Cerebral Blood Flow and Glucose Metabolism Measured With Positron Emission Tomography Are Decreased in Human Type 1 Diabetes

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Subclinical systemic microvascular dysfunction exists 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 (CBF) and metabolism in these patients. We performed dynamic [^{15}O]H_2O and [^{18}F]-fluoro-2-deoxy-D-glucose brain positron emission tomography scans to measure CBF and cerebral glucose metabolism (CMR_{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 coregistered magnetic resonance images and full kinetic analysis was performed. Plasma glucose and insulin levels were higher in patients versus controls. Total gray matter CBF was 9%, whereas CMR_{glu} was 21% lower in type 1 diabetic subjects 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, which is only partially explained by the decreased CBF. These findings suggest that mechanisms other than generalized microangiopathy account for the altered CMR_{glu} observed in well-controlled type 1 diabetes. ***Diabetes*** **62:2898–2904, 2013**

Long-standing hyperglycemia in type 1 diabetes is associated with well-known clinical microvascular and macrovascular complications that are preceded by changes in microvascular function 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. Altered cerebral function, metabolism (4,5), and structure (6), as well as cognitive function (7), were 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 because, 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 uses 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 attributable to microvascular changes resulting from both abnormal glucose and insulin levels. 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_{glu}) can be measured in vivo using positron emission tomography (PET) and the tracers [^{15}O]H_2O and [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), respectively (16–20). Only two studies have directly compared type 1 diabetic subjects and healthy subjects using [^{15}O]H_2O or [^{18}F]FDG PET; however, these studies have yielded conflicting results. Using [^{18}F]FDG PET, Ziegler et al. (21) found decreased CMR_{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 semiquantitative approach to the calculation of CMR_{glu} was used. In another PET study (22) using [^{15}O]H_2O and [1-^{11}C]glucose, no differences were found in CBF or blood-to-brain glucose transport between those with poorly controlled type 1 diabetes and healthy volunteers. This study was performed under hyperinsulinemic clamp conditions, during which insulin levels were artificially and acutely increased by an intravenous infusion of insulin and 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. Because 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 CBF and glucose metabolism between groups. Therefore, the purpose of the current study was to simultaneously measure and compare CBF and CMR_{glu} in those with well-controlled type 1 diabetes and healthy men under normal daily conditions with ambient glucose and insulin levels.

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RESEARCH DESIGN AND METHODS

This cross-sectional study consisted of a screening visit to assess eligibility for participation and two endpoint visits, during which magnetic resonance imaging (MRI) and PET scans were acquired. Data were collected in men with well-controlled type 1 diabetes for at least 1 year 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 18–60 years and BMI 18–35 kg/m^2) were recruited from the outpatient clinic of the VU University Medical Center, from neighboring 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 past 3 months, inability to undergo MRI scanning, and 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 person using terbutaline because of mite allergy. All type 1 diabetic patients were treated for a period of at least 10 weeks before PET scanning with NPH insulin once or twice daily and insulin aspart at meal times; in addition, three patients were treated with antihypertensive medication (one patient used an angiotensin II receptor antagonist (angiotensin receptor blocker [ARB]), one used an ACE inhibitor and an ARB, and one patient used an ACE inhibitor, an ARB, a diuretic, and a calcium antagonist), three patients used cholesterol-lowering medication, and one patient used acetylsalicylic acid. Two patients had stable hypothyroidism treated with thyroxin, one patient used incidental salmeterol/fluticason/salbutamol inhalation for asthma, and one patient 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 patient had peripheral neuropathy (Toronto score of 9/19 and a vibration perception threshold of >25 V at 5 of 12 locations). The study was approved by the local Medical Ethics Review Committee and was conducted according to the Declaration of Helsinki.

Patient preparation. Before the imaging visit, participants were instructed to refrain from food, alcohol, and coffee from 10:00 P.M. the day before scanning. All subjects arrived at the hospital at 7:15 A.M. and blood glucose was measured and adjusted if necessary (when blood glucose was <5 mmol/L and declining) by the infusion of 20% glucose. Intravenous catheters were placed in the antecubital vein for blood collection and tracer injection. Two patients consumed two to five glucose tablets after waking because of hypoglycemia; at arrival to the hospital, blood glucose levels were 7.8 and 10.3 mmol/L, respectively. In two patients, glucose at arrival was <5 mmol/L (in one of two even though he consumed an apple after awaking); 10 and 35 mL 20% glucose were administered intravenously, respectively, to prevent hypoglycemia during scanning. Patients remained fasted during the entire imaging procedure. After checking for collateral circulation and administration of local anesthesia using intradermal 1% lidocain, the radial artery was cannulated by an experienced anesthesiologist. Both cannules were kept patent by 3 IU/mL 0.9% NaCl heparin solution. All scans were performed between 9:30 A.M. and 12:00 P.M. to minimize diurnal variations.

Data acquisition. Three-dimensional (3D) structural MRI images were acquired on a 3.0-T GE Signa HDxt scanner (General Electric, Milwaukee, WI) using a T1-weighted fast spoiled gradient echo sequence. Gray matter volume assessments were made using FSL Sienax (25,26). White matter lesions were scored visually by an experienced neuroradiologist based on T2 or fluid-attenuated inversion recovery sequences using the Fazekas criteria (27).

PET scans were performed using an HRRT (Siemens/CTI, Knoxvillle, TN) PET scanner, as described previously (28). The protocol consisted of a [^{15}O]H_2O scan to measure CBF and an [^{18}F]FDG scan to measure CMR_{glu}. Before or immediately after the [^{15}O]H_2O scan, a transmission scan was acquired. For the CBF study, a bolus of 800 MBq [^{15}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}F]FDG (29). During both scans, arterial concentrations were monitored continuously using a dedicated online blood sampler (30) to measure radioactivity. In addition, manual samples were taken for cross-calibration of the measured input function. Samples obtained during the [^{18}F]FDG scan (15, 35, and 55 min postinjection) also were used to measure arterial plasma glucose levels.

Data analyses

Image processing. List mode emission data were histogrammed into multiframe sinograms (28), which were normalized and corrected for random, 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 × 256 voxels and a voxel size of 1.22 × 1.22 × 1.22 mm^3. The effective spatial resolution of the reconstructed images was 3 mm full-width at half maximum.

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

CBF. Using nonlinear regression, appropriately weighted [^{15}O]H_2O time activity curves were fitted to the standard one-tissue compartment model (36) to obtain CBF values.

CMR_{glu}. Using a standard nonlinear regression algorithm, appropriately weighted [^{18}F]FDG time activity curves were fitted to an irreversible two-tissue compartment model with three rate constants and blood volume as fit parameters. Next, the net rate of FDG influx, K_i, was calculated as $K_1 k_3/(k_2+k_3)$, 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_{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 because of hypoglycemia (37), for example. CMR_{glu} was calculated using two different approaches for the LC: assuming a fixed LC of 0.81 (38) or using a variable LC based on its reported relationship with plasma glucose in rats (39) (for details and a third LC approach, see Supplementary Fig. 1). Values obtained from the second approach were scaled to those from the first approach by assuming an average LC of 0.81 for the group of healthy volunteers.

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

Biochemical analyses. Capillary blood glucose for safety purposes was measured using a blood glucose meter (OneTouch ultra easy; LifeScan, Milpitas, CA). 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 ANCOVA, without and with adjustment for age, BMI, A1C, glucose, and insulin level. Univariate correlations (Pearson r) were used to examine associations of age, A1C, insulin, BMI, and diabetes duration with changes in CBF and CMR_{glu}. Analyses were performed using SPSS for Windows 20.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

Based on an expected difference in CMR_{glu} of 2 ± 2 μmol/100 g/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 subjects 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, CMR_{glu} was available in 28 type 1 diabetic patients (one patient was excluded because of problems with arterial sampling and the other was excluded because of mild hypoglycemia during the scan that needed to be treated with a glucose infusion) and nine healthy volunteers (one scan was

TABLE 1
Subject characteristics

	T1D patients	Healthy controls
N	30	12
Age, years	36.8 ± 9.7	35.2 ± 13.2
Diabetes duration, years	13.4 ± 8.5	NA
Age of diabetes onset, years	23.4 ± 11.5	NA
BMI, kg/m^2	25.3 ± 2.6	25.1 ± 3.0
Systolic blood pressure, mmHg	113 ± 10	115 ± 7
Diastolic blood pressure, mmHg	75 ± 7	77 ± 7
Heart rate, s	66 ± 9	68 ± 10
A1C, % (mmol/mol)	7.4 ± 0.6* (57 ± 6.6)	5.4 ± 0.2 (36 ± 2.2)
Total cholesterol, mmol/L	4.5 ± 0.6	4.6 ± 1.0
HDL cholesterol, mmol/L	1.5 ± 0.4	1.4 ± 0.3
LDL cholesterol, mmol/L	2.5 ± 0.6	2.7 ± 1.0
Triglycerides, mmol/L	1.1 ± 0.5	1.2 ± 0.5
Albumin:creatinine ratio, mg/mmol	1.1 ± 2.8	0.4 ± 0.2
Daily insulin dose of NPH insulin, IU/day	26.8 ± 11.3	NA
Daily insulin dose of insulin aspart, IU/day	32.0 ± 11.6	NA
Gray matter volume, mL†	791 ± 57	810 ± 70

Data are mean ± SD. T1D, type 1 diabetes. *$P < 0.001$ between-group difference. †Measured with MRI.

excluded because of subject movement, one was excluded because of sampler problems, and one was excluded because of technical problems). Similarly, CBF measurements were available in 23 type 1 diabetic patients (for three patients no [^{15}O]H_2O was available and in four patients there were problems with arterial sampling) and in all 11 healthy volunteers who had a [^{15}O]H_2O scan (for one subject no [^{15}O]H_2O was available). Groups were well-matched for age, BMI, blood pressure, and lipid levels. No significant differences were found in gray 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.

CBF. In type 1 diabetic patients ($n = 23$), total gray matter CBF was 9% lower than in healthy volunteers ($n = 11$; $P = 0.06$; Table 2 and Fig. 1*A*). 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-hand-dominant subjects ($n = 1$ patient and $n = 2$ controls), results remained unchanged. Age was negatively correlated with total gray 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.

CMR_{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_1 decreased with increasing glucose levels. Furthermore, k_3 and K_i were significantly lower in patients than in controls; for k_2, a trend toward an increase was observed in type 1 diabetic patients (Table

TABLE 2
Experimentally determined parameters during PET scanning

Parameters	T1D patients	HC	*P*
Fasting parameters for T1D ($n = 30$) and HC ($n = 12$)			
Serum insulin level, pmol/L	88.8 ± 40.0	58.0 ± 24.4	0.02
Arterial plasma glucose, mmol/L	10.4 ± 3.0	5.5 ± 0.2	<0.001
[^{15}O]H_2O PET measurements for T1D ($n = 23$) and HC ($n = 11$)			
CBF, mL/cm^3/min	0.31 ± 0.05	0.34 ± 0.05	0.06
[^{18}F]FDG PET measurements for T1D ($n = 28$) and HC ($n = 9$)			
K_1, mL/cm^3/min	0.044 ± 0.01	0.062 ± 0.007	<0.001
k_2, min	0.098 ± 0.02	0.080 ± 0.03	0.06
k_3, min	0.037 ± 0.01	0.065 ± 0.02	0.001
K_i, mL/cm^3/min	0.013 ± 0.005	0.028 ± 0.003	<0.001
CMR_{glu}, μmol/cm^3/min; LC = 0.81	0.15 ± 0.02	0.19 ± 0.02	<0.001
CMR_{glu}, μmol/cm^3/min; LC from Schuier et al. (39)	0.16 ± 0.02	0.19 ± 0.02	<0.001
Combined FDG and H_2O PET measurements for T1D ($n = 21$) and HC ($n = 8$)			
FDG extraction fraction, %	15 ± 4	18 ± 1	0.07
PS product, mL/cm^3/min	0.050 ± 0.01	0.070 ± 0.007	0.001

Data are expressed as mean values ± SD. T1D, type 1 diabetes; HC, healthy controls; GM, gray matter.

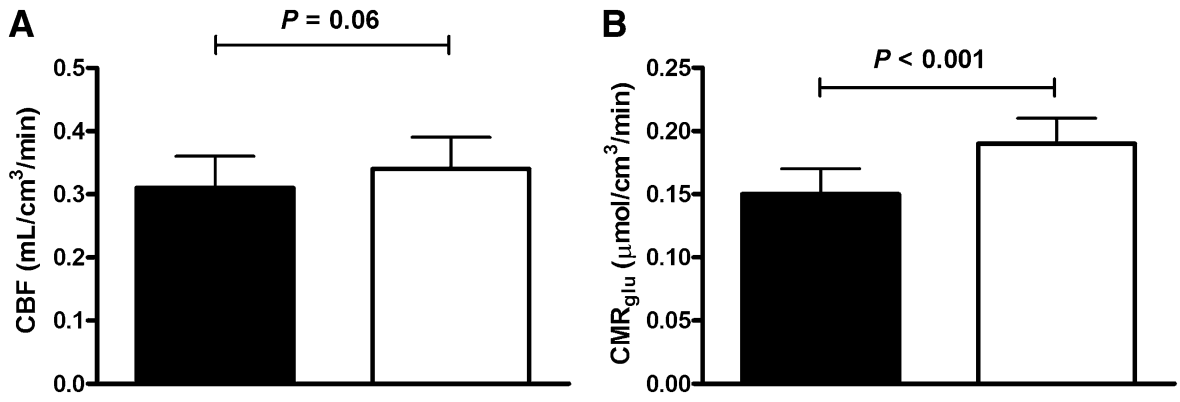

FIG. 1. *A*: Mean CBF in total gray matter in type 1 diabetic patients (black bar; $n = 23$) vs. healthy controls (white bar; $n = 11$). *B*: Mean CMR_{glu} (LC = 0.81) in total gray matter in type 1 diabetic patients (black bar; $n = 28$) vs. healthy controls (white bar; $n = 9$).

2). Calculation of CMR_{glu} resulted in 16% (LC scenario 2) to 21% (LC scenario 1) (Supplementary Table 1) lower gray matter values in patients compared with healthy volunteers (Table 2 and Fig. 1*B*). Exclusion of left-hand-dominant 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 who had received a glucose infusion before scanning to prevent hypoglycemia, results were similar as well. A negative correlation was found between age and total gray matter CMR_{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 gray matter CMR_{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 because glucose is already part of the calculation of CMR_{glu} and additional correction for glucose therefore would result in overadjustment. In addition, a negative correlation of diabetes duration and CMR_{glu} was found ($R = -0.53$; $P = 0.004$). We did not find a significant correlation of BMI with CMR_{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 gray matter CBF and CMR_{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 gray matter CBF and CMR_{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 microvascular 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 $[^{15}O]H_2O$ and [^{18}F]FDG scans, decreases in CMR_{glu} and, to a lesser extent, in CBF were observed in type 1 diabetic patients compared with healthy volunteers. This study is the first to simultaneously quantify CBF and CMR_{glu} in two well-defined populations using state-of-the-art PET methodology, including full kinetic modeling using an online sampled arterial input curve and a high-resolution PET scanner.

So far, only one study has reported a direct comparison of CMR_{glu} using [^{18}F]FDG PET between type 1 diabetic patients and healthy volunteers (21). In line with the present data, decreased CMR_{glu} in patients with well-controlled type 1 diabetes was found. This finding, however, was not statistically significant, probably because of the small sample size of the patient group ($n = 6$). Using D-[U-^{11}C]glucose, a decreased CMR_{glu} in well-controlled type 1 diabetic patients was found compared with healthy controls (42), and using [1-^{11}C]glucose no difference in CMR_{glu} was observed between patients with poorly controlled type 1 diabetes 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 current study) and hypoglycemic (2.8 and 3.7 mmol/L, respectively, compared with 10.4 mmol/L in the current study) levels. In addition, [^{11}C]glucose is a more difficult tracer, because it requires a correction term for regional egress of ^{11}C-labeled metabolites. Based on these studies and the present data, it can be concluded that under ambient real-life glucose and insulin levels, CMR_{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 be quantified only by 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 to 16.4 mmol/L), which was higher than in fasting healthy subjects (plasma glucose levels ranging from 5.1 to 5.7 mmol/L). In diabetic patients, both steps in uptake of FDG were altered because, 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_1 at increased glucose levels was in accordance with Michaelis-Menten 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 current study. It should be noted that hypoglycemic conditions (i.e., plasma glucose <3.8 mmol/L) would have imposed a different problem, because the transport step would then become a limiting factor because of the limited glucose supply, resulting in a change in LC (37). The k_3 is probably decreased because of 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_1, 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 nonlinear 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 mechanisms other than generalized microangiopathy account for the altered CMR_{glu} observed in well-controlled type 1 diabetes.

With respect to CBF, only one human PET study using $[^{15}O]H_2O$ has compared type 1 diabetic patients with healthy volunteers (22) and no differences were observed between both groups. As mentioned, however, this study was performed under hyperinsulinemic clamp conditions, with almost eight-fold higher insulin levels. In contrast to the present findings, increased CBF in patients with well-controlled type 1 diabetes was found using inhaled $[^{11}C]H_3F$ and PET, but these measurements were also performed under clamped conditions (insulin 667 pmol/L) (44). More importantly, in line with preclinical 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). With data from all studies taken together, it may be concluded that with 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 gray matter CBF.

In the current study, groups were well-matched except for glucose and insulin levels during scanning, both of which were higher in patients because of the real-life nature of the study protocol. This made differentiation between effects of hyperglycemia and hyperinsulinemia and diabetes 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_{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 current study, may have masked the decrease in CBF and CMR_{glu} in diabetic patients in previous studies using such a clamp. Concerning the higher glucose levels, CMR_{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, because these lower K_1 values compensate for the higher plasma glucose levels.

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 current study, decreased CMR_{glu} was observed in diabetic patients using either a fixed (scenario 1) or a hyperglycemia-adjusted (scenario 2) LC (Supplementary Data); 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 (Supplementary Data). It should be noted that the equation adopted in LC scenario 2 was derived from data obtained from hyperglycemic rats and not humans. Furthermore, LC scenario 2 was based on measurements using $[^{14}C]$DG and not $[^{18}F]$FDG. Nevertheless, because the LC takes into account the differences between FDG and glucose, and because 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 attributable to a reduced brain volume, i.e., atrophy, or white matter lesions, both of which previously have been described in type 1 diabetic patients (54). However, both CBF and CMR_{glu} are expressed per volume of gray matter tissue. Therefore, differences in gray matter volume could have affected our results only indirectly via partial volume effects between groups but, in the current study, gray matter volumes as well as white matter lesions were similar between groups. This is probably attributable 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, sex-specific difference with respect to CBF (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 comorbidities were present. In additional analyses, however, differences between patients and controls were similar after exclusion of subjects with comorbidities. Third, it is important to note that the CBF and CMR_{glu} measurements were not obtained simultaneously, because 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 acclimatization period of at least 20 min. Therefore relevant changes in CMR_{glu} or CBF during the 25 min between the CBF and CMR_{glu} measurements are highly unlikely to occur.

In conclusion, both CBF and CMR_{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 only can be evaluated in large-scale prospective studies in well-characterized type 1 diabetic cohorts.

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L.W.v.G. participated in the design of the study, performed the study, performed PET analyses and statistical analyses, and drafted the manuscript. M.C.H. supervised all data quality control and data analyses, supervised PET analyses, and critically commented on the manuscript. R.G.I. clinically supervised the study and critically commented on the manuscript. N.J.H. performed data acquisition. L.A.S. performed all radial artery punctures. A.A.L. participated in the design of the study, supervised PET analyses, and critically commented on the manuscript. M.D. participated in the design of the study, clinically supervised the study, and critically commented on the manuscript. All authors reviewed the text and made crucial revisions to the manuscript. M.C.H., R.G.I., A.A.L., and M.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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