Cerebral white matter integrity and resting-state functional connectivity in middle-aged patients with type 2 diabetes

Short running title: Brain structural and functional connectivity

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ABSTRACT

Early detection of brain abnormalities at the pre-clinical stage can be useful for developing preventive interventions to abate cognitive decline. We examined whether middle-aged type 2 diabetic patients show reduced white matter integrity in fiber tracts important for cognition, and whether this abnormality is related to pre-established altered resting-state functional connectivity in the default-mode network. Diabetic and non-diabetic participants underwent fMRI and cognitive assessment. Multiple diffusion measures were calculated using streamline tractography, and correlations with default-mode network functional connectivity were determined. Diabetic patients showed lower fractional anisotropy (a measure of white matter integrity) in the cingulum bundle and uncinate fasciculus -- fiber tracts that connect frontal, temporal, and parietal regions. Controls showed stronger functional connectivity than patients between the posterior cingulate and both left fusiform and medial frontal gyri. Fractional anisotropy of the cingulum bundle was correlated with functional connectivity between the posterior cingulate and medial frontal gyrus for combined groups. Thus, middle-aged patients with type 2 diabetes show white matter abnormalities that correlate with disrupted functional connectivity in the default-mode network, suggesting that common mechanisms may underlie both structural and functional connectivity. Detecting brain abnormalities in middle age enables implementation of therapies to slow progression of neuropathology.
Type 2 diabetes mellitus is characterized by insulin resistance and hyperglycemia (1), which can increase risk for brain abnormalities including stroke (2), dementia (3; 4), white matter lesions (5), and cognitive impairment (6; 7). By using magnetic resonance imaging (MRI) techniques to identify brain abnormalities in middle-aged patients, clinicians can introduce cognitive training or other therapies to slow and/or prevent cognitive decline.

Resting state functional connectivity, measured using functional MRI (fMRI), can be used to assess brain health. Disruptions in resting-state functional connectivity in the default mode network (DMN), a network of brain regions most active during rest, are often associated with current and future cognitive decline. Reduced connectivity and hypometabolism in these regions has been observed during early to mid-adulthood in people at genetic risk for Alzheimer’s disease (8), and in people with mild cognitive impairment (9).

Diffusion tensor imaging (DTI) has been used to quantify microstructural alterations in white matter (10) that may also impact cognition (11). DTI techniques measure the magnitude and directionality of random water movement to provide information about tissue integrity of the physical connections between different brain regions. Fractional anisotropy (FA) and mean diffusivity are the primary DTI-derived metrics believed to reflect overall white matter health, maturation and organization (10; 12). In addition to these primary DTI measures, axial diffusivity, which reflects axon integrity, and radial diffusivity, which reflects myelin sheath integrity, can be useful in understanding the underlying physiology (13).

Diabetes researchers have recently used DTI techniques to assess white matter integrity using whole-brain methods (14-16). Fiber tractography can be used to evaluate white matter integrity in isolated tracts (17) that may be especially impacted by diabetes. To date, only one study (11) has used fiber tractography in type 2 diabetes. These authors reported reduced fiber
integrity in superior and inferior longitudinal fasciculi, corpus callosum, and uncinate fasciculus in elderly patients (mean age 71 years).

The current study examined whether a relationship exists between functional and structural connectivity using resting-state fMRI and fiber tractography. Specifically, we evaluated a potential relationship between resting-state functional connectivity in the DMN and white matter integrity in the cingulum bundle (CB), a fiber tract that connects key regions of the DMN (18; 19). Our group recently reported altered functional connectivity in the DMN in type 2 diabetes (20), and coincident disruptions have been found in both the CB and the DMN in Alzheimer’s disease (21). In this report, we expand our previous finding in a separate group of middle aged participants with type 2 diabetes, and assess whether a correlation exists between the strength of connectivity within the DMN (functional connectivity) and FA in the CB (structural connectivity). Evaluating the nature of relationships between brain function and structure during middle age can be helpful for developing therapies to prevent progression of cognitive decline and other neuropathology.

A secondary goal of our study was to measure structural integrity in the uncinate and superior longitudinal fasciculi (UF and SLF, respectively), which interconnect frontal, temporal, and parietal regions. These regions exhibit cerebral atrophy and diminished cerebral perfusion and vasoreactivity in type 2 diabetes (22), and are associated with specific cognitive domains, such as memory and executive function (23), that are often affected in type 2 diabetes (7).

Our study enables identification of specific neurological abnormalities in both structure and function during midlife that may lead to clinically significant cognitive decline decades later. A number of longitudinal studies have shown that health profiles at middle age are predictive of
brain health later in life (24; 25). Thus, therapies introduced during midlife, before disease progression peaks, may be more effective.

**RESEARCH DESIGN AND METHODS**

The study was approved by local Institutional Review Boards at the Joslin Diabetes Center and Beth Israel Deaconess Medical Center. Following a full description of the study to the participants, written informed consent was obtained.

**Subjects**

Participants were recruited from the Joslin Diabetes Center, advertisements in its newsletter, mailings to clinic patients from the Joslin’s database, and advertisements in local papers. Eighteen individuals with type 2 diabetes, and 19 non-diabetic healthy control subjects were included in this study (see Table 1). Diabetic patients were all previously diagnosed with type 2 diabetes, except for one person who was initially recruited as a control subject but whose 2-hour oral glucose tolerance test (OGTT) glucose exceeded 200 mg/dL. All controls had fasting glucose < 100 mg/dL and 2 hour OGTT glucose < 140 mg/dL. Participants were between the ages of 45-65 years (mean±SD= 54.6±6.4 years; disease duration 10.5±6.9 years). Because insulin resistance was of particular interest in this study, diabetic subjects could not be treated with insulin sensitizing medications such as metformin or thiazolidinediones. One diabetic patient had moderate nonproliferative retinopathy, two diabetic patients had moderate neuropathy, and two diabetic patients had nephropathy. Patients were group-matched on age, education, gender, and BMI. We controlled for IQ given its relationship to cognition.

Medical history and medication use was gathered from medical records and
questionnaires. Chronic glycemic control was calculated by grouping HbA1c results in 4-year increments and averaging the four-year means (26). Exclusion criteria included 1) history of stroke or myocardial infarction; 2) unstable or current episode of a DSM Axis I disorder, including major depression, bipolar disorder, schizophrenia, and panic disorder; 3) sleep disorder, eating disorder, or learning disorder; 4) sensorimotor handicap, central nervous system neurological disorder, or medical illness that significantly affects neurological function; 5) history of substance abuse (including alcohol, excluding nicotine); 6) left-handedness (excluding ambidextrity, determined by the Edinburgh Handedness Inventory (27)); and 7) BMI > 40 kg/m². One control participant was eliminated after a structural brain abnormality was identified by the Magnetic Resonance Technician and study radiologist (both blind to group status).

Eligible subjects visited the Joslin Diabetes Center after an 8-10 hour fast, and patients on insulin were asked to withhold their morning dose until after study blood samples were obtained. All subjects had a medical history and physical examination, during which blood pressure, height, weight, and BMI were recorded. Type 2 diabetic patients had only fasting laboratories assessed, whereas controls also underwent a 2-hour 75-gram OGTT to determine glucose tolerance and insulin resistance. Insulin resistance (HOMA-IR, homeostatic model assessment – insulin resistance) was calculated using the following formula: HOMA-IR = [fasting glucose (mmol/L)*fasting insulin (µU/L)/22.5]. Because HOMA-IR may not accurately reflect insulin sensitivity in patients who require insulin or who have minimal β cell function (28), diabetic patients treated with insulin were not included in the calculation of insulin resistance.

Table 1 about here
Cognitive Assessment

All study participants completed the Wechsler Abbreviated Scale of Intelligence (WASI) (29), two subtests in the Delis-Kaplan Executive Function System (DKEFS, verbal fluency and trail making number-letter switching) (30), the Rey Auditory Verbal Learning Test (RAVLT) (31), and the Grooved Pegboard (32). This battery assesses general intelligence, executive function, memory, and psychomotor speed, respectively.

MRI Image Acquisition

Within two weeks of the cognitive assessment, MRIs were acquired using an 8-channel head coil on a 3T GE Signa HDxt system (General Electric Medical Systems, Milwaukee, WI, United States). All subjects were screened for foreign metal in their body, pacemakers, pregnancy, and claustrophobia prior to scanning. All image analysts were blinded to group status.

Diffusion-Weighted Images

Fifty-six axial slices parallel to the anterior commissure-posterior commissure line covering the whole brain were acquired at a resolution of 0.9375mm x 0.9375mm x 2.6mm (30 gradient directions at b=1000 s/mm$^2$, 5 baseline scans at b=0 s/mm$^2$, TR 16000ms, TE 84ms, FOV 24 cm, 256x256 matrix). The acquisition time was 9 minutes and 20 seconds.

Functional Images

Functional image parameters included gradient-echo planar sequence sensitive to blood oxygen level-dependent (BOLD) contrast (repetition time = 3,000 ms, echo time = 30 ms, flip angle = 90°), whole brain volumes with 26 contiguous 5 mm-thick transverse slices, no interslice gap,
and 3.125 x 3.125 mm in-plane resolution (33). Patients lay still in the scanner with their eyes closed but remained awake.

**DTI Analysis**

All DTI analyses were performed with the 3DSlicer software package (http://slicer.org, Surgical Planning Laboratory, Brigham and Women’s Hospital, Boston, MA). The diffusion data were corrected for motion and artifacts using affine registration with a reference volume [FLIRT; FMRIB Software Library (FSL), Oxford, UK, www.fmrib.ox.ac.uk/fsl] and Rician denoising (34). Maps of FA and color-by-orientation were calculated for each voxel using 3DSlicer.

The three white matter tracts of interest were defined using a region-of-interest (ROI) approach. ROI placements were guided by standardized white matter atlases and delineation criteria previously described (35). See Figure 1 for a detailed description. In short, four ROI sets were strategically placed on color-by-orientation maps to reconstruct the dorsal and ventral portions of the CB. For the UF, two sets of ROIs were drawn on the coronal slice adjacent to the most anterior point of the fornix, and placed in the stem of the anterior temporal lobe and around the white matter of the anterior floor of the external/extreme capsule. By retaining only those tracts whose paths connected both regions of interest (the “AND” approach), we ensured that fibers were selected that exclusively belonged to the UF. For the SLF, the most dorsal part was identified in the axial view on color-by-orientation maps, usually one or two slices above the body of the corpus callosum. The SLF was then outlined from superior to inferior direction in approximately five consecutive axial slices.

The voxels defined by the ROIs were used as seed voxels for streamline tractography, a method described in previous publications (36; 37). See Figure 2 for tractography results of a
representative subject in this study. In short, streamline tractography was initiated for each ROI and followed the direction defined by the principal eigenvector, based on a Runge-Kutta fourth-order protocol. The stopping criteria for this tractography method were Westin’s linear measure (38) below 0.15 and rapid change of direction angle above 45 degrees. Westin’s linear measure, instead of fractional anisotropy, was used to terminate tractography, in order to avoid the confounding effect of using the same measure to construct fiber tracts and to quantify their integrity. A length criterion was also employed and fibers shorter than 20 mm were excluded. To ensure that only fibers of the CB, UF, or SLF were extracted, exclusion ROIs were introduced, i.e., fibers were excluded if they passed through the exclusion ROI or if they did not pass through the inclusion ROI. To quantify microstructural white matter abnormalities, we computed several DTI measures, including fractional anisotropy (FA), trace (=mean diffusivity*3), axial diffusivity (AD), and radial diffusivity (RD).

Tractography was conducted by multiple raters. On a random subset of 10 cases, excellent inter-rater reliability was established (left and right CB FA: intraclass correlation>0.92, p<0.001).

Figure 1 and Figure 2 about here

fMRI Image Processing and Analysis

We assessed functional connectivity between the posterior cingulate (PCC) and all other regions in the brain using BrainVoyager QX as described elsewhere (18). The PCC is a key component of the DMN that is often used as a seed region for such analyses (39). For DMN regions showing
between-group differences in strength of connectivity to the PCC, we computed the correlation between beta weights and FA in the CB.

**Statistical Analyses**

Group differences in demographic, clinical, and cognitive variables involving continuous data were analyzed with Student t-tests or Mann-Whitney U-tests as deemed appropriate. Differences in medication use, smoking history, gender, and race distributions were analyzed using Fisher’s exact tests or Chi-square tests as deemed appropriate.

To test for inter-hemispheric and intra-tract differences in the CB, a repeated measures analysis of covariance (ANCOVA) was conducted with study cohort as the between-group factor, hemisphere and CB subdivision (dorsal, ventral) as the within-group factors, and age as a covariate. To test for the effects of hemisphere on the UF and SLF, a separate repeated measures ANCOVA was performed with study cohort as between-subject factor, hemisphere as within-group factor, and age as a covariate. White matter tracts that showed no hemispheric or intra-tract differences in FA were merged into a single ROI, and DTI values were recalculated to obtain single measures per tract.

Diffusion values (FA, trace, AD, RD) were compared between groups for all white matter tracts studied (CB, UF, SLF) using multivariate analysis of covariance (MANCOVA), controlling for age. Multivariate analysis was also controlled for multiple comparisons with Bonferroni correction. Significant between-group differences in MANCOVA (adjusted for age and multiple comparisons) were further tested using univariate ANCOVA with additional model covariates.
A general linear model was used to assess functional connectivity strengths (using beta-weights). The linear relationships between diffusion measures and beta-weights, fasting glucose, lifetime HbA1c, demographics, clinical characteristics, and cognitive scores were analyzed with Spearman’s correlation (ρ). All tests were conducted with PASW Statistics Release 17.0.2 (SPSS Inc., Chicago, IL) using a two-sided α-level of 0.05.

RESULTS

Demographic and Clinical Results

Clinical and demographic characteristics for the diabetic and control groups are summarized in Table 1. There were no significant differences between groups in age, years of education, gender distribution, race, Hamilton Depression score, smoking history, BMI, blood pressure, total cholesterol, HDL-C, LDL-C, or fasting insulin, but triglycerides (p=0.01) and creatinine (p=0.03) were higher in type 2 diabetic patients. As expected, HbA1c (p<0.001), fasting plasma glucose levels (p<0.001), and HOMA-IR (p=0.03) were also elevated in diabetic patients. The diabetic group was more frequently prescribed cholesterol medications (p=0.046), but not blood pressure medications. Diabetes duration was not correlated with any clinical measure.

Table 1 about here

Neuropsychological Results

Cognitive results are summarized in Table 2. Both groups performed within the normal range on all tests, but the diabetic group scored lower than the control group on full-scale IQ (t=2.19, df=35, p=0.04, Cohen’s d=0.74), verbal fluency (t=2.41, df=35, p=0.02, Cohen’s d=0.82), and
RAVLT immediate recall ($t=2.06$, df=35, $p=0.047$, Cohen’s $d=0.63$); thus, we controlled for these variables in statistical analyses. Diabetes duration and HbA1c were not significantly correlated with performance on any cognitive test.

Table 2 about here

**fMRI Results**

When combining both groups, the following regions were functionally connected to the PCC ($\beta>0$, $p<0.05$, corrected for multiple comparisons) (40): left medial frontal gyrus, right superior frontal gyrus, left superior parietal lobule, bilateral middle temporal gyrus, and right precuneus.

In a second-level analysis, we used a random-effects two sample t-test to determine whether there were group differences in strength of functional connectivity for these regions (41). Similar to our previous report, we found that control subjects showed stronger connectivity within the DMN than the diabetic patients in several regions including the left fusiform gyrus (Talairach coordinates: -49, -65, -12; $p$’s<.02), which is often considered part of the DMN (42; 43), and the left medial frontal gyrus (-4, 55, 12; $p=0.08$, trend). Connections between the PCC and both the left superior parietal lobule and left middle temporal gyrus approached significance ($p$’s<0.10).

**DTI Results: Effects of Hemisphere and CB Subdivision**

Following repeated measures ANCOVA for bilateral tracts, no main effect of hemisphere ($F<0.39$, $p>0.54$), or hemisphere by group interaction ($F<0.20$, $p>0.66$) was found for any tract. The cingulum bundle was further tested for intra-tract differences: FA in the dorsal subdivision
measured higher than in the ventral subdivision, but did not reach statistical significance (F=3.39, p=0.074). Furthermore, no subdivision by hemisphere (F=0.16, p=0.22), or subdivision by hemisphere by group interactions (F=0.03, p=0.87) were found. Since no inter-hemispheric or intra-tract differences in FA were found for any tract in this study, each tract was merged into a single ROI and DTI values were recalculated to obtain one value per tract.

**DTI Results: Between-Group Differences**

The DTI results are summarized in Figure 3 and Table 3. Following multivariate analyses adjusted for age and multiple comparisons, we observed a significant between-group effect for CB FA (F=7.76, df=1, 34, p=0.027) and UF FA (F=7.89, df=1, 30, p=0.027). Patients with type 2 diabetes exhibited on average 6.0% lower FA in the CB and 4.7% lower FA in the UF compared to controls. Although type 2 diabetic patients also showed 4.6% lower FA in the SLF, this finding did not reach statistical significance (F=4.15, df=1, 33, p=0.15). Furthermore, we found a trend between-group difference for CB AD (F=4.95, df=1, 30, p=0.099), with the diabetic group exhibiting 2.3% lower AD. We did not find significant between-group effects for trace or RD in any tract (p>0.05).

Significant between-group effects in multivariate analyses were further tested in univariate analyses adjusting for age, gender, IQ, verbal fluency, and immediate memory. FA remained significantly reduced in patients with type 2 diabetes compared to controls for both the CB (F=4.76, df=1, 30, p=0.037; Adjusted-model R-squared=0.20) and UF (F=4.76, df=1, 26, p=0.038; Adjusted-model R-squared=0.23).

Of further note, the number of fibers or total tract volume did not differ between groups for any white matter tract (p’s>0.05), but the average fiber length of each tract studied
approached statistical significance for being shorter in diabetic patients compared to control subjects (CB: t=2.00, df=35, p=0.053; UF: t=1.83, df=31, p=0.077; and SLF: t=1.81, df=34, p=0.079).

Figure 3 and Table 3 about here

**Correlational Analyses**

Correlations were evaluated between DTI measures and beta-weights representing functional connectivity between the PCC and other DMN regions. Importantly, CB FA was correlated with the beta-weight between the PCC and the left medial frontal gyrus (ρ=0.38, p=0.02) for combined groups (See Figure 4). Given the between-group differences in DTI results, correlations between white matter integrity and study variables were evaluated for FA in the CB and UF. Age, education, diabetes duration, and insulin resistance (HOMA-IR) were not correlated with FA (CB and UF) (p>0.05), but a positive correlation was found between HbA1c and UF FA in diabetic patients (ρ=0.569, p=0.034) and controls (ρ=0.541, p=0.020). In both diabetic patients and controls, lower UF FA was associated with slowing of information processing speed (grooved pegboard time – dominant hand) (patients: ρ=-0.587, p=0.027; controls ρ=-0.486, p=0.035). Among diabetic patients but not controls, lower UF FA was associated with higher serum creatinine level (ρ=-0.644, p=0.013), and lower CB FA was associated with higher BMI (ρ=-0.482, p=0.043) and higher delayed memory scores (ρ=-0.498, p=0.035). We did not observe any correlation between either CB or UF FA and lifetime HbA1c (p>0.246). However, when both groups were combined, fasting glucose was negatively correlated with CB FA (ρ=-0.362, p=0.028), but not UF FA (p=0.493).
DISCUSSION

We investigated a relationship between structural and functional connectivity in type 2 diabetic patients and controls. Using fiber tractography, we demonstrated reduced FA in the CB and reduced functional connectivity within the DMN in middle-aged diabetic patients. We also observed a correlation between CB FA and the strength of functional connectivity between the medial frontal gyrus and the PCC, suggesting that these modalities may be supported by a common neural mechanism (18; 19). This finding has important implications, as it suggests that therapies that improve one modality (i.e. structure or function) may impact the other as well. For example, it is possible that cognitive training, which improves FA (44), may also improve functional connectivity. Further, combined data obtained from both structural and functional measures have been used to more accurately classify people with and without dementia than either variable used alone (8). Our study suggests that such combined data may be useful in determining which diabetic patients are most at risk for clinically significant cognitive decline.

In this study, compared to non-diabetic control subjects, patients with type 2 diabetes showed reduced FA in all white matter tracts, which was statistically significant for the CB and UF corrected for multiple comparisons and adjusted for age, gender, IQ, verbal fluency, and immediate memory. We also observed a negative correlation between FA and fasting glucose across both subject groups. This finding is consistent with recent reports showing that high glucose, even in the normal range, is associated with brain abnormalities (4; 45). Reduced FA is
generally thought to reflect loss of white matter integrity that may reflect demyelination or axon membrane damage, or perhaps reduced axonal packing density, and/or reduced axonal coherence (see review in (46)). Consistent with our FA findings, previous reports have found reduced FA in type 2 diabetes in various brain regions, including fronto-temporal regions (14), cingulate, cerebral peduncle, temporal stem (16), and bilateral frontal lobe (15). Our finding of reduced FA in the UF extends a previous result reported in the only other study known to us that examined fiber tracts in older type 2 diabetic patients using a similar tractography method (11). The UF connects regions responsible for executive function and memory, and microstructural abnormalities in this fiber have predicted DMN hypometabolism in Alzheimer’s disease (21). In our study, reduced UF FA was correlated with slower processing speed, which is common in type 2 diabetes (47). The present study demonstrates that reduced FA is present in middle-aged adults and not confined to an older population that is more prone to cognitive decline and other co-morbidities. This finding is of great clinical significance, as identifying white matter abnormalities in middle age may allow for proactive treatments that can prevent or reduce future cognitive decline.

This is also the first tractography study in type 2 diabetes to report reduced FA in the CB and its relationship to DMN functional connectivity, confirming previous findings that cerebral white matter abnormalities are widespread in type 2 diabetes, and suggesting that they are related to functional aberrations. In addition to our FA findings, we observed numerically lower AD in the CB, in the absence of significant RD changes, which may suggest axonal damage. Only two other groups (11; 15) have previously examined microstructure specific to either myelin or axon pathology in type 2 diabetes. These methods are new and not fully understood; thus, it remains unclear whether microstructural white matter abnormalities in type 2 diabetes reflect myelin or
axon pathology. Further efforts with larger study samples are needed to shed more light on the underlying physiology that causes reduced cerebral white matter integrity in type 2 diabetes. Although potential relationships between clinical variables and white matter changes have not been studied in detail, associations between white matter integrity and variations in blood pressure have been previously reported in healthy, normotensive, older adults (48). In the present study, we found similar correlations between various clinical variables (creatinine, BMI, and HbA1c) and diffusion values, suggesting that certain clinical variables that affect vascular health or metabolism and are often comorbid with type 2 diabetes, are potential contributors to impaired white matter integrity.

We found a positive correlation between UF FA and HbA1c. Although this was unexpected, we previously reported that current HbA1c levels in type 1 diabetic patients in poor control were unrelated to brain glutamate/glutamine levels (49). Thus, current HbA1c may not be the best predictor of brain health, and perhaps this finding is due to a separate metabolic or vascular abnormality present in type 2 diabetes, or to a potential compensatory mechanism in UF fiber integrity that requires further investigation.

Our study is limited by its relatively small sample size (n=37) and cross-sectional design; therefore, our results should be interpreted cautiously. Further, although our patients did not differ significantly from controls on most clinical and demographic variables, there were some variables in which the diabetic patients had worse profiles than controls. Thus, certain clinical variables such as hypertension may also contribute to the brain differences observed in this study (50). Additional studies are needed to investigate the effects of long-term glucose control and number of severe hypoglycemic and/or hyperglycemic events on diffusion measures. Further, there are limitations to DTI tractography. In our study, seeds for tractography relied on manual
tracing of regions-of-interest, which is prone to anatomical misplacement. However, we believe these were kept to a minimum because excellent white matter atlases (35) were used by highly trained tracers blind to group, and inter-rater reliability was high. Finally, although the ROI-based approach in this study enabled *a priori* testing and reduced the risk of multiple comparisons, further studies are needed to explore other cerebral white matter tracts that are potentially affected in type 2 diabetes.

In conclusion, diffusion tensor tractography revealed lower FA in our sample of type 2 diabetic subjects, indicative of microstructural white matter abnormalities in major cerebral white matter tracts, including the UF and CB, the latter of which is correlated with functional connectivity between key regions of the DMN. Future studies should aim to elucidate the underlying physiology of these structural and functional changes. Also, interventions aimed at improving diabetes control or reversing early neuropathology in order to prevent and/or reduce cognitive decline should be further explored.
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W.S.H. and G.M. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript. V.L.F., T.J.M., S.H., H.P.E., J.S.S., N.R.B., D.C.S., A.M.J., M.K., and M.E.S researched data, contributed to discussion, and reviewed and edited the manuscript. W.S.H. and G.M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The authors report no potential conflicts of interest for this manuscript.

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### Table 1

Demographic and clinical characteristics of the study sample.

|                                | Type 2 diabetic subjects (N=18) | Control subjects (N=19) | P-value |
|--------------------------------|---------------------------------|-------------------------|---------|
|                                | Mean    | SD    | Mean    | SD    |         |
| Age, years                     | 56.2    | 6.3   | 53.1    | 6.2   | 0.12    |
| Education, years               | 14.9    | 2.2   | 16.4    | 2.9   | 0.10    |
| HbA1c, % (mmol/mol)            | 7.5 (58) | 1.8 (19.7) | 5.6 (38) | 0.3 (3.3) | <0.001 |
| Lifetime HbA1c*                | 7.1     | 1.3   | -       | -     | -       |
| Fasting plasma glucose, mg/dL  | 158     | 89    | 81      | 7     | <0.001  |
| Fasting serum insulin, µU/mL   | 12.1    | 9.7   | 8.3     | 8.1   | 0.10    |
| HOMA-IR, non-insulin users only| 4.7     | 5.1   | 1.7     | 1.7   | 0.03    |
| Diabetes duration, years       | 10.5    | 6.9   | -       | -     | n/a     |
| Body mass index, kg/m²         | 30.8    | 4.9   | 28.3    | 4.0   | 0.11    |
| BP systolic, mmHg              | 128     | 13    | 120     | 13    | 0.07    |
| BP diastolic, mmHg             | 72      | 9     | 72      | 9     | 0.71    |
| Serum creatinine               | 1.20    | 0.93  | 0.84    | 0.27  | 0.03    |
| Cholesterol                    | 181     | 29    | 182     | 38    | 0.73    |
| Triglycerides                  | 119     | 42    | 100     | 82    | 0.01    |
| HDL-C                          | 52      | 16    | 54      | 15    | 0.67    |
| LDL-C                          | 108     | 28    | 101     | 32    | 0.54    |

N | % | N | % | P value
| Table: Diabetes Characteristics |
|--------------------------------|
| Blood pressure lowering medications | 6 | 33 | 2 | 11 | 0.12 |
| Cholesterol lowering medications   | 4 | 22 | 0 | 0  | 0.05 |
| History of smoking                 | 10| 50 | 9 | 47 | 0.75 |
| Female/Male (% Female)             | 7/11| 39 | 8/11| 42 | 0.55 |
| Race/ethnicity                     |                |    |    |    | 0.20 |
| African American                   | 2 | 11 | 5 | 26 |
| Asian                              | 2 | 11 | 1 | 5 |
| Caucasian                          | 10| 56| 13| 68 |
| Hispanic                           | 2 | 11 | 0 | 0 |
| More than one                      | 2 | 11 | 0 | 0 |

Abbreviations: HOMA-IR, homeostatic model assessment-insulin resistance; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

* Lifetime HbA1c was obtained by calculating four-year means starting with the date of diagnosis, and averaging the four-year means.
Table 2
Cognitive scores and depressive symptoms in this study sample.

|                                      | Type 2 diabetic subjects (N=18) | Control subjects (N=19) | P-value |
|--------------------------------------|---------------------------------|--------------------------|---------|
|                                      | Mean | SD  | Mean | SD  |         |
| WASI full scale-IQ                   | 104.8| 13.0| 113.6| 11.4| 0.04    |
| DKEFS Verbal fluency, scaled        | 10.5 | 3.1 | 12.9 | 2.9 | 0.02    |
| DKEFS trail making number-letter    | 9.2  | 3.8 | 11.0 | 2.8 | 0.11    |
| switching, scaled                   |      |     |      |     |         |
| RAVLT immediate recall T-score      | 46.8 | 10.7| 53.3 | 10.4| 0.05    |
| RAVLT delayed recall T-score        | 49.0 | 9.3 | 51.6 | 9.6 | 0.41    |
| Grooved Pegboard, dominant hand,    | 90.0 | 21.8| 81.7 | 11.9| 0.16    |
| time in seconds                     |      |     |      |     |         |
| Hamilton’s depression rating scale  | 5.0  | 4.0 | 2.9  | 4.0 | 0.14    |

Abbreviations: WASI, Wechsler Abbreviated Scale of Intelligence; DKEFS, Delis-Kaplan Executive Function System; RAVLT, Rey Auditory Verbal Learning Test.
Table 3

DTI values for the cingulum bundle, uncinate fasciculus, and superior longitudinal fasciculus in people with type 2 diabetes and healthy control subjects.

|                          | Type 2 diabetic subjects | Control subjects | Statistical analyses |
|--------------------------|--------------------------|------------------|----------------------|
|                          | Mean  SD                  | Mean  SD         | MANCOVA*  | ANCOVA†  |
|                          |                          |                  | F         | Adjusted p-value | F         | Adjusted p-value |
| **Cingulum Bundle**      |                          |                  |           |               |           |               |
| FA                       | 0.332  0.021             | 0.356  0.029     | 7.76      | 0.027         | 4.76      | 0.037†         |
| Trace (x10^3 µm^2/ms)    | 2.47  0.09               | 2.46  0.11       | 0.02      | 1.00          | n/a       | n/a            |
| AD (x10^3 µm^2/ms)       | 1127  36                 | 1153  43         | 4.95      | 0.099         | n/a       | n/a            |
| RD (x10^3 µm^2/ms)       | 669   32                 | 654   41         | 1.04      | 0.948         | n/a       | n/a            |
| **Uncinate Fasciculus**  |                          |                  |           |               |           |               |
| FA                       | 0.335  0.014             | 0.352  0.017     | 7.89      | 0.027         | 4.76      | 0.038†         |
| Trace (x10^3 µm^2/ms)    | 2.65  0.10               | 2.62  0.08       | 0.53      | 1.00          | n/a       | n/a            |
| AD (x10^3 µm^2/ms)       | 1220  41                 | 1226  33         | 0.18      | 1.00          | n/a       | n/a            |
| RD (x10^3 µm^2/ms)       | 714   32                 | 698   28         | 1.86      | 0.549         | n/a       | n/a            |
| **Superior Longitudinal |                          |                  |           |               |           |               |
| Fasciculus               |                          |                  |           |               |           |               |
| FA                       | 0.362  0.022             | 0.379  0.027     | 4.15      | 0.150         | n/a       | n/a            |
| Trace (x10^3 µm^2/ms)    | 2.38  0.11               | 2.35  0.09       | 0.76      | 1.00          | n/a       | n/a            |
|                  | AD (×10⁻³ μm²/ms) | 1117 | 46  | 1119 | 31  | 0.09 | 1.00 | n/a | n/a |
|------------------|-------------------|------|-----|------|-----|------|------|-----|-----|
| RD (×10⁻³ μm²/ms)| 632               | 36   | 614 | 36   | 1.91| 0.528| n/a  | n/a | n/a |

Abbreviations: FA, fractional anisotropy; AD, axial diffusivity; RD, radial diffusivity; MANCOVA, multivariate analysis of covariance; ANCOVA, analysis of covariance; SD, standard deviation.

* MANCOVA with group as between-subject factor, controlling for age. Corrected for multiple comparisons (Bonferroni).
† Follow-up ANCOVA with group as between-subject factor and with age, gender, IQ, verbal fluency, and memory performance as covariates. Only significant between-group effects in MANCOVA were further tested with ANCOVA.
‡ Degrees of freedom = 1, 30; Model adjusted R-squared = 0.20
§ Degrees of freedom = 1, 26; Model adjusted R-squared = 0.23
Schematic overview of region-of-interest (ROI) placement and tract reconstruction. For the cingulum bundle (CB), four ROI sets were strategically placed on color-by-orientation maps to reconstruct the dorsal and ventral portions of the CB in each hemisphere. The anterior dorsal ROI (#1) is outlined on three consecutive coronal slices in which the anterior commissure is most notable. The posterior dorsal ROI (#2) and posterior ventral ROI (#3) are outlined in the same view on two consecutive coronal slices at the posterior disjunction of the corpus callosum (CC) in the first two slices in which the CC appears disjoint. Finally, the first coronal slice showing the middle cerebellar peduncle was selected for outlining the anterior ventral ROI (#4).

Tractography was filtered through a midline exclusion region to exclude any interhemispheric fibers (mostly corpus callosum).

For the uncinate fasciculus (UF), two ROI sets were drawn on fractional anisotropy maps on a single coronal slice adjacent to the most anterior point of the fornix, and placed in the stem of the anterior temporal lobe and around the white matter of the anterior floor of the external/extreme capsule. By retaining only those tracts whose paths connected both regions of interest (the “AND” approach), we ensured that fibers were
selected that exclusively belonged to the UF. For the superior longitudinal fasciculus (SLF), the most dorsal part was identified in the axial view on color-by-orientation maps, usually one or two slices above the body of the corpus callosum. The SLF was then outlined from superior to inferior direction in approximately five consecutive axial slices.
Tractography results of the cingulum bundle (red color), uncinate fasciculus (green color), and superior longitudinal fasciculus (blue color) of a representative subject in this study.
Fractional anisotropy (FA) of the cingulum bundle (CB), uncinate fasciculus (UF), and superior longitudinal fasciculus (SLF) in non-diabetic healthy controls (white bars) and people with type 2 diabetes (black bars). Error bars represent Mean±SD. Compared to controls, people with type 2 diabetes showed lower fractional anisotropy in all tracts, statistically significant for the CB and UF corrected for multiple comparisons and adjusted for age, gender, IQ, verbal fluency, and memory performance. *p<0.05.
Correlation between fractional anisotropy (FA) of the cingulum bundle (CB) and strength of functional connectivity (β weight) between the medial frontal gyrus (MedFG) and the posterior cingulate cortex (PCC), components of the default-mode network. Groups are combined and each point represents one individual. Spearman’s $\rho=0.387$, $p=0.019$.  
403x292mm (72 x 72 DPI)