Global and Regional Effects of Type 2 Diabetes Mellitus on Brain Tissue Volumes and Cerebral Vasoreactivity

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Running title: Regional perfusion and brain volumes in diabetes

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ABSTRACT

OBJECTIVE: The aims of this study were to evaluate the regional effects of type 2 diabetes and associated conditions on cerebral tissue volumes and cerebral blood flow (CBF) regulation.

RESEARCH DESIGN AND METHODS: CBF was examined in 26 diabetic (age 61.6 ± 6.6 years) and 25 control subjects (age 60.4 ± 8.6 years) using continuous arterial spin labeling (CASL) imaging during baseline, hyperventilation, and CO₂ rebreathing. Regional gray (GM) and white matter (WM), cerebrospinal fluid (CSF), and white matter hyperintensities (WMHs) volumes were measured on T1-weighted inversion recovery fast gradient echo and fluid-attenuation inversion recovery MRI at 3 Tesla.

RESULTS: The diabetic group had smaller global WM (p=0.006) and GM (p=0.001) and larger CSF (36.3%, p<0.0001) volumes than the control group. Regional differences were observed for WM (-13.1%, p=0.0008) and CSF (36.3%, p<0.0001) in the frontal region, for CSF (20.9%, p=0.0002) in the temporal region, and for GM (-3.0%, p=0.04) and CSF (17.6%, p=0.01) in the parieto-occipital region. Baseline regional CBF (p=0.006) and CO₂ reactivity (p=0.005) were reduced in diabetic group.

Hypoperfusion in the frontal region was associated with GM atrophy (p<0.0001). Higher hemoglobin A1C was associated with lower CBF (p<0.0001) and greater CSF (p=0.002) within temporal region.

CONCLUSIONS: Type 2 diabetes is associated with cortical and subcortical atrophy involving several brain regions and with diminished regional cerebral perfusion and vasoreactivity. Uncontrolled diabetes may further contribute to hypoperfusion and atrophy. Diabetic metabolic disturbance and blood flow dysregulation that affects preferentially frontal and temporal regions may have implications for cognition and balance in elderly with diabetes.

Abbreviations: CBF, cerebral blood flow ● WM, white matter ● GM, gray matter ● CSF, cerebrospinal fluid, ● CASL, continuous arterial spin labeling ● WMHs, white matter hyperintensities
Diabetes mellitus is a prevalent condition associated with substantial morbidity due to vascular complications (1). Diabetes alters endothelial function (2) and permeability of the blood-brain barrier, thus affecting microcirculation and regional metabolism (3). Studies in type 1 diabetes have shown that the fronto-temporal cortex and periventricular white matter (3) are more affected by diabetic metabolic disturbance. SPECT studies suggest that chronic hyperglycemia alters cerebral blood flow (CBF) in the frontal, temporal, parietal, occipital, and cerebellar regions of interest (ROI) (4,5). Vasoreactivity to acetazolamide is not homogeneous, with a majority of hypoperfused and some hyperperfused ROIs (6). White matter hyperintensities (WMHs) on T2-weighted MRI (7) have been associated with arteriolosclerosis, arising as consequences of aging, diabetes, and other cardiovascular risk factors (8,9). Vascular and neurodegenerative changes in these structures have consequences for regional perfusion, cognitive impairment, and balance in elderly people with type 2 diabetes (10,11). We hypothesize that type 2 diabetes is associated with microvascular disease, manifesting as WMHs and CBF dysregulation, and neuronal loss affecting preferentially fronto-temporal regions. We aimed to determine the effects of type 2 diabetes on regional brain volumes and vasoreactivity using anatomical imaging and continuous arterial spin labeling (CASL) blood flow MRI at 3 Tesla (12,13).

**RESEARCH DESIGN AND METHODS**

**SUBJECTS**

Studies were conducted in the Syncope and Falls in the Elderly Laboratory and at the Magnetic Resonance Imaging Center at the Beth Israel Deaconess Medical Center using a quadrature head coil. All subjects were recruited consecutively and provided informed consent, approved by the Institutional Review Board. The study control groups consisted of 25 healthy subjects who were normotensive and were not treated for any systemic disease and 26 subjects with type 2 diabetes (Table 1). All subjects were screened with a medical history and physical and laboratory examinations. Diabetic subjects were treated with insulin (9), oral glucose-control agents (sulfonylurea and second generation agents (7), meglitinides (1), thiazolidinediones (4), and biguanides (8)) and their combinations (13) or diet (4) and for hypertension when clinically diagnosed (8). Diabetic retinopathy was diagnosed in 10 diabetic patients with the Joslin Vision Network video-digital retinal imaging (14). Statins were used in 4 control and 8 diabetic subjects. Urinary albumin, creatinine, and their ratio were not different between the groups. Diabetic subjects reported symptoms of dizziness (4) and syncope (2), orthostatic hypotension (2), numbness (8), and peripheral neuropathy (3). Subjects with a history of stroke, myocardial infarction, congestive heart failure, and other clinically important cardiac diseases, arrhythmias,
significant nephropathy, kidney or liver transplant, renal or congestive heart failure, carotid artery stenosis, and neurological or other systemic disorders were excluded. Subjects with MRI incompatible metal implants, pacemakers, arterial stents and claustrophobia were also excluded.

Magnetic resonance imaging
Anatomical imaging protocol included: T1-weighted inversion-recovery fast gradient echo (IR-FGE): $T_1/T_E/T_R=600/3.3/8.1$ ms, 24 cm × 19 cm field of view (FOV), 256 × 192 matrix size, 3 mm slice thickness (no skip); fluid-attenuated inversion recovery (FLAIR): $T_1/T_E/T_R=2250/161/11000$ ms, 24 cm × 24 cm FOV, 256 × 160 matrix size, 5 mm slice thickness (1.5 mm skip). CASL MRI was used for blood flow measurements (12,13). An echo planar imaging sequence was applied with $T_E=31$ ms, 24 cm × 24 cm FOV, 64 × 64 matrix size, 5 mm slice thickness (5 mm skip) for 8 slices starting at the level of ventricles. Tagged and control images were collected over 5-minute periods of normal breathing, CO$_2$ rebreathing of 95% air and 5% CO$_2$, and hyperventilation. Images were obtained every 8 seconds and averaged for each condition. End-tidal CO$_2$ was continuously monitored and averaged over 15-second intervals for all conditions. A CBF map was reconstructed for each condition, as previously described (ml / 100 g / min) (12,13), and corrected for the effect of hematocrit on T1 (15).

Image analysis
All image data were processed on a Linux workstation, using tools developed in the IDL programming environment (Research Systems, Boulder Co). Figure 1A presents an example of IR-FGE (I1) and FLAIR (F1) images and a T2 reference image used for CBF map reconstruction (C1). First, a 3D ROI corresponding to the parenchymal brain was extracted using the Brain Extraction Tools algorithm (16) on the IR-FGE and the FLAIR images and by simple thresholding of the T2 reference image for the CBF maps. Each ROI was divided into eight regions. On each axial slice, an ellipse was fitted to the edge of the brain using a non-linear least squares method. The medial axis of the smallest rectangle to enclose the ellipse was computed with the ellipse parameters. This first step allowed the delineation of six regions on all axial slices: the frontal, temporal, and parieto-occipital for each hemisphere separated using the ellipse major axis (Figure 1A, I2). Supraventricular slices were analyzed as one region that was named the cortical region. This region definition was proposed by Dahl et al (17) for quantification of regional perfusion using SPECT. It was applied to outline the same eight regions on all ROIs without any registration step, as illustrated on Figure 1A (I2, F2 and C2). Regions outline major intracranial vascular territories and the frontal, temporal, and parieto-occipital lobes. A relative limitation is that these regions do not follow the precise anatomical boundaries, and therefore may include small areas with different functions. Accordingly, our goal was to quantify the global differences in distribution of CBF and vascular reactivity to CO$_2$ rather than responses to local
The expectation-maximization (EM) algorithm was used to assess gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) volumes by segmenting the IR-FGE ROI into 3 classes (Figure 1A, I3). The EM method estimates iteratively the parameters of a model of the image histogram (3 Gaussian distributions) by maximizing the likelihood of the distribution. On the FLAIR ROI, WMH seeds were identified using thresholding of hyperintense pixels. Borders of WMHs were then detected using a simple region growing method applied on WMH seeds, yielding a connected WMH cluster for each seed (Figure 1A, F3). The volumes of the whole brain and of the 8 regions were computed on the IR-FGE images. GM, WM, and CSF volumes were normalized for the volume of each region on the segmented IR-FGE images (Figure 1A, I4). WMHs volume was normalized for the volume of each region on the segmented FLAIR images (Figure 1A, F4). Each CBF map (Figure 1A, C3) was averaged within each region for each breathing exercise (Figure 1A, C4). CO₂ reactivity was computed in each region as the slope of the linear fit between end tidal CO₂ and CBF values for each condition. Relative reactivity was also calculated as a percentage change in CBF and CO₂ between hyperventilation and rebreathing.

Statistical Analysis
Descriptive statistics were used to summarize all variables. Demographic and laboratory variables were compared between groups using one-way ANOVA and Fisher’s exact test. The volumes (whole brain and in regions), the normalized GM, WM, CSF, and WMHs regional volumes were compared between the control and diabetic groups using the least square models with adjustments for age, gender, regions, and hemisphere side. CBF and CO₂ reactivity comparisons between the groups and conditions (baseline, CO₂ rebreathing, and hyperventilation) also include hematocrit and body mass index (BMI) as co-variants. Step-wise multiple regression and least square models were also used to test the effects of hemoglobin A1C, triglycerides, systolic BP, and BMI as continuous variables in the model. The effects of diabetic retinopathy, hypertension, and neuropathy were included as nominal variables and evaluated using same approach.

RESULTS
Demographic and laboratory measures
Table 1 compares the demographic characteristics and intracranial brain volume of the control and the diabetes groups. Age, gender, and race did not differ between the groups. Diabetic subjects had higher BMI (p=0.01), hemoglobin A1C (p<0.0001), blood glucose (p=0.002), systolic blood pressure (p=0.04), and cholesterol (p=0.007) but lower hematocrit (p=0.02).

Regional intracranial volumes
The regional volumes were similar between the groups, except for the cortical region, which was smaller in the diabetes group (p=0.007). Figures 1B-E show normalized...
volumes of WM, GM, CSF, and WMHs in the frontal, temporal, parieto-occipital, and cortical regions for both groups. Over all regions, the diabetes group had smaller WM volume (p=0.006, Figure 1B) and GM matter volume (p=0.001, Figure 1C) but larger CSF volume (p < 0.0001, Figure 1D) than the control group. This difference was also observed for WM (-13.1%, p=0.0008) and CSF (36.3%, p < 0.0001) in the frontal region, for CSF (20.9%, p=0.0002) in the temporal region, and for GM (-3.0%, p=0.04) and CSF (17.6%, p=0.01) in the parieto-occipital region.

In the control and diabetes groups, normalized WMHs volume differed between regions (p < 0.0001). WMHs volume was greater in the temporal (p < 0.0001) and frontal regions, and smaller in the parieto-occipital (p=0.03) and cortical regions (p < 0.0001) (Figure 1E). WMHs volume increased with age (p < 0.0001, r=0.49) and was higher in the left hemisphere (p=0.004).

Presence of diabetic retinopathy was associated with greater CSF within the temporal region (r=0.58, p=0.0003). Diabetics with hypertension had increased CSF volume (p < 0.0001) and decreased GM volume (p=0.008), and greater CSF volume in frontal (p < 0.0001) and temporal (p=0.03) regions and GM volume in the frontal region (p=0.05) compared to with normotensive diabetics. In the diabetes group, A1C was associated with more atrophy (CSF: r=0.72, p=0.002), within the temporal region.

Cerebral blood flow and CO₂ reactivity
Figure 2 shows T2 reference images and the corresponding CBF maps for baseline, CO₂ rebreathing, and hyperventilation for a control subject (A,B,C,D) and a diabetic subject (E,F,G,H). CBF differed among test conditions (p < 0.0001) in both groups. During baseline rest, the regional CBF was higher in the parieto-occipital (p < 0.0001) and lower in the frontal (p=0.0005) and temporal (p=0.02) regions in both groups (Figure 2I). Over all regions, CBF was lower in the diabetic group than in the control group during normal breathing (p=0.006) and CO₂ rebreathing (p=0.001), but was similar during hyperventilation. Figure 2J shows group CO₂ reactivity values that were lower in the diabetic group than in the control group (p=0.005) over all regions. Relative CO₂ reactivity values were also lower in the diabetic group (p=0.02). For all conditions, CO₂ values did not differ between groups (control: 36.1 ± 5.3 mm Hg, diabetes: 37.0 ± 4.3 mm Hg during baseline rest).

CBF during baseline was positively associated with regional GM volume (r=0.77, p < 0.0001) reflecting CBF decline in frontal (p < 0.0001) and increase in the parieto-occipital regions (p=0.003) in both groups. In the frontal region, steeper slope of regression (4.99 vs. 2.7) indicated greater CBF decline in diabetes group. WMHs were associated with reduced CO₂ reactivity (r=-0.54, p=0.0004) in the control group and contributed to regional differences in vasoreactivity (p=0.001) in the diabetes group. In the diabetes group, retinopathy and hypertension were associated with lower CBF during hypercapnia (r=-0.61, p=0.0008 and r=-0.61, p=0.002) and hypocapnia (r=-0.77, p=0.05 and r=-0.80 p=0.003). These associations were observed
within the temporal region, during hypercapnia for retinopathy ($p=0.03$), and during hypercapnia ($p=0.02$) and hypocapnia ($p=0.04$) for hypertension. Higher BMI was associated with lower CBF in both groups ($r=-0.66$, $p < 0.01$). In the diabetic group, higher A1C was associated with lower CBF ($r=-0.78$, $p < 0.0001$) and lower CO$_2$ reactivity ($r=-0.45$, $p=0.009$). Higher baseline systolic BP was associated with lower CBF ($r=-0.41$, $p < 0.002$). Triglycerides had no significant effect on CBF. Retinopathy was associated with lower CO$_2$ reactivity ($r=-0.47$, $p=0.03$).

**DISCUSSION**

This study demonstrated that cortical and subcortical atrophy in type 2 diabetes is associated with diminished regional cerebral perfusion and vasoreactivity. Brain atrophy, as indicated by CSF volume, was prominent in the frontal and temporal regions. Hypoperfusion during baseline rest and diminished CBF response to hypercapnia affected all regions. Hemoglobin A1C levels and diabetic retinopathy were further associated with CBF reduction, altered vasoreactivity and atrophy, that was most prominent in the temporal region. This study focused on neuroasymptomatic subjects, and so those with significant late complications of diabetes and history of cerebrovascular accidents were excluded. Cerebromicrovascular disease and endothelial dysfunction affect multiple brain regions and vascular territories and are associated with CBF dysregulation beyond the brain atrophy.

Hyperglycemia is a unifying mechanism for diabetic tissue damage in the brain as glucose utilization requires transport through the blood-brain barrier and metabolism, to provide energy supply (20). Altered glucose transporter regulation results in intracellular hyperglycemia in endothelial cells and neurons (21),(22). Signaling of oxidative stress (23),(24) in mitochondria and the endoplasmic reticulum leads to activation of four major cell damaging pathways. Superoxide also triggers an inflammatory response through the release of pro-inflammatory cytokines tumor necrosis factor alpha, endothelin 1, and interleukins and decreased synthesis of nitric oxide. These cascades activate pro-apoptotic pathways and ultimately cause neuronal cell damage and death. The finding that N-acetyl aspartate (an indicator of functional neuronal mass), was reduced in hypertensive diabetic patients in the areas affected by WMHs, supports the notion that neuronal loss in frontal and temporal associative areas may contribute to functional decline in type 2 diabetes (25). In our study, WMHs were associated with regional differences in white matter volume, vasoreactivity and higher hemoglobin A1C. These results support the theory that hyperglycemia in association with other mechanisms (i.e., those related to oxidative stress) may contribute to neurodegeneration in diabetic brain in addition to endothelial dysfunction.

Our results demonstrating reduced CBF in type 2 diabetes are consistent with CBF abnormalities reported in earlier studies. Semi-quantitative assessments of regional CBF using SPECT detected the lower ratio between regions with normal vs.
areas of reduced CBF in type 1 diabetics (4), (5). The ratio was inversely correlated with systolic BP, total cholesterol, and atherogenic index, and it was positively correlated with high-density lipoprotein and cholesterol. These observations suggest that the age-related CBF reduction may be accelerated by a combination of hyperglycemia plus other risk factors for arteriosclerosis (26,27). The regional differences in cerebral metabolic capacity may explain increased sensitivity to hyperglycemia in the cerebral cortex (28).

Previous studies described reduced or normal CO\textsubscript{2} reactivity in diabetes (32,33). This study applying CASL for evaluation of CBF and CO\textsubscript{2} vasoreactivity demonstrated that CASL is a reliable tool for assessment of flow reserve in an elderly diabetic population.

There may be some limitation to this approach. Due to the short decay time of the CASL label (about 1 s), CBF measurements might be affected for longer arterial transit time at low flow as during hypocapnia. It is important to mention that CBF measurements that reflect mainly flow in the gray matter were normalized for the volume of the ROI on which they were computed and were also adjusted for any variability associated with age, gender, hematocrit, and BMI to adjust for the effects of tissue loss and possible effects of other variables and risk factors. Therefore, the observed CBF changes are unlikely to be affected by a partial volume effects due to an increased CSF. Our region selection approach allowed comparisons among different brain areas anatomical and CBF images. As the method did not require any registration step, the definition of identical regions on all ROIs was performed regardless of the differences in matrix and voxel size between IR-FGE, FLAIR, and CASL images. We validated our automated method of WMHs quantification, and we found excellent correlations between WMHs volume and clinical rating scale (35).

The segmentation method of the IR-FGE image allows accurate classification of noisy pixels and thus reliable measurements of CSF volume that has been validated using the phantom model (36). Regional intensity variation caused by an inhomogeneous RF coil and low signal to noise ratio, however, may reduce the accuracy of the brain tissue classification. The EM based segmentation method was selected because it can estimate the intensity inhomogenities. In addition, the voxel classification is influenced primarily by the neighboring voxels, reducing the difficulties encountered in noisy regions of the images (37). The relatively thick, 5 mm slices and 1.5 mm gaps in the FLAIR images may be a contributor to errors in the measurement of the WMHs. In order to estimate this uncertainty a high-resolution model of the WMHs was created with the same parameters. Then, an axial offset to the slices' position was introduced and the volume was calculated as a function of this offset. It is estimated that a ± 6% uncertainty in the measured WMHs volume is introduced by the selected geometry of the FLAIR images (36).

Microvascular and macrovascular disease and
This study addressed an important question about the effects of type 2 diabetes on regional cerebral blood flow distribution and vasomotor reserve in older adults, and provided evidence for reduced perfusion beyond gray and white matter atrophy. Higher levels of hemoglobin A1C were associated with lower CBF and greater CSF volume in the temporal region. Reduced resting blood flow may reflect combined effects of microvascular disease and metabolic tissue damage affecting preferentially frontal and temporal regions. These findings are clinically relevant for functional outcomes, such as cognition and balance of elderly people with diabetes. Further study of the mechanisms relating prospectively hypoperfusion to neurodegeneration and functional outcomes in type 2 diabetes is merited.

Acknowledgements

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Reference List


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Tables:

**Table 1: Demographic characteristics of the control and the diabetes groups**

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<th>Group</th>
<th>Control</th>
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<tr>
<td>Age (years)</td>
<td>60.4 ± 8.6</td>
<td>61.6 ± 6.6</td>
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<td>Gender (M, F)</td>
<td>13, 12</td>
<td>13, 13</td>
<td>NS</td>
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<tr>
<td>Race (W, A, AA)</td>
<td>22, 1, 2</td>
<td>21, 2, 3</td>
<td>NS</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>24.7 ± 2.5</td>
<td>27.4 ± 4.6</td>
<td>0.01</td>
</tr>
<tr>
<td>DM duration (years)</td>
<td></td>
<td>12.9 ± 11.3</td>
<td></td>
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<tr>
<td>Hemoglobin A1C (%)</td>
<td>5.5 ± 0.4</td>
<td>7.1 ± 1.0</td>
<td>&lt; 0.0001</td>
</tr>
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<td>Glucose</td>
<td>79.1 ± 16.5</td>
<td>133.6 ± 77.5</td>
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<td>Systolic BP</td>
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<td>Diastolic BP</td>
<td>65.9 ± 10.4</td>
<td>66.4 ± 9.5</td>
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<tr>
<td>Hyperlipidemia (yes,no)</td>
<td>7,15</td>
<td>10,18</td>
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<td>Total cholesterol (mg/dL)</td>
<td>226.5 ± 45.7</td>
<td>189.9 ± 42.7</td>
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<tr>
<td>Urinary Albumin (mg/dL)</td>
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<td>Diabetic retinopathy (yes, no, MV)</td>
<td>0, 21, 4</td>
<td>10, 15, 1</td>
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<td>Hematocrit (%)</td>
<td>40.4 ± 2.9</td>
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<td>Whole brain (cm³)</td>
<td>982.8 ± 20.6</td>
<td>971.5 ± 17.6</td>
<td>NS</td>
</tr>
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</table>

Data are presented as mean ± SD. p denotes between group comparisons
Race: W - White, A - Asian, AA: African-American, MV = missing values
Figure Legends:
Figure 1A: Brain regions partitioning method. The first column on the left presents an original axial slice at the level of the ventricles for the IR-FGE (I1), the FLAIR image (F1) and for the CASL acquisition (T2 reference image, C1). The second column illustrates the 6 regions computed on the images showed on column 1: the left (L) and right (R) side of the frontal (F), parieto-occipital (PO) and temporal (T) regions (as indicated on I2). The last 2 columns illustrate the processing/reconstruction performed on the images shown in column 1 and the assessment of spatial distribution for the computed parameters. The regions were applied to the segmented IR-FGE image (I3) to assess the regional distribution of gray matter (GM), white matter (WM), and cerebral blood flow (CSF) volumes (I4), to the segmented FLAIR image (F3) to assess white matter hyperintensities (WMHs) volume distribution (F4). After reconstruction of the (CBF) maps (an example CO\textsubscript{2} rebreathing map is shown on C3), CBF values were averaged over each region (C4) allowing CO\textsubscript{2} reactivity computation for each region.

GM (B), WM (C), CSF (D) and WMHs (E) volumes in the frontal (F), temporal (T), parieto-occipital (PO) and cortical (C) regions for both groups, normalized for region volume in control and diabetes mellitus (DM) groups (mean ± SE). † indicates between region comparisons for both groups p < 0.0001. ‡ indicates between hemispheres comparisons for both groups p = 0.004. # indicates between group comparisons over all regions p ≤ 0.006. *** indicates between group comparisons within regions p ≤ 0.0008, ** p = 0.01, * p = 0.04.
Figure 2: Cerebral blood flow (CBF) maps reconstructed from the continuous arterial spin labeling acquisition for a control subject (A-D) and a diabetes mellitus (DM) subject.
(E-G). A and E represent an axial slice of a T2 reference image at the level of the ventricles used for the CBF map reconstruction. B and F show the CBF maps at baseline, C and G for CO2 rebreathing, and D and H for hyperventilation. The perfusion ranges from 0 to 125 ml / 100 g / min on all CBF maps.

Cerebral blood flow during the first baseline, the CO2 rebreathing and hyperventilation periods (I) and the calculated CO2 reactivity (J) in the frontal (F), temporal (T), parieto-occipital (PO) and cortical (C) regions in control and DM groups (mean ± SE). †† indicates between region comparisons for both groups $p < 0.0001$, † $0.001 \leq p \leq 0.006$. ## indicates between group comparisons over all regions $p = 0.001$, # $0.005 \leq p \leq 0.006$. 