Ventral Striatum, but not Cortical Volume Loss, Is Related to Cognitive Dysfunction in Type 1 Diabetic Patients With and Without Microangiopathy

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OBJECTIVE

Patients with longstanding type 1 diabetes may develop microangiopathy due to high cumulative glucose exposure. Also, chronic hyperglycemia is related to cerebral alterations and cognitive dysfunction. Whether the presence of microangiopathy is conditional to the development of hyperglycemia-related cerebral compromise is unclear. Since subcortical, rather than cortical, volume loss was previously related to cognitive dysfunction in other populations, we measured these brain correlates and cognitive functions in patients with longstanding type 1 diabetes with and without microangiopathy.

RESEARCH DESIGN AND METHODS

We evaluated differences in subcortical volume and cortical thickness and volume in type 1 diabetic patients with ($n=51$) and without ($n=53$) proliferative retinopathy and 49 control subjects and related volume differences to cognitive dysfunction. Analyses were corrected for age, sex, systolic blood pressure, and A1C.

RESULTS

Putamen and right thalamic volume loss was noted in both patients with and without proliferative retinopathy compared with control subjects (all $P<0.05$). Additionally, in patients with proliferative retinopathy relative to control subjects, volume loss of the bilateral nucleus accumbens was found (all $P<0.05$). No differences were observed between the two patient groups. Cortical thickness and volume were not different between groups. In pooled analyses, lower left nucleus accumbens volume was associated with cognitive dysfunction ($P<0.035$).

CONCLUSIONS

This study shows subcortical, but not cortical, volume loss in relation to cognitive dysfunction in patients with long-standing type 1 diabetes, irrespective of microangiopathy. The time-course, pathophysiology, and clinical relevance of these findings need to be established in longitudinal and mechanistic studies.

Longstanding type 1 diabetes mellitus may lead to development of microangiopathy, mediated by cumulative hyperglycemic exposure (1). Cognitive dysfunction is also related to prolonged hyperglycemia (2,3). Proliferative retinopathy, the presence of which has been found to be independently related to cognition and cerebral alterations

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in type 1 diabetes (4–8), has been identified as a surrogate marker for cumulative hyperglycemic exposure (9).

However, whether the presence of microangiopathy is conditional to the development of hyperglycemia-related cerebral compromise is unclear. It has been shown that alterations in neuronal communication (i.e., functional connectivity) and white matter integrity (i.e., structural connectivity) were most pronounced in type 1 diabetic patients with proliferative retinopathy, but were also noted in those without microvascular complications (6–8,10). These cerebral alterations were directly associated with type 1 diabetes–related cognitive decrements (6–8,10).

Earlier studies have found evidence of limited cortical volume loss in patients relative to control subjects (5,11–14), which correlated poorly with cognition (12,15). From studies in healthy elderly, patients with Alzheimer disease, and patients with multiple sclerosis, it is known that volume loss of subcortical structures (e.g., amygdala, hippocampus, thalamus, and nucleus accumbens) strongly correlates with cognitive dysfunction, independent of cortical changes (16–18). Subcortical alterations, however, have not been systematically studied in type 1 diabetes. So far, one study assessing hippocampal volume did not find a difference between type 1 diabetic patients and control subjects (19). Another study, on cortical volume differences, noted lower left thalamus volume relative to control subjects (13).

It could be hypothesized that high cumulative hyperglycemic exposure, mediated by microangiopathy, is associated with subcortical volume loss in type 1 diabetic patients, which in turn relates to cognitive dysfunction in this population. The cortical mantle may be relatively spared. To this end, we studied subcortical and cortical measures in patients with longstanding type 1 diabetes with and without proliferative retinopathy, which serves as a proxy for hyperglycemic exposure, and non-diabetic control subjects.

**RESEARCH DESIGN AND METHODS**

**Participants**

This study was conducted in accordance with the Declaration of Helsinki and approved by the medical ethics committee of the VU University Medical Center. Written informed consent was obtained from all participants. This study assessed the effect of type 1 diabetes and concomitant proliferative retinopathy, as a marker of cumulative hyperglycemic exposure, on cognitive functioning, brain volume, and functional and structural connectivity (8). Study design, inclusion and exclusion criteria, and demographic and medical measures are described in more detail elsewhere (8). In short, 51 patients with proliferative retinopathy, 53 patients without clinically manifest microangiopathy, and 51 matched non-diabetic control subjects were included. Participants were between 18 and 56 years of age, right-handed, and patients had type 1 diabetes for at least 10 years. Exclusion criteria were psychiatric disorders, history of or current alcohol (men >21; women >14 units a week) or drug use, head trauma, cerebro- or cardiovascular disease, magnetic resonance imaging (MRI) contraindications, and centrally acting medication use. Proliferative retinopathy was ascertained by fundus photography and rated according to the EURODIAB classification (20); microalbuminuria as a 24-h urine albumin/creatinine ratio >2.5 mg/mmol for men or >3.5 mg/mmol for women; neuropathy status was based on the results of the annual checkup patients receive, which includes assessment of vibration perception using a 128-Hz tuning fork and tactile perception with the 10-g Semmes-Weinstein monofilament, and is incorporated into the medical records (n = 92) or self-report if not available (n = 12) (8). Lifetime severe hypoglycemic events were self-reported, based on Diabetes Control and Complications Trial criteria (21). After a 15-min rest, in a sitting position, blood pressure was measured three times with 5-min intervals at the left arm. Measures were averaged, and hypertension was defined as a systolic blood pressure ≥140 mmHg, a diastolic blood pressure >90 mmHg, or the use of antihypertensive drugs. Hypertension was an exclusion criterion for control subjects. Current depressive symptoms in the past week were evaluated with the Center for Epidemiological Studies Depression scale.

**MRI Protocol**

MRI scanning was performed on a 1.5T whole-body MR-system (Siemens Sonata; Siemens, Erlangen, Germany) using an eight-channel phased-array head coil. For this study, a high-resolution T1-MPRAGE (repetition time 2.700 ms, echo time 5.17 ms, inversion time 95 ms, flip angle 8°, 248 × 330 mm2 field of view, and 1.0 × 1.0 × 1.5 mm voxel size) was used. All scans were corrected for scanner-specific geometric distortions.

**Subcortical Volume Analysis**

For subcortical structures, Oxford Centre for Functional MRI of the Brain’s Integrated Registration and Segmentation Tool (FSL-FIRST) was used (22,23). First, excessive neck signal was removed by registration of a Montreal Neurological Institute standard brain (MNI-152) to each participant’s T1-MPRAGE, thereby identifying the lower border of the brain, to increase reliability of the analyses. A detailed description of FSL-FIRST is found elsewhere (22). In short, FSL-FIRST models the outer surface of the bilateral hippocampus, thalamus, amygdala, nucleus accumbens, caudate nucleus, globus pallidus, putamen, and the brain stem including the 4th ventricle (Supplementary Fig. 1) by creating a vertex based mesh for each image. Then each voxel within the images is assigned the label of the structure to which that voxel belongs, taking into account individuals’ variations in surface shape of each structure, as well as the presence of neighboring structures. From this, in the participant’s native space, volume of each subcortical structure is calculated. All segmentations were manually checked, and no errors occurred.

**Whole-Brain Cortical Thickness Analysis**

Cortical thickness was calculated using the surface-based stream of FreeSurfer5.1. A detailed description is provided elsewhere (24–26). In short, brain images were linearly registered to Talairach space to compute seed points, the bias field homogeneity was corrected, the skull stripped, and the white matter segmented using volumetric classification. Both hemispheres were separated using cutting planes derived from Talairach space. Based on the resulting white matter segmentation, an initial white matter surface was created for each hemisphere. These surfaces were subsequently nuded into the direction of the gradient to find the white/gray matter and pial surface. The
test for categorical variables. Normality was checked using the Kolmogorov-Smirnov test and histogram analysis. Cortical thickness was analyzed using FreeSurfer5.1 vertex-wise general linear modeling by ANCOVA tested for the main effects of group and sex on cortical thickness. Age, systolic blood pressure, and A1C were entered as covariates. Data were smoothed with a 10-mm full width at half maximum Gaussian kernel. Cluster-wise correction for multiple comparisons was performed using Monte Carlo Z simulation while thresholding vertex-wise statistical maps at $P < 0.001$, setting the cluster-level threshold at $P < 0.05$, using 5,000 iterations. To minimize the risk of type 1 errors, normalized subcortical and cortical volume were each compared between groups using two individual multivariate ANCOVA models, corrected for age, sex, systolic blood pressure, and A1C. The overall multivariate $F$ test for group tests the null hypothesis that there is no effect of group on any of the dependent variables (30–32). Thus, only if this overall multivariate $F$ test for group was statistically significant post hoc statistical significance of the univariate ANCOVAs was checked. Only for those dependent variables showing an effect of group, between-group differences with Bonferroni correction for multiple comparisons were tested. Confounding factors were identified as those factors that were found to have an overall effect on the statistical model or a main effect on any of the dependent variables within that model. As control subjects with hypertension were excluded from participation, this factor could not be taken into account as a confounding factor in the analyses that included those participants.

In all type 1 diabetic patients, correlations between cognition and potentially differing subcortical and cortical structures were analyzed using a stepwise linear regression model. First, the structures that showed between-group differences were included, and the best predictors were determined using a forward regression model. This model was then corrected for estimated IQ, age, sex, systolic blood pressure, A1C, proliferative retinopathy, diabetes duration, self-reported lifetime hypoglycemic events, and additionally for current smoking status, total cholesterol, BMI, and depressive symptoms. To determine demographic, anthropometric, and medical correlates of structural alterations, those were entered in a forward linear regression, including diabetes duration, onset age, lifetime self-reported severe hypoglycemic events, microvascular complications, lipid profile, A1C, albumin/creatinine ratio, age, sex, BMI, systolic blood pressure, current smoking status, and estimated IQ.

All analyses were performed using IBM SPSS Statistics 20 (IBM SPSS, Chicago, IL) or FreeSurfer5.1. A $P$ value $<0.05$ was considered to be statistically significant.

RESULTS

Participants

For two nondiabetic control subjects, the T1-MPRAGE scan could not be used due to artifacts. Patients with proliferative retinopathy were significantly older and reported higher levels of depressive symptoms relative to the other groups. Systolic blood pressure was higher in these patients compared with control subjects. Disease onset age was earlier, disease duration longer, and albumin/creatinine ratio higher in patients with proliferative retinopathy relative to their counterparts with uncomplicated type 1 diabetes (all $P < 0.05$; Table 1).

Subcortical Volume

The overall MANCOVA $F$ test for subcortical volumes was statistically significant after correction for age, sex, systolic blood pressure, and A1C ($F[2,131] = 1.958; P = 0.023$), allowing post hoc testing. Group effects were statistically significant for the bilateral nucleus accumbens (left: $P = 0.016$; right: $P = 0.031$), putamen (left: $P = 0.014$; right: $P = 0.011$), right caudate nucleus ($P = 0.045$), and right thalamus ($P = 0.019$). Between-group testing revealed, after Bonferroni correction for multiple comparisons, volume loss of bilateral nucleus accumbens (part of the ventral striatum), putamen (part of the dorsal striatum), and right thalamus in patients with proliferative retinopathy relative to control subjects ($P < 0.05$; Fig. 1A–C and Table 2 for volume differences and effect sizes). Bilateral putamen and right thalamus also showed significant volume loss in patients without microangiopathy compared with control
Table 1—Baseline characteristics of patient groups and control subjects

<table>
<thead>
<tr>
<th></th>
<th>T1DM with proliferative retinopathy</th>
<th>T1DM without microangiopathy</th>
<th>Control subjects</th>
<th>Overall $P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>51</td>
<td>53</td>
<td>49</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.5 ± 7.2**†</td>
<td>37.8 ± 9.2</td>
<td>36.7 ± 11.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex [men/women (% men)]</td>
<td>21/30 (41.2)</td>
<td>20/33 (37.7)</td>
<td>19/30 (38.8)</td>
<td>0.935</td>
</tr>
<tr>
<td>Estimated IQ</td>
<td>110.2 ± 13.4</td>
<td>106.9 ± 11.2</td>
<td>108.5 ± 11.7</td>
<td>0.399</td>
</tr>
<tr>
<td>Depressive symptoms (minimum–maximum)§</td>
<td>8.0 (0–42)*‡</td>
<td>5.0 (0–31)</td>
<td>4.0 (0–37)</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.6 ± 4.1</td>
<td>24.8 ± 3.5</td>
<td>24.3 ± 3.5</td>
<td>0.189</td>
</tr>
<tr>
<td>A1C (mmol/mol)</td>
<td>64.7 ± 14.1</td>
<td>61.7 ± 9.8</td>
<td>34.2 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>8.1 ± 1.3</td>
<td>7.8 ± 0.9</td>
<td>5.3 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133.6 ± 17.3*</td>
<td>129.1 ± 14.7</td>
<td>123.6 ± 11.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76.0 ± 8.7</td>
<td>78.2 ± 9.5</td>
<td>77.4 ± 7.2</td>
<td>0.426</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.5 ± 0.7</td>
<td>4.7 ± 0.7</td>
<td>4.5 ± 0.9</td>
<td>0.217</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.8 ± 0.5*</td>
<td>1.8 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.4 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.8</td>
<td>0.593</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.9 ± 0.4*</td>
<td>1.0 ± 0.9</td>
<td>1.2 ± 0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>6 (11.8)</td>
<td>10 (18.9)</td>
<td>11 (22.4)</td>
<td>0.371</td>
</tr>
<tr>
<td>Cholesterol medication (%)</td>
<td>11 (20.8)</td>
<td>16 (31.4)</td>
<td>—</td>
<td>0.226</td>
</tr>
<tr>
<td>Blood pressure medication (%)</td>
<td>32 (62.7)</td>
<td>6 (11.3)</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (%)‡</td>
<td>33 (64.7)*</td>
<td>13 (24.5)</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin/creatinine ratio [mg/mmol (minimum–maximum)]</td>
<td>0.91 (0–33.17)*</td>
<td>0.39 (0–3.19)</td>
<td>—</td>
<td>0.003</td>
</tr>
<tr>
<td>Daily insulin units [IU (minimum–maximum)]</td>
<td>46.0 (19–90)</td>
<td>47.5 (23–102)</td>
<td>—</td>
<td>0.384</td>
</tr>
<tr>
<td>BG before MRI (mmol/L)</td>
<td>9.2 ± 3.7</td>
<td>10.5 ± 4.7</td>
<td>—</td>
<td>0.124</td>
</tr>
<tr>
<td>BG before NPA (mmol/L)</td>
<td>8.6 ± 4.0</td>
<td>8.4 ± 4.0</td>
<td>—</td>
<td>0.777</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>34.3 ± 7.9*</td>
<td>21.6 ± 9.3</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes onset age (years)</td>
<td>10.2 ± 7.2*</td>
<td>16.2 ± 9.7</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes early-onset age (%)§‡</td>
<td>18 (35.3)</td>
<td>10 (18.9)</td>
<td>—</td>
<td>0.077</td>
</tr>
<tr>
<td>Severe hypoglycemic events (minimum–maximum)§‡</td>
<td>2.0 (0–40)</td>
<td>2.0 (0–50)</td>
<td>—</td>
<td>0.701</td>
</tr>
<tr>
<td>Neuropathy (%)§§</td>
<td>25 (49)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Microalbuminuria (%)§§§</td>
<td>14 (27.5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SD, median with minimum, and maximum or absolute numbers with percentages. BG, blood glucose; NPA, neuropsychological assessment; T1DM, type 1 diabetes. *Statistically different from control subjects. †Statistically different from patients without proliferative retinopathy. ‡Estimated IQ was measured using the Dutch version of the National Adult Reading Test. §Depressive symptoms were measured using the Center for Epidemiological Studies scale for Depression. ¶Hypertension was defined as a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥90 mmHg, or the use of antihypertensive drugs. Control subjects with hypertension were excluded from the study. #Diabetes early-onset age was defined as an onset age <7 years. ††Severe hypoglycemic events were self-reported and defined as events for which the patient needed assistance from a third person to recuperate as a result of loss of consciousness or seriously deranged functioning, coma, or seizure owing to low glucose levels. §§Neuropathy was based on medical records or, in case they were not available, self-report. ¶¶¶Microalbuminuria was defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and >3.5 mg/mmol for women.

subject, (P < 0.05; Fig. 1A–C). No between-group differences were noted for the right caudate nucleus.

To adjust for potential confounding due to differences in type 1 diabetes disease duration, disease onset age, and current age, we performed additional analyses in which groups were matched for these variables. In this analysis, 27 patients with proliferative retinopathy (mean age: 41.9 ± 7.8 years; mean disease duration: 29.0 ± 5.7 years; and mean onset age: 12.9 ± 7.5 years), 29 patients without microangiopathy (mean age: 40.3 ± 8.6; mean disease duration: 27.7 ± 8.2 years; and mean onset age: 12.7 ± 8.4 years), and 38 control subjects (mean age: 40.8 ± 9.0 years) were included. This matched analysis showed a similar pattern of volume loss in patients regardless of complication status as compared with the whole-group analysis (Supplementary Fig. 3A–C). Due to the smaller mean difference, sample size, and discriminative power, in the presence of increased SD, the differences reached statistical significance for the bilateral putamen only.

Whole-Brain Cortical Thickness and Region-of-Interest Cortical Volume

There were no cortical thickness differences noted between the diabetic groups and control subjects (P > 0.05). The overall general linear model F test for cortical volume did not yield statistical significance (F[2,137] = 1.043; P = 0.407). Hence, no post hoc testing was performed.

Association Between Cognitive Domains and Subcortical Structures

We previously reported poorer general cognitive ability, information-processing speed, and attention in patients relative to control subjects (8). For these three domains, a linear regression approach was performed in all type 1 diabetic patients irrespective of group allocation. Lower left nucleus accumbens volume
was associated with poorer general cognitive ability (β = 0.284, \(P = 0.004\)), poorer information-processing speed (β = 0.275, \(P = 0.006\)), and lower attention performance (β = 0.306; \(P = 0.002\)), adjusted for estimated IQ, age, sex, systolic blood pressure, A1C, proliferative retinopathy, diabetes duration, and self-reported lifetime hypoglycemic events. These associations remained significant after additional correction for current smoking status, total cholesterol, BMI, and depressive symptoms (general cognitive ability: β = 0.205, \(P = 0.026\); information-processing speed: β = 0.205, \(P = 0.034\); and attention: β = 0.233, \(P = 0.021\)).

### Determinants of Subcortical Volume Loss

In all patients, higher systolic blood pressure and longer diabetes duration were associated with lower right nucleus accumbens volume. Longer disease duration and male sex correlated with lower thalamus volume (Table 3). Linear regression showed that higher age was related to lower volume of the right putamen and left thalamus and nucleus accumbens. Lower left putamen volume was related to older age and male sex. The association between longer disease duration and thalamus/nucleus accumbens volume loss was not mediated by proliferative retinopathy. As untreated hypertension may exert a negative effect on subcortical volume, we replaced, in an additional analysis, systolic blood pressure for hypertension. Hypertension was not related to volume loss in any of the subcortical structures.

### CONCLUSIONS

Volume loss of the bilateral putamen and right thalamus were found in type 1 diabetic patients both with and without proliferative retinopathy. Bilateral nucleus accumbens volume loss was noted in patients with proliferative retinopathy only. In contrast, cortical structures were not affected in this cohort of well-phenotyped patients. Left nucleus accumbens volume loss was independently related to cognitive dysfunction in type 1 diabetic patients. Subcortical volume loss was related to longer disease duration, but not mediated by proliferative retinopathy, suggesting a direct effect of cumulative hyperglycemic exposure unrelated to the development of microangiopathy. This is contrary to our hypothesis.

Volume loss of the left nucleus accumbens, which is part of the ventral striatum within the basal ganglia, was independently associated with type 1 diabetes–related cognitive dysfunction. Similar findings have been reported in healthy elderly, elderly with memory complaints and dementia, and patients with multiple sclerosis (16–18), suggesting that this structure is of vital importance for retaining cognitive functioning. No associations...
were noted between other subcortical nuclei showing volume loss and cognitive decrements. This could indicate that other pathophysiological processes are related to cognitive dysfunction. In previous studies, it was shown that integrity of white matter and functional connectivity are related to cognitive dysfunction (7,8,10). Indeed, processing speed, the hallmark of type 1 diabetes-related decrements, may rely on the integration of activity in multiple brain regions and their interconnections. In this study, we aimed to identify the association between brain volume alterations and cognitive decrements that were previously found in this group of type 1 diabetic patients. Given this hypothesis, correlation analyses between (sub)cortical structures and cognitive domains that did not show alterations were not assessed. Although some associations may exist, this would lead to an increased risk of type 1 errors in this study. Future studies should assess the role of other subcortical structures in cognition in this patient population.

Subcortical volume loss was noted in both patient groups, and proliferative retinopathy did not further exacerbate this. This is contrary to earlier structural and functional connectivity findings in this cohort, in which proliferative retinopathy was independently correlated with these measures, and decrements were exacerbated by proliferative retinopathy (6–8). The results presented in this study indicate that cumulative hyperglycemic exposure may be related to striatal and thalamic volume loss, but that presence of clinically manifest microangiopathy does not lead to increased volume loss rates. This is supported by the data from the matched analysis (Supplementary Fig. 3A–C), which showed that the absolute difference in volume between patients with and without microangiopathy was less pronounced when disease duration was equal in both groups, while the differences in volume between patients and control subjects remained similar to the full-sample analysis. Indeed, exposure to the similar cumulative hyperglycemic load does not affect the same organ systems, including the microvasculature, to the same extent in all patients. Thus, although proliferative retinopathy has previously been found to be a proxy for type 1 diabetes-related cerebral and cognitive changes (4,7,8), this does not seem to be the case for subcortical structures.

In this cohort, no differences in the cortical structure were noted between patients and control subjects, whereas previous studies showed small gray matter differences (5,11–14). Two studies, which used similar analysis techniques, found lower cortical thickness in the frontal and posterior regions in patients (11,12). Another study, using a different segmentation technique, observed frontal volume loss in patients (14). The effects noted, however, were small. Further studies with relevant sample size are needed to add to these findings. However, given the absence of cortical differences in this study and the small effects in the other studies, it may be hypothesized that the cortex is relatively spared in type 1 diabetes.

The exact mechanisms underlying MRI-measured volume loss cannot be determined in this study. Possible contributors include neuronal loss, axonal damage, cell death, and loss of interstitial fluids. In animal models of type 1 diabetes, pericyte loss and microvascular rarefaction are typically noted in retinopathy (95% CI).

Data are means ± SD in milliliters unless otherwise indicated. T1DM, type 1 diabetes.
diabetes, cerebral volume loss was related to synaptic loss and loss of myelin (33). Neuronal loss was only seen in animals with both type 1 diabetes and hypertension (33). In our patient group, we previously reported axonal and myelin damage measured with diffusion tensor imaging (7), which could account for subcortical volume loss. Due to the real-life nature of the study protocol, participants with and without diabetes and potentially contributing factors to brain alterations, including current smokers as well as subjects who were overweight (BMI <35 kg/m²), and patients with elevated blood pressure were included in this study. These factors have been shown to impact cognition and brain volume. Indeed, elevated systolic blood pressure, which is a common comorbidity in patients with longstanding type 1 diabetes, is related to lower right nucleus accumbens volume. However, the absence of a correlation between systolic blood pressure and volume of other subcortical structures suggests that systolic blood pressure is not the driving force of subcortical volume loss in these patients with type 1 diabetes. Both current smoking status and BMI were not correlated to subcortical volume loss in the multivariate models, nor were there univariate correlations (Supplementary Table 1). Additionally, a recently published study did not find an effect of current or former smoking on brain volume in a type 1 diabetic cohort with similar characteristics as this cohort (14). Thus, it seems unlikely that these factors have contributed to the brain alterations found in this study, as there are no between-group differences on these variables. For studies that aim to detail the mechanisms underlying brain alterations, animal models of diabetes would be preferred.

Study limitations include the older age, higher systolic blood pressure, longer disease duration, and earlier onset age of our subgroup of patients with proliferative retinopathy. These group differences are most likely due to the fact that diabetic patients with complications have a longer disease duration, as it takes many years to develop proliferative retinopathy, which may also lead to other diabetes-related comorbidities, including elevated blood pressure. Older age and increased systolic blood pressure may have influenced the volume differences between patients with proliferative retinopathy and control subjects. Indeed, in the matched analysis, the differences are no longer statistically significant. Loss of power and increase of variance may also contribute to this result. Age and systolic blood pressure were treated as confounding factors in all analyses. However, statistically controlling for the effect of these variables may not be sufficient to fully rule out these effects. Since there were no statistically significant volume differences between both patient groups, the variables that were not fully matched had no influence in those analysis. A second potential limitation of this study is the risk of false positive findings (type 1 errors) due to the large number of a priori selected regions of interest. This approach was chosen to establish the potential differential effects of type 1 diabetes and concomitant proliferative retinopathy on subcortical structures and the cortex. To reduce the risk of false-positive findings, we corrected the cortical thickness analyses using family-wise error correction, the current gold standard in neuroimaging (34). For the region-of-interest analyses, we choose a multivariate ANCOVA design that aims to reduce the amount of tests by applying an overall multivariate F test (30–32). Post hoc testing was only performed in case of a significant overall F test for the group, which was the case for the subcortical regions of interest. Multivariate regression models have often been found to produce less reliable estimates of effects in the presence of insufficient sample size for the amount of variables entered in the equation (35). In this study, the number of variables was in proportion with the sample size, suggesting reliable effect estimations (35).

To conclude, this study showed striatal and thalamic volume loss in type 1 diabetic patients, related to longer disease duration, but not to peripheral microvascular complications, in particular the presence of proliferative retinopathy. These findings suggest that selective subcortical structures are prone to the effect of cumulative hyperglycemic exposure. Additionally, striatal volume
loss may play a part in cognitive decre- 
ments, which is commonly observed in 
type 1 diabetic patients. Future longitu-
dinal studies need to determine the 
clinical implications and causal associa-
tions of subcortical volume loss. Prefer-
ably, studies in children and adolescents 
should be performed to determine 
whether subcortical deficits are a 
marker of early type 1 diabetes–related 
brain deficits.

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