caudate nucleus (hCaud), putamen, globus pallidus, thalamus, and four cortical regions: rostral and dorsal anterior cingulate cortex, dorsolateral prefrontal cortex, and lateral orbitofrontal cortex were examined. Compared with healthy controls, patients with T2DM had significantly lower magnetization transfer ratio (MTR) in bilateral anterior cingulate and hCaud. Reduced MTRs in the above regions showed correlations with T2DM-related clinical measures, including hemoglobin A1c level and vascular risk factors, and neuropsychological task performance in the domains of Learning & Memory, Executive Function, and Attention & Information Processing. The impaired biophysical integrity of brain macromolecular protein pools and their local micro-environments in T2DM patients may provide insights into the neurological pathophysiology underlying diabetes-associated clinical and cognitive deficits.

Keywords: type 2 diabetes mellitus; magnetization transfer imaging; magnetization transfer ratio; anterior cingulate; caudate nucleus; frontal-subcortical circuits
Introduction

Type 2 diabetes mellitus (T2DM) is associated with metabolic, macro- and microvascular complications in multiple organ systems including the brain (1). T2DM is also associated with behavioral aberrations including mood disorders, cognitive impairment, and increased risk of dementia in the elderly (2-5). This may be due in part to neuroanatomical alterations as revealed by structural magnetic resonance imaging (MRI), including atrophy in prefrontal cortex and the anterior cingulate (6-8). Findings from diffusion tensor imaging (DTI) studies indicate decreased fractional anisotropy (FA) in frontal white matter (9), cingulum bundle, uncinate fasciculus (10), and anterior limb of internal capsule (ALIC) (11) in patients with T2DM relative to non-diabetic controls. Moreover, recent resting-state functional MRI studies demonstrated disrupted functional connectivity within the default mode network (DMN) and decreased spontaneous brain activity in the occipital lobe and postcentral gyrus in T2DM patients compared to controls (10;12;13). However, it is not clear whether macromolecular protein pools in these regions and circuits are also impaired in T2DM. Investigating the integrity of macromolecular protein pools in gray and white matter may provide complementary information and would aid in our understanding of the mechanisms underlying T2DM-related brain alterations and the neurobiology of diabetes.

Magnetization transfer (MT) imaging exploits magnetization exchange between protons bound to macromolecules and free protons in tissue water. In MT imaging, an off-resonance prepulse is applied to selectively saturate bound protons, and magnetization is then transferred from saturated bound protons to free protons through chemical exchange and direct dipolar coupling. This magnetization transfer leads to decreased MR signal from free protons. The
contrast between MT images with and without the saturation prepulse is defined as “magnetization transfer ratio (MTR)” (14), which reflects the biophysical integrity of macromolecular protein pools and their local micro-environment. Post mortem MT and histopathology studies of multiple sclerosis revealed that in white matter lower MTR is associated with axonal loss and myelin compromise (15;16). The origins of MTR changes in gray matter are more complex and heterogeneous and may reflect multiple neurobiological aberrations (17-20). In gray matter cell membrane proteins and phospholipids contribute to macromolecular density (19). Therefore, damage to cell membranes, reduction in dendritic density and neuronal size and number may independently or collectively lead to decreased MTR (19). Wallerian degeneration, secondary to proximal and/or distal axonal damage, has also been implicated as a mechanism contributing to lower MTR (18;20). Although lacking of specificity in its origins, MTR imaging provides an innovative way to probe the integrity of macromolecular proteins and phospholipids in the brain.

Despite the unique features of MT imaging, there are very few MT studies on T2DM in the literature. The only published MT study was on T2DM and major depression in combination, which focused on subcortical regions and found compromised macromolecular protein pools in the head of the caudate nucleus (hCaud) in T2DM patients (21). However, it is unknown whether there are accompanied changes in macromolecular protein pools in the connected cortical regions.

Fronto-striato-thalamic circuits have been implicated in the pathophysiology of several mood and associated cognitive disorders (22;23). T2DM is consistently associated with
disturbances in mood and cognition (4;24). Particularly, individuals with T2DM are at a greater risk for depression (25;26). However, neurosubstrates underlying the increased risk of mood disturbance and/or cognitive impairments in T2DM are unclear. In this study, we specifically evaluated the biophysical integrity of macromolecular protein pools in fronto-striato-thalamic circuits in patients with T2DM without comorbid mood or cognitive disturbances. Such a study design allows us to elucidate T2DM-specific influence on brain circuits implicated in mood and cognitive functions, which may potentially inform mechanisms underlying increased vulnerability to mood and cognitive disorders in T2DM.

More specifically, this study investigated brain regions/structures associated with the dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate frontal-subcortical circuits – circuits that are neurobiologically relevant in human behavior including cognitive control, decision making, action planning and execution, learning and working memory, attention, and emotional processing (22). Additionally, we were interested in examining the relationships between altered macromolecular protein pools and T2DM-related clinical measures as well as neuropsychological metrics in different cognitive domains. We hypothesized that MTR would be lower at nodes of fronto-striato-thalamic circuits in T2DM patients compared with healthy controls. We additionally hypothesized that MTR in these node regions in patients with T2DM would be negatively correlated with clinical measures reflecting vascular comorbidities and blood sugar regulation and positively correlated with neuropsychological measures.

**Research Design and Methods**

**Subjects**
The subject population consisted of 20 T2DM patients without mood disorders (age=65.05±11.95 years) and 26 non-diabetic healthy controls (HC) (age=62.92±12.71 years). Subjects were selected from a larger sample of a research program on diabetes and depression at the University of Illinois at Chicago (UIC). All participants were age 30 and older and recruited from the greater Chicago area through flyers, local advertisements, and relevant outpatient clinics. The study was approved by the UIC Institutional Review Board and written informed consent was obtained from all participants.

The diagnosis of T2DM in patients was made by their primary care physicians and was confirmed using the American Diabetes Association guidelines (27) (an elevated non-fasting hemoglobin A1c (HbA1c) level (> 6.5% (48 mmol/mol)) or the use of anti-diabetic medications (oral hypoglycemic and/or insulin) when enrolled for this study. T2DM patients reported using oral hypoglycemic medications and/or insulin for glycemic control (6 patients with one oral hypoglycemic medication or insulin; 7 with two or more hypoglycemic medications; 2 with both medications and insulin; 5 without any hypoglycemic medication or insulin). With respect to diabetic vascular complications (28), 5 patients had diabetic microvascular complications (diabetic nephropathy, neuropathy, and retinopathy, etc.), 1 with diabetic macrovascular complications (coronary artery disease, peripheral arterial disease, and myocardial infarction, etc.), 2 with both, and 12 without any vascular complications to their diabetes. Healthy control subjects were free of diabetes and had HbA1c levels within normal limits. All participants received the Mini-Mental State Examination (MMSE) (29), Structured Clinical Interview for DSM-IV (30), and 17-item Hamilton Depression Rating Scale (HAM-D) (31), administered by a trained research assistant and a board certified (AK) or board eligible (OA) psychiatrist. Both
control and patient subjects denied a history of depressed mood, obtained scores of 8 or lower on the HAM-D score, were free of unstable medical conditions, and were of comparable age and gender.

Exclusion criteria included: any current or past history of neurological and psychiatric disorders (i.e., dementia, stroke, seizure, transient ischemic attack, depression, etc.), learning disability or attention deficit hyperactivity disorder, psychotropic medication, current or past history of substance abuse or dependence, history of head injury or loss of consciousness, a MMSE score less than 24, or any contraindication to MRI scan such as metal in the body, surgically implanted devices containing metal, claustrophobia, and pregnancy.

All participants were assessed for medical comorbidity using the Cumulative Illness Rating Scale (CIRS) (32) and for vascular comorbidities using the Framingham Stroke Risk Profile (FSRP) score (33). We also designed a modified Framingham Stroke Risk Profile (mFSRP) score in which the contribution of T2DM was removed to represent vascular complications only. All participants received a non-fasting blood draw to document HbA1c levels, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and to verify the participants’ diabetic status. The systolic and diastolic blood pressure (SBP and DBP) and body mass index (BMI) were documented on each participant. All participants were also assessed for IQ using the Wechsler Test of Adult Reading (WTAR) (34). Demographic and T2DM-related clinical measures are summarized in Table 1. Obesity, hypertension, vascular diseases, and/or elevated bad cholesterol levels are often seen in patients with T2DM and each of these comorbidities has been reported to exert their own effects on the human brain. It must be noted
that in this study these comorbidities, including the following variables: BMI, SBP, DBP, mFSRP, and LDL cholesterol, were of comparable levels between two groups (p’s > 0.20). So the possible effects of these comorbidities on the group difference in MTR have been minimized and the findings from this study are primarily attributable to T2DM.

Neuropsychological Tests

A neuropsychological battery was conducted on each participant across three domains: Learning & Memory (California Verbal Learning Test–2nd Edition immediate total recall and long delay free recall (35); Wechsler Memory Scale–3rd Edition (WMS-III) Logical Memory I and II and Visual Reproduction I and II (36)); Attention & Information Processing (Stroop Color and Word trials (37), Trail Making Test A and Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) Digit-Symbol Coding (38)); Executive Function (Delis-Kaplan Executive Function System Category Switching (39), Trail Making Test B (40), Stroop Interference Score (37), WAIS-III Backwards Digit Span (38), and Self-Ordered Pointing Task Total Errors (41)). Raw scores from the neuropsychological battery were standardized using the healthy control sample mean and standard deviation. Relevant scores were reversed so that high scores consistently reflected better performance. Composite Z scores were calculated for each domain and the Cronbach’s alphas (α) suggested that each variable measured a unidimensional latent construct (Learning & Memory, α=.88; Attention & Information Processing, α=.85; Executive Function, α=.76).

Magnetization Transfer Image Data Acquisition

Magnetic resonance imaging (MRI) was performed on a Philips Achieva 3T scanner (Philips Medical Systems, Best, The Netherlands) with a body coil for transmission and an 8-element
phased-array (Philips’ SENSE-Head-8) coil for reception. Subjects were equipped with soft ear plugs, positioned comfortably in the head coil using custom-made foam pads to minimize head motion, and instructed to remain still. MT images were acquired using a three-dimensional (3D) spoiled gradient-echo sequence with multi-shot echo-planar imaging (EPI) readout and the following parameters: TR/TE = 64/15 ms, flip angle = 9°, FOV (field of view) = 24 cm, 67 axial slices, slice thickness/gap = 2.2 mm/no gap, EPI factor = 7, reconstructed voxel size = 0.83 × 0.83 × 2.2 mm³, with a nonselective five-lobed Sinc-Gauss off-resonance MT prepulse (B₁ / Δf / dur = 10.5µT / 1.5kHz / 24.5ms) optimized for maximum white matter/gray matter contrast (42).

Image slices were parallel to the anterior commissure–posterior commissure line. Parallel imaging was utilized with a reduction factor of 2, and the total duration of MT data acquisition was close to 5 min. Before the MT scan, high resolution 3D T₁-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) images were acquired with the following sequence parameters: TR/TE = 8.4/3.9 ms, flip angle = 8°, FOV = 24 cm, 134 axial slices/no gap, reconstructed voxel size = 0.83 × 0.83 × 1.1 mm³. In addition, T₂-weighted fluid-attenuated inversion recovery (FLAIR) images were also acquired using turbo spin echo sequence with sequence parameters: TR/TI/TE=11000/2800/68 ms, FOV = 24 cm, 67 axial slices without gap, and reconstructed voxel size = 0.83 × 0.83 × 2.2 mm³, for delineation of hyperintense areas in the brain.

**Image Processing**

For each participant, T₁-weighted MPRAGE image, T₂-weighted FLAIR image, and MT images (with and without the off-resonance MT prepulse: Mₛ and M₀) were co-registered. MTR values were calculated on a voxel-by-voxel basis using co-registered M₀ and Mₛ with the formula MTR
= (M₀-M₄)/M₀. ROIs were placed on the co-registered high-resolution T₁-weighted image at the nodes of fronto-striato-thalamic circuits (22), including four subcortical regions, i.e., hCaud, putamen, globus pallidus, and thalamus, and four cortical regions, i.e., rostral and dorsal anterior cingulate cortex (rACC and dACC), dorsolateral prefrontal cortex (DLPFC), and lateral orbitofrontal cortex (lOFC) in both hemispheres (see Fig. 1). Care was taken to ensure consistent placement of the subcortical ROIs for the MTR analysis. The slice displaying the most anterior margin of the genu of the corpus callosum (Montreal Neurological Institute (MNI), (x, y, z) coordinates: (1, 32, 6) mm) was chosen as the reference slice for placing the following subcortical ROIs: hCaud, putamen, and thalamus, because this landmark could be easily and consistently identified across subjects and these ROIs are visible at this slice level (4) (see Fig. 1a). In the case that putamen was not completely visualized on this slice, the next inferior slice was used as the reference slice (4). For the ROI of globus pallidus, the slice clearly displaying the anterior commissure (MNI coordinates: (0, 2, -4) mm) was chosen as the reference slice (see Fig. 1b). During the placement of the subcortical ROIs, the co-registered FLAIR image was closely examined to ensure the ROIs were not placed in hyperintense areas. Moreover, we used constant volumes of ROIs in all of the defined subcortical regions, i.e., the volume was fixed to 73.3 mm³ for hCaud, putamen, and thalamus and was fixed to relatively smaller 55 mm³ for globus pallidus. These two volumes were selected so that the MTR calculation in each subcortical ROI could be devoid of any partial volume effects from adjoining brain regions and/or cerebrospinal fluid (CSF) on all the involved subjects. For the four cortical ROIs, i.e., rACC, dACC, DLPFC, and lOFC, we used the FreeSurfer package (https://surfer.nmr.mgh.harvard.edu/) to parcellate these structures and evaluated MTR within cortical white matter to minimize partial volume effects from the CSF. To better illustrate the
anatomical location of each cortical region, both gray (green) and white matter areas (red) are displayed in Figs. 1c-e (the region of dACC is not shown for simplicity). Generation of ROI masks and calculation of MTR were performed using in-house developed programs.

**Statistical Analysis**

Clinical and demographic measures were analyzed using univariate analysis of variance (ANOVA) for continuous variables and Chi-squared tests for categorical variables. Group differences in MTR in bilateral regions were assessed using a mixed model analysis with diagnostic group as the between-group factor and hemisphere as a within-subject factor. Group differences in MTR in individual regions on each hemisphere were further analyzed using univariate analysis of covariance (ANCOVA) controlling for age. Correlations between MTR and FSRP, mFSRP, HbA1c level, or other related clinical variables were analyzed using partial Pearson’s product-moment correlations controlling for age. In the correlation analysis of MTR and HbA1c, a natural logarithm transformation was first performed on HbA1c (i.e., log-transformed HbA1c) to reduce the skewness of this variable when the sample was combined from both HC and T2DM groups. Correlations between MTR and neuropsychological task performance were also analyzed using partial Pearson’s product-moment correlations controlling for age and MMSE as initial covariates. Multiple comparison correction was performed using false discovery rate (FDR) approach (43-45) with the maximum acceptable FDR set at 0.15. All statistical analyses were carried out using SPSS version 18 (SPSS Inc., Chicago, IL, USA).

**Results**

**Demographic and Clinical Measures**
Table 1 summarizes the demographic and clinical characteristics of the T2DM and HC groups. There were no significant group differences in age, sex, race, handedness, years of education, IQ (WTAR), MMSE, HAM-D (both groups were free of depression, with mean scales < 2), BMI, systolic and diastolic blood pressure (SBP and DBP), LDL cholesterol, and mFSRP. As expected, there were significant group differences in the diabetes-related clinical measures (HDL cholesterol: $F = 19.406$, $df = 1,44$, $p<0.001$; CIRS: $F = 15.812$, $df = 1,44$, $p<0.001$; FSRP: $F = 8.848$, $df = 1,44$, $p=0.005$; HbA1c: $F = 24.503$, $df = 1,44$, $p<0.001$).

**Neuropsychological Tests**

There was no significant group difference in neuropsychological task performance in the three domains: Learning & Memory, Executive Function, and Attention & Information Processing ($p’s > 0.09$) (see Table 2) (the neuropsychological data of one control subject was not recorded; one diabetic subject was color blind and did not perform the Stroop tasks; and another diabetic subject’s “Trail Making Test A” task was not scored).

**Group Differences in MTR**

The mixed model analysis (with diagnostic group as the between-group factor and hemisphere as a within-subject factor) showed that among the ROIs examined, MTR was significantly lower in bilateral dACC ($F = 6.082$, $df = 1,44$, $p = 0.018$) and bilateral hCaud ($F = 5.085$, $df = 1,44$, $p = 0.029$) and there was a trend of lower MTR in bilateral rACC ($F = 2.767$, $df = 1,44$, $p = 0.10$) in T2DM patients compared with non-diabetic controls (see Fig. 2). The above significant results remained significant after the FDR multiple comparison correction. In the above regions, there were no significant hemispheric differences in MTR (dACC: $F = 1.040$, $df = 1,45$, $p = 0.313$;
hCaud: $F = 0.548$, df = 1,45, $p = 0.463$; and rACC: $F = 1.451$, df = 1,45, $p = 0.235$). ANCOVA analysis further revealed that the dACC MTRs in both left and right hemispheres were significantly lower in T2DM patients than non-diabetic controls (right: $F = 4.847$, df = 1,43, $p = 0.033$; left: $F = 5.787$, df = 1,43, $p = 0.021$) and right hCaud MTR was significantly lower in T2DM patients ($F = 5.416$, df = 1,43, $p = 0.025$) while no significant difference was found in left hCaud MTR ($F = 1.974$, df = 1,43, $p = 0.167$) despite a lower mean value in T2DM patients than controls. In the following sections, we limited our further statistical analysis to the regions showing group differences in MTR, i.e., bilateral dACC, bilateral rACC, and right hCaud.

**Correlation between MTR and T2DM-Related Clinical Measures**

When groups were combined, MTR was negatively correlated with the log-transformed HbA1c level in bilateral dACC (left: $r = -0.401$, df = 43, $p = 0.006$ and right: $r = -0.412$, df = 43, $p = 0.005$) (see Fig. 3a-b), bilateral rACC (left: $r = -0.350$, df = 43, $p = 0.018$ and right: $r = -0.329$, df = 43, $p = 0.027$), and right hCaud ($r = -0.323$, df = 43, $p = 0.031$). These results remained significant after the FDR multiple comparison correction.

MTRs in bilateral rACC were negatively correlated with the FSRP score across the entire sample combining both groups (left: $r = -0.344$, df = 43, $p = 0.021$; right: $r = -0.371$, df = 43, $p = 0.012$). If examining MTR in each group, significant correlation between right rACC MTR and FSRP was found in the T2DM group but not in the control group (T2DM: $r = -0.589$, df = 17, $p = 0.008$; HC: $r = -0.158$, df = 23, $p = 0.450$) (see Fig. 4a). The above significant results remained significant after the FDR multiple comparison correction. We repeated the above correlation analysis using the mFSRP score and the results remained significant in bilateral rACC across the
entire sample (left: r = -0.304, df = 43, p = 0.043; right: r = -0.357, df = 43, p = 0.016) and in right rACC in the T2DM group (T2DM: r = -0.599, df = 17, p = 0.007; HC: r = -0.158, df = 23, p = 0.450).

There was no significant correlation between MTR in the examined regions and the duration of T2DM (p’s > 0.07), SBP (p’s > 0.08), DBP (p’s > 0.13), BMI (p’s > 0.39), HDL cholesterol (p’s > 0.19), and LDL cholesterol (p’s > 0.12) in T2DM patients.

**Correlation between MTR and Neuropsychological Task Performance**

The Learning & Memory Z-score was positively correlated with bilateral rACC MTR in the T2DM group but not in the control group (left: T2DM: r = 0.595, df = 16, p = 0.009; HC: r = -0.166, df = 21, p = 0.449, and right: T2DM: r = 0.811, df = 16, p < 0.001; HC: r = -0.100, df = 21, p = 0.651). The significant correlation in right rACC remained significant after the FDR multiple comparison correction (see Fig. 4b). Furthermore, this correlation remained significant after adding the HbA1c level (r = 0.722, df = 15, p = 0.001) or mFSRP score (r = 0.648, df = 15, p = 0.005) as an additional covariate besides age and MMSE. In addition, the right rACC MTR was also positively correlated with the Executive Function Z-score in the T2DM group but not in the control group (T2DM: r = 0.540, df = 15, p = 0.025; HC: r = -0.097, df = 21, p = 0.659).

Finally, the Attention & Information Processing Z-score was positively correlated with right hCaud MTR in the T2DM group only (T2DM: r = 0.549, df = 14, p = 0.028; HC: r = -0.209, df = 21, p = 0.339). While the significant correlations between right rACC MTR and the Z-score of Executive Function and between hCaud MTR and the Attention & Information Processing Z-score remained significant after adding the HbA1c levels or mFSRP score as an additional
covariate besides age and MMSE, these two correlations did not survive the FDR multiple comparison correction.

**Discussion**

The primary finding of the present study is that biophysical integrity of macromolecular protein pools are compromised at node regions of frontal-subcortical circuits, i.e., bilateral anterior cingulate and head of the right caudate nucleus, in T2DM patients compared with non-diabetic controls. Reduced MTR in these regions correlated with T2DM-related clinical measures, including hemoglobin A1c level and increased vascular risk factors, and neuropsychological task performance in the domains of Learning & Memory, Executive Function, and Attention & Information Processing.

Our findings of decreased MTRs in bilateral anterior cingulate and head of the right caudate nucleus are consistent with recent reports of reduced FA and increased radial diffusivity (RD) (suggestive of possible demyelination) in the frontal white matter and associations between disease duration and increased RD in the cingulate white matter gyrus and the right caudate nucleus in T2DM patients (9). Also in line with our findings, significantly lower glutamate and glutamine concentrations in the caudate nucleus and higher *myo*-inositol concentrations in the frontal white matter were observed in patients with T2DM relative to healthy controls (2). Further, a trend toward significant white matter impairment in the fibers of anterior limb of internal capsule (ALIC) was observed in T2DM, which was associated with elevated HbA1c in a recent DTI tractography study (11). The ALIC contains the anterior thalamic radiation,
prefrontal corticopontine tracts, and thalamo-striate and striate-striate tracts, providing reciprocal connections between the frontal lobe, stratum and thalamus (11;46;47). Our study extended the finding of altered WM integrity in ALIC fibers and demonstrated that the biophysical integrity of macromolecular protein pools in brain regions connected through these white matter fibers are also impaired in T2DM.

Head of the caudate nucleus and the anterior cingulate are important nodes of cortico-striatal-pallidal-thalamic (CSPT) circuits that are involved in cognitive functions such as learning and memory, attention and information processing, and executive function (22). Specifically, the caudate nucleus is extensively connected with cortical and subcortical structures in well-characterized circuits (4;22) that subserve the complex regulation of motor functions, cognition, and mood. Among five primary cortical-subcortical circuits (prefrontal, striatal, pallidal, thalamic, and prefrontal circuits), three circuits, i.e., the oculomotor, dorsolateral prefrontal, and lateral orbitofrontal circuits, have direct connections from the prefrontal regions to the caudate nucleus (4;22). In agreement with these circuitry functions, our results demonstrated significant association between cognition and the macromolecular integrity of node regions of frontal-subcortical circuits. The positive correlations between MTRs and neuropsychological task performance in distinct domains are consistent with and further expand the reported association between caudate MTR and global cognition (24). Changes in the caudate and prefrontal regions have been associated with a broad spectrum of behavioral aberrations (4;11;22). Our results demonstrated that the biophysical integrity of the CSPT circuits was compromised in T2DM patients in the absence of significant cognitive impairments or mood disturbances. T2DM-related changes in these circuits may contribute in part to increased susceptibility of cognitive
deficits and/or mood disorders in patients with T2DM and potentially inform the underlying substrates linking T2DM and cognitive/mood disorders.

Further, MTRs in bilateral dACC, rACC and right hCaud were significantly correlated with the HbA1c level, and decreased MTRs in bilateral rACC were associated with increased vascular risk factors. While correlations do not prove causality, these correlations may suggest that both hyperglycemia and vascular diseases may be contributing to the abnormalities observed in our clinical sample. In contrast, there was no significant association between decreased MTRs and duration of T2DM. The HbA1c is known to reflect an average level of blood sugar over the last three months. A possible explanation is that reduced MTRs may in part reflect the intermediate or dynamic impact of T2DM on macromolecular protein pools of these regions.

Several limitations of the present study should be considered. The study is limited by its relatively small sample size (20 patients with T2DM and 26 healthy controls), which may have limited the power to detect subtle changes in some regions of the fronto-striato-thalamic circuits in T2DM patients. Furthermore, the relatively small sample size restricted our ability to examine specific task-level cognitive processes. Thus, a composite Z-score approach was used to protect against multiple comparisons. In addition, a cross-sectional instead of longitudinal design was used in this study. The cross-sectional design is inherently more vulnerable to inter-subject variance and cohort effects. Therefore, future studies are needed to increase the sample size and to investigate the relationship between biophysical integrity of macromolecular protein pools and specific cognitive functions.
In conclusion, our study found compromised macromolecular proteins pools in bilateral anterior cingulate and head of the right caudate nucleus in T2DM patients compared to comparison controls. Moreover, the compromised biophysical properties of macromolecular proteins pools represented by lower MTR were correlated with T2DM-related clinical measures and neuropsychological task performance in distinct domains. This study is the first to report macromolecular impairment in frontal-subcortical circuits and its clinical and cognitive correlates in patients with T2DM. These findings contribute to the growing literature in brain alterations in T2DM and have important implications for the underlying neurobiology of diabetes.

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S.Y. performed the investigations, designed the imaging protocol, acquired and analyzed the data, and wrote the manuscript. O.A. designed the study and reviewed/edited the manuscript, M.W. analyzed the data and wrote the manuscript. M.L. reviewed/edited the manuscript, A.K. designed the study and wrote the manuscript. A.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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All the authors have no conflict of interest to disclose.

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Table

### TABLE 1. Demographic and Clinical Measures across Subject Groups

| Measures                    | HC (N=26)       | T2DM (N=20)     | Statistics |
|-----------------------------|-----------------|-----------------|------------|
|                             | Mean  | SD  | Mean  | SD  | F       | df  | p      |
| Age (years)                 | 62.92 | 12.71 | 65.05 | 11.95 | 0.333  | 1,44 | 0.567  |
| Sex                         | 11M/15F | 12M/8F |  |  | $\chi^2=1.415$ | 1 | 0.234 |
| Race                        | 10B/2H/1A/11W/2O | 11B/1H/0A/8W/0O |  |  | $\chi^2=3.125$ | 4 | 0.537 |
| Handedness                  | 25R/1L/0M | 16R/3L/1M |  |  | $\chi^2=3.248$ | 2 | 0.197 |
| Education (years)           | 14.54 | 1.86 | 14.80 | 2.28 | 0.184  | 1,44 | 0.670  |
| WTAR                        | 102.52 | 11.09 | 99.95 | 16.54 | 0.387  | 1,44a | 0.537 |
| MMSE                        | 28.92 | 0.98 | 28.35 | 1.18 | 3.241  | 1,44 | 0.079  |
| HAM-D                       | 0.96  | 1.311 | 1.65  | 1.843 | 2.193  | 1,44 | 0.146  |
| BMI (kg/m²)                 | 30.18 | 17.54 | 31.43 | 5.91  | 0.092  | 1,44 | 0.763  |
| SBP (mm Hg)                 | 136.38 | 14.05 | 139.30 | 17.87 | 0.384  | 1,44 | 0.539  |
| DBP (mm Hg)                 | 79.85 | 10.06 | 83.70 | 10.30 | 1.627  | 1,44 | 0.209  |
| T2DM Duration (months)      | -     | -    | 115.15 | 85.19 | -     | -    | -     |
| HDL Cholesterol             | 72.88 | 22.21 | 48.15 | 13.27 | 19.406 | 1,44 | <0.001 |
| LDL Cholesterol             | 93.27 | 23.99 | 88.00 | 22.56 | 0.574  | 1,44 | 0.453  |
| CIRS                        | 3.69  | 2.83  | 7.15  | 3.05  | 15.812 | 1,44 | <0.001 |
| FSRP                        | 8.81  | 4.61  | 13.00 | 4.90  | 8.848  | 1,44 | 0.005  |
| mFSRP (T2DM removed)        | 8.81  | 4.61  | 10.60 | 5.14  | 1.544  | 1,44 | 0.221  |
| HbA1c (%) (mmol/mol)        | 5.70 (39) | 0.36 (3.9) | 7.37 (57) | 1.67 (18.3) | 24.503 | 1,44 | <0.001 |

HC, healthy controls; T2DM, patients with type 2 diabetes mellitus
Sex: M=male, F=female; Race: B=black, H=hispanic, A=asian, W=white, O=other; Handedness: R=right, L=left, M=mixed; WTAR, Wechsler Test of Adult Reading; MMSE, Mini-Mental Status Exam; HAM-D, Hamilton Depression Rating Scale; BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; CIRS, Cumulative Illness Rating Scale; FSRP, Framingham Stroke Risk Profile; mFSRP (T2DM removed), modified Framingham Stroke Risk Profile after removing contribution of T2DM; HbA1c, Hemoglobin A1c

*a One control subject’s WTAR was not recorded
TABLE 2. Neuropsychological Task Performance (in Composite Z score) in Three Domains across Subject Groups

| Domain                          | HC (N=26) | T2DM (N=20) | Statistics |
|---------------------------------|-----------|-------------|------------|
|                                 | Mean  SD  | Mean  SD    | F  df  p   |
| Learning & Memory (LM)          | Refa 0.785| -0.280 0.779| 1.217 1.43 0.276|
| Executive Function (EF)         | Refa 0.715| -0.372 0.673| 2.972 1.42 0.092|
| Attention & Information Processing (AIP) | Refa 0.826| -0.278 0.861| 1.348 1.41 0.252|

a Raw score of each specific cognitive task on each subject was standardized (called “Z score”) using the mean and standard deviation of the HC group.
Figure Legends

Figure 1. Regions of interest for magnetization transfer ratio (MTR) analysis.

lOFC, lateral orbitofrontal cortex; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex

Figure 2. Scatterplots of magnetization transfer ratios (MTRs) in (a) hCaud, (b) dACC, and (c) rACC.

hCaud, head of the caudate nucleus; dACC, dorsal anterior cingulate cortex; rACC, rostral anterior cingulate cortex
(a): (F = 5.085, df = 1,44, p = 0.029)
(b): (F = 6.082, df = 1,44, p = 0.018)
(c): (F = 2.767, df = 1,44, p = 0.10)

Figure 3. Representative scatterplots of correlations between magnetization transfer ratios (MTRs) and log-transformed HbA1c level in (a) left dACC and (b) right dACC.

HbA1c, hemoglobin A1c; dACC, dorsal anterior cingulate cortex
(a): (r = 0.401, df = 43, p = 0.006)
(b): (r = 0.412, df = 43, p = 0.005)

Figure 4. Representative scatterplots of correlations between magnetization transfer ratios (MTRs) in right rACC and (a) vascular risk factor assessed by FSRP score and (b) neuropsychological task performance (in Z-score) in Learning & Memory.

rACC, rostral anterior cingulate cortex; FSRP, Framingham stroke risk profile
(a): (T2DM: r = 0.589, df = 17, p = 0.008; HC: r = 0.158, df = 23, p = 0.450)
(b): (T2DM: r = 0.811, df = 16, p < 0.001; HC: r = 0.100, df = 21, p = 0.651)
Figure 1. Regions of interest for magnetization transfer ratio (MTR) analysis.

IOFC, lateral orbitofrontal cortex; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex

215x407mm (300 x 300 DPI)
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HbA1c, hemoglobin A1c; dACC, dorsal anterior cingulate cortex
(a): (r = -0.401, df = 43, p = 0.006)
(b): (r = -0.412, df = 43, p = 0.005)
Figure 4. Representative scatterplots of correlations between magnetization transfer ratios (MTRs) in right rACC and (a) vascular risk factor assessed by FSRP score and (b) neuropsychological task performance (in Z-score) in Learning & Memory.

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