Original Article

Microvascular Disease in Type 1 Diabetes Alters Brain Activation

A Functional Magnetic Resonance Imaging Study

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Individuals with type 1 diabetes have mild performance deficits on a range of neuropsychological tests compared with nondiabetic control subjects. The mechanisms underlying this cognitive deterioration are still poorly understood, but chronic hyperglycemia is now emerging as a potential determinant, possibly through microvascular changes in the brain. In 24 type 1 diabetic patients, we tested at euglycemia and at acute hypoglycemia whether the presence of proliferative diabetic retinopathy, as a marker of microvascular disease, adversely affects the ability of the brain to respond to standardized hypoglycemia, using functional magnetic resonance imaging with a cognitive task. Patients with retinopathy, compared with patients without, showed less deactivation (hence, an increased response) in the anterior cingulate and the orbital frontal gyrus during hypoglycemia compared with euglycemia ($P < 0.05$). Task performance and reaction time were not significantly different for either group. We conclude that microvascular damage in the brain of patients with retinopathy caused this increased brain response to compensate for functional loss. *Diabetes* 55:334–340, 2006

Individuals with type 1 diabetes have mild performance deficits on a range of neuropsychological tests compared with nondiabetic control subjects, particularly in learning and memory, problem solving, and mental and motor speed (1–4). The mechanisms underlying cognitive deterioration in diabetes are poorly understood and raise the question of to what extent they are related to structural and/or functional changes in the brain.

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BOLD, blood oxygenation level dependent; CBF, cerebral blood flow; fMRI, functional magnetic resonance imaging; NART, National Adult Reading Test; WAIS, Wechsler Adult Intelligence Scale.

Although retrospective studies in adult patients with type 1 diabetes have demonstrated an association between a history of recurrent severe hypoglycemia and a modest degree of cognitive impairment (2,5,6), two large prospective studies did not find such an association (7,8). Reanalyses of the Diabetes Control and Complications Trial findings confirmed the latter conclusion (9).

The potential effect of chronic hyperglycemia on brain function is receiving increasing attention. Some evidence for a damaging effect of chronic hyperglycemia on brain function is now emerging (10,11). Hyperglycemia may lead to an accumulation of potentially toxic glucose metabolites, oxidative stress, accelerated formation of advanced glycation end products, and microvascular changes in the brain. The evidence for this is, however, limited and includes one neuropathological study (12). To test the hypothesis that the presence of microvascular disease adversely affects the regional vasodilatory responses in the brain, we induced a standardized hypoglycemia as a mode to test brain response, using functional magnetic resonance imaging (fMRI). Increases in cerebral blood flow (CBF) during brain activation are accompanied by decreased blood deoxyhemoglobin levels, which can be visualized during fMRI with the blood oxygenation level–dependent (BOLD) contrast technique. The BOLD mechanism makes use of regional vasodilatory responses in the brain after neuronal stimulation. In this study, we tested whether the BOLD fMRI response to a working memory task with increasing demand is different in diabetic patients with and without microvascular disease during euglycemia and hypoglycemia.

RESEARCH DESIGN AND METHODS

Ten right-handed type 1 diabetic patients (World Health Organization criteria [1999]) with microvascular disease, using the presence of proliferative diabetic retinopathy as a marker (this is grade 4–5 diabetic retinopathy according to the EURODIAB classification [13]), and 14 type 1 diabetic patients free of retinopathy (no diabetic retinopathy; maximum three microaneurysms) participated in this study (Table 1). Groups were matched for age and sex. All subjects had a visual acuity of >0.3 as measured with the Snellen method (14) and were normotensive (<140/90 mmHg; range 100–140/60–82). None of the no–diabetic retinopathy patients had a micro- or macrovascular diabetes complication. Four diabetic retinopathy patients were known with nephropathy; two other patients were known with nephropathy and neuropathy. Those with metal implants or BMI >35 kg/m^2 were excluded. Exclusion criteria were hypertension (blood pressure >160/90 mmHg); previous alcohol or drug abuse; history of psychiatric disease/treatment; history of severe head trauma accompanied by loss of consciousness; stroke; epilepsy; history of severe, recurrent hypoglycemia (defined as >5 episodes that required external assistance for recovery) (15); pregnancy; and other diseases that could

TABLE 1
Characteristics of participants

	No retinopathy*	Retinopathy*	*P* values
n	14	10	
Age (years)	40.1 ± 4.1	39.0 ± 2.1	0.43
Age range (years)	33–49	36–43	
Age at diagnosis (years)	16.6 ± 7.9	10.9 ± 4.7	0.06
Age range at diagnosis (years)	4–32	3–18	
Sex (men:women)	6:8	4:6	0.89
HbA_{1c} (%)	8.2 ± 0.9	7.8 ± 1.1	0.26
Range of HbA_{1c} (%)	7.0–9.8	5.4–9.4	
BMI (kg/m^2)	23.9 ± 2.9	26.0 ± 3.0	0.10
Duration of diabetes (years)	23.5 ± 9.8	28.2 ± 5.4	0.15
Range of duration of diabetes (years)	5–36	22–40	
NART scores (IQ ± SD)	95.4 ± 10.1	90.5 ± 16.1	0.41
Range of NART scores (IQ)	75–112	73–112	

Data are means ± SD. *Two pictures of the retina, one with the macula and one with the optic disc in the center (using a Topcon nonmydriatic camera) of each eye were made. NART standard score: 100 ± 15 (means ± SD).

possibly affect cognitive function or CBF. Written consent was obtained from all subjects, and the study was approved by the local medical ethics advisory committee.

All patients were admitted to the hospital the evening before testing to avoid nocturnal hypoglycemia and to ensure euglycemia. At admission, an intravenous Teflon cannula was inserted for insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) and glucose infusion (5% glucose) into the antecubital vein of the dominant right arm. Patients were instructed to omit their customary dosage of long-/intermediate-acting insulin and were not allowed to drink alcohol or perform vigorous physical exercise the day before testing. The night before, an intravenous infusion of insulin with a syringe pump was given (50 mU · kg^{-1} · h^{-1}) with hourly blood glucose testing using a One Touch glucose meter (LifeScan, Beerse, Belgium). Patients maintained a euglycemic level until the induction of hypoglycemia the following day (±12 h euglycemia). Cognitive tests (National Adult Reading Test [NART] [16] and Wechsler Adult Intelligence Scale [WAIS] digit span [17]) were administrated the evening before the experimental test session. The *n*–letter back task (18) was practiced to stable performance.

MRI setup. In the morning, subjects were admitted to the neuroimaging center and made comfortable in a supine position on the scanner table. A second Teflon cannula for blood sampling was inserted in a retrograde direction distally in the left forearm and kept patent with a slow infusion of 0.9% sodium chloride to obtain arterialized blood. The left hand was placed in the hand box at 55°C (serie 134410, Kobold TD 1300 D), resulting in an oxygen saturation in the venous blood during euglycemia and hypoglycemia of 97%. During scanning, plasma glucose was kept at a euglycemic level (72–108 mg/dl) using the hyperinsulinemic-euglycemic clamp technique as described previously (19,20). Hypoglycemia (~45 mg/dl) was reached by a one-step decrease of blood glucose. A maximum of 30 min was taken for blood glucose values to drop to the hypoglycemic level. The test session was started a few minutes after hypoglycemia was established, and participants were hypoglycemic for ~12–15 min. The blood glucose concentrations were measured using a HemoCue (Lake Forest, CA). Glucose was infused using an IMED Gemini PC-1 infusion pump (Alaris Medical Systems, San Diego, CA), and insulin was infused using a Terumo Pilot A2 pump (Terumo, Burgdorf, Switzerland) by 9-m-long infusion lines. Both pumps were located in the control room so that magnetic materials did not enter the magnetic resonance room. During scanning, blood samples were transported from the magnetic resonance room to the control room through a radio frequency sealed wave guide. Blood glucose values were measured every 5 min during euglycemia and every 2 min during hypoglycemia.

Magnetic resonance acquisition. Imaging was performed on a 1.5-T Siemens Sonata (Siemens, Erlangen, Germany) scanner using a standard circularly polarized head coil with foam padding to restrict head motion. A localizer scan was first performed for positioning of the image planes, followed by an automated shim procedure to improve magnetic field homogeneity. For fMRI, an echo planar imaging echo planar imaging sequence was used with the following imaging parameters: interpulse interval = 2,100 ms; echo time = 60 ms; flip angle = 90°. Twenty-one slices with a field of view of 210 mm^2, 64 × 64 matrix, and 5-mm slice thickness (1-mm interslice gap) were acquired in the transversal orientation, covering the whole brain (286 volumes). The cognitive task was performed twice during scanning: during euglycemia and hypoglycemia.

Stimuli were generated by a Pentium PC and projected on a screen at the back end of the scanner bore. The projected image was seen through a mirror positioned above the participant's head. The subjects had to use an MRI-compatible four-key response box (Lumitouch; Lightwave Medical Industries, Richmond, British Columbia, Canada) with their right hand to record their performance and reaction times. An inversion time–weighted structural MRI-scan was also acquired (MPRAGE; inversion time = 950 ms, interpulse interval = 2,700 ms; echo time = 5.15 ms; flip angle = 8°; 160 coronal slices with an field of view of 210 mm^2).

The study conditions were in fixed order (i.e., euglycemic test condition before hypoglycemic test condition), and participants were not blinded to the experimental condition. After the hypoglycemic test, the insulin infusion was stopped, and the plasma glucose was restored to euglycemia. The subject was removed from the scanner and provided with a meal. Blood glucose monitoring continued until euglycemia was maintained spontaneously, then all lines were withdrawn, and the subject went home.

Cognitive function tests. Immediate and working memory was assessed with an expanded version of the WAIS digit span test (17), requiring the subject to repeat and reverse digit series of increasing length. Additionally, the subjects were administered the Dutch version of the NART (16,21). This vocabulary test was used as an estimate of crystallized intelligence and has good correlations with verbal IQ.

The *n*–letter back task is a working memory task, used during fMRI scanning. The task was randomly presented in blocks: instruction, a 0-back condition, a 1-back condition, and a 2-back and 3-back condition (corresponding to increased working memory load). Each of the conditions started with an instruction that was presented visually for 10 s. Letters were then presented sequentially: every 2 s, one letter was presented (1.5 s for presentation, followed by black screen for 0.5 s). In the 0-back condition, subjects responded to a single prespecified target letter (e.g., X). In the 1-back condition, the target was any letter identical to the one immediately preceding it (i.e., one trial back). In the 2-back and 3-back condition, the target was any letter that was identical to the one presented two or three trials back, respectively. Subjects responded to each stimulus with their dominant (right) hand. Conditions were randomly repeated in 12 different blocks (20 letters per block; block duration 40 s; hit rate 1 in 3). Reaction times and numbers of true positive errors were recorded.

Statistical analyses. Statistical analysis was performed using SPSS version 11.0 (SPSS, Chicago, IL). The relationships between demographic variables, neuropsychological performance, and laboratory results were examined using independent means *t* tests. χ^2 tests were used for categorical variables. Repeated-measures ANOVA was used to assess the interaction effect of task condition and disease status on cognitive performance. We made pairwise comparisons for the three main effects (group, condition, and *n* back) using a Bonferroni adjustment.

Magnetic resonance data analysis. fMRI analysis was carried out using fMRI Expert Analysis Tool (FEAT) version 5.4, part of fMRIB's Software Library (www.fmrib.ox.ac.uk/fsl). Prestatistical processing consisted of motion correction, nonbrain removal, spatial smoothing using a Gaussian kernel of FWHM 8 mm, mean-based intensity normalization of all volumes by the same factor, and high-pass temporal filtering (Gaussian-weighted LSF straight-line fitting, with σ = 100.0 s). Time-series statistical analysis was done with local autocorrelation correction (22). fMRI images were registered to the individual's structural scan, which was registered to standard space images (23). These transformations were applied to images of parameter estimates and variances to put them in standard space.

Higher level analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME) stage 1 only (i.e., without the final MCMC-based stage) (22). *Z* (Gaussianized T/F) statistic images were thresholded using clusters determined by $Z > 3.1$ and a corrected cluster significance threshold of $P = 0.05$. For the differences between groups, the analysis was limited to regions of activation only. A $P < 0.001$ uncorrected and a minimum cluster size of 50 voxels (2 × 2 × 2 mm in standard space) was used as a threshold for significant brain activation.

Areas activated were assessed by contrasting 0-back to 1-back (1-back>0-back and 0-back>1-back), 0-back to 2-back (2-back>0-back and 0-back>2-back), and 0-back to 3-back (3-back>0-back and 0-back>3-back). For each comparison, we calculated the activation (1-back>0-back; 2-back>0-back; and 3-back>0-back) and deactivation (0-back>1-back; 0-back>2-back; and 0-back>3-back), using repeated-measures ANOVA.

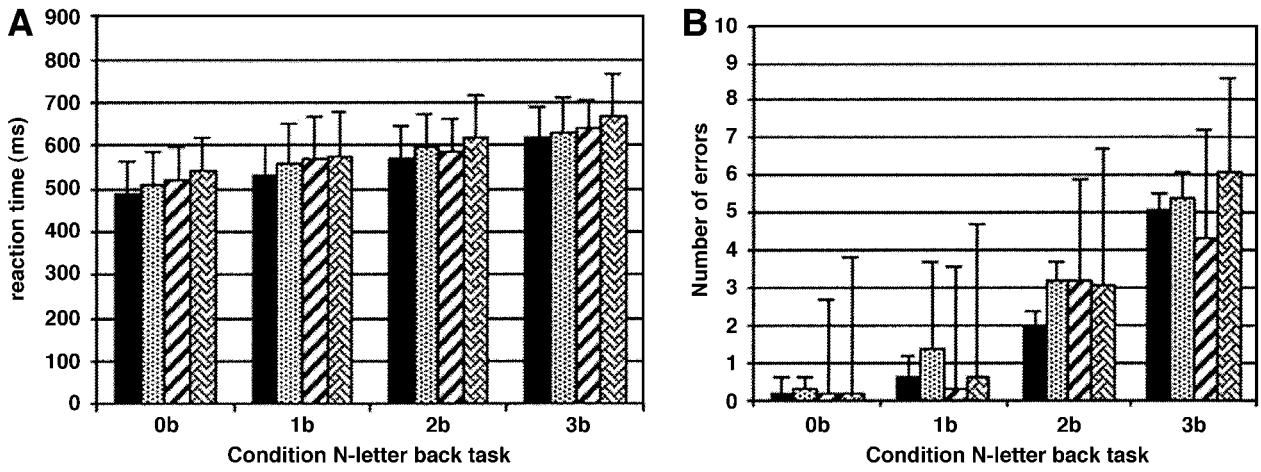

FIG. 1. Reaction times (*A*) and errors (*B*) during performance of the *n*–letter back task. Data are means ± SE. ■, euglycemic NDRP; ▒, hypoglycemic NDRP; ▨, euglycemic DRP; and , hypoglycemic DRP.

Comparisons were made for both groups contrasting hypoglycemia and euglycemia (hypoglycemia>euglycemia; euglycemia>hypoglycemia). We were also interested in the effect of microvascular disease on brain (de)activation (diabetic retinopathy>no diabetic retinopathy; no diabetic retinopathy>diabetic retinopathy) and the interaction of these effects: does the diabetic retinopathy group respond differently in the euglycemic or hypoglycemic condition compared with the no diabetic retinopathy group?

RESULTS

Hypoglycemic clamp. The blood glucose values at the beginning of the euglycemic test session were 100.5 ± 9.3 mg/dl for the no–diabetic retinopathy group and 104.2 ± 10.9 mg/dl for the diabetic retinopathy group ($P = 0.39$). At the end of the euglycemic test session, blood glucose values were 87.0 ± 9.2 and 88.7 ± 8.9 mg/dl ($P = 0.66$) for the no–diabetic retinopathy and the diabetic retinopathy group, respectively. The second session of the *n*–letter back task started with a blood glucose value of 41.9 ± 5.4 (no–diabetic retinopathy group) and 43.0 ± 5.5 mg/dl (diabetic retinopathy group) ($P = 0.63$). At the end of this test session, blood glucose values were 39.5 ± 5.6 (no–diabetic retinopathy group) and 40.5 ± 7.2 mg/dl (diabetic retinopathy group) ($P = 0.70$). The mean euglycemic (no diabetic retinopathy, 93.8 ± 6.5 mg/dl; diabetic retinopathy, 96.5 ± 7.2 mg/dl) and hypoglycemic (no diabetic retinopathy, 40.7 ± 5.1 mg/dl; diabetic retinopathy, 41.8 ± 5.7 mg/dl) blood glucose levels during the scanning sessions did not differ significantly between the groups ($P = 0.35$ and 0.64, respectively).

Cognitive ability. No significant differences were observed on task performance between the groups on the WAIS digit span test. Moreover, NART scores did not significantly differ between both groups, suggesting similar levels of crystallized intelligence.

Performance of the *n*–letter back task declined ($F[1.7, 37.1] = 44.1$; $P < 0.001$) and reaction time increased ($F[2.1, 45.1] = 26.8$; $P < 0.001$) when task load increased. Furthermore, performance declined significantly ($F[1, 22] = 5.2$; $P = 0.03$) and reaction time increased significantly ($F[1, 21] = 6.9$; $P = 0.02$) when patients became hypoglycemic (compared with euglycemia), but these deteriorations were not related to task level and patient group (Fig. 1).

There was no significant interaction effect between task load and condition (hypoglycemia/euglycemia) on perfor-

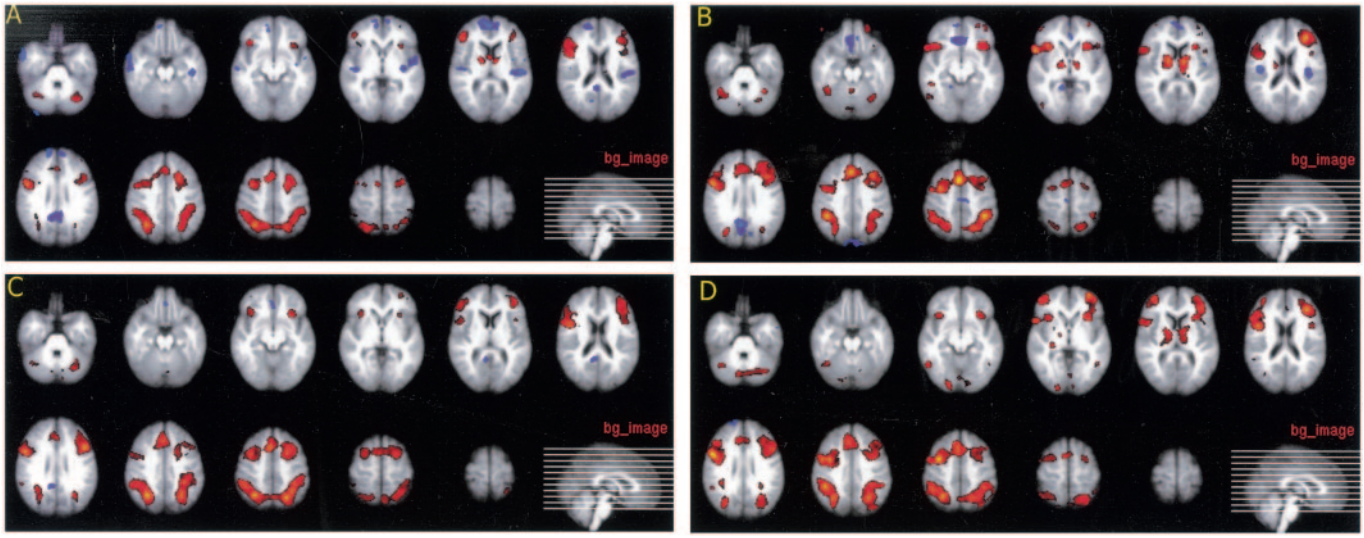

FIG. 2. Red to yellow represents activation, and light blue to dark blue represents deactivation of brain activity. *A*: (de)activation no–diabetic retinopathy euglycemia 2-back>0-back. *B*: (de)activation diabetic retinopathy euglycemia 2-back>0-back. *C*: (de)activation no–diabetic retinopathy hypoglycemia 2-back>0-back. *D*: (de)activation diabetic retinopathy hypoglycemia 2-back>0-back.

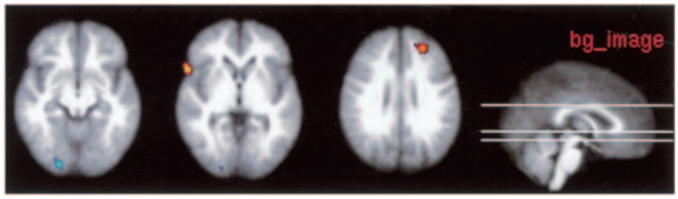

FIG. 3. Diabetic retinopathy euglycemia/hypoglycemia>no–diabetic retinopathy euglycemia/hypoglycemia 2-back>0-back. Blue (left occipital lobe): diabetic retinopathy hypoglycemia>no–diabetic retinopathy hypoglycemia. Red (right superior frontal gyrus): diabetic retinopathy euglycemia>no–diabetic retinopathy euglycemia.

mance (F[2.2, 49.2] = 1.08; $P > 0.3$) and on reaction time (F[3, 63] = 0.15; $P > 0.9$). There were no significant interaction effects between task level, glycemic condition, and patient group on performance (F[2.2, 49.2] = 2.6; $P > 0.1$) and on reaction time (F[3, 63] = 0.31; $P > 0.8$). This indicates that performance and reaction time on the n–letter back task were unaffected by glycemia and presence of retinopathy.

Imaging

Main effects. Because the n–letter back task consists of levels with increasing cognitive load, increasing activity over these levels were expected. In all three contrasts (1-back>0-back, 2-back>0-back, and 3-back>0-back), the same brain areas were activated, and task load–related increases in activity were observed. Effects of group and condition on brain activation were only seen in the 2-back>0-back and 0-back>2-back contrasts, and therefore our description is limited to this contrast. Main effects (independent of group) during euglycemia and hypoglycemia involved activation in bilateral parietal areas, bilateral frontal areas, bilateral temporal areas, bilateral thalamus, and bilateral cerebellum (Fig. 2) but also deactivation (that is 0-back>2-back) in the insula, anterior cingulate gyrus, posterior cingulate gyrus, bilateral parietal areas, and bilateral frontal areas during euglycemia (Fig. 2*A* and *B*).

Group differences. During hypoglycemia, deactivation in the no–diabetic retinopathy group was seen in the anterior and posterior cingulate gyrus and left medial frontal gyrus (Fig. 2*C*). The diabetic retinopathy group showed no deactivation during hypoglycemia (Fig. 2*D*). In comparison with the no–diabetic retinopathy group, the diabetic retinopathy group showed an increase in activation in the right superior frontal gyrus (during euglycemia) and left occipital lobe (during hypoglycemia) (Fig. 3).

Effect of condition. The no–diabetic retinopathy group showed no differences between euglycemia and hypoglycemia in activated or deactivated brain regions. The diabetic retinopathy group showed less deactivation in the left anterior cingulate, right orbital frontal gyrus, and left parietal lobe during hypoglycemia compared with euglycemia (Fig. 4).

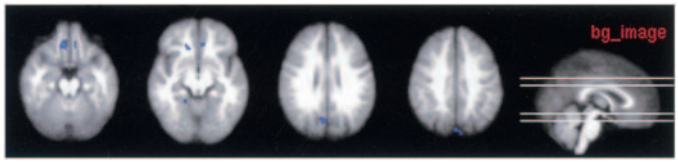

FIG. 4. Diabetic retinopathy hypoglycemia > euglycemia 2-back>0-back.

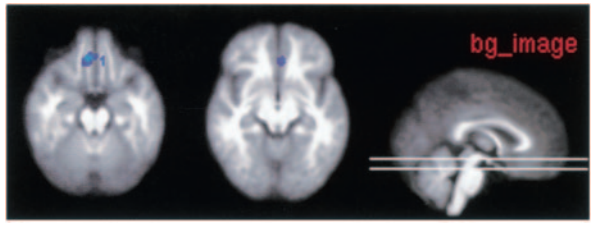

FIG. 5. Diabetic retinopathy>no–diabetic retinopathy (hypoglycemia> euglycemia) 2-back>0-back.

Interaction effect. Only the left anterior cingulate gyrus and right orbital frontal gyrus showed an interaction effect of diabetic group and condition (Figs. 5 and 6). These areas appeared to be less deactivated during hypoglycemia in the diabetic retinopathy group.

DISCUSSION

This study demonstrates the effect of the presence of diabetic retinopathy on the brain response to a standardized hypoglycemia in type 1 diabetic patients. We found significant differences in BOLD response between the two groups when enhancing the cognitive demands during the

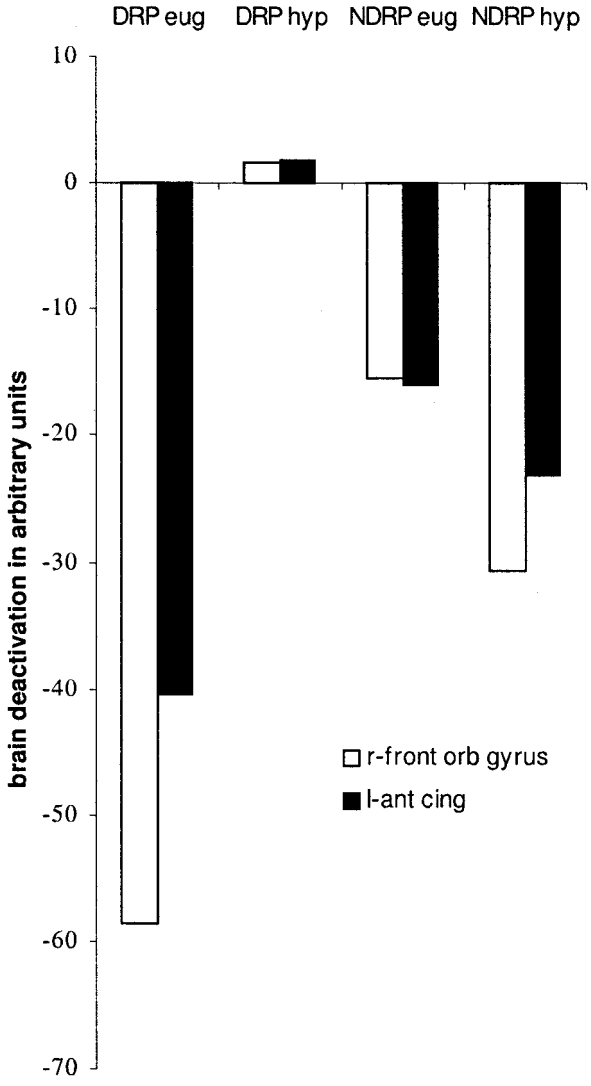

FIG. 6. Diabetic retinopathy>no–diabetic retinopathy (hypoglycemia> euglycemia) 2-back>0-back.

2-back relative to the 0-back condition. During euglycemia, the diabetic retinopathy group compared with the no diabetic retinopathy group showed a higher BOLD response in the right superior frontal gyrus. During hypoglycemia, a higher BOLD response was seen in the left occipital lobe. Furthermore, during hypoglycemia, less deactivation in the left anterior cingulate, left parietal lobe, and right orbital frontal gyrus was seen in the diabetic retinopathy group compared with the no–diabetic retinopathy group. When tested for interaction, we found the left anterior cingulate and the right orbital frontal gyrus to be less deactivated during hypoglycemia in the diabetic retinopathy group. Task performance and reaction time were not significantly different for either groups.

In the 1-back>0-back and 0-back>1-back and the 3-back>0-back and 0-back>3-back contrast, no group differences and no differences between the two conditions on the BOLD response were observed. This is probably because the cognitive demands were too low and too high, respectively. The 1-back condition may not challenge the brain network hard enough (given the fact that hardly any errors are made in the 1-back condition) to enable the detection of changes in brain activation. In the 2-back condition, the stress on the working memory network is increased, whereas the number of errors is still very small (that is, increased working memory load with still efficient processing). Therefore this condition may be most optimal to detect changes in brain activation. In the most difficult (3-back) condition, a significant decline in task performance was observed. This may indicate less efficient working memory activation, which may explain why in this condition, no interactions were found. Other studies provide support for this explanation: decreases in brain activation at highest working memory load have been reported to coincide with a significant decrement in performance (24,25). Furthermore, the capacity-limited fMRI response to increasing working memory load has been replicated in two additional cohorts of healthy volunteers (26,27). In these studies, loci within the prefrontal cortex also peaked at 2-back.

The anterior cingulate and the orbitofrontal gyrus showed a pattern of deactivation after stimulation of a cognitively demanding working memory task. This is often observed in functional imaging studies (28). These areas are part of an organized network referred to as "default mode of brain function." This network is active during rest and suspended during performance of externally cued tasks. The implications of this network for behavior are not yet identified, but evidence suggests that it has a role in attending to environmental stimuli, both internally and externally generated (28). Moreover, it has been suggested that the network mediates processes such as reviewing past knowledge in preparing for future actions (29) and episodic memory processing (30). Externally cued tasks with high cognitive demand have been shown to modulate the network, contributing to the phenomenon of task-related decreases in brain activity (28,31); when task-related attention is required, activation decreases in that network. In young adults, greater task difficulty is usually associated with increased deactivation (32). Changes in deactivation patterns have been reported in populations in which brain function differs from that of healthy young adults, including patients with amnesia and schizophrenia (33,34). Hence, abnormalities in deactivation patterns, as seen in the diabetic retinopathy group during hypoglycemia, are considered manifestations of pathology.

There are at least two possible explanations regarding the functional differences between the diabetic retinopathy group and the no–diabetic retinopathy group. First, there is the possibility of altered neurovascular coupling in the diabetic retinopathy group; i.e., the process by which neural activity influences the surrounding vasculature. Studying differences in brain activity between individuals relies on the assumption of comparable neurovascular coupling, and any alteration in the cerebrovascular system could affect neurovascular coupling. Considering vascular changes in diabetes, it is known that structural abnormalities at the microvascular level include thickening of capillary basement membranes and decreased capillary density (35). This can lead to decreased vasoreactivity and therefore altered BOLD signal.

Second, functional microvascular alterations in type 1 diabetic patients include regional alteration in CBF, and this regional alteration in CBF in the diabetic retinopathy group might be responsible for the altered BOLD response. There are several somewhat contradictory reports dealing with cerebrovascular reactivity in type 1 diabetic patients. Fulesdi et al. (36) found that the increase in blood flow after administration of a dilatory stimulus is impaired, and this impairment appeared to be most pronounced in subjects with other complications such as retinopathy and nephropathy. In a single-photon emission tomography study, Keymeulen et al. (37) could not find a correlation between the presence of proliferative retinopathy or microalbuminuria and alterations in CBF, but the number of included patients with proliferative retinopathy was very small. Increases in CBF in response to acute hypoglycemia and studies using single-photon emission tomography demonstrated blood flow increases to the frontal lobes (38–40). In diabetic patients who have a history of recurrent, severe hypoglycemia, the regional blood flow changes were observed to be present during normoglycemia and may represent a cerebral adaptation to recurring hypoglycemic insults (39,40). In a study performed by Chabriat et al. (41), no differences were found in neuropsychological test performance and rate of oxygen metabolism measured by position emission tomography scanning in patients with a history of >10 hypoglycemic comas compared with those without a history of comas. Patients with angiopathic complications were excluded. Obviously, because patients with a history of hypoglycemia were excluded from our study and patients with angiopathic complications were included, it is uncertain whether these findings can be held accountable for the functional differences between the two groups. However, it is unlikely that previous severe hypoglycemia exposure by our participants will confound our results.

Cerebral vasoreactivity and accompanying changes in blood flow are important compensatory mechanisms during hypoglycemia, and loss of these compensatory mechanisms may therefore result in the changes in BOLD response.

We found the anterior cingulate and the orbitofrontal gyrus to be less deactivated during hypoglycemia in the diabetic retinopathy group. The functional role of the anterior cingulate is not yet clear, but there is evidence to suggest that the anterior cingulate translates intentions to actions, participates in the willed control of behavior, and suppresses inappropriate responses (42). The orbitofrontal cortex on the other hand, plays a specific role in controlling voluntary goal-directed behavior (43,44) and executive functions (45,46). Different hypotheses may be

postulated on why the activation of the anterior cingulate and the orbitofrontal gyrus is altered. Interpretation of altered (de)activation of certain brain areas need to be considered in relation with the changes in other brain regions that co-activate or deactivate with these regions. If a pathological process alters the response in a certain region, this will affect the activation in another region to compensate for functional loss. Although we did not find significant decrease in the activated network related to pathology, this explanation is consistent with our findings. In that case, the integrity of the anterior cingulate and the orbitofrontal gyrus would be intact, while other brain regions are affected. An alternative explanation is that the anterior cingulate and the orbital frontal gyrus themselves are affected and have, for example, a decreased resting-state metabolism. This would also explain a decrease in deactivation during the task. Methods to measure resting state metabolism and perfusion would allow further study of this possibility. The observed decreased deactivation may serve as a compensatory mechanism (because task performance and reaction time were not significantly different for either groups). These areas remain "active " to achieve equal task performance, to maintain task performance, or to compensate for functional loss. At this point, it is a successful mechanism, but it could fail with aging and/or if disease complications progress.

Our results are in line with those of Rosenthal et al. (47), who also found region specific changes in brain activation during hypoglycemia. However, we did not find task-specific changes in brain activation. Furthermore, the changes in brain function we found were primarily seen in the deactivation network not in the activation network. For these reasons, it is difficult to compare the results.

There are several limitations regarding this study: no follow-up of the cognitive status was done; therefore, we were not able to assess whether cognitive performance will deteriorate over time in our patients. Future research should help to clarify this issue, taking into account the impact of various degrees of retinopathy/microvascular disease. We did not find differences in task performance and reaction time between the groups, probably because of the small sample size of both groups. Larger sample sizes might have shown differences in cognitive abilities between groups.

Complex studies concerning brain structure and functioning are now more feasible, because it is possible to control the glucose values in patients with diabetes during MRI studies. In that sense, our study was unique in its design and showed that manipulation of glucose level during an fMRI experiment is feasible.

Our results indicating that type 1 diabetic patients with microvascular disease respond differently to a cognitive test during standardized hypoglycemia with preserved cognitive performance may only hint at a compensatory mechanism. Further studies, including larger sample sizes, are warranted to establish the exact mechanisms underlying these differential brain responses.

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