

# Altered Hippocampal White Matter Connectivity in Type 2 Diabetes Mellitus and Memory Decrements

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## ORIGINAL ARTICLE

## Altered Hippocampal White Matter Connectivity in Type 2 Diabetes Mellitus and Memory Decrements

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Type 2 diabetes mellitus is associated with cognitive decrements. Specifically affected cognitive domains are learning and memory, for which the hippocampus plays an essential role. The pathophysiological mechanism remains to be revealed. The present study examined whether local hippocampal microstructure and white matter connectivity are related to type 2 diabetes and memory performance. Forty participants with type 2 diabetes and 38 participants without type 2 diabetes underwent detailed cognitive assessment and 3-Tesla diffusion magnetic resonance imaging (MRI). Diffusion MRI was performed to assess microstructure (fractional anisotropy and mean diffusivity) and white matter connectivity (tract volume) of the hippocampus, which were compared between participants with and without type 2 diabetes. No differences in hippocampal microstructure were observed. Participants with type 2 diabetes had fewer white matter connections between the hippocampus and frontal lobe ( $P = 0.017$ ). Participants who scored lower on memory function, regardless of type 2 diabetes, had fewer white matter connections between the hippocampus and temporal lobe ( $P = 0.017$ ). Taken together, type 2 diabetes and memory decrements appear to be associated with altered hippocampal white matter connectivity.

**Key words:** cognition, memory, diffusion, white matter connectivity, hippocampus

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Type 2 diabetes mellitus is associated with cognitive decrements (1), accelerated cognitive decline (2) and an increased risk for developing dementia and Alzheimer's disease (2–5). In particular, learning and memory are the most prominently and specifically affected cognitive domains (6,7). It is well known that the hippocampus plays an essential role in learning and memory processes. Previous studies, which focused on conventional structural magnetic resonance imaging (MRI), demonstrated hippocampal atrophy in type 2 diabetes patients with memory problems (8–10). Using functional MRI, Zhou *et al.* (11) showed reduced functional connectivity between the hippocampus and other parts (i.e. frontal, temporal and parietal) of the brain. Therefore, there is a need to investigate whether the intrinsic hippocampal microstructure and the white matter connectivity to other brain regions are affected in type 2 diabetes and related to memory decrements.

Diffusion MRI (dMRI) is a non-invasive advanced MRI technique that provides greater insights into cerebral white matter abnormalities (i.e. microstructure and white matter connectivity) by measuring the hindered diffusion of water molecules. The most commonly used diffusion measures are: (i) fractional anisotropy (FA), which describes the preferred diffusion directionality of water molecules and alterations in the microstructural organisation of the white matter, and (ii) mean diffusivity (MD), which represents the mean magnitude of water diffusivity and reflects tissue density (12,13). In addition, the spatial organisation of white matter fibre bundles (i.e. tract volume) between brain regions can be derived from the directional information of the diffusing water molecules. Note that the intrinsic microstructure (FA and MD) and white matter connectivity measures are not completely independent properties. For example, locally increased FA focuses the directionality of white matter tracts

and might therefore influence the connectivity. However, because the two concepts describe different aspects of microstructure, it is relevant to report on both.

Previous dMRI studies on type 2 diabetes demonstrated reduced FA and increased MD in different brain regions (14–16) or in white matter tracts connecting frontal, parietal and temporal regions (17). Other type 2 diabetes studies have related increased MD in the parahippocampal gyrus to lower memory performance (18) and reduced FA in the cingulum bundle to higher memory performance (19). To identify microstructural correlates of cognitive decrements, it is important to focus on white matter connectivity because cognitive function depends on the transfer of information between different brain regions via white matter fibre bundles. Besides the commonly used local diffusion measures of specific white matter fibre bundles, it is important to investigate whether white matter connectivity (i.e. total tract volume of white matter fibres) differs and correlates with cognitive performance in type 2 diabetes.

To the best of our knowledge, there are no type 2 diabetes studies that focus specifically on the hippocampus and memory performance using (hippocampal) white matter connections. Therefore, the present study aimed to examine whether hippocampal microstructural abnormalities and white matter connections are related to type 2 diabetes and memory performance.

## Materials and methods

### Study population

Forty-seven participants with type 2 diabetes and 41 participants without type 2 diabetes were recruited from the first 866 participants of The Maastricht Study for additional brain MRI measurements. The Maastricht Study is an ongoing observational prospective population-based cohort study that focuses on the aetiology, pathophysiology, complications and comorbidities of type 2 diabetes. Participants are aged between 40–75 years and live in the southern part of The Netherlands (20). Participants are considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication, or if they have a fasting blood glucose  $\geq 7.0$  mmol/l and/or a 2-h blood glucose  $\geq 11.1$  mmol/l after an oral glucose tolerance test. Participants without type 2 diabetes are characterised by fasting blood glucose  $< 6.1$  mmol/l and a 2-h blood glucose  $< 7.8$  mmol/l. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements and blood sampling. A detailed overview of all procedures is provided in Schram *et al.* (20). After the baseline measurements of The Maastricht Study, participants were invited to participate in the present MRI study.

Participants with the highest and lowest cognitive scores were selected from the first 866 participants to increase the probability of finding MRI differences associated with cognitive decrements (Table 1). A detailed selection procedure is provided in van Bussel *et al.* (21). In brief, the division of participants in a low and high cognition group was based on a cumulative score of three neuropsychological tests covering the domains of verbal memory, attention and flexibility, and executive functioning (Table 1). Scores were adjusted for age, sex and education level using linear regression. Exclusion criteria for participants were: (i) a known history of stroke or neurological disease; (ii) a time span between enrollment in The Maastricht Study and MRI  $> 1.5$  years; (iii) incomplete cognitive assessments; (iv) type 1 diabetes mellitus; (v) an impaired fasting blood glucose level, in participants without type 2 diabetes; (vi) mild cognitive impairment or dementia; (vii) the metabolic syndrome; (viii) colour blindness; and (ix) unknown diabetes status. The

**Table 1.** Characteristics of the Two Cognition Groups.<sup>a</sup>

	Lower cognition (n = 40)	Higher cognition (n = 38)	P-value
Type 2 diabetes (%), n	55.0 (n = 22)	47.4 (n = 18)	0.500 <sup>b</sup>
Age (years)	61.1 $\pm$ 9.5	62.7 $\pm$ 6.7	0.367
Sex (male, %), n	57.5 (n = 23)	52.6 (n = 20)	0.666 <sup>b</sup>
Education			0.769 <sup>b</sup>
Low (%), n	15.0 (n = 6)	21.1 (n = 8)	
Middle (%), n	47.5 (n = 19)	42.1 (n = 16)	
High (%), n	37.5 (n = 15)	36.8 (n = 14)	
WLT total score	37.1 $\pm$ 10.0	50.1 $\pm$ 9.0	$< 0.001$
Stroop (s)	63.3 $\pm$ 35.2	34.9 $\pm$ 13.1	$< 0.001$
Verbal fluency	20.3 $\pm$ 4.9	27.3 $\pm$ 5.7	$< 0.001$
Cumulative cognition score	-2.30 $\pm$ 2.18	2.10 $\pm$ 1.25	$< 0.001$

WLT, (verbal memory) Word Learning Test. <sup>a</sup>Only participants who were included in the final analysis; independent samples t-test. <sup>b</sup>Pearson's chi-squared test.

low and high cognition groups were matched on age, sex and education level, and display a similar distribution of participants with and without type 2 diabetes (Table 1).

After taken into account those individuals who declined the invitation and exclusion of participants with MRI contraindications, a total of 47 and 41 participants with and without type 2 diabetes were included, respectively.

Prior to MRI, these participants underwent a general cognitive function test [Mini-Mental State Examination, MMSE (22)] to assess clinically significant differences in cognitive performance compared to the baseline cognitive tests at enrollment in The Maastricht Study. Structural and dMRI brain scans were obtained from all participants. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), The Netherlands, and all participants provided their written informed consent. The study is registered at: <http://www.clinicaltrials.gov> (with identifier NCT01705210).

### MRI

MRI data were acquired on a 3T scanner (Achieva TX; Philips Healthcare, Best, The Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation [including T1-, T2-, T2\*-weighted and fluid attenuated inversion recovery (FLAIR) sequences] and high angular resolution diffusion imaging (HARDI). A three-dimensional T1-weighted (T1) fast field echo sequence (TR/TE 8.1/3.7 ms, 1.00 mm isotropic voxel size, 170 continuous slices, matrix size of 240  $\times$  240; 7 min 56 s acquisition time) and FLAIR (TR/TE/TI 4800/276/1650 ms, 1.12 mm isotropic voxel size, matrix size of 224  $\times$  224; 4 min 53 s acquisition time) were acquired. HARDI data were obtained using an echo-planar imaging (EPI) sequence (TR/TE 6980/84 ms, 2.4 mm isotropic voxel size, 128 diffusion sensitising gradient directions, a *b*-value of 1500 s/mm<sup>2</sup>; 15 min 56 s acquisition time). In addition, a single minimally diffusion-weighted image (b0-scan) was acquired (23).

### Data analysis

The T1 images were automatically segmented to obtain both hippocampal volumes, intracranial volume, subcortical grey matter and the cortical areas using *Freesurfer* (Martinos Center for Biomedical Imaging, Boston, MA, USA) (24) and the segmentations were inspected visually. dMRI data analysis

(preprocessing, tractography, connectivity analyses) was performed with the diffusion MR toolbox *EXPLOREDTI*, version 4.8.2 (25). In brief, the preprocessing steps included: (i) visual image quality assessment; (ii) correction of dMRI images for eddy current induced geometric distortions and head motion; (iii) correction of dMRI images for EPI distortions and transformation to T1 space; and, finally, (iv) estimation of the diffusion tensor for calculating the FA and MD maps.

After preprocessing, the local diffusion measures (FA and MD) were extracted from both hippocampi as derived from *FREESURFER*. Subsequently, fibre orientation distributions (FOD) were estimated using constrained spherical deconvolution with a maximum harmonic degree of 8 (26), which allows fibre tracking through regions with crossing fibres. The FOD represents the local fibre orientation. Whole brain probabilistic tractography was performed using FOD sampling (27) with a seed point resolution of 1 mm<sup>3</sup>, a step size of 1 mm, and an FOD and maximum deflection angle threshold of 0.1 and 30°, respectively, yielding approximately 4.3 million streamlines for each dataset. Next, connectivity analysis was performed to obtain white matter tracts (tract volumes) from the individually segmented hippocampi, used as seed region (i.e. include tracts that only go through the hippocampus) to the segmented grey matter. The segmented grey matter was subdivided into five regions: frontal lobe, parietal lobe, temporal lobe, occipital lobe and sub-cortical grey matter (28,29). Examples of white matter tracts between both hippocampi and the frontal lobe are provided in Fig. 1. Subsequently, the tract volumes seeded from both hippocampi to each region were normalised to the intracranial volume to reduce inter-individual variation (30). A previous study from our group showed that white matter tracts (tract volumes) are reproducible (31). The local diffusion measures (FA and MD) were averaged and tract volumes were added up over the left and right hippocampus, respectively. Tract volumes are interpreted as a measure for connectivity (32).

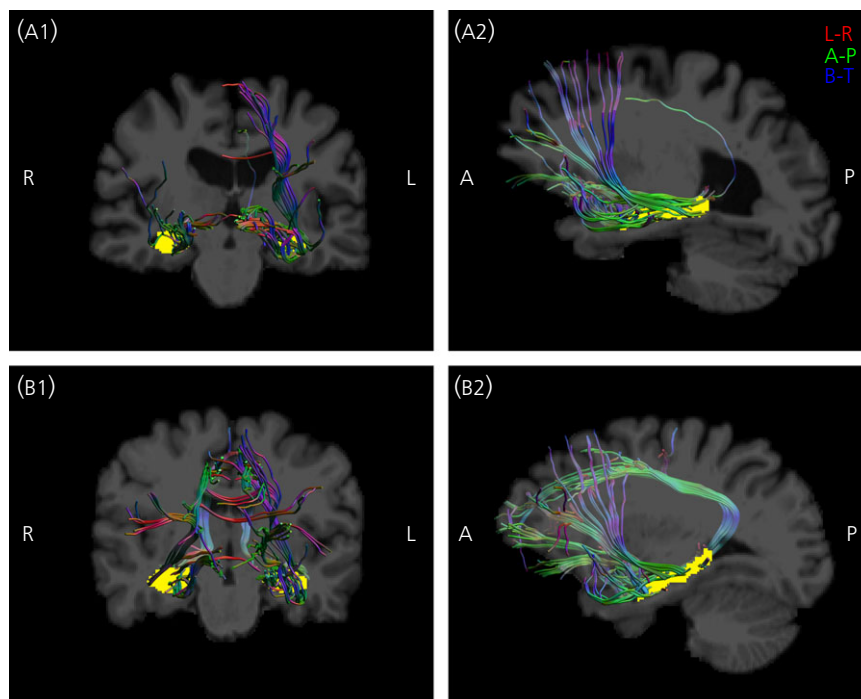
After careful analyses, data from forty type 2 diabetes participants and 38 participants without type 2 diabetes remained suitable for final analysis. Data from nine participants were excluded as a result of incomplete data ( $n = 1$ ), claustrophobia ( $n = 2$ ), nondiabetes participants with impaired fasting blood glucose levels ( $n = 2$ ), Parkinsonism ( $n = 1$ ), brain injury because of an accident ( $n = 1$ ), an incidental finding (i.e. tumour,  $n = 1$ ) and susceptibility artefacts ( $n = 2$ ).

## Cognition

As described in Schram *et al.* (20), participants completed an extensive cognitive battery at enrollment in The Maastricht Study. Global cognitive functioning was measured using the MMSE. Verbal memory was assessed by the 15-Word Learning Test (WLT) total score, in which 15 words are presented in five subsequent trials. Immediately after each trial, participants recall as many words as they are able. The maximum score that could be reached, was 75.

## Covariates

Educational level was assessed by interview and classified into eight levels commonly used in The Netherlands: (1) no education; (2) primary education; (3) lower vocational education; (4) intermediate general secondary education; (5) intermediate vocational education; (6) higher general secondary education; (7) higher vocational education; and (8) university degree. For the present study, educational level was subdivided into three groups: low (level 1–3), middle (level 4–6) and high (level 7–8). Office blood pressure was assessed three times on the right arm after a 10-min rest, using a non-invasive blood pressure monitor (Omron 705IT; Omron, Kyoto, Japan). A



**Fig. 1.** White matter fibre bundles seeded from both hippocampi (yellow regions of interest) to the frontal lobe of representative participants with (A) and without (B) type 2 diabetes (A1 and B1, coronal view; A2 and B2, sagittal view), projected on a semitransparent T1-weighted image. The colours of the tracts represent the left–right (red), anterior–posterior (green) and bottom–top (blue) directions. Depicted coronal and sagittal levels are purely for illustrative purposes; quantitative analyses was performed independent of the visual presentation selected.

fourth measurement was performed when the difference between measurement two and three exceeds more than 10 mmHg. Here, we used the averaged blood pressure values over all the available measurements (20). White matter lesions were automatically segmented using T1 and FLAIR in accordance with the method described by de Boer *et al.* (33).

### Statistical analysis

Descriptive participants' characteristics and diffusion measures are reported as the mean  $\pm$  SD. Group characteristics were tested using independent samples t-tests and Pearson's chi-squared tests using SPSS, version 20 (IBM Corp., Armonk, NY, USA).

Linear regression analyses, adjusted for age, sex, education level, body mass index, systolic blood pressure, relative (to intracranial) hippocampal volume and relative white matter lesion load to correct for differences in clinical characteristics between groups, were performed to assess the association of the hippocampal diffusion measures (FA, MD and tract volumes) with type 2 diabetes status and memory performance. In addition, a correction for multiple testing was applied according to Benjamini and Hochberg (34) using a false discovery rate of 10%.

To investigate the combined effect of type 2 diabetes and memory on the diffusion measures, an interaction term between type 2 diabetes and memory performance (WLT total score) was added to the same linear regression model. The use of continuous fasting blood glucose and HbA<sub>1c</sub> values instead of the dichotomous diabetes status was also tested.

### Results

Table 1 shows the baseline characteristics of the low and high cognition groups because participants were selected based on cognitive status. The groups were matched on age, sex and education and participants with type 2 diabetes were divided equally over the two groups. Table 2 shows the clinical characteristics of participants with type 2 diabetes. Type 2 diabetes participants had higher fasting blood glucose levels, higher HbA<sub>1c</sub> levels, higher body mass index, higher diastolic and systolic blood pressure, and larger white matter lesion loads, compared to healthy controls (Table 2). With respect to cognition, type 2 diabetes participants scored significantly lower on the WLT total score ( $P = 0.004$ ) and on baseline MMSE score ( $P = 0.008$ ) compared to nondiabetes participants. Baseline and repeated MMSE did not differ between participants with and without type 2 diabetes ( $P = 0.317$ ).

For the hippocampus, the local FA was decreased in type 2 diabetes compared to nondiabetes participants ( $0.11 \pm 0.01$  and  $0.12 \pm 0.02$ , respectively;  $P = 0.033$ ), whereas the MD was increased in type 2 diabetes participants compared to nondiabetes participants ( $0.98 \pm 0.06$  and  $0.93 \pm 0.05 \times 10^{-3} \text{ mm}^2/\text{s}$ , respectively;  $P < 0.001$ ). However, after adjustment for covariates, multivariable linear regression revealed no significant associations between any of the local diffusion measures and type 2 diabetes or memory performance.

Figure 2 shows quantitative boxplots of the relative tract volumes seeded from both hippocampi to the various brain regions. Tract volumes from the hippocampi to the frontal lobe ( $P = 0.005$ ), temporal lobe ( $P = 0.010$ ) and subcortical grey matter ( $P = 0.031$ ) were decreased in type 2 diabetes participants. After adjustment for covariates, multivariable linear regression (Fig. 2 and Table 3) revealed a decreased relative tract volume from the hippocampi to

**Table 2.** Clinical Characteristics of Participants with Type 2 Diabetes.

	Participants with type 2 diabetes (n = 40)	Participants without type 2 diabetes (n = 38)	P-value
Type 2 diabetes-related variables			
Duration of diabetes (years)	9.9 $\pm$ 6.8	–	
Fasting blood glucose (mmol/l)	7.5 $\pm$ 1.2	5.1 $\pm$ 0.3	< 0.001
HbA <sub>1c</sub> (%)	6.7 $\pm$ 0.5	5.6 $\pm$ 0.4	< 0.001
HbA <sub>1c</sub> (mmol/mol)	50.3 $\pm$ 4.9	38.1 $\pm$ 4.4	< 0.001
Type 2 diabetes medication			
None (%)	12.5	100	< 0.001 <sup>a</sup>
Oral medication (%)	75.0	–	
Insulin (%)	2.5	–	
Insulin and oral medication (%)	10.0	–	
Clinical variables			
BMI (kg/m <sup>2</sup> )	29.2 $\pm$ 3.5	24.7 $\pm$ 2.9	< 0.001
SBP (mmHg)	152 $\pm$ 18	131 $\pm$ 18	< 0.001
DBP (mmHg)	83 $\pm$ 11	76 $\pm$ 13	0.014
White matter lesion volume (cm <sup>3</sup> )	6.0 $\pm$ 10.5	2.0 $\pm$ 1.5	0.021
Left hippocampal volume (cm <sup>3</sup> )	3.8 $\pm$ 0.5	4.0 $\pm$ 0.4	0.076
Right hippocampal volume (cm <sup>3</sup> )	4.0 $\pm$ 0.5	4.1 $\pm$ 0.4	0.332
Cognitive score			
Baseline MMSE total score	28.6 $\pm$ 1.5	29.4 $\pm$ 0.9	0.008
WLT total score	39.8 $\pm$ 10.8	47.2 $\pm$ 11.1	0.004

HbA<sub>1c</sub>, glycated haemoglobin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; (baseline/repeated) MMSE, (The Maastricht Study/before MRI) Mini-Mental State Examination; WLT, (verbal memory) Word Learning Test. Data are the mean  $\pm$  SD. Independent samples t-test. <sup>a</sup>Pearson's chi-squared test.

the frontal lobe in type 2 diabetes participants ( $P = 0.017$ , which remains significant after correction for multiple testing). For participants (regardless of type 2 diabetes) who scored lower on memory performance, multivariable linear regression (Table 3) revealed a decreased relative tract volume to the temporal lobe ( $P = 0.017$ , which remains significant after correction for multiple testing).

Additional multivariable linear regression including the interaction term (type 2 diabetes times WLT total score) showed no significant interaction ( $P > 0.15$ ). Analyses with fasting blood glucose levels and HbA<sub>1c</sub> both showed a trend: high fasting blood glucose levels and high HbA<sub>1c</sub> levels are associated with a decreased relative tract volume from the hippocampi to the frontal lobe ( $P = 0.069$  and  $P = 0.081$ , respectively).

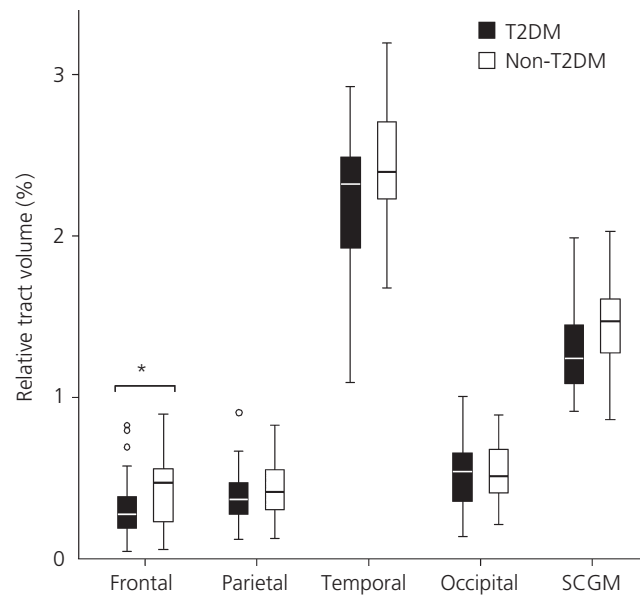
### Discussion

The present study investigated whether hippocampal microstructural abnormalities are related to type 2 diabetes and verbal memory. To our knowledge, this is the first study to investigate white matter connections from the hippocampus to different brain lobes in type 2 diabetes. The results of the present study demonstrate fewer white matter connections to the frontal lobe in participants with type 2 diabetes compared to nondiabetic participants. For



participants who scored relatively low on memory performance, we observed fewer white matter connections to the temporal lobe.

In the present study, participants with type 2 diabetes revealed decreased white matter connections between the hippocampi and the frontal lobe. As a result of decreased white matter connectivity, the white matter appears to be less well organised to transfer and integrate information in participants with type 2 diabetes. Therefore, the affected white matter connectivity, rather than the intrinsic microstructure of the tracts in participants with type 2 diabetes, might underlie the memory decrements. The fact that the frontotemporal connection was affected is in close agreement with the type of cognitive decrements (i.e. memory, executive functioning,



**Fig. 2.** Boxplots of the relative hippocampal tract volumes to the frontal lobe, parietal lobe, temporal lobe, occipital lobe and subcortical grey matter (SCGM) between participants with and without type 2 diabetes. \*Tract volumes were significantly different between groups after multivariable linear regression analysis ( $P < 0.05$ ) (Table 3). T2DM, type 2 diabetes mellitus.

processing speed) in type 2 diabetes (8), as well as previous studies reporting frontal and/or temporal structural alterations (11,35).

Only two other studies (17,19) have previously investigated white matter connectivity (tract volumes) in participants with type 2 diabetes and focused, not on the hippocampal, but other white matter tracts (including the superior longitudinal fasciculus and the uncinate fasciculus), for which they did not observe any differences in volume. Hoogenboom *et al.* (19) related cognitive decline to reduced FA of the uncinate fasciculus bundle. Reijmer *et al.* (17) showed microstructural abnormalities in specific white matter bundles. Another study by Reijmer *et al.* (36) observed disruptions of the more global white matter network in patients with type 2 diabetes, which was related to cognitive decline. The decreases in hippocampal white matter connections in type 2 diabetes observed in the present study likely contribute to those global network disruptions.

For participants who scored relatively low on memory performance, we observed fewer hippocampal white matter connections to the temporal lobe. The temporal lobe plays an important role in memory and our results potentially indicate that there is less optimal transfer and integration of information between the hippocampus and the temporal lobe, which could play a role in the underlying memory decrements. The involvement of the temporal lobe in memory performance that we observed is in line with studies by Yau *et al.* (18,37), who showed that white matter abnormalities in the temporal stem (decreased FA) and parahippocampal gyrus (increased MD) explained the lower memory performance in type 2 diabetes. It was concluded that type 2 diabetes has a deleterious effect on the vulnerability of the temporal lobe memory networks (37), especially the hippocampus and parahippocampal gyrus (18).

The intrinsic microstructure measures (FA and MD) were not different between participants with and without type 2 diabetes or were not associated with memory performance after controlling for the study characteristics. Falvey *et al.* (15) observed increased MD for type 2 diabetes in both hippocampi after controlling for age, sex and race. When using the same statistical model as Falvey *et al.*

**Table 3.** Relationship Between Relative (to Intracranial) White Matter Connections (Tract Volumes) Seeded from Both Hippocampi and Type 2 Diabetes Status and Memory Performance.

Relative tract volumes	Hippocampus					
	Type 2 diabetes status			Memory performance		
	$\beta$	95% CI	P-value	$\beta$	95% CI	P-value
To frontal lobe	-0.720	-1.310 to -0.130	0.017*	-0.105	-0.356 to 0.146	0.408
To parietal lobe	-0.517	-1.124 to 0.089	0.093	-0.268	-0.527 to -0.010	0.042
To temporal lobe	0.118	-0.332 to 0.567	0.603	0.235	0.043 to 0.427	0.017*
To occipital lobe	0.022	-0.549 to 0.594	0.938	-0.137	-0.380 to 0.107	0.267
To SCGM	-0.024	-0.487 to 0.438	0.916	-0.081	-0.278 to 0.116	0.416

SCGM, subcortical grey matter. Standardised  $\beta$  [95% confidence interval (CI)] indicates increments/decrements of the tract volumes with type 2 diabetes status or memory performance. Model: adjusted for age, sex, education level, body mass index, systolic blood pressure, corresponding relative (to intracranial) hippocampal volume and relative (to intracranial) white matter lesion volume. \*Significant after correction for multiple comparisons.

(15), we observed similar results (i.e. increased hippocampal MD in participants with type 2 diabetes;  $P = 0.014$ ), which illustrates the importance of the inclusion of clinical characteristics as covariates to prevent superfluous results. Previously, in the same population, we showed an association between increased hippocampal MD and memory performance, but not specifically for type 2 diabetes, for which we found differences in microvasculature (21). In that study, we applied intravoxel incoherent motion imaging, another diffusion technique, which distinguishes water diffusion of the intrinsic microstructure (parenchyma) from that of the microvasculature. Such a distinction cannot be made using standard dMRI. Other studies have also reported local microstructural white matter abnormalities in adolescents with type 2 diabetes (16) and in patients with type 2 diabetes (14); however, these results were not specific to the hippocampus. Other nondiabetic studies, specific to the hippocampus, have shown associations between higher hippocampal MD and memory performance in nondemented participants and in elderly participants with cerebral small vessel disease, respectively (38,39).

In the present study, type 2 diabetes participants scored lower on memory performance and had less hippocampal white matter connectivity to the frontal lobe. However, we did not observe a synergistic effect (interaction) of type 2 diabetes and memory decrements on the white matter connectivity between the hippocampus and frontal lobe. This might be attributable to the relatively healthy diabetes population engaged in the present study, which showed no obvious abnormalities or volume reductions of the hippocampus (Table 2) and was only mildly affected in terms of memory performance, and may be under good treatment control regarding glucose levels. Moreover, the memory scores of participants with type 2 diabetes were in the range of normal performance (40) and hence potentially not sufficiently strong to detect a synergistic effect of type 2 diabetes and memory decrements on the white matter connectivity between the hippocampus and the frontal lobe. In addition, it might be that other connections, either involving the hippocampus or not, are related to memory performance, although these connections were not considered in the present study. The observed effect might therefore represent relatively early signs of developing brain abnormalities related to type 2 diabetes and decrements in cognition.

The strengths of the present study include: first, the extensive characterisation of the participants. Second, both hippocampi that were used as seed for the connectivity analyses and the cortical areas were automatically (and thus operator-independent) parcellated with *FREESURFER*, which reduces the risk and variability of anatomical misplacements. Third, the scan protocol included high quality structural and dMRI data. The dMRI data were obtained using a high number (128) different gradient directions. Fourth, whole brain tractography yielded approximately 4.3 million tracts, which were used for the connectivity analyses from the hippocampus to the rest of the brain.

A number of issues limit the conclusions. First, the study had a cross-sectional design. Therefore, the results should be interpreted cautiously. Nevertheless, the initial results are promising and open directions for future (longitudinal) studies. Second, the time span

between enrollment for The Maastricht Study (i.e. baseline, in which cognitive tests were performed) and the subsequent MRI assessment was  $16.4 \pm 3.1$  months, which might have limited the validity of the subject characteristics and the long-term validity of the WLT score at the time of the MRI evaluation. Nevertheless, the baseline and repeated MMSE did not differ, which is indicative of no clinically significant cognitive differences within this time frame. Third, tract volume was used as a measure for white matter connection (32). In theory, alterations in axon diameters or myelination can also lead to differences in tract volume at the same time as maintaining the number of actual connections, although this explanation is less likely. Finally, the present study does not consider specific tracts, such as the cingulum bundle or the uncinate fasciculus (17,19), and therefore cannot provide details of specific effects on these tracts. However, the applied approach, which considers all connections from the hippocampus to other cerebral regions, facilitates a more global assessment of hippocampal connectivity, which might be more sensitive to effects of type 2 diabetes and cognition.

Future longitudinal studies will provide additional insights, favourably including not only participants with type 2 diabetes, but also potentially participants with pre-diabetes, such as the metabolic syndrome or diabetes with mild cognitive impairment. Furthermore, better memory performance in type 2 diabetes has been shown by improvement in fasting plasma glucose levels (41). An important question to be addressed in future (longitudinal) studies is whether improvement in glycaemic control leads to less affected white matter connectivity, and whether specific tracts (e.g. cingulum) are affected. These extensions could clarify whether the decrease in white matter connectivity is possibly an early brain tissue biomarker for verbal memory decrements.

In conclusion, dMRI tractography revealed reduced white matter connectivity between the hippocampus and the frontal lobe in type 2 diabetes. For participants who scored lower on memory performance, tractography revealed reduced white matter connectivity between the hippocampus and the temporal lobe. Memory decrements in participants with type 2 diabetes appear to be associated with altered hippocampal white matter connectivity. The findings of the present study contribute to a better understanding of diabetes-associated memory performance, although the exact mechanism remains to be revealed in future studies.

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