

Brain Activation During Working Memory Is Altered in Patients With Type 1 Diabetes During Hypoglycemia

Nicolas R. Bolo,^{1,2,3} Gail Musen,^{3,4} Alan M. Jacobson,^{3,4,5} Katie Weinger,^{3,4} Richard L. McCartney,⁴ Veronica Flores,⁴ Perry F. Renshaw,^{1,3,6} and Donald C. Simonson^{7,8}

OBJECTIVE—To investigate the effects of acute hypoglycemia on working memory and brain function in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Using blood oxygen level–dependent (BOLD) functional magnetic resonance imaging during euglycemic (5.0 mmol/L) and hypoglycemic (2.8 mmol/L) hyperinsulinemic clamps, we compared brain activation response to a working-memory task (WMT) in type 1 diabetic subjects ($n = 16$) with that in age-matched nondiabetic control subjects ($n = 16$). Behavioral performance was assessed by percent correct responses.

RESULTS—During euglycemia, the WMT activated the bilateral frontal and parietal cortices, insula, thalamus, and cerebellum in both groups. During hypoglycemia, activation decreased in both groups but remained 80% larger in type 1 diabetic versus control subjects ($P < 0.05$). In type 1 diabetic subjects, higher HbA_{1c} was associated with lower activation in the right parahippocampal gyrus and amygdala ($R^2 = 0.45$, $P < 0.002$). Deactivation of the default-mode network (DMN) also was seen in both groups during euglycemia. However, during hypoglycemia, type 1 diabetic patients deactivated the DMN 70% less than control subjects ($P < 0.05$). Behavioral performance did not differ between glycemic conditions or groups.

CONCLUSIONS—BOLD activation was increased and deactivation was decreased in type 1 diabetic versus control subjects during hypoglycemia. This higher level of brain activation required by type 1 diabetic subjects to attain the same level of cognitive performance as control subjects suggests reduced cerebral efficiency in type 1 diabetes. *Diabetes* 60:3256–3264, 2011

Acute episodes of hypoglycemia are a rate-limiting adverse effect in the treatment of type 1 diabetes. When severe, they can lead to seizures and coma (1). Even mild to moderate hypoglycemia is known to impair cognitive functions, such as working memory (2,3). Working memory is used to actively maintain and manipulate information over a brief period of time and to allocate attentional resources among competing subtasks (4,5). Traditionally, working-memory performance

is thought to depend primarily on a network of brain regions, including portions of the frontal and parietal lobes, thalamus, precuneus, cerebellum, and insula (6,7).

Using blood oxygen level–dependent (BOLD) functional magnetic resonance imaging (fMRI), we evaluated how diabetes impacts these neural processes under euglycemic and hypoglycemic conditions when subjects were presented with a working-memory task (WMT). Diabetes is known to negatively affect working memory (8). This task evaluates functional effects that might reflect changes in brain structure and/or presage decreases in cognitive performance. A better understanding of the brain's metabolic and physiological mechanisms underlying the cognitive functions implicated in working memory could lead to improved treatment strategies to help maintain cortical function in patients with diabetes during hypoglycemia (9).

BOLD fMRI is a well-established method for examining regional brain activation in response to physiological, pharmacological, sensory, or cognitive tasks (10). Studies that have examined brain activation in response to sensory stimulation or cognitive challenges using BOLD fMRI during hypoglycemic conditions in nondiabetic subjects (11–13) have shown that hypoglycemia reduces regional brain BOLD activation. This reduction in BOLD response during hypoglycemia has been attributed to low glucose levels causing decreases in neuronal activity, glucose oxidative metabolism, cerebral blood flow, neurovascular coupling, and/or neuronal recruitment (12).

Whether cognitive function in patients with type 1 diabetes is affected by hypoglycemia in the same manner as in nondiabetic individuals remains unclear because few studies using functional neural imaging have directly compared diabetic and nondiabetic subjects during the performance of cognitive tasks (14,15). If brain glucose transport or metabolism are altered in type 1 diabetes, as has been suggested in recent studies by our group (16) and others (17), then one would expect that the BOLD activation response during hypoglycemia may differ between diabetic patients compared with nondiabetic control subjects. On the basis of these findings, we hypothesized that 1) patients with type 1 diabetes would have greater BOLD activation during the performance of a WMT during hypoglycemia when compared with nondiabetic control subjects, 2) cognitive performance would deteriorate during hypoglycemia in both groups, and 3) among type 1 diabetic patients, better glycemic control (lower HbA_{1c}) would correlate with BOLD activation responses to the WMT during hypoglycemia. We also conducted exploratory analyses to examine deactivation patterns in the default-mode network (DMN), the regions of the brain that are more active during rest (18), because of other research by our group examining the effects of diabetes on deactivation patterns during cognitive tasks and previous research suggesting that DMN function may

From the ¹Brain Imaging Center, McLean Hospital, Belmont, Massachusetts; the ²Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, Massachusetts; the ³Department of Psychiatry, Harvard Medical School, Boston, Massachusetts; the ⁴Research Division, Joslin Diabetes Center, Boston, Massachusetts; the ⁵Research Institute, Winthrop-University Hospital, Mineola, New York; the ⁶Department of Psychiatry, University of Utah, Salt Lake City, Utah; the ⁷Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Boston, Massachusetts; and the ⁸Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Corresponding author: Nicolas R. Bolo, nbolo@bidmc.harvard.edu.

Received 14 April 2011 and accepted 25 August 2011.

DOI: 10.2337/db11-0506

© 2011 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

be altered in diseases that affect cognition, such as Alzheimer's disease (19).

RESEARCH DESIGN AND METHODS

The study sample consisted of 16 patients with type 1 diabetes and 16 healthy control subjects from an ongoing study of brain function during hypoglycemia in type 1 diabetes. Data from five type 1 diabetic and five control subjects were included in a previous publication (20). Demographic and clinical characteristics of the subjects are presented in Table 1. The patients' number of self-reported hypoglycemic episodes (plasma glucose <3.9 mmol/L with concomitant symptoms) in the month preceding the initial visit averaged eight episodes (range 2–28). Patients with autonomic neuropathy (assessed by standard criteria) (21), painful peripheral neuropathy, urinary albumin levels >300 mg/day, or proliferative retinopathy by review of medical records, physical exam, or self-report were excluded from the study. Other exclusion criteria were a history of psychosis or schizophrenia; cocaine, heroin, or alcohol dependence; and any contraindications to MRI, such as metallic implants, pregnancy, or claustrophobia.

Following approval from the institutional review boards of both the Joslin Diabetes Center (where patients were recruited) and the McLean Hospital (where the MRI was performed), patients provided the following information during screening: self-report of their lifetime experience of severe hypoglycemic events leading to unconsciousness (22); psychiatric history; handedness; medical history; and current medications.

Of 18 control participants who were eligible for the study, 2 were excluded from analysis because of excessive head motion during the fMRI by applying exclusion criteria of translations in excess of 3 mm in *x*, *y*, or *z* directions or rotations in excess of 3° around the *x*, *y*, or *z* axes. No diabetic subjects were excluded for head motion.

Experimental protocol. The experimental protocol is described elsewhere (20) and briefly reviewed below. On the day before the study, patients with type 1 diabetes had a continuous glucose monitor (CGM System Gold; Medtronic, Northridge, CA) inserted. If the continuous glucose monitor showed glucose <3.3 mmol/L, the study was postponed to a later date. The experiment used the insulin clamp technique with four successive time periods corresponding to different plasma glucose levels: baseline (30 min); euglycemic clamp (40 min, target glucose 5.0 mmol/L); declining glycemia (40 min, plasma glucose reduced from 5.0 to 2.8 mmol/L); and hypoglycemic clamp (30 min, target glucose 2.8 mmol/L). Anatomical MRI was performed during baseline, and fMRI was performed during the euglycemic and hypoglycemic periods while the WMT was administered (Fig. 1).

Insulin clamp technique. An intravenous catheter was inserted into an antecubital vein for the administration of insulin and glucose, and a second catheter was inserted into a distal forearm or hand vein for the withdrawal of blood samples. A heated gel pack was used to warm the hand to arterialize the venous blood. After the baseline period, regular human insulin was infused at 12

TABLE 1
Demographic and clinical characteristics of study subjects

	Type 1 diabetic subjects	Control subjects
<i>n</i>	16	16
Age (years)	33.8 ± 9.9 (19–50)	31.3 ± 10.0 (19–50)
Sex (men/women)	8/8	13/3
Diabetes duration (years)	16.7 ± 4.9 (7.9–26.7)	—
BMI (kg/m ²)	24.8 ± 2.9 (20.5–30.7)	25.1 ± 3.1 (20.2–31.1)
HbA _{1c} (%)	7.4 ± 1.1 (5.7–9.7)	5.2 ± 0.3 (4.4–5.5)
Fasting plasma glucose (mmol/L)	6.0 ± 1.4 (4.1–8.7)	5.0 ± 0.3 (4.4–5.6)
Education (years)	16.3 ± 1.9 (13–20)	16.8 ± 2.7 (12–21)

Data are means ± SD (range).

pmol/kg per min for 110 min. The plasma glucose levels were maintained at the desired level by infusion of 20% dextrose using a negative-feedback algorithm, as previously described (23–25). After the euglycemic clamp period (40 min), the glucose infusion rate was reduced to allow the plasma glucose level to decline by 2.2 mmol/L (from 5.0 to 2.8 mmol/L) over the next 40 min, followed by the 30-min hypoglycemic clamp period. During the entire clamp protocol, glucose levels were measured every 5 min, and counterregulatory hormones (epinephrine, cortisol, growth hormone, and glucagon) were measured every 10 min. At the end of the protocol, the insulin infusion was discontinued, the glucose infusion was increased to restore euglycemia, and the subjects were given a meal and discharged.

Hormone and substrate assays. Plasma glucose was measured using the glucose oxidase method. Serum insulin and growth hormone and plasma epinephrine were measured by enzyme-linked immunosorbent assay. Plasma glucagon and cortisol were measured by radioimmunoassay.

WMT. The task stimuli were administered using Presentation software (Neurobehavioral Systems, Albany, CA). The images were projected on a backlit screen that was visualized from within the magnet bore by a mirror mounted on the head coil. Response times and errors were collected using a magnetic resonance-compatible hand-held four-button fiberoptic response pad (FORP; Current Designs, Philadelphia, PA) connected to the PC by an optical cable interface. The stimulus presentation was synchronized with the fMRI acquisition sequence at the beginning of each trial by the interface that responded to scanner-generated trigger signals. The WMT was administered 15–20 min after the beginning of the euglycemic period and again at 15–20 min after the

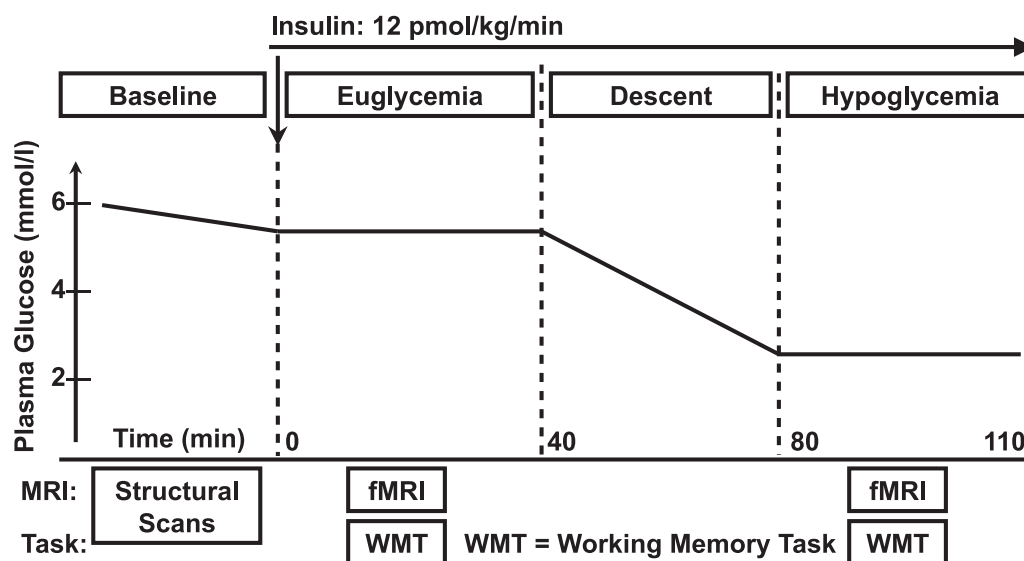


FIG. 1. Study protocol. Time line of structural and functional scans, WMTs, and periods of euglycemia (nominal 5.0 mmol/L), glucose descent, and hypoglycemia (nominal 2.8 mmol/L).

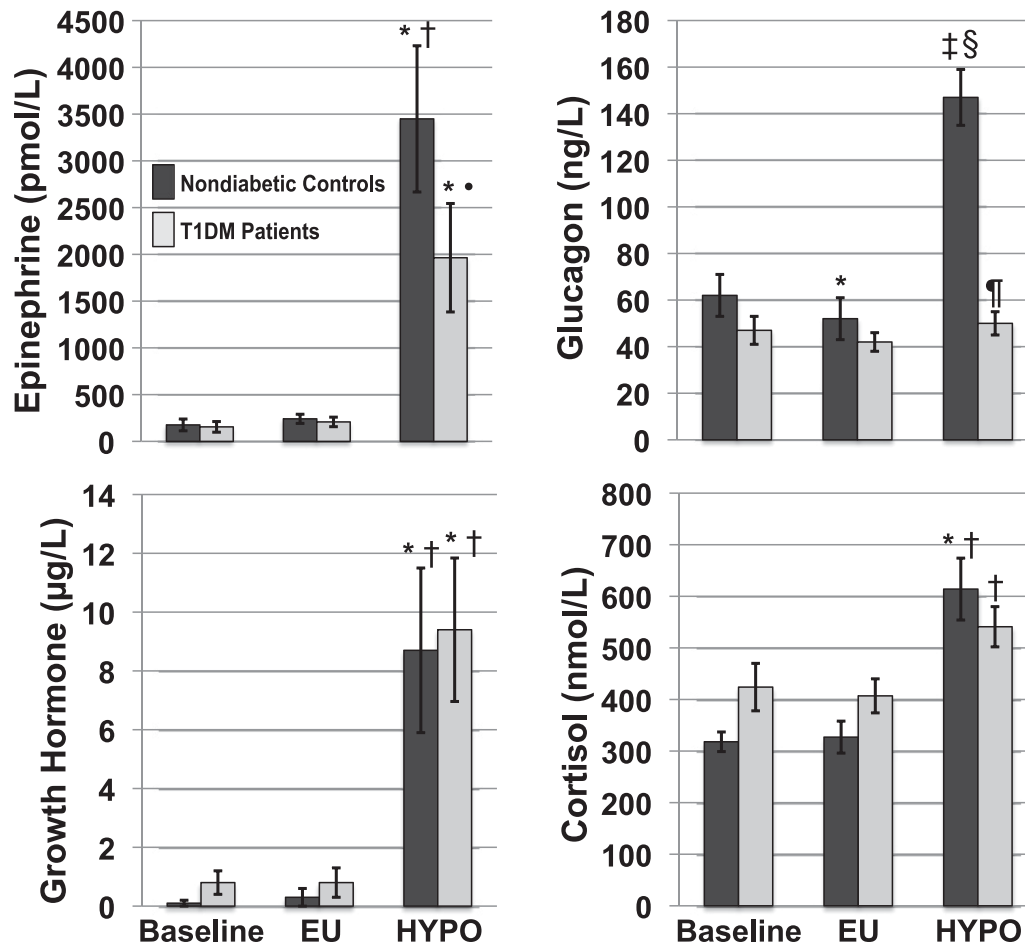


FIG. 2. Plasma levels of counterregulatory hormones (error bars \pm SE) during the baseline and euglycemic periods and at the end of the hypoglycemic period in control subjects and patients with type 1 diabetes (T1DM). EU, euglycemia; HYPO, hypoglycemia. *P* values for statistically significant comparisons: **P* < 0.05 vs. baseline; †*P* < 0.05 vs. euglycemia; ‡*P* < 0.01 vs. baseline; §*P* < 0.01 vs. euglycemia; •*P* < 0.05 vs. control subjects; ¶*P* < 0.001 vs. control subjects.

beginning of the hypoglycemic period (Fig. 1). Subjects performed practice tests before the study to achieve familiarity with the test procedure, and we used randomly assigned counterbalanced forms of the test during euglycemia and hypoglycemia to minimize learning.

During the WMT, adapted from Rypma et al. (26), participants viewed a string of six digits for 1,200 ms followed by a 2,000-ms unfilled retention interval (blank screen). Then, one digit was displayed on the computer monitor for 1,500 ms, and the subject had 1,300 ms to decide whether the digit was a member of the previously seen string. Using the same timing parameters, a matched control task was used in which subjects viewed six percent symbols (%) on the screen, followed by the unfilled retention interval, followed by a right- or a left-pointing arrow. The participants were asked to press the response key corresponding to the direction of the arrow. In the rest task, participants fixated on a plus (+) sign for 30 s. Each of these three tasks (WMT, matched control, and rest) was presented four times in a block design for a total of 6 min of testing. There were 5 memory-scanning trials per block for a total of 20 memory-scanning trials.

MRI acquisition methods. All magnetic resonance images were acquired with a 3.0 Tesla Siemens Trio scanner (Siemens, Erlangen, Germany), using a circularly polarized birdcage radiofrequency head coil tuned to the proton frequency. Global field uniformity was adjusted at the beginning of each scanning session using Siemens' automated shimming routines. A three-dimensional, T1-weighted anatomical image was acquired using a magnetization-prepared, rapid-acquisition gradient echo sequence (TR/TE = 2,100/2.74 ms; spatial resolution = 1 × 1 × 1.3 mm³; matrix size = 256 × 256 × 128) and used for functional image registration. BOLD fMRI images covering the whole brain were acquired in the axial plane using an echo planar imaging (EPI) sequence (TR/TE = 3,000/30 ms; 26 slices acquired in interleaved scanning order; slice thickness = 5 mm; field of view = 200 × 200 mm²; matrix size = 64 × 64), with an in-plane resolution of 3.125 × 3.125 mm². One multislice EPI volume covering the whole brain was acquired every 3 s for a total of 120 volumes for the 6-min fMRI run.

Image processing. Image processing and analyses were performed using the FSL software package (Analysis Group, FMRIB, Oxford, U.K.) running on a Mac-Pro Quad-Core Intel-Xeon computer (Apple, Cupertino, CA). All brain images were registered to the standard MNI-152 brain (Montreal Neurologic Institute) to allow multisubject analyses in standardized space and identification of all regions of interest. The first two EPI volumes of each functional run were discarded to allow for T1 equilibration. Prestatistical processing of EPI time series consisted of motion correction, slice scan-time correction, nonbrain removal, spatial smoothing with a 6-mm full-width at half-maximum three-dimensional Gaussian filter, linear trend removal, and a temporal high-pass filter with a cutoff of 120 s. Functional data were overlaid on the MNI-152 brain. **Data analyses.** Regional brain activations and deactivations in response to the WMT were examined by performing a first-level general linear model (GLM) multiple regression analysis for each subject and glycemic condition. Model predictors for BOLD signal time courses were the boxcar time courses for each task stimulus paradigm convolved with a γ function to account for hemodynamic response. Predictors for the temporal derivatives of the task paradigm were added to the design matrix to improve the fit to the data by allowing for potential misspecification of the hemodynamic delay (27). The model for the baseline rest condition predictor was a constant function.

To compare activation and deactivation patterns between groups and conditions, higher-level multisubject mixed-effects GLM analyses compared multiple regression correlation coefficients associated with each model predictor for each brain voxel for each subject under each condition. Activations were computed by contrasting the WMT task to its control task. Deactivations were computed by contrasting the WMT task to the rest period. These higher-level analyses were performed using FSL's local analysis of mixed effects stage 1. Resulting Z score (i.e., Gaussianized T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $P = 0.05$ (28). These statistical parametric maps yield the clusters of adjacent brain voxels of regional activation or deactivation from which we

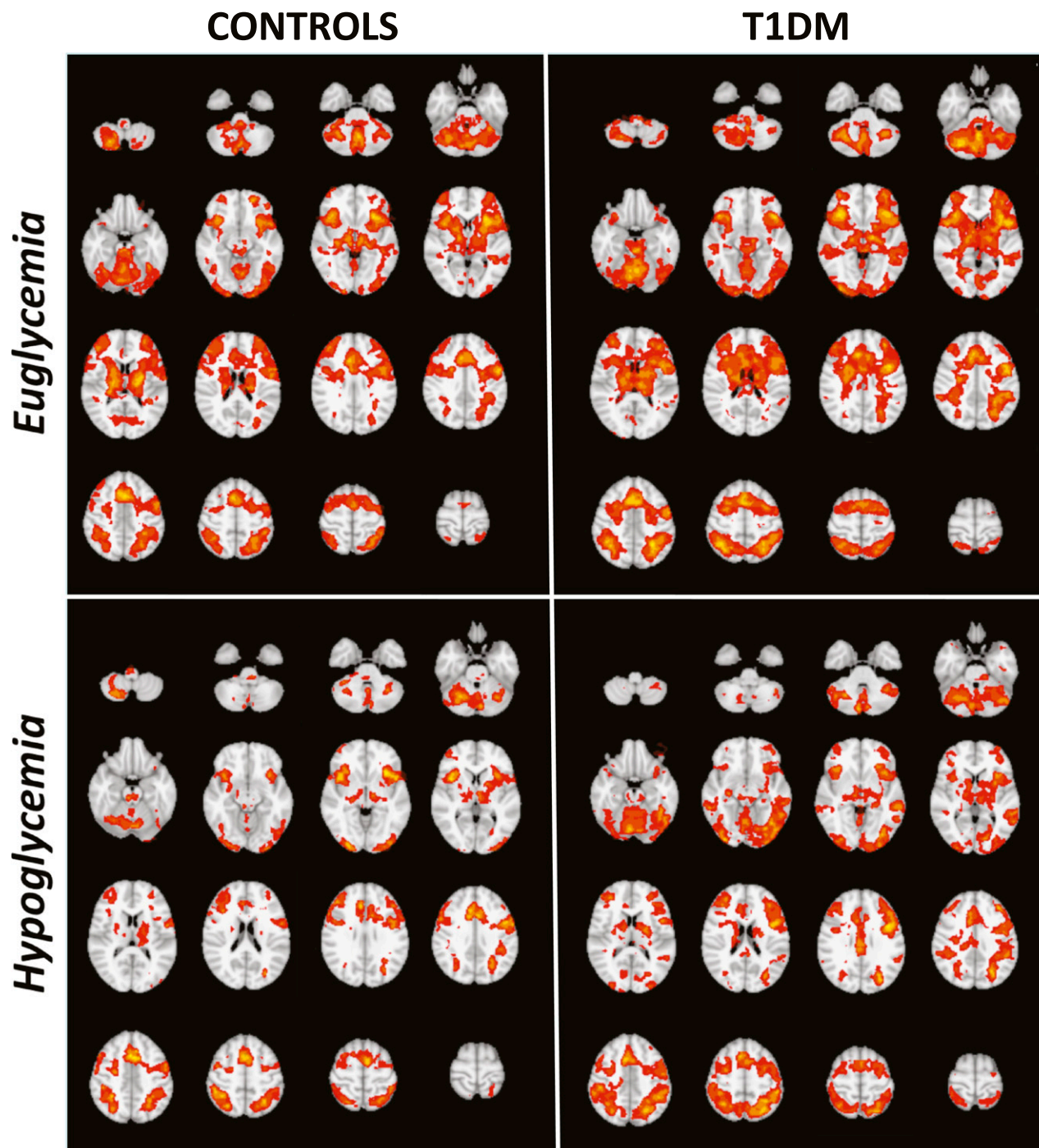


FIG. 3. BOLD activation during the WMT in control and type 1 diabetic (T1DM) subjects during euglycemia and hypoglycemia. Statistical parametric maps of regions of greatest activation during WMTs vs. control tasks for each subject group and glycemic condition. Functional activations (red-to-yellow color scale) are overlaid on the MNI-152 standard brain anatomy (gray scale). The threshold for activation was $P < 0.05$ after correction for multiple comparisons using the cluster-based threshold method (see RESEARCH DESIGN AND METHODS). During hypoglycemia, type 1 diabetic subjects exhibit greater activation than control subjects during the WMT. (A high-quality digital representation of this figure is available in the online issue.)

computed a single brain volume of activation or deactivation at the set statistical significance threshold ($P < 0.05$).

To investigate the variation of the regional percent BOLD signal change, we applied a region-of-interest (ROI) analysis in the superior parietal lobule (SPL) region, which had significant activation differences during hypoglycemia. To define the ROI, we created a binary mask of the SPL region provided by the probabilistic Harvard-Oxford Cortical Structural Atlas (29) thresholded at the 50% probability level. The ROI analysis was performed using FSL's FEATQUERY tool to extract the average time courses of parameter estimates of the percent BOLD signal change from all voxels contained in the SPL mask.

To investigate the association of WMT activation and deactivation to glycemic control in type 1 diabetes, we performed the mixed-effects GLM group analyses while including HbA_{1c} values as a covariate in the design matrix. To compute the regional coefficients of correlation of activation to HbA_{1c} , the average percent BOLD activation values were extracted from the regions of significant correlation in type 1 diabetes during hypoglycemia using the FEATQUERY tool.

Standard statistical tests, including t tests for paired and unpaired data as appropriate, and ANOVA were used to compare glucose and counter-regulatory hormone levels between diabetic and nondiabetic subjects during euglycemia and hypoglycemia. All data are presented as means \pm SEM, unless

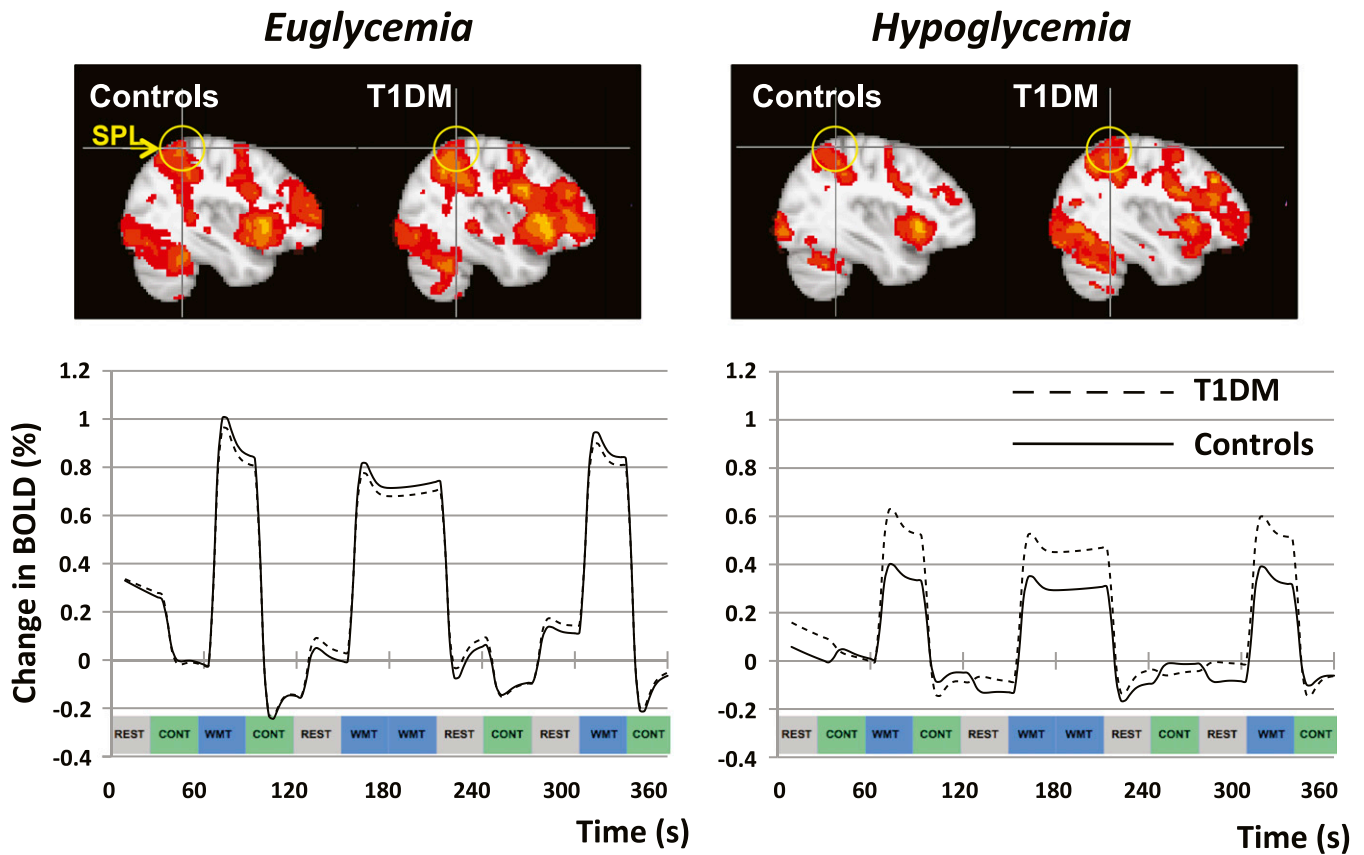


FIG. 4. Percent BOLD activation by ROI analysis during the WMT during euglycemia (left) and hypoglycemia (right). Upper: Location of the superior parietal lobule (SPL, association cortex including Brodmann areas 7 and 40) region used for ROI analysis is indicated at the intersection of the crosshairs on the activation maps for each subject group overlaid on the MNI-152 standard brain. Lower: Plots of the time courses of change in percent BOLD activation in the SPL ROI during the fMRI WMT. Percent change was measured relative to BOLD activation during the control task and averaged over all pixels in the ROI and all subjects within each subject group. The colored strip above the horizontal time axis shows the time sequence of task conditions. CONT, control task; REST, resting state task. (A high-quality digital representation of this figure is available in the online issue.)

otherwise specified, and statistical tests were conducted using a two-sided α level of 0.05.

RESULTS

Plasma glucose and counterregulatory hormones.

Average plasma glucose levels during the euglycemic period for nondiabetic control and type 1 diabetic subjects were 5.0 ± 0.4 mmol/L and 5.0 ± 0.6 mmol/L, respectively. During the hypoglycemic period, average glucose levels for the two groups were 2.8 ± 0.2 mmol/L and 2.7 ± 0.1 mmol/L, respectively. There was no significant difference between groups and no significant interaction between the glycemic condition and group. Mean counterregulatory hormone levels during baseline and euglycemia and peak levels attained during hypoglycemia are shown in Fig. 2. Glucagon and epinephrine secretion were lower in the type 1 diabetic patients during hypoglycemia compared with control subjects.

Cognitive performance. Type 1 diabetic and control subjects did not differ in accuracy or reaction time during the WMT at either euglycemia or hypoglycemia. During euglycemia, control subjects achieved $85 \pm 2\%$ correct, with a reaction time of $1,133 \pm 54$ ms, whereas type 1 diabetic patients had $85 \pm 4\%$ correct, with a reaction time of $1,200 \pm 57$ ms. At hypoglycemia, control subjects had $84 \pm 5\%$ correct, with a reaction time of $1,122 \pm 57$ ms, whereas type 1 diabetic patients had $85 \pm 4\%$ correct, with

a reaction time of $1,204 \pm 58$ ms. There were no significant within-subject differences for either group when comparing the performance between glycemic conditions and no interaction between group and condition.

Functional imaging. Brain activations in response to the WMT for each subject group during each glycemic condition are shown in Fig. 3. The regions of greater BOLD activation for the WMT relative to the control task ($P < 0.05$, corrected for multiple comparisons using the cluster-based threshold method) (28) are indicated in a red-to-yellow color scale overlaid on the gray scale standard MNI-152 T1-weighted anatomical brain atlas. For both subject groups during both glycemic conditions, the WMT activated brain regions located in bilateral medial superior frontal gyrus, left precentral gyrus, bilateral SPL, bilateral middle frontal gyrus, bilateral anterior cingulate cortex, bilateral insula, left supramarginal gyrus, bilateral thalamus, bilateral inferior occipital cortex, and bilateral cerebellum.

During euglycemia, the overall extent of activation was similar in the diabetic patient and control groups, with activated brain volumes of 613 and 498 cm³, respectively. During hypoglycemia, the extent of regional BOLD activation decreased relative to euglycemia in both subject groups; however, it decreased less in patients with diabetes than in control subjects ($P < 0.05$). The regional patterns of activation also differed between groups. In type 1 diabetes, activation decreased mostly in the insula,

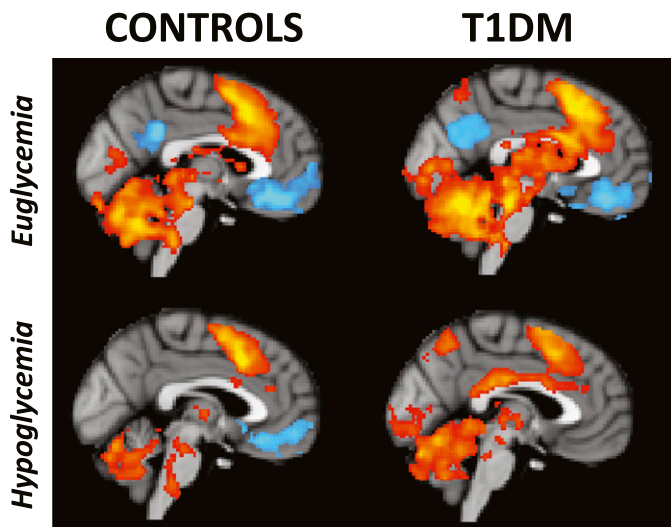


FIG. 5. BOLD activation and deactivation during the WMT in type 1 diabetic (T1DM) and control subjects during euglycemia and hypoglycemia. Statistical parametric maps of regions of greatest activation during WMTs vs. control tasks (functional activations in the red-to-yellow color scale) and regions of decreased activation during the WMTs vs. resting tasks (functional deactivations in the blue color scale) are overlaid on the MNI-152 standard brain anatomy (gray scale). The threshold for activations and deactivations was $P < 0.05$ after correction for multiple comparisons using the cluster-based threshold method (see RESEARCH DESIGN AND METHODS). During hypoglycemia, patients with type 1 diabetes exhibit less deactivation than control subjects. (A high-quality digital representation of this figure is available in the online issue.)

whereas for control subjects, activation decreased mostly in the cerebellum. Regions with higher activation in type 1 diabetes during hypoglycemia include the bilateral SPL, bilateral posterior supramarginal gyrus, bilateral anterior and posterior cingulate gyrus, left inferior frontal gyrus and posterior middle temporal gyrus, right hippocampus, and the cerebellum. Thus, during hypoglycemia, activation volumes were 80% larger in type 1 diabetic versus control subjects, with activation volumes of 431 and 239 cm^3 , respectively ($P < 0.05$). The extended activation volume in type 1 diabetic relative to control subjects during hypoglycemia was mainly a result of maintenance of a higher percent BOLD activation during the WMT, as illustrated in Fig. 4, which compares the time courses of the percent BOLD signal estimates averaged over the SPL region for each subject group under each glycemic condition.

Regional brain deactivations in response to the WMT for each subject group during each glycemic condition are shown in Fig. 5. Regions of lesser BOLD activation (or more deactivation) for WMT relative to the resting task ($P < 0.05$, corrected for multiple comparisons using the cluster-based threshold method) (28) are indicated in a dark blue-to-light blue color scale overlaid on the standard MNI-152 T1-weighted anatomical brain atlas in gray scale, along with activations in red to yellow. During euglycemia, WMT deactivated brain regions located in bilateral medial frontal cortex, posterior cingulate cortex, and superior lateral occipital cortex for both groups. The extent of deactivation was similar in the type 1 diabetic and control groups, with only a 10% difference between groups. Overall, deactivated brain volumes were 45 and 41 cm^3 , respectively. During hypoglycemia, the extent of regional BOLD deactivation decreased relative to euglycemia in both subject groups; however, it decreased more for patients with type 1 diabetes

($P < 0.05$) (i.e., they were unable to significantly deactivate these regions).

The regional patterns of decreased deactivation also differed between groups (Fig. 5). For patients with type 1 diabetes, deactivation decreased mostly in the medial frontal and posterior cingulate cortices, whereas for control subjects, deactivation decreased mostly in the posterior cingulate cortex. Control subjects also had small deactivation volumes in the cerebellum (right crus II) during hypoglycemia only. Thus, during hypoglycemia, the extent of deactivation was almost 70% smaller in type 1 diabetic relative to control subjects, with deactivation volumes of 5 cm^3 located in the superior lateral occipital cortex for patients and 17 cm^3 located mainly in the medial frontal and superior lateral occipital cortices for control subjects ($P < 0.05$).

Finally, we examined the relationship of HbA_{1c} levels with activation patterns in type 1 diabetes by entering HbA_{1c} as a covariate into the GLM. We found that lower HbA_{1c} levels were associated with higher activation in the right parahippocampal gyrus and the amygdala (Fig. 6) during hypoglycemia. We also found that HbA_{1c} levels were not associated with deactivation.

DISCUSSION

This study demonstrates that patients with type 1 diabetes show a different pattern of brain activation in response to a WMT than do nondiabetic control subjects during hypoglycemia. Specifically, we found that for patients with type 1 diabetes during hypoglycemia, WMT-related activation responses were increased in several cortical regions, including the parietal and frontal cortices, hippocampus, and cerebellum. Task-induced deactivations, typically observed in the DMN during cognitive effort, were significantly suppressed during hypoglycemia in bilateral medial-frontal and posterior cingulate cortices for type 1 diabetic patients compared with control subjects. Activation and deactivation patterns were similar across groups during euglycemia. Behavioral performance on the WMT was similar across groups and conditions. Finally, HbA_{1c} was inversely correlated with WMT activation during hypoglycemia in the right parahippocampal gyrus and amygdala, two areas that have been reported to activate in memory-disordered populations as a form of compensatory recruitment (30–32).

The regional BOLD activations we observed in response to the WMT were compatible with those found in other fMRI studies of similar WMTs (5,26,33–35). Although the main regions that help govern working memory are the dorsolateral and medial prefrontal cortices and anterior cingulate cortex, other regions, such as the parietal lobe (36) and cerebellum (37), are known to play supplementary roles. These regions were activated more in type 1 diabetic patients than in control subjects during hypoglycemia, suggesting that supplementary brain regions may have been recruited to help preserve cognitive performance. The failure to suppress activation in the DMN also is consistent with an interpretation that type 1 diabetic patients need to recruit more brain resources for cognitive preservation (11). Similar patterns of increased activation and decreased deactivation have been observed with mild cognitive impairment (30) and in older individuals at risk for Alzheimer's disease (38), suggesting, in these cases, a compensatory response to accumulating pathology.

Cognitive performance was not altered by hypoglycemia in either subject group. Although some studies have shown similar results for less challenging WMTs (39), others have

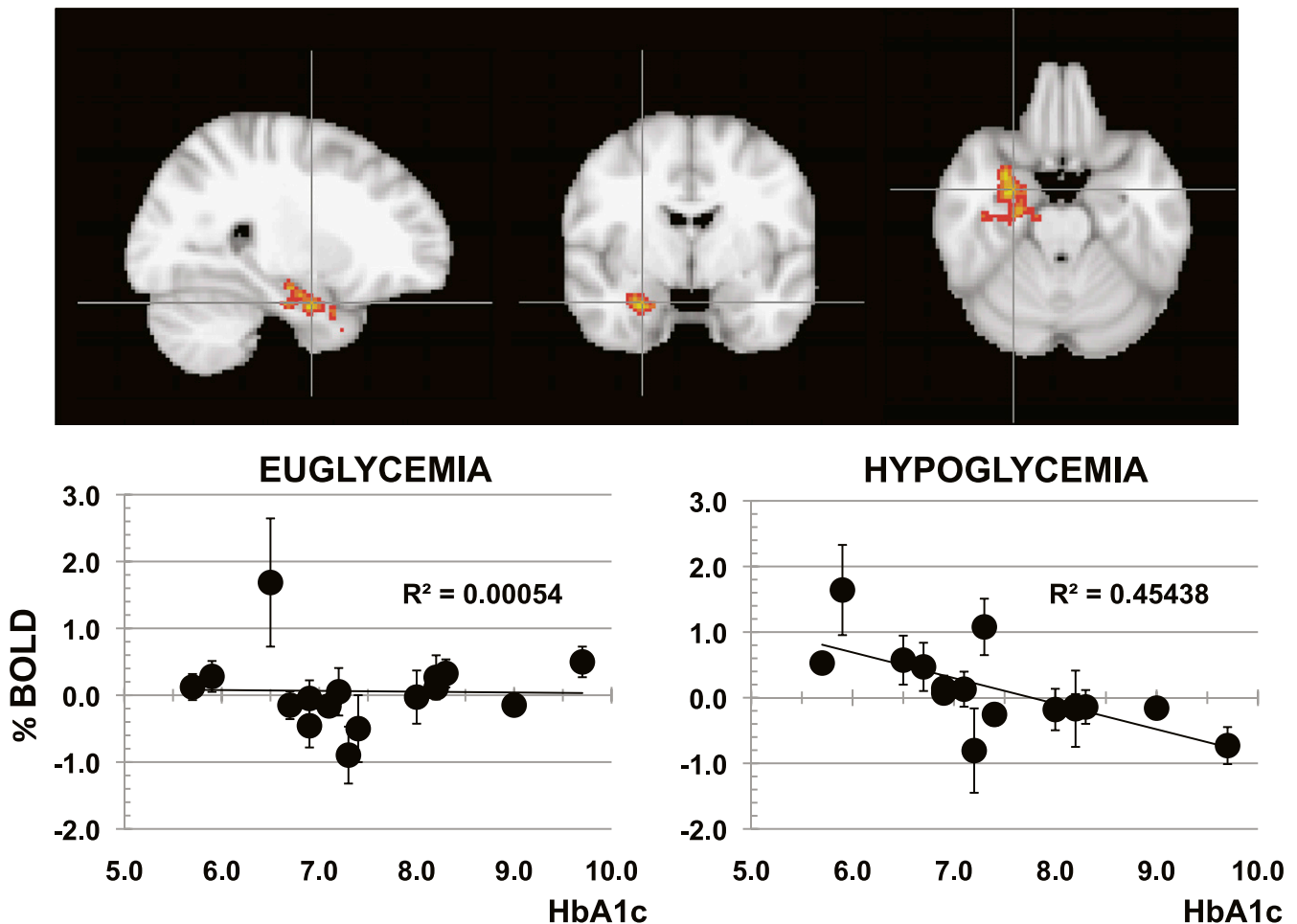


FIG. 6. Regions of BOLD activation inversely correlated with HbA_{1c} in patients with type 1 diabetes. *Upper:* Statistical parametric map of the correlation of WMT activation with HbA_{1c} in patients with type 1 diabetes during hypoglycemia ($P < 0.05$). Sagittal, coronal, and axial slices (from left to right) showing activation correlation in a red-to-yellow scale overlaid on the MNI-152 standard brain anatomy (gray scale). These HbA_{1c}-correlated activation clusters were identified in the right parahippocampal gyrus and amygdala using the Harvard-Oxford Cortical Structural Atlas. *Lower:* Plots of regional percent BOLD activation vs. HbA_{1c} for patients with type 1 diabetes during euglycemia (*left*) and hypoglycemia (*right*). Filled circles with error bars: average percent BOLD activation for each patient \pm SD within the region of activation. The line is the linear regression best fit of the data with its correlation coefficient R^2 . (A high-quality digital representation of this figure is available in the online issue.)

shown severe impairment during hypoglycemia for highly challenging WMTs involving reasoning (2). Of importance, a number of studies have used the same Sternberg WMT used here to evaluate differences in brain activation patterns across different populations (40,41). In our study, different brain activation patterns, despite similar cognitive performance across groups, suggest unique strategies of brain recruitment used across groups to augment performance during the glycemic challenge.

Our results showing hyperactivation of brain regions in type 1 diabetic patients with low HbA_{1c} and hypoactivation in patients with higher HbA_{1c} could reflect upregulation of glucose transport in the brain, as seen in patients with good glycemic control (42,43). Type 1 diabetic patients may engage more brain regions to maintain the same performance to compensate for cerebral inefficiency attributed to reduced brain resources (44). These results also are consistent with the patterns observed in many physiologic systems in which a period of compensatory hyperfunction precedes functional decline and ultimate organ-system failure.

In an earlier report from our group, we demonstrated that type 1 diabetic patients showed reduced gray-matter density

in the parahippocampal gyrus associated with higher HbA_{1c} (45). One possible explanation may be that gray-matter loss in this temporal region prevents its participation in the brain's response to moderate acute hypoglycemia. Wessels et al. (46) also observed abnormal brain activity patterns in patients with diabetes retinopathy, which is more likely to be associated with elevated HbA_{1c} levels. However, our studies differed in both design and data analytic methodology, making a direct comparison difficult.

The differences in the hypoglycemic BOLD response between patients and control subjects found in this study may be attributed to a variety of mechanisms, including preservation of global brain glucose uptake in diabetes as a result of adaptation to hypoglycemia (12,47), differences in neurovascular coupling, resting cerebral blood flow, neuronal activity linked to oxidative metabolism, increased brain glycogen stores (48), or a tendency to use nonglucose substrates to support higher neuronal activity (49). In addition, changes in brain glucose transport or metabolism, resulting in increased brain glucose levels, might occur as a result of recurrent hypoglycemia. We reported such a finding along with accompanying increases in glutamate

that were correlated with decreases in memory and executive function (16). It may follow that abnormal glutamate metabolism contributed to altered neurovascular coupling in patients in our study. Although these adaptive mechanisms in type 1 diabetes may in the short-term allow compensation for altered brain activity patterns during a hypoglycemic challenge, they may presage long-term maladaptive or adverse consequences.

Although our study demonstrates greater activation during hypoglycemia in type 1 diabetes along with reduced deactivation of the DMN, the small sample size may limit the implications of our results. However, the regional BOLD activations we observed in response to the WMT were compatible with regional activations found in other fMRI studies of similar WMTs (5,26,33–35). The reductions in BOLD response observed during hypoglycemia were also compatible with other reports showing reduced brain BOLD activation in primary or association cortex in response to sensory, motor, or cognitive tasks in nondiabetic subjects (11,12,14,47). Also, this study was unable to resolve whether the alterations in brain activation were secondary to chronic hyperglycemia or recurrent hypoglycemia. Future studies can help resolve this issue.

In summary, patients with type 1 diabetes activate more brain regions than control subjects during hypoglycemia by maintaining activity from euglycemia to hypoglycemia in task-relevant regions and by failing to suppress activation in the DMN. This suggests that type 1 diabetic patients may need to recruit more brain resources to preserve cognitive performance. The pattern of hyperactivation of both the DMN and task-relevant regions is consistent with findings in disease states with impaired cognition, such as mild cognitive impairment and Alzheimer's disease (38). There has been persistent concern about the consequences of recurrent hypoglycemia on brain structure and cognitive function. There are minimal long-term effects of recurrent hypoglycemia on cognition into middle age (50), but it is not clear whether this resiliency will last throughout the aging process. Future research should evaluate our findings as an early manifestation and warning of future clinically relevant cognitive decline. This research may guide the development of treatment regimens to enhance symptom recognition or to stabilize the neurochemical response to hypoglycemia to reduce the impact of glucose deprivation on cognitive function.

ACKNOWLEDGMENTS

This study was supported in part by National Institutes of Health grants 5R01-DK073843-03, DK-60754, DK-62218-01A1, and P30-DK-36836 (Joslin Diabetes and Endocrinology Research Center); the Herbert Graetz Fund; and grant 5M01-RR001032-32 to the Beth Israel Deaconess General Clinical Research Center.

No potential conflicts of interest relevant to this article were reported.

N.R.B., G.M., A.M.J., and K.W. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript. R.L.M. researched data, contributed to discussion, and reviewed and edited the manuscript. V.F. researched data and reviewed and edited the manuscript. P.F.R. contributed to discussion and reviewed and edited the manuscript. D.C.S. researched data, contributed to discussion, and wrote, reviewed, and edited the manuscript.

Parts of this article were presented in abstract form at the 70th Scientific Sessions of the American Diabetes

Association, Orlando Florida, 25–29 June 2010, and at the 71st Scientific Sessions of the American Diabetes Association, San Diego, California, 24–28 June 2011.

The authors thank Judi Lauerman, RN, Brigham and Women's Hospital, Boston, Massachusetts, and Karen Branch, RN, Joslin Diabetes Center, Boston, Massachusetts, for their expert nursing assistance.

REFERENCES

1. Cryer PE. Hypoglycaemia: the limiting factor in the glycaemic management of type I and type II diabetes. *Diabetologia* 2002;45:937–948
2. Deary IJ, Sommerfield AJ, McAulay V, Frier BM. Moderate hypoglycaemia obliterates working memory in humans with and without insulin treated diabetes. *J Neurol Neurosurg Psychiatry* 2003;74:278–279
3. Sommerfield AJ, Deary IJ, McAulay V, Frier BM. Moderate hypoglycemia impairs multiple memory functions in healthy adults. *Neuropsychology* 2003;17:125–132
4. Wager TD, Smith EE. Neuroimaging studies of working memory: a meta-analysis. *Cogn Affect Behav Neurosci* 2003;3:255–274
5. Baddeley A. Working memory: looking back and looking forward. *Nat Rev Neurosci* 2003;4:829–839
6. LaBar KS, Gitelman DR, Parrish TB, Mesulam M-M. Neuroanatomic overlap of working memory and spatial attention networks: a functional MRI comparison within subjects. *Neuroimage* 1999;10:695–704
7. Smith EE, Jonides J. Working memory: a view from neuroimaging. *Cognit Psychol* 1997;33:5–42
8. Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP. The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes Care* 2005;28:726–735
9. Bingham E, Rosenthal JM, Amiel SA. Cognitive function in acute hypoglycaemia 2. *Diabetes Nutr Metab* 2002;15:363–367
10. Ogawa S, Tank DW, Menon R, et al. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci USA* 1992;89:5951–5955
11. Rosenthal JM, Amiel SA, Yáñez L, et al. The effect of acute hypoglycemia on brain function and activation: a functional magnetic resonance imaging study. *Diabetes* 2001;50:1618–1626
12. Anderson AW, Heptulla RA, Driesen N, et al. Effects of hypoglycemia on human brain activation measured with fMRI. *Magn Reson Imaging* 2006;24:693–697
13. Warren RE, Sommerfield AJ, Greve A, Allen KV, Deary IJ, Frier BM. Moderate hypoglycaemia after learning does not affect memory consolidation and brain activation during recognition in non-diabetic adults. *Diabetes Metab Res Rev* 2008;24:247–252
14. Driesen NR, Goldberg PA, Anderson AW, et al. Hypoglycemia reduces the blood-oxygenation level dependent signal in primary auditory and visual cortex: a functional magnetic resonance imaging study. *J Neurosci Res* 2007;85:575–582
15. Wessels AM, Rombouts SA, Simsek S, et al. Microvascular disease in type 1 diabetes alters brain activation: a functional magnetic resonance imaging study. *Diabetes* 2006;55:334–340
16. Lyoo IK, Yoon SJ, Musen G, et al. Altered prefrontal glutamate-glutamine- γ -aminobutyric acid levels and relation to low cognitive performance and depressive symptoms in type 1 diabetes mellitus. *Arch Gen Psychiatry* 2009;66:878–887
17. Criego AB, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER. Brain glucose concentrations in patients with type 1 diabetes and hypoglycemia unawareness. *J Neurosci Res* 2005;79:42–47
18. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci USA* 2001;98:676–682
19. Celone KA, Calhoun VD, Dickerson BC, et al. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. *J Neurosci* 2006;26:10222–10231
20. Musen G, Simonson DC, Bolo NR, et al. Regional brain activation during hypoglycemia in type 1 diabetes. *J Clin Endocrinol Metab* 2008;93:1450–1457
21. American Diabetes Association, American Academy of Neurology. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy: summary and recommendations. *Diabetes Care* 1992;15:1104–1107
22. The Diabetes Control and Complications Trial Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 1997;46:271–286

23. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes* 1988;37:901–907
24. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214–E223
25. Widom B, Simonson DC. Glycemic control and neuropsychologic function during hypoglycemia in patients with insulin-dependent diabetes mellitus. *Ann Intern Med* 1990;112:904–912
26. Rypma B, Prabhakaran V, Desmond JE, Glover GH, Gabrieli JD. Load-dependent roles of frontal brain regions in the maintenance of working memory. *Neuroimage* 1999;9:216–226
27. Beckmann CF, Jenkinson M, Woolrich MW, et al. Applying FSL to the FIAC data: model-based and model-free analysis of voice and sentence repetition priming. *Hum Brain Mapp* 2006;27:380–391
28. Friston KJ. Testing for anatomically specified regional effects. *Hum Brain Mapp* 1997;5:133–136
29. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006;31:968–980
30. Dickerson BC, Salat DH, Bates JF, et al. Medial temporal lobe function and structure in mild cognitive impairment. *Ann Neurol* 2004;56:27–35
31. Rosenbaum RS, Furey ML, Horwitz B, Grady CL. Altered connectivity among emotion-related brain regions during short-term memory in Alzheimer's disease. *Neurobiol Aging* 2010;31:780–786
32. Yetkin FZ, Rosenberg RN, Weiner MF, Purdy PD, Cullum CM. FMRI of working memory in patients with mild cognitive impairment and probable Alzheimer's disease. *Eur Radiol* 2006;16:193–206
33. Smith EE, Jonides J. Neuroimaging analyses of human working memory. *Proc Natl Acad Sci USA* 1998;95:12061–12068
34. Smith EE, Jonides J. Storage and executive processes in the frontal lobes. *Science* 1999;283:1657–1661
35. Duncan J, Owen AM. Common regions of the human frontal lobe recruited by diverse cognitive demands. *Trends Neurosci* 2000;23:475–483
36. Jonides J, Schumacher EH, Smith EE, et al. The role of parietal cortex in verbal working memory. *J Neurosci* 1998;18:5026–5034
37. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain* 1998;121:561–579
38. Pihlajamäki M, Sperling RA. Functional MRI assessment of task-induced deactivation of the default mode network in Alzheimer's disease and at-risk older individuals. *Behav Neurol* 2009;21:77–91
39. Hvidberg A, Fanelli CG, Hershey T, Terkamp C, Craft S, Cryer PE. Impact of recent antecedent hypoglycemia on hypoglycemic cognitive dysfunction in nondiabetic humans. *Diabetes* 1996;45:1030–1036
40. Kim MA, Tura E, Potkin SG, et al.; FBIRN. Working memory circuitry in schizophrenia shows widespread cortical inefficiency and compensation. *Schizophr Res* 2010;117:42–51
41. Meda SA, Stevens MC, Folley BS, Calhoun VD, Pearlson GD. Evidence for anomalous network connectivity during working memory encoding in schizophrenia: an ICA based analysis. *PLoS ONE* 2009;4:e7911
42. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ. Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 1995;333:1726–1731
43. McCall AL. Cerebral glucose metabolism in diabetes mellitus. *Eur J Pharmacol* 2004;490:147–158
44. Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. *J Int Neuropsychol Soc* 2002;8:448–460
45. Musen G, Lyoo IK, Sparks CR, et al. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes* 2006;55:326–333
46. Wessels AM, Simsek S, Remijnse PL, et al. Voxel-based morphometry demonstrates reduced grey matter density on brain MRI in patients with diabetic retinopathy. *Diabetologia* 2006;49:2474–2480
47. Kennan RP, Takahashi K, Pan C, Shamooh H, Pan JW. Human cerebral blood flow and metabolism in acute insulin-induced hypoglycemia. *J Cereb Blood Flow Metab* 2005;25:527–534
48. Choi IY, Seaquist ER, Gruetter R. Effect of hypoglycemia on brain glycogen metabolism in vivo. *J Neurosci Res* 2003;72:25–32
49. Mason GF, Petersen KF, Lebon V, Rothman DL, Shulman GI. Increased brain monocarboxylic acid transport and utilization in type 1 diabetes. *Diabetes* 2006;55:929–934
50. Jacobson AM, Ryan CM, Cleary P, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group. Long-term effect of diabetes and its treatment on cognitive function. *N Engl J Med* 2007;356:1842–1852