On the Interplay of Microvasculature, Parenchyma, and Memory in Type 2 Diabetes

Citation for published version (APA):

Document status and date:
Published: 01/05/2015

DOI:
10.2337/dc14-2043

Document Version:
Publisher's PDF, also known as Version of record

Document license:
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Please check the document version of this publication:
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Download date: 05 Jul. 2024
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Diabetes Care 2015;38:876–882 | DOI: 10.2337/dc14-2043

OBJECTIVE
Type 2 diabetes is associated with accelerated cognitive decline, especially regarding memory for which the hippocampus plays an essential role. The pathophysiological mechanisms still remain to be elucidated. The purpose of this study is to examine whether hippocampal microvascular and microstructural changes are related to type 2 diabetes (based on status or based on fasting blood glucose [FBG] levels) and verbal memory performance.

RESEARCH DESIGN AND METHODS
Thirty-nine participants with type 2 diabetes (64.5 ± 6.1 years old) and 34 participants without type 2 diabetes (58.3 ± 9.2 years old) underwent detailed cognitive assessments and 3-Tesla MRI using intravoxel incoherent motion (IVIM) MRI. Multivariate regression analyses controlling for age, sex, education level, BMI, systolic blood pressure, hematocrit level, and relative hippocampal volume were performed to examine associations between hippocampal IVIM measures, type 2 diabetes (status and FBG), and memory performance.

RESULTS
For the microvasculature, blood perfusion volume \( f \) was larger in participants with type 2 diabetes, \( fD^* \) and blood flow \( fD^\star \) increased with higher FBG levels, and microvascular pseudodiffusion \( D^* \) and \( fD^* \), which are indicative of altered microvasculature, were higher in participants with both relatively high FBG levels and low memory performance. In addition, \( fD^* \) increased with lower memory performance. For the parenchymal microstructure, the diffusion \( D \), indicative of injured microstructure, was higher with reduced memory performance.

CONCLUSIONS
In addition to the parenchymal microstructure, especially the microvascular properties of the hippocampus are altered in participants with both type 2 diabetes and memory problems and possibly hint at an underlying vascular mechanism.

Type 2 diabetes is associated with cognitive deficits, accelerated cognitive decline, and an increased risk of dementia and Alzheimer disease (AD) (1–3). A prominently affected cognitive domain in type 2 diabetes is memory (4), for which the hippocampus plays an essential role. In patients with type 2 diabetes and memory problems, reduced hippocampal volumes have previously been reported (5). Furthermore, chronic hyperglycemia has been shown to be associated with altered hippocampal microstructure and memory performance in elderly individuals without diabetes (6). Unfortunately, the exact mechanism underlying hyperglycemia and
memory impairment is not fully understood. In patients with stroke, it was shown that chronic hyperglycemia might be involved in the remodeling of the cerebral microvasculature (7). Possibly the microvasculature is also affected in hippocampal areas in patients with type 2 diabetes. However, advanced and detailed MRI analyses with respect to hippocampal microstructure (parenchyma) as well as microvasculature to explain memory problems in patients with type 2 diabetes have not been conducted so far.

The noninvasive intravoxel incoherent motion (IVIM) MRI technique, which was introduced by Le Bihan et al. (8), enables a detailed assessment of hippocampal microstructure and microvasculature through 1) the diffusion coefficient (D), which is a measure for neural tissue integrity and reflects the diffusion of water molecules in tissue; 2) the pseudodiffusion coefficient (D*), which reflects the incoherent motion of water molecules in the microvasculature; 3) the perfusion fraction (f), a measure for blood perfusion volume; and 4) blood flow (fD*). The latter two (f and fD*) are potentially sensitive to microvascular pathology. It has recently been shown by Federau et al. (9) that IVIM in the brain yields clinically relevant parameters, e.g., for acute stroke and gliomas. An interesting advantage of the IVIM technique is that it allows the simultaneous (noninvasive) assessment of tissue microstructure and microvasculature, and therefore the interplay between brain tissue and vessels.

The purpose of this study is to examine whether hippocampal microvascular and microstructural changes, derived from an IVIM MRI measurement, are correlated with type 2 diabetes (based on status or based on fasting blood glucose [FBG] levels), verbal memory decline, and the potential interaction between type 2 diabetes and memory performance.

**RESEARCH DESIGN AND METHODS**

**Study Population**

A total of 47 participants with type 2 diabetes and 41 participants without type 2 diabetes were recruited from the first 866 participants (total subject pool) of the Maastricht Study. The Maastricht Study is an ongoing observational, prospective, population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes. Participants are between 40 and 75 years of age and live in the southern part of the Netherlands (10). Participants are considered to have diabetes according to the World Health Organization 2006 criteria if they use diabetes medication or if they have an FBG ≥7.0 mmol/L or a 2-h blood glucose ≥11.1 mmol/L. Participants without type 2 diabetes are characterized by FBG <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, for example. A detailed overview is provided in Schram et al. (10). After their baseline measurements of the Maastricht Study, participants were invited to participate in this MRI study.

Participants with a broad range of cognitive performances were selected from the total subject pool to increase the probability of finding MRI differences associated with cognitive decrements (see the Supplementary Data for the detailed selection procedures). This selection was based on a cumulative score of neuropsychological tests: 1) verbal memory (verbal word learning (11)); 2) executive functioning, attention, and flexibility (Stroop test (12)); and 3) fluency test (13). Scores were adjusted for age, sex, and education level using linear regression. Exclusion criteria for participants were 1) a known history of stroke or neurological disease, 2) if the time span between enrollment in the Maastricht Study and MRI assessment was >1.5 years, 3) incomplete cognitive assessments, 4) type 1 diabetes, 5) an impaired FBG level in participants without type 2 diabetes, 6) mild cognitive impairment (MCI), 7) participants with the metabolic syndrome, 8) participants with color blindness, and 9) participants with an unknown diabetes status. After declining the invitation or 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, all participants underwent a general cognitive function test (Mini-Mental State Examination [MMSE] (14)) to assess clinically significant differences in cognitive performance compared with the baseline cognitive tests at enrollment in the Maastricht Study. Structural and IVIM brain scans were obtained from all participants. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), and all participants gave written informed consent. This 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-, and T2*-weighted and fluid-attenuated inversion recovery [FLAIR] sequences) and an IVIM scan. A three-dimensional T1-weighted (T1) fast-field echo sequence (TR/TE of 8.1/3.7 ms, 1.00 mm isotropic voxel, 170 continuous slices, matrix size of 240 × 240, and 7:56 min acquisition time) was acquired, and IVIM data were obtained using a single-shot spin-echo planar imaging sequence (TR/TE of 6,800/84 ms, 2.4 mm isotropic voxel, inversion time of 2,230 ms, and 5:13 min acquisition time). Inversion prepulses were applied to suppress the signal contamination of cerebrospinal fluid (15). Images were acquired at multiple b values (0, 5, 7, 10, 15, 20, 30, 40, 50, 60, 100, 200, 400, 700, 1,000, and 1,500 s/mm²) in the phase-encoding direction, and the number of signal averages was 1, except for the largest three b values which were 2, 3, and 3, respectively, to improve the signal-to-noise ratio.

**Data Analysis**

The T1 images were automatically segmented to obtain both hippocampal volumes using FreeSurfer (16). As we expect that microvasculature measures are most strongly indicative of gray matter, analyses were restricted to the gray matter. The IVIM data were preprocessed with the diffusion MR toolbox ExploreDTI (v.4.8.2) (17). In brief, the preprocessing steps included 1) visual image quality assessment, 2) eddy current induced geometric distortions and head motion corrections, and, finally, 3) echo-planar imaging distortion correction.
The IVIM model describes the quantitative relationship between signal intensity and the applied diffusion sensitization (see Supplementary Eq. 1). In this model, the diffusion motion of parenchymal and intravascular water molecules can be distinguished. A quantitative description of the model then provides the following physiological measures (see Supplementary Data): the blood perfusion $f$, the diffusion coefficient of parenchymal water $D$, and the so-called pseudodiffusion coefficient of intravascular water $D^*$, which resembles both diffusion and convection of blood water ($B$). We also consider $fD^*$ (i.e., $f$ times $D^*$), which has previously been shown to be related to the classical cerebral blood flow (CBF) (9, 18).

IVIM measures were derived after fitting the IVIM model to a biexponential formula, using a two-step approach as described by Federau et al. (19) (see Supplementary Data for the detailed procedures). A fitting example of the two-step approach is given in Supplementary Fig. 2.

After careful analyses, data from 39 and 36 participants with type 2 diabetes (for right and left hippocampus, respectively) and 34 participants without type 2 diabetes (both hippocampi) remained suitable for final analysis (see Supplementary Data).

**Statistical Analysis**

Descriptive participant characteristics and IVIM measures are reported as mean ± SD. Group characteristics were tested using independent samples Student t tests or Pearson $\chi^2$ tests using SPSS (version 20; IBM, Chicago, IL). Linear regression analyses, adjusted for age, sex, education level, BMI, systolic blood pressure, hematocrit level, and relative (to intracranial) hippocampal volume, were performed to assess the association of the hippocampal IVIM measures ($f$, $D$, $D^*$, and $fD^*$) with type 2 diabetes (model 1, dichotomous status; model 2, FBG levels) and memory performance (total score of 15-word learning [verbal] memory task, WLT total score) was added to both models, to investigate the possible interaction of type 2 diabetes and memory performance. The resulting interaction term (model 2) was devised to be high in participants with both high FBG levels and poor memory performance.

Additionally, linear regression analyses with glycated hemoglobin (HbA1c) levels instead of FBG in model 2 were performed.

**RESULTS**

Table 1 shows the baseline characteristics of the participants. The groups were different with regard to age, sex, and education. Participants with type 2 diabetes had higher FBG levels; higher HbA1c levels, a measure for long-term blood glucose control; higher BMI; and higher diastolic as well as systolic blood pressure. With respect to cognition, participants with type 2 diabetes scored worse on the WLT total score ($P = 0.016$) and on baseline MMSE ($P = 0.023$), but not on the repeated MMSE ($P = 0.366$), and the recall words learning (verbal) memory task (WLT recall score, $P = 0.178$) compared with participants without type 2 diabetes. Baseline and repeated MMSE did not differ between participants with and without type 2 diabetes ($P = 0.314$), which indicates no signs of severe cognitive decline over a period of $16.7 ± 2.7$ months.

Table 1 also shows the descriptive hippocampal IVIM measures of the

<table>
<thead>
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<td><strong>Participants with type 2 diabetes (n = 39)</strong></td>
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<td>$D^*$ (10⁻³ mm²/s)</td>
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<td>$fD^*$ (10⁻³ mm²/s)</td>
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</table>

Data are mean ± SD. Baseline/repeated MMSE, the Maastricht Study/before MRI MMSE; DBP, diastolic blood pressure; SBP, systolic blood pressure. *Independent samples Student t test, $P < 0.05$. **Independent samples Student t test, $P ≤ 0.001$. $^*$Pearson $\chi^2$ test, $P = 0.005$. $^{d}$Pearson $\chi^2$ test, $P ≤ 0.001$. 
participants. The order of magnitude of the IVIM measures in the current study was consistent with previously published data (19). Figure 1 shows the rather heterogeneous pixel-by-pixel distribution of the IVIM measures in the delineated hippocampus.

Linear regression (Table 2, model 1) revealed an increased perfusion fraction $f$ for participants with type 2 diabetes status ($P = 0.022$). The perfusion fraction $f$ was also increased in participants with lower hematocrit levels ($\beta = -0.252, P = 0.047$). The diffusion coefficient $D$ increased with lower memory performance ($P = 0.021$) (Table 2) and age ($\beta = 0.344, P = 0.018$). No significant associations were observed for $D^*$. Blood flow–related $fD^*$ increased with higher FBG levels ($P = 0.020$) and with lower memory performance ($P = 0.041$) (Table 2).

Additional linear regression analyses with the interaction term (type 2 diabetes status times memory performance) did not show any significant interaction (data not shown).

Linear regression (model 2) with FBG levels revealed an increased perfusion fraction $f$ with higher FBG levels ($P = 0.031$) (Table 2) and in participants with lower hematocrit levels ($\beta = -0.266, P = 0.037$). The diffusion coefficient $D$ increased with lower memory performance ($P = 0.025$) (Table 2) and age ($\beta = 0.366, P = 0.013$). No significant associations were observed for $D^*$. Blood flow–related $fD^*$ increased with higher FBG levels ($P = 0.020$) and with lower memory performance ($P = 0.041$) (Table 2).

The interaction analysis (FBG levels times memory performance) revealed a significant interaction for $D^*$ ($\beta = 0.273, P = 0.038$) and $fD^*$ ($\beta = 0.278, P = 0.018$). Additional analyses with HbA1c did not show any significant associations with IVIM measures.

**CONCLUSIONS**

The current study simultaneously examined the microvasculature and parenchymal microstructure of the hippocampus in relation to diabetes, FBG levels, and memory performance in participants with and without type 2 diabetes using IVIM. To the best of our knowledge, this is the first study to investigate the microvascular properties of the hippocampus in participants with type 2 diabetes. For the microvasculature, under the model considered, the blood perfusion volume ($f$) was higher in participants with type 2 diabetes, and blood perfusion volume ($fD^*$) increased with higher FBG levels, blood flow ($fD^*$) increased with poorer verbal memory performance, and pseudodiffusion coefficient of vascular water ($D^*$) and blood flow ($fD^*$) were higher in participants with both higher FBG levels and lower memory performance. Regarding the hippocampal parenchymal microstructure, under the model considered, the microstructural diffusion coefficient ($D$) increased with poorer verbal memory performance.

**Microvasculature**

We demonstrated an increased blood perfusion volume in participants with type 2 diabetes, in participants with higher FBG levels, and an increased blood flow with higher FBG in the hippocampus (Table 2). Currently, no other hippocampal perfusion studies are available in participants with type 2 diabetes, and global (whole brain) perfusion studies show inconclusive results (20,21). Moreover, insulin administration in participants with type 2 diabetes leads to reduced cerebral perfusion in the insular cortex and improved cognitive performance (22).

A previous study found that patients with MCI had higher hippocampal CBF (which is related to blood flow $fD^*$ [9]) compared with healthy control subjects (23,24), whereas the hippocampal CBF was lower in AD patients with more severe cognitive impairment than MCI patients (23). Hauser et al. (23) hypothesized that the increased hippocampal CBF in MCI patients may be a result of an early compensatory mechanism, possibly due to inflammation, elevation of neuronal activation, or production of vasodilators (24). This hypothesis can also be extended to our study results, because the cognitive performance of participants with type 2 diabetes is still better but more similar to the cognitive performance of MCI patients than that of AD patients.

The current study revealed that high FBG levels are associated with increased
blood perfusion volume and high blood flow in the hippocampus, suggesting that a glucose mechanism might underlie microvascular alterations. In a recently published study by Crane et al. (25), the authors showed that higher blood glucose levels are associated with a higher risk of developing dementia. As participants with type 2 diabetes are associated with an increased risk of dementia, our results might suggest that the blood perfusion volume might be an early MRI biomarker for dementia and is mediated by vascular pathology. As no participants with dementia or MCI were included in this study, future studies are needed to fully elucidate the interplay of vascular pathology and blood glucose levels in dementia.

The significantly higher blood perfusion volume in participants with lower hematocrit levels is in accordance with findings by Thomas et al. (26).

### Interplay of Blood Glucose, Microvasculature, and Memory Performance

We observed a higher blood flow with lower memory performance (Table 2), and a higher microvascular pseudodiffusion and blood flow in participants with both higher FBG levels and lower memory performance. A higher microvascular pseudodiffusion and blood flow could indicate that the hippocampal microvasculature is altered or more leaky, which is in agreement with previously reported increased blood-brain barrier permeability in patients with type 2 diabetes (27). To investigate the full extent of leaky microvasculature, future studies using, for example, dynamic contrast-enhanced MRI are warranted (28).

### Microstructure

The increased microstructural diffusion with lower memory performance in the hippocampus, as found in our study, hints at a mechanism where an injured microstructure might underlie the memory decline. These results are consistent with a study by Falvey et al. (29), who observed greater hippocampal mean diffusivity in participants with diabetes.

Kerti et al. (6) showed a significant correlation between lower FBG levels and decreased microstructural diffusion within the hippocampus. Nevertheless, in the current study, no such relationship was observed (Table 2), possibly due to the fact that we applied the IVIM technique, distinguishing diffusion of the parenchymal microstructure from that of the microvasculature, whereas Kerti et al. (6) applied diffusion tensor imaging, in which this distinction cannot be made. The observation of an association between microstructural diffusion and age in the hippocampus is in line with results demonstrated by Pereira et al. (30) and suggests that microstructural diffusion is sensitive to age-related degeneration.

### Limitations

Several limitations to the study need to be discussed. First, the study has a cross-sectional design. Therefore, it was not possible to investigate whether the higher blood perfusion volume is indeed an early compensatory mechanism and/or whether it will decrease at a later stage. Still, the first results are promising and open directions for future studies. Second, the study population was relatively heterogeneous due to the selection procedure but reflects typical participants with and without type 2 diabetes, and therefore the analyses were corrected for age, sex, and education. After correcting for covariates, group differences became significant. Third, the time span between enrollment for the Maastricht Study (baseline, in which cognitive tests were performed) and the subsequent MRI assessment was 16.7 ± 2.7 months, which might limit 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 in this time window. Fourth, to limit the scan time, the diffusion was only measured in one direction (phase-encoding direction). Therefore, we investigated the diffusion properties of the hippocampus only in the gray matter, where diffusion is not or less dependent on the directions compared with the white matter.

### Clinical Perspectives

Our results show that increased blood perfusion volume (f) is associated with high blood glucose and is therefore possibly an early biomarker for hyperglycemia-associated impairment of cognitive performance (25). Moreover,
microvascular pseudodiffusion ($D^*$) and blood flow ($J^*$) are indicative of the added effect of hyperglycemia on memory impairment. Previously, it has already been shown that improvement in glycemic control is associated with improved cognition in participants with type 2 diabetes (31). Thus, it is plausible that higher FBG levels lead to cerebral microvascular alterations, which could explain the impaired cognitive performance. An important next question to be addressed in future studies is how the IVIM measures relate to cognitive performance in individuals with the metabolic syndrome or impaired glucose metabolism (prediabetic stages) (32). In addition, the results of this study indicate that, next to improvement of hyperglycemia, treatment to improve vascular function (e.g., with antihypertensive or antiplatelet drugs) could be beneficial in patients with type 2 diabetes and impaired cognition. This should be explored in other studies. Future (longitudinal) studies are therefore needed to elucidate whether IVIM measures provide an early biomarker for memory impairment and dementia in the context of (pre)diabetes. Validating these potential biomarkers as indicators of biological alternations might open new avenues to monitor therapeutic/lifestyle interventions for improvement of cognition and prevention of cognitive decline.

Conclusion

This study indicated that especially microvascular properties of the hippocampus are altered in participants with type 2 diabetes and memory problems, which hints at a possible contribution of an underlying vascular mechanism. The IVIM measures have the potential to be good candidates as MRI biomarkers for memory impairment in type 2 diabetes.

Acknowledgments. The authors acknowledge Marc Geerlings and Jos Slentor (Department of Radiology, Maastricht University Medical Center) for continuous hardware and software support.

Funding. J.F.J. was funded by VENI research grant 916.11.059 from the Netherlands Organisation for Scientific Research and the Netherlands Organisation for Health Research and Development. Additionally, this work was supported by the Stichting De Weijerhorst foundation.

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

Author Contributions. F.C.v.B., W.H.B., and J.F.J. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript; P.A.H., R.J.V.O., A.G.K., M.P.V.B., M.T.S., C.D.S., and J.E.W. contributed to discussion and critically reviewed and edited the manuscript. All authors gave final approval of the version of the manuscript to be published and agreed to be listed as authors. F.C.v.B. and J.F.J. 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 as an abstract and as an oral presentation at the Joint Annual Meeting of the International Society for Magnetic Resonance in Medicine and the European Society for Magnetic Resonance in Medicine and Biology, Milan, Italy, 10–16 May 2014.

References

27. Starr JM, Wardlaw J, Ferguson K, MacLullich A, Deary IJ, Marshall I. Increased blood-brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. J Neurol Neurosurg Psychiatry 2003;74:70–76