Title: Positron emission tomography measured cerebral blood flow and glucose metabolism are decreased in human type 1 diabetes

Running title: Cerebral blood flow and glucose metabolism

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Abstract

Subclinical systemic microvascular dysfunction exists already in asymptomatic patients with type 1 diabetes. We hypothesized that microangiopathy, resulting from long-standing systemic hyperglycemia and hyperinsulinemia, may be generalized to the brain, resulting in changes in cerebral blood flow and metabolism in these patients. We performed dynamic $^{15}$O$\text{H}_2\text{O}$ and $^{18}$F-fluoro-2-deoxy-D-glucose ($^{18}$F$\text{FDG}$) brain positron emission tomography (PET) scans to measure cerebral blood flow (CBF) and cerebral glucose metabolism (CMR$\text{glu}$), respectively, in 30 type 1 diabetic patients and 12 age-matched healthy controls after an overnight fast. Regions of interest were automatically delineated on co-registered MRI images and full kinetic analysis was performed. Plasma glucose and insulin levels were higher in patients versus controls. Total grey matter CBF was 9%, whereas CMR$\text{glu}$ was 21% lower in type 1 diabetic versus control subjects. We conclude that at real-life fasting glucose and insulin levels, type 1 diabetes is associated with decreased resting cerebral glucose metabolism, that is only partially explained by the decreased CBF. These findings suggest that other mechanisms than generalized microangiopathy account for the altered CMR$\text{glu}$ observed in well-controlled type 1 diabetes. (NCT00626080)
Introduction

Longstanding hyperglycemia in type 1 diabetes mellitus is associated with well-known clinical micro- and macrovascular complications, that are preceded by changes in microvascular function and/or structure in multiple organ systems, including the retina (1), kidney (2) and myocardium (3). There is increasing evidence that the brain may be susceptible to the effects of hyperglycemia as well. Indeed, altered cerebral function, metabolism (4;5) and structure (6), as well as cognitive function (7) was demonstrated in type 1 diabetic patients, especially in those with peripheral microvascular complications, suggesting that diabetes-related microangiopathy is a generalized phenomenon. Insulin may play a role in the vascular and metabolic changes, as, under physiological conditions, insulin stimulates glucose uptake and promotes vasodilation in peripheral tissues (8;9). Although type 1 diabetes is characterized by insulinopenia, exogenous insulin administration results in supraphysiological systemic insulin levels. In healthy humans, the brain mainly utilizes glucose as an energy substrate in an insulin-independent manner, but insulin sensitive regions have been identified (10). Furthermore, the existence of central insulin resistance has been proposed (11). Although it is currently unknown whether elevated plasma insulin levels in human type 1 diabetic patients also result in higher insulin concentrations in the brain, it could be hypothesized that observed changes in brain function and structure in these patients may be the result of altered cerebral blood flow and metabolism due to microvascular changes resulting from both abnormal glucose and insulin levels. Indeed, several tracer studies in rats have shown that both acute (intraperitoneal glucose injection) and chronic (single streptozotocin injection) hyperglycemia may result in decreased blood to brain glucose transport, in the presence of decreased (12-14) or unaltered (15) blood flow.

Cerebral blood flow (CBF) and glucose metabolism (CMR$_{\text{glu}}$) can be measured in vivo using positron emission tomography (PET) and the tracers $[^{15}\text{O}]\text{H}_2\text{O}$ and $[^{18}\text{F}]$-2-fluoro-2-
deoxy-D-glucose ([\(^{18}\)F]FDG), respectively (16-20). Only two studies have directly compared type 1 diabetic and healthy subjects using \([^{15}\text{O}]\text{H}_2\text{O}\) and/or \([^{18}\text{F}]\text{FDG}\) PET, however, these studies have yielded conflicting results. Using \([^{18}\text{F}]\text{FDG}\) PET, Ziegler et al (21) found decreased \(\text{CMR}_{\text{glu}}\) in type 1 diabetic patients with neuropathy, but this decrease was not statistically significant in patients without diabetes-related complications. Groups, however, were small and a semi-quantitative approach to the calculation of \(\text{CMR}_{\text{glu}}\) was used. In another PET study (22), using \([^{15}\text{O}]\text{H}_2\text{O}\) and \([1-^{11}\text{C}]\text{glucose}\), no differences were found in CBF or blood-to-brain glucose transport between poorly controlled type 1 diabetic patients and healthy volunteers. This study was performed under hyperinsulinemic clamp conditions during which insulin levels were artificially and acutely raised by an intravenous infusion of insulin, whilst glucose levels were clamped at a mildly hypoglycemic (~3.6 mmol/l) level. Although clamp methodology is often used to impose an isometabolic state, it does not represent the real-life situation in type 1 diabetic patients, who usually have higher and, more importantly, fluctuating glucose and insulin levels. Since both glucose and insulin levels affect the brain and differ between type 1 diabetic and healthy subjects, a clamp situation could mask the potential differences in cerebral blood flow and glucose metabolism between groups. Therefore, the purpose of the present study was to simultaneously measure and compare CBF and \(\text{CMR}_{\text{glu}}\) in well-controlled type 1 diabetic patients and healthy men under normal daily conditions with ambient glucose and insulin levels.
Research design and methods

This cross-sectional study consisted of a screening visit to assess eligibility for participation and two end-point visits, during which MRI and PET scans were acquired. Data were collected in men with well-controlled type 1 diabetes of at least one year duration, and in healthy men in whom glucometabolic abnormalities were excluded by a 75 g oral glucose tolerance test. Groups were matched for age and BMI. Participants (age 18 to 60 years and BMI 18-35 kg/m²) were recruited from the outpatient clinic of the VU University Medical Center (VUMC), from neighbouring hospitals and through advertisements in local newspapers. After giving written informed consent, all participants underwent a screening visit, consisting of a medical history, physical examination and fasting blood and urine analyses. Exclusion criteria for all participants were a history of cardiovascular, renal or liver disease, severe head trauma, neurological or psychiatric disorders, endocrine diseases not well-controlled for the last three months, inability to undergo MRI scanning, substance abuse or the use of anticoagulants, oral steroids or any centrally acting agent. Exclusion criteria for type 1 diabetic patients were A1C > 8.5% (69 mmol/mol), proliferative retinopathy, a history of recurrent severe hypoglycemia (defined as an episode that requires external assistance to aid recovery), or a medical history of hypounawareness. Peripheral sensorimotor polyneuropathy was tested by the Toronto Clinical neuropathy scoring system (23) and the vibration perception threshold was measured by a biothesiometer (24). Participating controls did not use any medication except for one person using omeprazol because of gastroesophageal reflux disease and one using terbutaline because of mite allergy. All type 1 diabetic patients were treated for a period of at least 10 weeks prior to PET scanning with NPH insulin once or twice daily and insulin aspart at mealtimes; in addition, three patients were treated with antihypertensive medication (1 patient used an angiotensine II receptor antagonist (ARB), 1 used an angiotensin converting enzyme inhibitor (ACEI) and an ARB, 1
patient used an ACEI, an ARB, a diuretic and a calcium antagonist), three patients used cholesterol lowering medication and one used acetylsalicylic acid. Two patients had stable hypothyroidism treated with thyroxin, one patient used incidental salmeterol/fluticason/salbutamol inhalation for asthma and one had stable ulcerative colitis treated with mesalazine. Stable microalbuminuria treated with an ARB was present in one patient, two patients had stable background retinopathy and one had peripheral neuropathy (Toronto score of 9/19 and a vibration perception threshold of > 25V at 5/12 locations). The study was approved by the local Medical Ethics Review Committee and was conducted according to the Declaration of Helsinki.

Patient preparation

Prior to the imaging visit, participants were instructed to refrain from food, alcohol and coffee from 10:00PM the day before scanning. All subjects arrived at the hospital at 7:15AM and blood glucose was measured and adjusted if necessary (when blood glucose was below 5 mmol/l and falling) by the infusion of 20% glucose. Intravenous catheters were placed in the antecubital vein for blood collection and tracer injection. Two patients consumed 2-5 glucose tablets after getting up because of hypoglycemia; at arrival in the hospital blood glucose levels were 7.8 and 10.3 mmol/l respectively. In two patients, glucose at arrival was lower than 5 mmol/l (in one of two even though he consumed an apple at getting up) and 10 and 35 ml 20% glucose was given intravenously respectively to prevent hypoglycemia during scanning. Patients remained fasted during the entire imaging procedure. After checking for collateral circulation and administration of local anaesthesia using intradermal 1% lidocain, the radial artery was cannulated by an experienced anaesthesiologist. Both cannules were kept patent by a 3IEL/ml 0.9% NaCl heparin solution. All scans were performed between 9:30AM and 12:00 (noon) to minimise diurnal variations.
Data acquisition

3D structural MRI images were acquired on a 3.0 T GE Signa HDxt scanner (General Electric, Milwaukee, Wisconsin, USA), using a T1-weighted fast Spoiled Gradient echo (FSPGR) sequence. Gray matter volume assessments were made using FSL Sienax (25;26). White matter lesions were scored visually by an experienced neuro-radiologist based on T2 or FLAIR sequences using the Fazekas criteria (27).

PET scans were performed using an HRRT (Siemens/ CTI, Knoxville, TN, USA) PET scanner, as described previously (28). The protocol consisted of a $[^{15}\text{O}]H_2O$ scan to measure CBF and an $[^{18}\text{F}]FDG$ scan to measure CMR$_{\text{glu}}$. Prior to or immediately after the $[^{15}\text{O}]H_2O$ scan, a transmission scan was acquired. For the CBF study, a bolus of 800 MBq $[^{15}\text{O}]H_2O$ was administered intravenously 10 s after starting a 10 min 3D dynamic emission scan. At least 10 min after the end of the CBF study, a 60 min 3D dynamic emission scan was started 30 s before the injection of 185 MBq $[^{18}\text{F}]FDG$ (29). During both scans, arterial concentrations were monitored continuously using a dedicated on-line bloodsampler (30) to measure radioactivity. In addition, manual samples were taken for cross-calibration of the measured input function. Samples obtained during the $[^{18}\text{F}]FDG$ scan (15, 35 and 55 min post-injection) were also used to measure arterial plasma glucose levels.

Data analyses

Image processing

List mode emission data were histogrammed into multi-frame sinograms (28) which were normalized and corrected for randoms, dead time, decay, scatter and attenuation. Next, fully corrected sinograms were reconstructed using the standard 3D OP-OSEM reconstruction algorithm (31-33) resulting in 207 image planes with 256 x 256 voxels and a voxel size of
1.22 x 1.22 x 1.22 mm³. The effective spatial resolution of the reconstructed images was 3 mm full width at half maximum.

MRI images were co-registered with the PET images using the software package VINCI (34). Both PET and MRI images were rebinned and cropped and subsequently saved as a 128 x 128 x 63 matrix consisting of isotropic voxels with a linear dimension of 2.44 mm. Regions of interest (ROIs) were delineated on the MRI scan using the template defined in PVElab (35). For every subject, the volume-weighted total grey matter region was projected onto all dynamic PET frames, resulting in a grey matter time activity curve (TAC) for each subject in analyses.

**CBF**

Using non-linear regression (NLR), appropriately weighted [¹⁵O]H₂O TACs were fitted to the standard one tissue compartment model (36) to obtain CBF values.

**CMR\textsubscript{glu}**

Using a standard NLR algorithm, appropriately weighted [¹⁸F]FDG TACs were fitted to an irreversible two tissue compartment model with 3 rate constants and blood volume as fit parameters. Next, the net rate of FDG influx, $K_i$, was calculated as $K_i k_3/(k_2+k_3)$ with $K_1$ being the rate of transport from blood to brain, $k_2$ the rate of transport from brain to blood, and $k_3$ the rate of phosphorylation by hexokinase. Finally, $K_i$ was multiplied with the plasma glucose concentration and divided by a lumped constant (LC) to obtain CMR\textsubscript{glu}. The LC is a linear scaling factor accounting for the differences in transport and phosphorylation between glucose and FDG. The LC is constant under normal physiological conditions, but can change due to e.g. hypoglycemia (37). CMR\textsubscript{glu} was calculated using 2 different approaches for the LC: 1) assuming a fixed LC of 0.81 (38); 2) using a variable LC based on its reported
relationship with plasma glucose in rats (39) (for details and a third LC approach, see Online Supplemental Data). Values obtained from approach 2 were scaled to those from 1 by assuming an average LC of 0.81 for the group of healthy volunteers.

Combined measurements

The rate constant $K_1$ of $[^{18}\text{F}]$FDG is the product of flow and extraction, i.e. $K_1 = E \cdot \text{CBF}$, providing a means to calculate the $[^{18}\text{F}]$FDG extraction fraction (E). According to the Renkin-Crone model (40;41) the extraction fraction is related to the permeability surface area product, PS, according to the, i.e. $E = 1 - \exp^{-\frac{\text{PS}}{\text{CBF}}}$, where $P$ is capillary permeability (cm/min) and $S$ capillary surface area (cm$^2$/g). This equation was used to derive PS values for $[^{18}\text{F}]$FDG.

Biochemical analyses

Capillary blood glucose for safety purposes was measured using a blood glucose meter (OneTouch ultra easy, LifeScan, Inc. Milpitas, CA, USA). Arterial glucose samples were measured using the hexokinase method (Glucoquant; Roche Diagnostics, Mannheim, Germany). A1C was measured by cation-exchange chromatography (reference value: 4.3–6.1% (23-43 mmol/mol); Menarini Diagnostics, Florence, Italy). Serum insulin concentrations were quantified using immunometric assays (Advia Centaur; Siemens Medical Solutions Diagnostics, Deerfield, IL). Urine microalbumin was quantified using immunonefelometry (Immage 800, Beckman).

Statistical analysis

Group data are expressed as mean ± SD. Group effects were assessed by analyses of (co)variance (AN(C)OVA), without and with adjustment for age, BMI, A1C, glucose and insulin level. Univariate correlations (Pearson’s $r$) were used to examine associations of age,
A1C, insulin, BMI and diabetes duration with changes in CBF and CMR$_{\text{glu}}$. Analyses were performed using SPSS for Windows, version 20.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

Based on an expected difference in CMR$_{\text{glu}}$ of $2 \pm 2$ µmol/100g/min between groups (21;22), we calculated that a sample size of 24 type 1 diabetic patients and 10 healthy volunteers would result in a statistical power of 80%. To account for a drop-out rate of ~20%, we included 30 diabetic and 12 healthy subjects in total.
Results

Subject characteristics are listed in Table 1. PET scans were performed in 30 type 1 diabetic patients and 12 healthy volunteers. After quality control, $\text{CMR}_{\text{glu}}$ was available in 28 type 1 diabetic patients (one patient was excluded due to problems with arterial sampling and the other because of mild hypoglycemia during the scan that needed to be treated with a glucose infusion) and 9 healthy volunteers (one scan was excluded due to subject movement, one due to sampler problems and one due to technical problems). Similarly, CBF measurements were available in 23 type 1 diabetic patients (for 3 patients no $[^{15}\text{O}]\text{H}_2\text{O}$ was available and in 4 there were problems with arterial sampling) and in all 11 healthy volunteers that had a $[^{15}\text{O}]\text{H}_2\text{O}$ scan (for one subject no $[^{15}\text{O}]\text{H}_2\text{O}$ was available). Groups were well matched for age, BMI, blood pressure and lipid levels. No significant differences were found in grey matter volume between groups (Table 1). One patient had score 2 according to Fazekas criteria (confluent white matter lesions). No white matter lesions were detected in healthy volunteers.

$\text{CBF}$

In type 1 diabetic patients ($n = 23$), total grey matter CBF was 9% lower than in healthy volunteers ($n = 11$; $P = 0.06$; Table 2, Fig. 1A). After exclusion of patients using antihypertensive medication ($n = 3$), statins ($n = 3$), thyroxin ($n = 2$), salmeterol/fluticason/salbutamol inhalation ($n = 1$) or mesalazine ($n = 1$) and after the exclusion of left-handed subjects ($n = 1$ patient and $n = 2$ controls), results remained unchanged. Age was negatively correlated with total grey matter CBF in diabetic patients ($R = -0.6$, $P = 0.001$); adjustment for age yielded similar results. BMI did not differ significantly between groups. No correlation of CBF with BMI was observed (pooled data: $R = -0.11$, $P = 0.5$) and after
adjustment for BMI differences between groups were similar. Adjustment for arterial plasma glucose, A1C and serum insulin resulted in higher $P$-values of 0.2, 0.4 and 0.2, respectively.

$\text{CMR}_{\text{glu}}$

Throughout the scanning period, mean arterial plasma glucose in all subjects remained stable within 10%, but, as expected, was higher in patients versus controls, as were insulin levels ($P < 0.001$, and $P = 0.02$ respectively; Table 2). As expected, $K_i$ decreased with increasing glucose levels. Furthermore, $k_3$ and $K_i$ were significantly lower in patients than controls, and for $k_2$, a trend towards an increase was observed in type 1 diabetic patients (Table 2). Calculation of $\text{CMR}_{\text{glu}}$ resulted in 16 (LC scenario 2) to 21% (LC scenario 1) lower grey matter values in patients compared with healthy volunteers (Table 2; Fig. 1B). Exclusion of left-handed subjects ($n = 2$ patients and $n = 2$ controls), patients using antihypertensive medication ($n = 3$), statins ($n = 3$), thyroxin ($n = 2$), salmeterol/ fluticason/ salbutamol inhalation ($n = 1$) or mesalazine ($n = 1$) yielded similar results (data not shown). After exclusion of both patients that had received a glucose infusion prior to scanning to prevent hypoglycemia, results were similar as well. A negative correlation was found between age and total grey matter $\text{CMR}_{\text{glu}}$ (all subjects: $R = -0.36$, $P = 0.03$); however, age did not have an effect on the difference between groups ($P$ for interaction, 0.7). In healthy volunteers a negative correlation was observed between A1C and total grey matter $\text{CMR}_{\text{glu}}$ ($R = -0.8$, $P < 0.01$). Differences between groups remained unaltered after adjustment for age, A1C and insulin - adjustment for glucose level was not performed since glucose is already part of the calculation of $\text{CMR}_{\text{glu}}$ and additional correction for glucose therefore would result in over-adjustment. In addition, a negative correlation of diabetes duration and $\text{CMR}_{\text{glu}}$ was found ($R = -0.53$, $P = 0.004$). We did not find a significant correlation of BMI with $\text{CMR}_{\text{glu}}$ (pooled data: $R = -0.12$, $P = 0.5$).
Combined measurements

Average FDG extraction trended to be lower in patients versus controls by 17% \((P = 0.07;\) Table 2). According to the Renkin-Crone model, PS was 29% lower \((P = 0.001;\) Table 2).

In type 1 diabetic patients \((n = 21)\), a positive correlation was observed between total grey matter CBF and CMR\textsubscript{glu} \((R = 0.5, P < 0.05)\), whereas this correlation did not reach statistical significance in healthy volunteers \((n = 8; R = 0.6, P = 0.1)\). Adjustment for glucose levels resulted in a stronger correlation of total grey matter CBF and CMR\textsubscript{glu} in both patients \((R = 0.6, P = 0.01)\) and controls \((R = 0.9, P = 0.01)\).
Discussion

In line with the well-known hyperglycemia-related micro- and macrovascular complications in patients with type 1 diabetes, hyperglycemia may affect the brain; an increased understanding of the underlying mechanisms could improve prevention and treatment strategies. Using combined $[^{15}\text{O}]\text{H}_2\text{O}$ and $[^{18}\text{F}]\text{FDG}$ scans, decreases in CMR$_{\text{glu}}$ and to a lesser extent in CBF were observed in type 1 diabetic patients compared to healthy volunteers. This study is the first to simultaneously quantify CBF and CMR$_{\text{glu}}$ in two well-defined populations using state-of-the-art PET methodology, including full kinetic modeling using an on-line sampled arterial input curve and a high resolution PET scanner.

So far, only one study has reported a direct comparison of CMR$_{\text{glu}}$ using $[^{18}\text{F}]\text{FDG}$ PET between type 1 diabetic patients and healthy volunteers (21). In line with the present data, decreased CMR$_{\text{glu}}$ in patients with well-controlled type 1 diabetes was found. This finding, however, was not statistically significant, probably due to the small sample size of the patient group (n = 6). Using D-[U-$^{11}\text{C}$]glucose, a decreased CMR$_{\text{glu}}$ in well-controlled type 1 diabetic patients was found compared with healthy controls (42), and using [1-$^{11}\text{C}$]glucose no difference in CMR$_{\text{glu}}$ was observed between poorly controlled type 1 diabetic patients and healthy volunteers (22). The latter studies were performed under artificially clamped hyperinsulinemic (mean insulin levels of 707 and 690 pmol/L, respectively, compared with 89 pmol/L in the present study) and hypoglycemic (2.8 and 3.7 mmol/L, respectively, compared with 10.4 mmol/L in the present study) levels. In addition, $[^{11}\text{C}]\text{glucose}$ is a more difficult tracer, as it requires a correction term for regional egress of carbon-$^{11}$ labeled metabolites. Based on these studies and the present data, it can be concluded that under ambient real-life glucose and insulin levels, CMR$_{\text{glu}}$ is decreased in patients with type 1 diabetes. It may be hypothesized that for compensation, the diabetic brain uses alternative substrates (21;22;42-45).
Metabolism of FDG involves two different steps, transport across the blood-brain barrier and phosphorylation by hexokinase. The parameters describing these successive steps can only be quantified using a dynamic scanning protocol together with full kinetic modeling and an arterial input function. It should be noted that the measured rate constants relate to FDG kinetics and not to glucose kinetics. In the calculation of CMR_{glu}, however, this is taken into account by the LC. Although diabetic patients were fasting, they showed mild to modest hyperglycemia (plasma glucose levels ranging from 5.0-16.4 mmol/L), which was higher than in fasting healthy subjects (plasma glucose levels ranging from 5.1-5.7 mmol/L). In diabetic patients both steps in uptake of FDG were altered as, apart from the net rate of influx $K_i$, both transport ($K_1$) and phosphorylation ($k_3$) parameters were significantly decreased. The decrease in $K_i$ at increased glucose levels was in accordance with Michaelis-Menten (MM) kinetics, which describes competition between glucose and FDG and is valid in both normoglycemia and hyperglycemia, i.e. for plasma glucose levels that are well within the range encountered in the present study. It should be noted that hypoglycemic conditions (i.e. plasma glucose <3.8 mmol/L) would have imposed a different problem, as the transport step would then become a limiting factor due to the limited glucose supply, resulting in a change in LC (37). $k_3$ is probably decreased due to a primary effect (reduced hexokinase activity) in diabetes. Note that $k_2$ was not affected by plasma glucose levels.

Based on the linear relationship between CMR_{glu} and $K_i$, it follows that CMR_{glu} is linearly related to $E \cdot CBF$, where $E = 1 - \exp^{PS/CBF}$ (40;41). In other words, the relationship between CMR_{glu} and CBF is non-linear and, especially at higher flow values, an increase in CBF will induce a smaller increase in CMR_{glu}. Similarly, a reduction in CBF will be accompanied by at most a similar reduction in CMR_{glu}. These findings indicate that the 21% decrease in CMR_{glu} cannot be explained by the 9% reduction in CBF, and therefore that other
mechanisms than generalized microangiopathy account for the altered CMR$_{\text{glu}}$ observed in well-controlled type 1 diabetes.

With respect to CBF, only one human PET study using $[^{15}\text{O}]\text{H}_2\text{O}$ has compared type 1 diabetic patients with healthy volunteers (22) and no differences were observed between both groups. As mentioned above, however, this study was performed under hyperinsulinemic clamp conditions, with almost eightfold higher insulin levels. In contrast to the present findings, increased CBF in well-controlled type 1 diabetic patients was found using inhaled $[^{11}\text{C}]\text{H}_3\text{F}$ and PET, but these measurements were also performed under clamped conditions (insulin 667 pmol/L) (44). More importantly, in line with pre-clinical data (46), studies that did not use clamping techniques found, in line with the present data, decreased perfusion in type 1 diabetic patients compared with healthy controls (47-49). Taken data from all studies together, it may be concluded that, under real-life ambient glucose and insulin levels, CBF is decreased in type 1 diabetic patients compared with healthy volunteers. This conclusion is supported by the fact that adjustment for A1C levels resulted in a smaller between-group difference in total grey matter CBF.

In the present study, groups were well matched except for glucose and insulin levels during scanning, both of which were higher in patients due to the real-life nature of the study protocol. This made differentiation between effects of hyperglycemia and hyperinsulinemia on one hand and diabetes on the other difficult, if not impossible. Nevertheless, diabetic patients are subject to these increased glucose and insulin levels most of the day. Moreover, under normal conditions, both CBF and CMR$_{\text{glu}}$ are expected to increase in response to higher insulin levels (50;51). Consequently, a hyperinsulinemic clamp, which increases insulin to much higher levels than those seen in the present study, may have masked the decrease in CBF and CMR$_{\text{glu}}$ in diabetic patients in previous studies using such a clamp. Concerning the higher glucose levels, CMR$_{\text{glu}}$ is only indirectly measured via FDG and as expected, $K_1$ values
in diabetic patients were lower than in healthy controls. It should be noted, however, that calculated CMR_{glu} values are still correct, as these lower K_1 values compensate for the higher plasma glucose levels.

In order to convert measured FDG derived parameters to CMR_{glu}, a LC is used, which takes into account differences in transport and phosphorylation between glucose and FDG. It has been shown that this LC can change under hyperglycemic and especially hypoglycemic conditions (37). In the present study, decreased CMR_{glu} was observed in diabetic patients using either a fixed (scenario 1) or a hyperglycemia adjusted (scenario 2) LC (see Online Supplemental Data for details); therefore the finding of a decreased CMR_{glu} most likely is a true reflection of altered cerebral metabolism in type 1 diabetes. Based on these arguments, LC scenario 2 may account best for differences in glucose between groups (Online Supplemental Data). It should be noted that the equation adopted in LC scenario 2 was derived from data obtained in hyperglycemic rats and not humans. Furthermore, LC scenario 2 was based on measurements using [^{14}C]DG and not [^{18}F]FDG. Nevertheless, as the LC takes into account the differences between FDG and glucose, and since absolute values between LC of [^{18}F]FDG and [^{14}C]DG do not significantly differ and behave similarly in humans and animals (52) it does not change interpretation of the data.

It has been suggested (53) that decreased CBF and CMR_{glu} in diabetes patients could be due to a reduced brain volume, i.e. atrophy, or white matter lesions, both of which have previously been described in type 1 diabetic patients (54). However, both CBF and CMR_{glu} are expressed per volume of grey matter tissue. Therefore, differences in grey matter volume could have affected our results only indirectly via partial volume effects between groups, but in the present study, grey matter volumes as well as white matter lesions were similar between groups. This is probably due to the fact that the patients studied were investigated relatively
early in the course of their disease and did not have clinical signs or symptoms of diabetes-related complications.

Our study has some limitations. First, the inclusion of only men resulted in a relatively homogenous group and avoided menstrual-cycle dependent effects (55) in women, but we acknowledge that our findings may not be readily extrapolated to women. Besides, gender-specific difference with respect to cerebral blood flow (56) and metabolism (57;58) were reported, and consequently, the size of the study would need to be doubled to address these issues. Second, as could be expected in patients with type 1 diabetes, several co-morbidities were present. In additional analyses however, differences between patients and controls were similar after exclusion of subjects with co-morbidities. Third, it is important to note that the CBF and CMR$_{\text{glu}}$ measurements were not obtained simultaneously, since this is not possible with the techniques used. Both scans were acquired on average only 25 min apart, but were performed under stable resting conditions after an acclimatisation period of at least 20 minutes. Therefore relevant changes in CMR$_{\text{glu}}$ and/or CBF during the 25 minutes between the CBF and CMR$_{\text{glu}}$ measurements are highly unlikely to occur.

In conclusion, both CBF and CMR$_{\text{glu}}$ were decreased in patients with well-controlled type 1 diabetes, when scanned at fasting (elevated) glucose and insulin levels. Assuming that in daily life, these alterations persist throughout the day, clinical consequences, particularly in the longer term, may be expected. However, these can only be evaluated in large-scaled prospective studies in well-characterized type 1 diabetic patient cohorts.
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LWvG, MD, and AAL participated in the design of the study. LWvG performed the study, PET analyses and statistical analyses and drafted the manuscript. MCH supervised all data quality control and data analyses. MCH and AAL supervised PET analyses and critically commented on the manuscript. RGIIJ and MD clinically supervised the study and critically commented on the manuscript. NJH performed data acquisition. LAS did all radial artery punctures. All authors reviewed the text and made crucial revisions to the manuscript.

MD, AAL, RIJ and MCH are the guarantors of this work and, as such, had full access to all the study material and take responsibility for integrity of the data and the accuracy of data analyses.

Conflicts of interest/ disclosures: MD: member advisory board: Abbott, Eli Lilly, Merck Sharp & Dohme (MSD), Novo Nordisk and Poxel Pharma; Consultant: Astra-BMS; Speaker: Eli Lilly, MSD, Novo Nordisk and Sanofi; through MD, the VU University Medical Center, receives research grants from Amylin/Eli Lilly, MSD, Novo Nordisk and Sanofi; MD receives no personal payments in connection to the above-mentioned activities, but all payments are directly transferred to the Institutional Research Foundation. The other authors declare no conflict of interest.
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### Table 1 Subject characteristics

<table>
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<th>Healthy controls</th>
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</tr>
<tr>
<td><strong>Systolic Blood Pressure (mmHg)</strong></td>
<td>113 ± 10</td>
<td>115 ± 7</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mmHg)</strong></td>
<td>75 ± 7</td>
<td>77 ± 7</td>
</tr>
<tr>
<td><strong>Heart rate (s)</strong></td>
<td>66 ± 9</td>
<td>68 ± 10</td>
</tr>
<tr>
<td><strong>A1C (%) (mmol/mol)</strong></td>
<td>7.4 ± 0.6* (57 ± 6.6)</td>
<td>5.4 ± 0.2 (36 ± 2.2)</td>
</tr>
<tr>
<td><strong>Total Cholesterol (mmol/l)</strong></td>
<td>4.5 ± 0.6</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mmol/l)</strong></td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td><strong>LDL Cholesterol (mmol/l)</strong></td>
<td>2.5 ± 0.6</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/l)</strong></td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td><strong>Albumin: creatinine ratio (mg/mmol)</strong></td>
<td>1.1 ± 2.8</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td><strong>Daily insulin dose (NPH insulin) (IU/day)</strong></td>
<td>26.8 ± 11.3</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Daily insulin dose (insulin aspart) (IU/day)</strong></td>
<td>32.0 ± 11.6</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Grey matter volume (ml)</strong></td>
<td>791 ± 57</td>
<td>810 ± 70</td>
</tr>
</tbody>
</table>

Mean ± SD; T1D, type 1 diabetes; *P < 0.001 between-group difference; † measured with MRI.
Table 2 Experimentally determined parameters during PET scanning

<table>
<thead>
<tr>
<th></th>
<th>T1D patients</th>
<th>Healthy controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting parameters for T1D (n= 30) and HC (n= 12)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum insulin level (pmol/l)</td>
<td>88.8 ± 40.0</td>
<td>58.0 ± 24.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Arterial plasma glucose (mmol/l)</td>
<td>10.4 ± 3.0</td>
<td>5.5 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>[¹⁵O]H₂O PET measurements for T1D (n= 23) and HC (n= 11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBF (mL/cm³/min)</td>
<td>0.31 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>[¹⁸F]FDG PET measurements for T1D (n= 28) and HC (n= 9)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_1$ (ml/cm³/min)</td>
<td>0.044 ± 0.01</td>
<td>0.062 ± 0.007</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$k_2$ (min⁻¹)</td>
<td>0.098 ± 0.02</td>
<td>0.080 ± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>$k_3$ (min⁻¹)</td>
<td>0.037 ± 0.01</td>
<td>0.065 ± 0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>$K_i$ (ml/cm³/min)</td>
<td>0.013 ± 0.005</td>
<td>0.028 ± 0.003</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CMR&lt;sub&gt;glu&lt;/sub&gt; (µmol/cm³/min);</td>
<td>0.15 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LC= 0.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMR&lt;sub&gt;glu&lt;/sub&gt; (µmol/ cm³/min);</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LC from Schuier (39)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Combined FDG and H₂O PET measurements for T1D (n= 21) and HC (n= 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDG extraction fraction (%)</td>
<td>15 ± 4</td>
<td>18 ± 1</td>
<td>0.07</td>
</tr>
<tr>
<td>PS product (mL/cm³/min)</td>
<td>0.050 ± 0.01</td>
<td>0.070 ± 0.007</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean values ± SD. T1D, type 1 diabetes; HC, healthy controls; CMR<sub>glu</sub>, cerebral metabolic rate of glucose; CBF, cerebral blood flow; GM, gray matter; $K_i$, net influx rate; $K_f$, rate of transport from blood to brain; $k_2$, rate of transport from brain to blood; $k_3$, rate of phosphorylation by hexokinase; LC, lumped constant; PS, permeability surface area product.
Figure Legends

Fig 1. A. Mean cerebral blood flow (CBF) in total grey matter in type 1 diabetes patients (black bar; n = 23) versus healthy controls (white bar; n = 11). B. Mean cerebral glucose metabolism (CMR_{glu}), LC = 0.81, in total grey matter, in type 1 diabetes patients (black bar; n = 28) versus healthy controls (white bar; n = 9).
Fig 1. A. Mean cerebral blood flow (CBF) in total grey matter in type 1 diabetes patients (black bar; n= 23) versus healthy controls (white bar; n= 11).

$P = 0.06$
B. Mean cerebral glucose metabolism (CMRglu), LC= 0.81, in total grey matter, in type 1 diabetes patients (black bar; n= 28 ) versus healthy controls (white bar; n=9 ).
Online Supplemental Data

LC scenarios

Based on existing data, the LC can be either considered as a constant factor, previously determined to be 0.52 (1) but in later studies assumed to be 0.81, as discussed in (2). A LC dependent on the plasma glucose level, i.e. increasing in hypoglycemia (3) and decreasing in hyperglycemia (4;5) has been used as well. Experimental data on the dependence of the LC on plasma glucose is exclusively available in rats (5-7). In clinical studies, a third approach, calculating the LC from experimentally derived rate constants, was developed by Phelps and co-workers (8-10). Therefore we calculated CMR\textsubscript{glu} using these 3 approaches for the LC:

1) Assuming a fixed LC of 0.81 (2).

2) Based on previously measured LC values as a function of plasma glucose (5;7), a linear relation for the glucose range relevant to our data (plasma glucose 4 to 15 mmol), was derived to be \( \text{LC} = -0.0043C_p + 0.48 \).

3) Based on the formula for the LC as a function of rate constants (10):

\[
\text{LC} = \frac{1}{\varphi} \cdot \frac{K_1^*/K_1 \cdot k_3^*/k_3 \cdot k_2^*/k_2 \cdot (1 + k_3^*/k_3)/(1 + k_3^*/k_3)}{1 + k_3^*/k_3},
\]

where \( k_3^*/k_3 = 0.50 \) and \( k_2^*/k_2 = 1.67 \), based on rat data (FDG and glucose parameters are marked with and without an asterisk, respectively) and \( \varphi \), the fraction of glucose that once phosphorylated is metabolized further, taken to be 1. Since transport of glucose over the blood brain barrier is a facilitated and saturable transport mechanism, it can be described by Michaelis-Menten kinetics for saturable transport (5), in which

\[
K_i = \frac{T_{\text{max}}}{(K_i + C_p)},
\]

where \( T_{\text{max}} \) is the maximal transport rate and \( K_i \) the plasma glucose concentration at which the transport rate is half maximal. For healthy subjects, Blomqvist et al. (11) reported values of \( T_{\text{max}} = 0.62 \mu\text{mol/g/min} \) and \( K_i = 4.1 \text{ mM} \). Assuming that these parameters also apply to type 1 diabetes patients, \( K_i \) values can be calculated, deriving \( K_i^*/K_i \). The LC was determined for each of the
subjects available in the study (n= 9 healthy volunteers and n= 28 diabetic patients).

The values obtained from approaches 2 and 3 were scaled to an average LC of 0.81 for the group of healthy volunteers. When applying scenario 3 to the present data, a large variation in calculated LC values (CoV 8.5% for scenario 3) of healthy volunteers was observed, despite stable arterial plasma glucose levels with an average of 5.5 ± 0.2 mmol/l (CoV 3%). For comparison, the CoV in LC for healthy volunteers using scenario 2 was only 0.16%. Therefore, approach 3 was discarded for further studies. Furthermore, the assumption of constant $k_3^*/k_3$ and $k_2^*/k_2$ ratios may not carry over directly from healthy subjects to diabetic patients. In Supplemental Figure 1, obtained LC values for all 3 scenarios are plotted as a function of plasma glucose. In Supplemental Table 1, LC values and resulting CMR$_{glu}$ values are listed.
Figure 1. Lumped constant (LC) as a function of plasma glucose level. Scenario 1, fixed LC (crosses); scenario 2, LC according to Schuier et al. (7) (open squares); scenario 3, LC according to Phelps et al. (10) (black dots).
Supplemental Figure 1.
Supplemental Table 1. LC and CMR\textsubscript{glu} values for scenarios 1, 2, and 3.

<table>
<thead>
<tr>
<th></th>
<th>T1D patients (n= 28)</th>
<th>Healthy controls (n= 9)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC scenario 1</td>
<td>0.81</td>
<td>0.81</td>
<td>1</td>
</tr>
<tr>
<td>LC scenario 2</td>
<td>0.77 ± 0.02</td>
<td>0.81 ± 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LC scenario 3</td>
<td>0.68 ± 0.09</td>
<td>0.81 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CMR\textsubscript{glu} scenario 1</td>
<td>0.15 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CMR\textsubscript{glu} scenario 2</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CMR\textsubscript{glu} scenario 3</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.006</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Mean ± SD. Values from scenarios 2 and 3 were scaled to those from scenario 1 by assuming an average LC of 0.81 for the group of healthy controls. T1D, type 1 diabetes; LC, lumped constant; CMR\textsubscript{glu}, cerebral glucose metabolism in total grey matter.
Reference List


