CHAPTER 8

Decreased cerebral blood flow in T2DM patients is not improved by treatment with liraglutide

Jennifer S. ten Kulve
Joost P.A. Kuijer
Liselotte van Bloemendaal
Frederik Barkhof
Michaela Diamant
Dick J. Veltman
Richard G. IJzerman

Submitted
ABSTRACT

Diabetes is a risk factor for cerebrovascular disease and is associated with increased risk for developing cognitive impairment. In patients with diabetes, reduced cerebral blood flow (CBF) may result from a combination of hyperglycemia with other risk factors for atherosclerosis. Currently, glucagon like peptide-1 (GLP-1) receptor agonists (GLP-1RA) are used for the treatment of diabetes. GLP-1 improves peripheral vascular perfusion, but the effects on CBF are unknown. We hypothesized that CBF is reduced in T2DM patients and that treatment with the GLP-1RA liraglutide enhances CBF. We included 20 healthy lean individuals and 20 obese T2DM patients. We measured CBF using a whole-brain three-dimensional pseudo-continuous arterial spin labelling technique at 3.0 Tesla and assessed whole-brain CBF and in predefined areas of interest and compared CBF between groups, also after correction for risk factors. T2DM patients participated in a crossover intervention study, comparing effects of treatment with liraglutide 1.8 mg and insulin glargine on CBF. CBF was decreased in T2DM patients in all areas compared to healthy lean individuals (P < 0.02). After correction for BMI, cholesterol levels or systolic blood pressure, similar differences remained (P < 0.09). However, after correction for HbA1c or fasting plasma glucose, differences between groups were no longer observed (P > 0.2, except for caudate nucleus). Liraglutide treatment did not enhance whole-brain CBF or CBF in the predefined areas.

We observed a generalized reduced CBF in T2DM patients, which was mainly associated with the hyperglycemic state. The reduced CBF in T2DM patients may contribute to the increased risk for the development of cognitive impairment. However, reduced CBF did not improve during liraglutide treatment.
INTRODUCTION
Diabetes is a major public health problem and is associated with substantial morbidity attributed to vascular complications, preceded by changes in microvascular function and/or structure in multiple organ systems, including the retina, kidney and myocardium (1). In addition, diabetes is associated with increased risk for developing mild cognitive impairment or progression of mild cognitive impairment to dementia (2;3). Several studies have shown that diabetes is related to impaired peripheral endothelial function, but also altered permeability of the blood-brain barrier with alterations in central blood flow (CBF) and regional metabolism (4-6). Since reduced CBF is associated with mild cognitive impairment (7), the observed reduced CBF in patients with T2DM may contribute to the associated increased risk for cognitive impairment in these patients. It has been suggested that reductions in CBF may be accelerated in diabetes due to various risk factors, i.e. hyperglycemia, dyslipidemia and hypertension (8), but it is unclear which risk factors mostly contribute.

During the last decades, glucagon-like peptide-1 (GLP-1) based therapies have been added to the armamentarium for the treatment of type 2 diabetes. GLP-1 is a gut-derived hormone, secreted after food ingestion from L-cells located in the intestine. GLP-1 is mainly known for its glucose lowering effects, as it augments meal-related insulin secretion (9). Interestingly, it was shown that GLP-1 has effects on vascular and endothelial function (10). In rodents and humans, infusion of GLP-1 improves peripheral endothelial function and increases skeletal and cardiac muscle microvascular blood flow (11-14), which may explain the observed improved myocardial function after GLP-1 infusion (12). GLP-1 and most GLP-1 receptor agonists (GLP-1RA) are able to cross the blood-brain barrier (15;16) and may therefore also affect cerebral perfusion. Indications for effects of GLP-1 and GLP-1RA in the central nervous system (CNS) mainly come from studies investigating the role of GLP-1 in the CNS regulation of feeding, as treatment with GLP-1RA is associated with reduced food intake and body weight. Studies in rodents showed that central administration of GLP-1 and GLP-1RA reduces food intake (17;18) and GLP-1RA administration in humans resulted in altered CNS activation in response to food stimuli (19;20). In addition, GLP-1 receptors and GLP-1 producing neurons have been found in the CNS (21-23). Furthermore, it was shown that GLP-1 has neuroprotective effects (24), but it is yet unclear if administration of GLP-1RA may also improve cerebral perfusion.

In the current study we investigated if obese T2DM patients, compared to healthy lean individuals, have decreased CBF in whole brain, in grey matter or in predefined areas involved in declarative memory and the regulation of feeding (i.e. hippocampus, insula, putamen and caudate nucleus). We also investigated which risk factors are associated to reduced CBF in obese T2DM patients. Finally, we investigated if treatment with the GLP-1RA liraglutide improves CBF in T2DM patients.

METHODS
Participants
This study (NTC 01363609) is part of a larger project investigating the effects of GLP-1 on the CNS (20;25) and was performed in accordance with the Helsinki Declaration and was approved by the Medical Ethics Committee of the VU University Medical Center. As described previously (20;25), we included 20 healthy lean individuals and 20 overweight or obese patients with T2DM, matched for gender and age. Participants were included after written informed consent was obtained.
and underwent a screening visit, consisting of a medical history, physical examination and fasting blood and urine analyses. Subjects were eligible if they were 40 to 65 years of age. Inclusion criteria for the healthy lean individuals included a body mass index (BMI) < 25 kg/m² and normoglycemia, defined by fasting plasma glucose < 5.6 mmol/l and 2-hour glucose < 7.8 following a 75g oral glucose tolerance test (OGTT). Inclusion criteria for the overweight or obese T2DM patients included BMI > 26 kg/m², HbA1c levels between 42 – 69 mmol/mol (6.0 – 8.5%). For the current treatment of diabetes only the oral glucose lowering agents metformin ± sulfonylurea derivatives were allowed. Exclusion criteria were a history of neurological, cardiovascular, renal or liver disease, malignancies, the use of any centrally acting agent or oral glucocorticoids.

In the group of T2DM patients, ten patients used antihypertensive medication and fifteen patients used cholesterol lowering agents.

**General experimental protocol**

Participants, both healthy lean individuals and T2DM patients, underwent an MRI scan in the morning after an overnight fast. T2DM patients continued in a randomized, cross-over, intervention study, which consisted of two treatment periods of 12 weeks each with a 12 week wash-out period in between, as described previously (20). During one period, patients were treated with liraglutide, which was injected in the evening. Patients underwent a dose-escalation period, starting at 0.6 mg once daily (q.d.), with weekly increments of 0.6 mg, if well tolerated, reaching a final dose of 1.8 mg q.d. by the end of the second week, which was maintained until the end of the treatment period. During the other period, patients were treated with an active comparator, i.e. insulin glargine, started at an initial dose of 10 IU q.d.. Patients were instructed to increase the daily dose based on their fasting self-monitored blood glucose levels according to a predetermined treat-to-target algorithm (26). Effects of treatment with liraglutide were evaluated and were also compared to treatment with insulin glargine, to evaluate the contribution of GLP-1 receptor activation per se, given the expected isoglycemic state in both treatment groups, thereby minimizing the possible effects of differences in glucose levels. In total, each T2DM patient underwent six test visits with MRI sessions. One at the start (baseline), one after 10 days (short-term) of each treatment and one after 12 weeks (long-term) of each treatment.

**MRI data acquisition**

MR imaging was performed on two 3.0 Tesla scanners (HDxt and MR750, General Electric, Milwaukee, Wisconsin, USA) using an eight-channel head coil. The HDxt scanner was replaced by the MR750 scanner halfway during the course of the study, with approximately equal numbers of patients and controls scanned on both scanner models. The effect of the scanner upgrade was controlled for in the statistical analysis. Structural imaging included a sagittal 3D T1-weighted inversion-recovery fast spoiled gradient echo sequence (inversion time (TI) 450 msec, repetition time (TR) 7.8 msec, echo time (TE) 3.0 msec, 176 slices, voxel size of 1.0x0.9x0.9 mm) for anatomical information. A sagittal 3D fluid-attenuated inversion-recovery sequence (fast-recovery fast spin-echo with variable flip angle refocusing, 8000/123.6/2351, echo train length of 230, acquisition matrix of 224 × 224, reconstruction matrix of 256 × 256, 132 sections, voxel size of 1.2 × 1 × 1 mm) was performed to determine the severity of high-signal-intensity areas in white matter by using the Fazekas scale (27).
Pseudo-continuous arterial spin labelling (ASL) (3D fast spin-echo acquisition with background suppression; label duration of 1.5 sec, post-label delay of 2.0 sec, TR 4.8 sec, TE 9 msec, spiral readout with eight arms x 512 samples, readout bandwidth 62.5 kHz, in-plane resolution 3.2 x 3.2 mm interpolated to 1.7x1.7 mm; 36 x 5.0 mm axial slices, 2 excitations, scan time approximately 4 min.) was performed to acquire perfusion images. An approximately proton-density (PD)-weighted image was obtained by a one-excitation saturation recovery (SR) acquisition, thereby correcting for coil sensitivities.

Pseudo-continuous ASL cerebral blood flow measures

After correcting T1-weighted and pseudo-continuous ASL images for gradient nonlinearities in all three directions, data-analyses were carried out using FSL (the FMRIB Software Library, version 4.1.9; http://www.fmrib.ox.ac.uk/fsl). Preprocessing of T1-weighted images consisted of non-brain tissue removal (28), linear registration to standard space (29), and tissue segmentation (30) yielding partial volume estimates and providing a brain mask. Pseudo-continuous ASL images were linearly registered to the brain-extracted T1-weighted images. CBF maps were calculated using a single tissue compartment model (31) after subtraction of labelled images from control images. CBF was described by using the following equation:

\[
\text{CBF} = \frac{\lambda (1-e^{-\frac{\tau}{T_{1\text{gm}}}}) x \frac{e^{\frac{w}{T_{1B}}}}{2T_{1B} (1-e^{-\frac{\tau}{T_{1B}}})} \Delta S}{S_0}
\]

with post-label delay \( w = 1.5 \) sec, labelling time \( \tau = 1.5 \) sec partition coefficient \( \lambda = 0.9 \), labelling efficiency \( \varepsilon = 0.8 \times 0.75 \) (label pseudo-continuous ASL x background suppression), \( T_1 \) of blood \( T_{1B} = 1.6 \) sec, SR time for PD image \( T_{\text{sat}} = 2.0 \) sec, and correction for SR in PD image \( T_{1\text{gm}} = 1.2 \) sec. \( \Delta S \) is the ASL difference image and \( S_0 \) the PD reference image.

The brain mask was used to calculate mean whole brain CBF. In addition, the MN1152 atlas and the Harvard-Oxford cortical atlas (both part of FSL) were used to create ROIs for the grey matter (frontal, parietal, temporal and occipital combined), the insula, caudate nucleus, putamen and hippocampus to extract mean regional CBF values.

Data analysis

Clinical group data and treatment effects were analyzed with the Statistical Package for the Social Sciences (SPSS) version 20. Data are expressed as mean ± SEM (unless otherwise stated). Differences in clinical characteristics between-groups were analyzed with independent Student t-tests. Differences in CBF between groups were analyzed with a linear regression corrected for MRI scanner model, both with and without correction for MRI scanner model, BMI, systolic blood pressure, cholesterol levels, HbA1c or fasting plasma glucose levels. To analyze the longitudinal difference between treatments, a generalized estimating equation approach was used. Results were considered statistically significant when \( P < 0.05 \).
CHAPTER 8

RESULTS

Clinical characteristics, blood glucose and plasma hormone levels

Table 1 summarizes the clinical characteristics of the healthy lean individuals and T2DM patients, as described previously (25). By design, the groups differed on weight, BMI and glucose levels (P < 0.001). Furthermore, systolic blood pressure was significantly higher in T2DM patients (P = 0.001). Whole brain volume and grey matter volume did not differ significantly between groups (P = 0.1, P = 0.2, respectively). High-signal-intensity areas in white matter, based on Fazekas score (27), were scored higher in T2DM patients compared to healthy lean individuals (P = 0.004). As described previously in the T2DM patients (20), treatment with liraglutide resulted in significant weight loss after 12 weeks compared to insulin glargine (Δ -3.3 kg, Δ +0.8 kg respectively, P < 0.001). HbA1c levels decreased during both treatments, however significantly more with liraglutide compared to placebo (Δ -8 mmol/l (-0.7 %); -3 mmol/l (-0.2 %) respectively, P < 0.001). Heart rate increased significantly after 10 days treatment with liraglutide, compared to insulin glargine, but this was not statistically significant after 12 weeks treatment (after 10 days; Δ +4 beats/min, Δ 0 beats/min, respectively, P = 0.04; after 12 weeks Δ +3 beats/min, Δ 0 beats/min, respectively, P = 0.1). Systolic and diastolic pressure did not differ between treatments (P > 0.4).

Due to a technical failure, one scan of a healthy lean individuals and one scan of a T2DM patient could not be used in the analysis.

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=20)</th>
<th>Obese patients with T2DM (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.3 ± 6.2</td>
<td>59.5 ± 4.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Gender, male/female (n)</td>
<td>10/10</td>
<td>11/9</td>
<td>0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.9 ± 11.2</td>
<td>95.4 ± 15.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.5 ± 1.7</td>
<td>32.0 ± 4.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 16</td>
<td>128 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.9 ± 11</td>
<td>78 ± 8</td>
<td>0.1</td>
</tr>
<tr>
<td>Hemoglobin (mmol/l)</td>
<td>8.9 ± 0.8</td>
<td>9.0 ± 0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>37 ± 1.7</td>
<td>56 ± 10.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.6 ± 0.5</td>
<td>8.7 ± 2.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.2 ± 0.9</td>
<td>4.5 ± 1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Albumin creatinin ratio</td>
<td>0.7 ± 0.5</td>
<td>0.9 ± 0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>-</td>
<td>7.8 ± 5.0</td>
<td>-</td>
</tr>
<tr>
<td>Blood pressure lowering medications (n)</td>
<td>0</td>
<td>10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol lowering medications (n)</td>
<td>0</td>
<td>15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WMH</td>
<td>0.45 ± 0.51</td>
<td>0.95 ± 0.51</td>
<td>0.004</td>
</tr>
<tr>
<td>Whole brain volume (ml)</td>
<td>1484 ± 510</td>
<td>1458 ± 469</td>
<td>0.1</td>
</tr>
<tr>
<td>Grey matter volume (ml)</td>
<td>801 ± 334</td>
<td>785 ± 359</td>
<td>0.2</td>
</tr>
<tr>
<td>Whole brain CBF (ml/100 g/min)</td>
<td>47.0 ± 10.0</td>
<td>42.2 ± 9.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Grey matter CBF (ml/100 g/min)</td>
<td>52.2 ± 10.7</td>
<td>46.2 ± 9.1</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Data are means ± SD or number of individuals (n). CBF, cerebral blood flow; T2DM, type 2 diabetes. WMH, high-signal-intensity areas in white matter (based on Fazekas score (27)).
T2DM patients have lower CBF compared to healthy lean individuals

T2DM patients showed decreased whole brain and grey matter CBF compared to healthy controls ((mean difference ± SEM) -7.5 ± 2.8 ml/100 g/min and -8.5 ± 3.0 ml/100 g/min, respectively, P < 0.01) (Figure 1). Furthermore, T2DM patients showed decreased CBF in the predefined areas involved in central reward and appetite circuits, i.e. the putamen, caudate nucleus and insula, ([10.1 to -7.7 ml/100 g/min], P < 0.02) and in the hippocampus (-10.1 ml/100 g/min, P = 0.004) (Figure 2). After adjustment for BMI, cholesterol level, or systolic blood pressure, the group effect on CBF remained similar in whole brain and grey matter (P ≤ 0.07) and in the predefined areas of interest (P ≤ 0.09). However, after correction for fasting plasma glucose or HbA1c level, the differences between groups were not statistically significant in all areas studied (P > 0.2), except for the caudate nucleus, which tended to remain similar (P = 0.09).

Treatment with liraglutide or insulin glargine did not affect CBF

To determine the effects of treatment with liraglutide and insulin, we analysed the effect of treatment after short-term treatment (10 days) and after longer-term treatment (12 weeks). In addition, we compared the effects of liraglutide with insulin glargine. We did not observe statistically significant changes during to liraglutide treatment after 10 days nor after 12 weeks in whole brain or grey matter CBF (P > 0.4). Also in the predefined areas of interest (i.e. the hippocampus, putamen, caudate nucleus and insula), CBF did not change significantly (P > 0.1), except in the hippocampus, where a small reduction in CBF was found after 12 weeks (-2.3 ml/100 g/min, P = 0.03). Insulin glargine also did not change CBF in all the areas studied after 10 days nor after 12 weeks (P > 0.1). Comparing the effects of liraglutide and insulin treatment, no differences in CBF were observed after 10 days or 12 weeks in all areas studied (Table 2).
Figure 2: CBF in healthy lean individuals and T2DM patients. CBF in whole brain, grey matter and four regions of interest (caudate nucleus, putamen, insula and hippocampus. *) P < 0.05; #) P < 0.01; T2DM, patients with type 2 diabetes.

Table 2: Effects of liraglutide and insulin glargine treatment on CBF

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10 days</th>
<th>12 weeks</th>
<th>P-value 10dy / 12wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>43.4 ± 8.7</td>
<td>42.8 ± 10.4</td>
<td>41.7 ± 11.5</td>
<td>0.4 / 0.6</td>
</tr>
<tr>
<td>insulin</td>
<td>42.9 ± 8.4</td>
<td>44.4 ± 10.4</td>
<td>42.9 ± 11.5</td>
<td></td>
</tr>
<tr>
<td>Grey matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>47.4 ± 9.0</td>
<td>46.9 ± 11.4</td>
<td>45.2 ± 12.5</td>
<td>0.5 / 0.7</td>
</tr>
<tr>
<td>insulin</td>
<td>47.0 ± 9.1</td>
<td>48.7 ± 11.5</td>
<td>47.0 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>34.9 ± 10.7</td>
<td>34.3 ± 10.0</td>
<td>34.2 ± 11.2</td>
<td>0.8 / 0.7</td>
</tr>
<tr>
<td>insulin</td>
<td>35.4 ± 9.7</td>
<td>35.0 ± 10.4</td>
<td>35.2 ± 10.5</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>44.0 ± 11.8</td>
<td>43.5 ± 12.9</td>
<td>42.9 ± 13.9</td>
<td>0.5 / 0.7</td>
</tr>
<tr>
<td>insulin</td>
<td>44.0 ± 11.6</td>
<td>45.0 ± 12.6</td>
<td>44.9 ± 13.4</td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>55.8 ± 10.6</td>
<td>53.9 ± 14.4</td>
<td>52.6 ± 15.2</td>
<td>0.5 / 0.7</td>
</tr>
<tr>
<td>insulin</td>
<td>54.6 ± 12.5</td>
<td>55.5 ± 13.4</td>
<td>54.7 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liraglutide</td>
<td>43.5 ± 9.7</td>
<td>43.2 ± 10.9</td>
<td>41.1 ± 10.9</td>
<td>0.7 / 0.2</td>
</tr>
<tr>
<td>insulin</td>
<td>42.6 ± 8.3</td>
<td>43.6 ± 10.2</td>
<td>43.1 ± 9.9</td>
<td></td>
</tr>
</tbody>
</table>

CBF mean ± SD in mL/100 g/min. P value for liraglutide compared to insulin after 10 days and 12 weeks of treatment.

DISCUSSION

In the current study we observed a generalized decrease in CBF in T2DM patients compared to healthy lean individuals. This effect remained or tended to remain similar after correction for important risk factors for atherosclerosis, i.e. BMI, cholesterol levels or systolic blood pressure. However, correction for HbA1c or fasting plasma glucose levels had a large effect on the differences between groups, suggesting an important role for glucose dysregulation in the observed decreases in CBF in obese T2DM patients. Compared to insulin glargine, short-term or long-term treatment with liraglutide did not improve CBF in T2DM patients in whole brain and in specific areas in the CNS, i.e. the hippocampus or areas involved in the central regulation of feeding. Our finding of decreased cerebral perfusion in patients with T2DM is in line with findings of others, using ASL or single photon emission computed tomography (SPECT) scans, showing reduced CBF in diabetes (5;6;32). This finding of reduced CBF in patients with T2DM may be important for cognitive performance, as mild cognitive impairment was shown to be associated with reduced CBF (7). The relationship between diabetes and the increased risk of cognitive impairment (2;3) may be mediated through cerebrovascular disease, since T2DM is well established as a risk factor for cerebrovascular disease. T2DM is not only associated with hyperglycemia, but also with
DECREASED CEREBRAL BLOOD FLOW IN T2DM PATIENTS IS NOT IMPROVED BY TREATMENT WITH LIRAGLUTIDE

hypothesis and dyslipidemia. In our study correction for risk factors for developing atherosclerosis (i.e. BMI, systolic blood pressure or cholesterol levels) did not significantly affect the difference between T2DM patients and healthy controls. However, the difference between obese T2DM patients and healthy lean individuals disappeared after adjustment for HbA1c or plasma glucose levels. We therefore suggest that reductions in CBF in T2DM patients are mainly driven by the hyperglycemic state in these patients. The important role for hyperglycemia is supported by findings of reduced CBF in patients with type 1 diabetes (5).

Although GLP-1RA have been shown to improve glucose control and to increase peripheral vascular perfusion (11-13;33), we did not observe enhanced CBF during treatment with liraglutide. Given the involvement and effects GLP-1RA in the central regulation of feeding and energy balance in humans (19), we hypothesized that treatment with GLP-1RA may affect perfusion in areas of the CNS involved in these regulations. However, we did not observe an effect of liraglutide treatment on CBF in these areas. A previous study in humans demonstrated effects of insulin on CBF in regions that are involved in the regulation of food intake (i.e. insula, putamen and caudate nucleus), using ASL (34), indicating that peripheral hormones are capable of inducing changes in CBF in humans. However, in this study, insulin was administered acutely and intranasally. The absence of liraglutide induced effects on CBF in our study could be explained by the different route of administration, as we administrated liraglutide peripherally, which may have resulted in lower levels compared with intranasal administration. Although liraglutide is able to cross the blood brain barrier (16), arguably levels of liraglutide were not high enough to induce potential vascular effects, which may lead to improved CBF.

The presence of endothelial dysfunction or structural microvascular damage, both associated with diabetes, may reduce the possibility to demonstrate effects of treatment with liraglutide on CBF. In line with this, studies showing effects of GLP-1 and GLP-1RA on endothelial function, peripheral microvascular recruitment and perfusion were mostly performed in rodents or non-diabetic subjects (10-14), whereas studies in T2DM patients did also not observe a significant vascular effects of GLP-1 and GLP-1RA (35;36). Interestingly, similar findings have been described for insulin mediated endothelial function and skeletal muscle blood flow, which was shown to be impaired in T2DM patients (37-39). Another mechanism which could hamper potential effects of liraglutide on CBF is cerebrovascular autoregulation, i.e. the property of cerebral blood vessels to act as a homeostatic mechanism that assures stable CBF despite variations in mean arterial pressure within a certain range (40). This mechanism may overrule potential effects induced by liraglutide.

In conclusion, we observed a generalized reduced CBF in T2DM patients which is mainly associated with the hyperglycemic state and not with other risk factors for the development of cardio- and/or cerebrovascular diseases. The reduced CBF in T2DM patients may contribute to the increased risk for the development of cognitive impairment. Although GLP-1RA are associated with improvement in peripheral microvascular perfusion, we did not found indications that treatment with GLP-1RA improves cerebral perfusion in T2DM patients.
REFERENCES


DECREASED CEREBRAL BLOOD FLOW IN T2DM PATIENTS IS NOT IMPROVED BY TREATMENT WITH LIRAGLUTIDE


