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Cerebral White Matter Integrity and Resting-State Functional Connectivity in Middle-aged Patients With Type 2 Diabetes

Early detection of brain abnormalities at the preclinical 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 preestablished altered resting-state functional connectivity in the default mode network (DMN). Diabetic and nondiabetic participants underwent diffusion tensor imaging, functional magnetic resonance imaging, and cognitive assessment. Multiple diffusion measures were calculated using streamline tractography, and correlations with DMN functional connectivity were determined. Diabetic patients showed lower fractional anisotropy (FA) (a measure of white matter integrity) in the cingulum bundle and uncinate fasciculus. Control subjects showed stronger functional connectivity than patients between the posterior cingulate and both left fusiform and medial frontal gyri. FA 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 DMN, suggesting that common mechanisms may underlie structural and functional connectivity. Detecting brain abnormalities in middle age enables implementation of therapies to slow progression of neuropathology.

Diabetes 2014;63:728–738 | DOI: 10.2337/db13-1219

COMPLICATIONS

Type 2 diabetes 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 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

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Received 8 August 2013 and accepted 25 October 2013.

brain regions most active during rest, are often associated with current and future cognitive decline. Reduced connectivity and hypometabolism in these regions have 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 (AD), which reflects axon integrity, and radial diffusivity (RD), 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). In addition, 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 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 fasciculi (UF) and superior longitudinal fasciculi (SLF), 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 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. After 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 database, and advertisements in local newspapers. The study included 18 individuals with type 2 diabetes and 19 nondiabetic healthy control subjects (Table 1). The 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-h oral glucose tolerance test (OGTT) glucose exceeded 200 mg/dL. All control subjects had a fasting glucose of $<$100 mg/dL and a 2-h OGTT glucose of $<$140 mg/dL. Participants were between the ages of 45 and 65 years (mean $\pm$ SD, 54.6 $\pm$ 6.4 years; disease duration, 10.5 $\pm$ 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 by age, education, gender, and BMI. We controlled for intelligence quotient (IQ) given its relationship to cognition.

Medical history and medication use were gathered from medical records and questionnaires. Chronic glycemic control was calculated by grouping HbA_{1c} results in 4-year increments and averaging the 4-year means (26). Exclusion criteria were *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 significantly affecting neurological function; *5*) history of substance abuse (including alcohol, excluding nicotine); *6*) left-handedness (excluding ambidexterity, determined by the Edinburgh Handedness Inventory [27]); and 7) BMI $>$40 kg/m^2. One control

Table 1—Demographic and clinical characteristics of the study sample

Characteristics	Type 2 diabetic subjects (n = 18)	Control subjects (n = 19)	P value
Age, years, mean (SD)	56.2 (6.3)	53.1 (6.2)	0.12
Education, years, mean (SD)	14.9 (2.2)	16.4 (2.9)	0.10
HbA_{1c}, %, mean (SD)	7.5 (1.8)	5.6 (0.3)	<0.001
HbA_{1c}, mmol/mol, mean (SD)	58 (19.7)	38 (3.3)	—
Lifetime HbA_{1c}, %, mean (SD)*	7.1 (1.3)	—	—
Fasting plasma glucose, mg/dL, mean (SD)	158 (89)	81 (7)	<0.001
Fasting serum insulin, μU/mL, mean (SD)	12.1 (9.7)	8.3 (8.1)	0.10
HOMA-IR, noninsulin users only, mean (SD)	4.7 (5.1)	1.7 (1.7)	0.03
Diabetes duration, years, mean (SD)	10.5 (6.9)	—	n/a
BMI, kg/m^2, mean (SD)	30.8 (4.9)	28.3 (4.0)	0.11
Blood pressure, mmHg, mean (SD)			
Systolic	128 (13)	120 (13)	0.07
Diastolic	72 (9)	72 (9)	0.71
Serum creatinine, mg/dL, mean (SD)	1.20 (0.93)	0.84 (0.27)	0.03
Cholesterol, mg/dL, mean (SD)			
Total	181 (29)	182 (38)	0.73
HDL	52 (16)	54 (15)	0.67
LDL	108 (28)	101 (32)	0.54
Triglycerides, mg/dL, mean (SD)	119 (42)	100 (82)	0.01
Blood pressure–lowering medications, n (%)	6 (33)	2 (11)	0.12
Cholesterol-lowering medications, n (%)	4 (22)	0 (0)	0.05
History of smoking, n (%)	10 (50)	9 (47)	0.75
Gender, n (%)			0.55
Female	7 (39)	8 (42)	
Male	11 (61)	11 (58)	
Race/ethnicity, n (%)			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)	

*Lifetime HbA_{1c} was obtained by calculating 4-year means starting with the date of diagnosis and averaging the 4-year means.

participant was eliminated after a structural brain abnormality was identified by the MRI technician and study radiologist (both blind to group status).

Eligible subjects visited the Joslin Diabetes Center after an 8–10 h fast, and patients who took 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. Only fasting laboratory values were assessed in type 2 diabetic patients, whereas control subjects also underwent a 2-h 75-g OGTT to determine glucose tolerance and insulin resistance. Insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR]) 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.

Cognitive Assessment

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

MRI Acquisition

Within 2 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). All subjects were screened for foreign

metal in their body, pacemakers, pregnancy, and claustrophobia before 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 0.9375 mm × 0.9375 mm × 2.6 mm (30 gradient directions at b = 1,000 s/mm^2, 5 baseline scans at b = 0 s/mm^2, repetition time = 16,000 ms, echo time = 84 ms, field-of-view = 24 cm, 256 × 256 matrix). The acquisition time was 9 min and 20 s.

fMRIs

fMRI parameters included gradient-echo planar sequence sensitive to blood oxygen level-dependent 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 mm × 3.125 mm in-plane resolution. 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, U.K., www.fmrib.ox.ac.uk/fsl) and Rician denoising (33). 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 (34). Figure 1 provides 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 with paths that 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 the 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 (35,36). Figure 2 shows the 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 (37) below 0.15 and rapid change of direction angle above 45°. Westin's linear measure, instead of FA, was used to terminate tractography to avoid the confounding effect of using the same measure to construct fiber tracts and to quantify their integrity. A length criterion was also used, 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 FA, trace (= mean diffusivity × 3), AD, and RD.

Tractography was conducted by multiple raters. On a random subset of 10 case subjects, excellent interrater reliability was established (left and right CB FA: intraclass correlation >0.92, $P < 0.001$).

fMRI Processing and Analysis

We assessed functional connectivity between the posterior cingulate cortex (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 (38). For DMN regions showing between-group differences in strength of connectivity to the PCC, we computed the correlation between β 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 exact tests or χ^2 tests as deemed appropriate.

To test for interhemispheric and intratract differences in the CB, a repeated-measures 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 the between-subject factor, hemisphere as the within-group factor, and age as a covariate. White matter tracts that showed no hemispheric or intratract 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 ANCOVA controlling for age. Multivariate analysis was also controlled for multiple

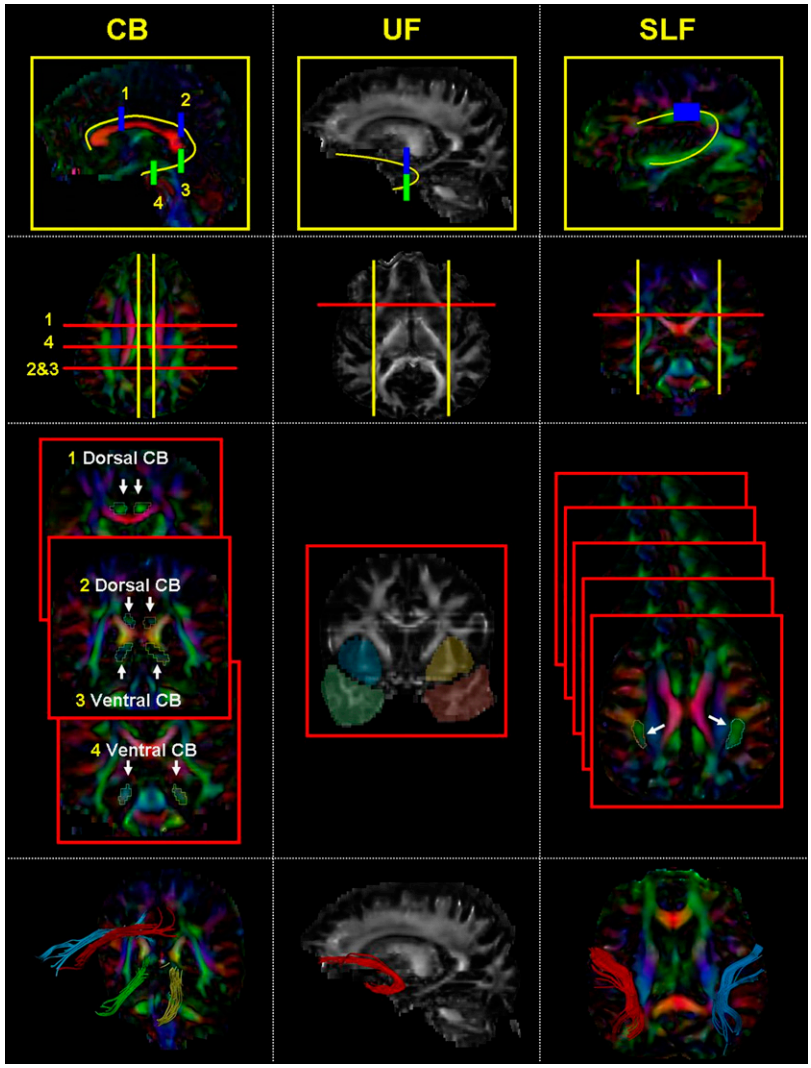

Figure 1—Schematic overview of ROI placement and tract reconstruction. For the CB, four ROI sets were strategically placed on color-by-orientation maps to reconstruct the dorsal and ventral portions of the CB in each hemisphere. The anterior dorsal ROI (#1) is outlined on three consecutive coronal slices 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 in the first two slices in which the corpus callosum appears disjointed. 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 UF, two ROI sets were drawn on FA 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 with paths that 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 the superior-to-inferior direction in approximately five consecutive axial slices.

comparisons with Bonferroni correction. Significant between-group differences in multivariate ANCOVA (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 β-weights). The linear relationships between diffusion measures and β-weights, fasting glucose, lifetime HbA_{1c}, 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

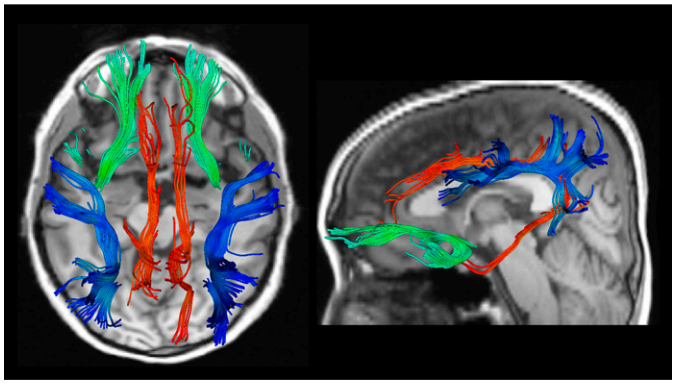

Figure 2—Tractography results of the CB (red color), UF (green color), and SLF (blue color) of a representative subject in this study.

triglycerides (P = 0.01) and creatinine (P = 0.03) were higher in type 2 diabetic patients. As expected, HbA_{1c} ($P < 0.001$), 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.

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 Rey Auditory Verbal Learning Test 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 HbA_{1c} were not significantly correlated with performance on any cognitive test.

fMRI Results

When both groups were combined, the following regions were functionally connected to the PCC ($\beta > 0$, $P < 0.05$, corrected for multiple comparisons) (39): 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 (40). 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: −37, −77, −12; and −49, −65, −12; $P < 0.02$), which is often considered part of the DMN (41,42) 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 ($P < 0.10$).

DTI Results: Effects of Hemisphere and CB Subdivision

After 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 CB was further tested for

Table 2—Cognitive scores and depressive symptoms in this study sample

	Type 2 diabetic subjects (n = 18)		Control subjects (n = 19)		
	Mean	SD	Mean	SD	P value
WASI full-scale IQ	104.8	13.0	113.6	11.4	0.04
D-KEFS					
Verbal fluency, scaled	10.5	3.1	12.9	2.9	0.02
Trail making number-letter switching, scaled	9.2	3.8	11.0	2.8	0.11
RAVLT					
Immediate recall T score	46.8	10.7	53.3	10.4	0.05
Delayed recall T score	49.0	9.3	51.6	9.6	0.41
Grooved Pegboard, dominant hand, time, s	90.0	21.8	81.7	11.9	0.16
Hamilton Depression Rating Scale	5.0	4.0	2.9	4.0	0.14

D-KEFS, Delis-Kaplan Executive Function System; RAVLT, Rey Auditory Verbal Learning Test; WASI, Wechsler Abbreviated Scale of Intelligence.

intratract 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. Because no interhemispheric or intratract differences in FA were found for any tract, 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 Fig. 3 and Table 3. After 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 with control subjects. 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 in the 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 with control subjects for the CB (F = 4.76; df = 1, 30; P = 0.037; adjusted-model R^2 = 0.20) and UF (F = 4.76; df = 1, 26; P = 0.038; adjusted-model R^2 = 0.23).

Of further note, the number of fibers or total tract volume did not differ between groups for any white matter tract ($P > 0.05$), but the average fiber length of each tract studied approached statistical significance for being shorter in diabetic patients than in 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).

Correlational Analyses

Correlations were evaluated between DTI measures and β-weights representing functional connectivity between the PCC and other DMN regions. Importantly, CB FA was correlated with the β-weight between the PCC and the left medial frontal gyrus (ρ = 0.38, P = 0.02) for combined groups (Fig. 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 HbA_{1c} and UF FA in diabetic patients (ρ = 0.569, P = 0.034) and control subjects (ρ = 0.541, P = 0.020). In diabetic patients and control subjects, lower UF FA was associated with slowing of information processing speed (Grooved Pegboard time − dominant hand) (patients: ρ = −0.587, P = 0.027; control subjects: ρ = −0.486, P = 0.035). Among diabetic patients, but not control subjects, 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 CB or UF FA and lifetime HbA_{1c} ($P > 0.246$). However, when both groups were combined, fasting glucose was negatively correlated with CB FA (ρ = −0.362, P = 0.028) but not with UF FA (P = 0.493).

DISCUSSION

We investigated a relationship between structural and functional connectivity in type 2 diabetic patients and

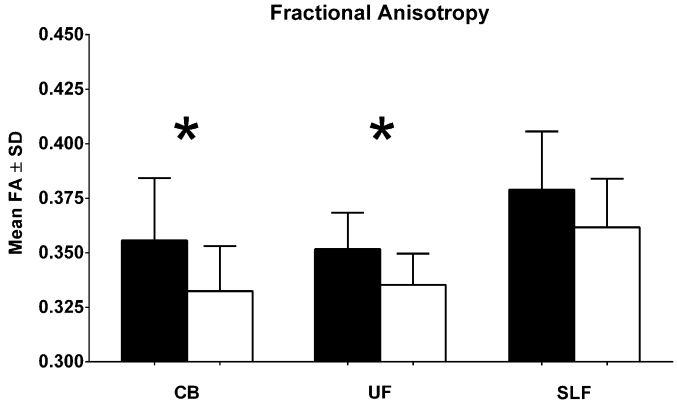

Figure 3—FA of the CB, UF, and SLF in people with type 2 diabetes (□) and nondiabetic healthy control subjects (■). The error bars represent mean ± SD. Compared with control subjects, people with type 2 diabetes showed lower FA in all tracts; statistically significant for the CB and UF corrected for multiple comparisons and adjusted for age, gender, IQ, verbal fluency, and memory performance. *$P < 0.05$.

Table 3—DTI values for the CB, UF, and SLF in people with type 2 diabetes and healthy control subjects

	Type 2 diabetic subjects		Control subjects		Statistical analyses			
					MANCOVA*		ANCOVA†	
	Mean	SD	Mean	SD	*F*	Adjusted *P*	*F*	Adjusted *P*
CB								
FA	0.332	0.021	0.356	0.029	7.76	0.027	4.76	0.037‡
Trace, $\times 10^3$ μm^2/ms	2.47	0.09	2.46	0.11	0.02	1.00	n/a	n/a
AD, $\times 10^3$ μm^2/ms	1,127	36	1,153	43	4.95	0.099	n/a	n/a
RD, $\times 10^3$ μm^2/ms	669	32	654	41	1.04	0.948	n/a	n/a
UF								
FA	0.335	0.014	0.352	0.017	7.89	0.027	4.76	0.038§
Trace, $\times 10^3$ μm^2/ms	2.65	0.10	2.62	0.08	0.53	1.00	n/a	n/a
AD, $\times 10^3$ μm^2/ms	1,220	41	1,226	33	0.18	1.00	n/a	n/a
RD, $\times 10^3$ μm^2/ms	714	32	698	28	1.86	0.549	n/a	n/a
SLF								
FA	0.362	0.022	0.379	0.027	4.15	0.150	n/a	n/a
Trace, $\times 10^3$ μm^2/ms	2.38	0.11	2.35	0.09	0.76	1.00	n/a	n/a
AD, $\times 10^3$ μm^2/ms	1,117	46	1,119	31	0.09	1.00	n/a	n/a
RD, $\times 10^3$ μm^2/ms	632	36	614	36	1.91	0.528	n/a	n/a

MANCOVA, multivariate ANCOVA; n/a, not applicable. *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. ‡df = 1, 30; model-adjusted R^2 = 0.20. §df = 1, 26; model-adjusted R^2 = 0.23.

control subjects. 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, because it suggests that therapies that improve one modality (i.e., structure or function) may affect the other as well. For example, it is possible that cognitive training, which improves FA (43), may also improve functional connectivity. Further, combined data obtained from structural and functional measures have been used to more accurately classify people with and without dementia than either variable used alone (8).

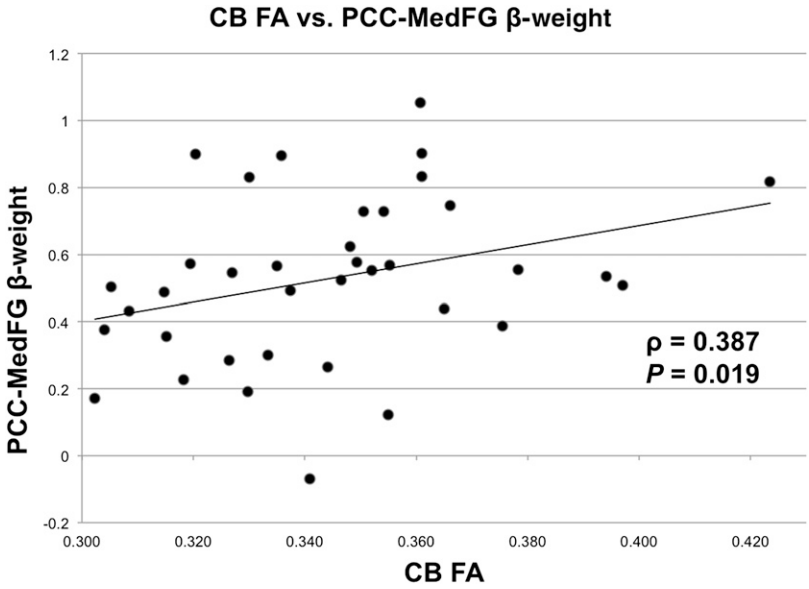

Figure 4—Correlation between FA of the CB and strength of functional connectivity (β weight) between the medial frontal gyrus (MedFG) and the PCC, components of the DMN. Groups are combined, and each point represents one individual.

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 with nondiabetic 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 (4,44). 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 [45]). Consistent with our FA findings, previous reports have found reduced FA in type 2 diabetes in various brain regions, including frontotemporal 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 (46). The current 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 comorbidities. This finding is of great clinical significance, because 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 myelin or axon pathology in type 2 diabetes. These methods are new and not fully understood; thus, whether microstructural white matter abnormalities in type 2 diabetes reflect myelin or axon pathology remains unclear. 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 (47). In the current study, we found similar correlations between various clinical variables (creatinine, BMI, and HbA_{1c}) 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 HbA_{1c}. Although this was unexpected, we previously reported that current HbA_{1c} levels in type 1 diabetic patients in poor control were unrelated to brain glutamate/glutamine levels (48). Thus, current HbA_{1c} 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 control subjects on most clinical and demographic variables, there were some variables in which the diabetic patients had worse profiles than the control subjects. Thus, certain clinical variables, such as hypertension, may also contribute to the brain differences observed in this study (49). Additional studies are needed to investigate the effects of long-term glucose control and the 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 ROIs, which is prone to anatomical misplacement. However, we believe these were kept to a minimum because excellent white matter atlases (34) were used by highly trained tracers blind to group, and interrater 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 to

prevent or reduce cognitive decline should be further explored.

Acknowledgments. The authors thank the study participants for their contribution to the study; Fotini Kourtelidis and Mike Amico for their help in scanning patients at Beth Israel Deaconess Medical Center throughout this research study; David Alsop, Director of MR Research at Beth Israel Deaconess Medical Center, for his advice on the DTI scan sequence in this imaging study; the research assistants at the Psychiatry Neuroimaging Laboratory (PNL) at Brigham and Women's Hospital for their help with DTI tractography; and Ryan Eckbo at the PNL for his technical assistance with the image postprocessing. The authors also thank the Specialized Assay Core at the Joslin Diabetes Center.

Funding. This study was supported in part by National Institutes of Health grants 5R01-AG-034165-A2 (G.M.) and P30-DK-36836 (to the Joslin Diabetes Research Center, Genetics Core) and by the Herbert Graetz Fund.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. W.S.H. and G.M. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript. T.J.M., V.L.F., 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.

Prior Presentation. Parts of this study were presented in abstract form at the 1st Annual Brigham and Women's Hospital Research Day, Boston, MA, 15 November 2012; at the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, PA, 8–12 June 2012; and at the 19th Annual Meeting of the Cognitive Neuroscience Society, Chicago, IL, 31 March–3 April 2012.

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