Impaired Awareness of Hypoglycemia Disrupts Blood Flow to Brain Regions Involved in Arousal and Decision Making in Type 1 Diabetes

Munachiso Nwokolo,[1,2] Stephanie A. Amiel,[1,2] Owen O'Daly,[3] Megan L. Byrne,[1] Bula M. Wilson,[1] Andrew Pernet,[1] Sally M. Cordon,[4] Ian A. Macdonald,[4] Fernando O. Zelaya,[3] and Pratik Choudhary[1,2]

Diabetes Care 2019;42:2127–2135 | https://doi.org/10.2337/dc19-0337

PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

Impaired awareness of hypoglycemia (IAH) affects one-quarter of adults with type 1 diabetes and significantly increases the risk of severe hypoglycemia. Differences in regional brain responses to hypoglycemia may contribute to the susceptibility of this group to problematic hypoglycemia. This study investigated brain responses to hypoglycemia in hypoglycemia aware (HA) and IAH adults with type 1 diabetes, using three-dimensional pseudo-continuous arterial spin labeling (3D pCASL) functional MRI to measure changes in regional cerebral blood flow (CBF).

RESEARCH DESIGN AND METHODS

Fifteen HA and 19 IAH individuals underwent 3D pCASL functional MRI during a two-step hyperinsulinemic glucose clamp. Symptom, hormone, global, and regional CBF responses to hypoglycemia (47 mg/dL [2.6 mmol/L]) were measured.

RESULTS

In response to hypoglycemia, total symptom score did not change in those with IAH ($P = 0.25$) but rose in HA participants ($P < 0.001$). Epinephrine, cortisol, and growth hormone responses to hypoglycemia were lower in the IAH group ($P < 0.05$). Hypoglycemia induced a rise in global CBF (HA $P = 0.01$, IAH $P = 0.04$) but was not different between groups ($P = 0.99$). IAH participants showed reduced regional CBF responses within the thalamus ($P = 0.002$), right lateral orbitofrontal cortex (OFC) ($P = 0.002$), and right dorsolateral prefrontal cortex ($P = 0.036$) and a lesser decrease of CBF in the left hippocampus ($P = 0.023$) compared with the HA group. Thalamic and right lateral OFC differences survived Bonferroni correction.

CONCLUSIONS

Responses to hypoglycemia of brain regions involved in arousal, decision making, and reward are altered in IAH. Changes in these pathways may disrupt IAH individuals' ability to recognize hypoglycemia, impairing their capacity to manage hypoglycemia effectively and benefit fully from conventional therapeutic pathways to restore awareness.

[1]Department of Diabetes, School of Life Course Sciences, King's College London, London, U.K.
[2]King's College Hospital NHS Foundation Trust, London, U.K.
[3]Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, U.K.
[4]School of Life Sciences, MRC Arthritis Research UK Centre of Excellence in Musculoskeletal Ageing, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, U.K.

Corresponding author: Munachiso Nwokolo, munachiso.nwokolo@kcl.ac.uk

Received 18 February 2019 and accepted 7 August 2019

Impaired awareness of hypoglycemia (IAH) affects one-quarter of people with type 1 diabetes (1,2). Individuals with IAH cannot reliably detect impending hypoglycemia, increasing their risk of severe hypoglycemia three- to sixfold (1–3) and doubling their risk of an ambulance call for hypoglycemia (4). Recurrent hypoglycemia attenuates symptomatic and hormonal responses that protect against falling blood glucose (5). Although IAH is more common in those with longer duration diabetes (1), the majority of those with long-standing type 1 diabetes do not have IAH. Indeed, some maintain awareness of hypoglycemia despite experiencing up to two episodes of hypoglycemia per week (6) and numerous episodes over their lifetime. This suggests that there may be other important contributors to the development of IAH. Behavioral factors such as low adherence to therapeutic advice and low concern regarding hypoglycemia have been associated with IAH (7,8). Whether this is due to adaptations in brain regions involved in behavior and decision making is unknown. Positron emission tomography (PET) and functional MRI (fMRI) have been used to investigate brain responses to hypoglycemia in individuals without diabetes and with type 1 diabetes; however, the described impacts of hypoglycemia awareness status are not conclusive (9–12). Data on global blood flow, an important possible mediator for differences in response, have been discrepant between studies (10,13). We used three-dimensional pseudo-continuous arterial spin labeling (3D pCASL) fMRI, a technique designed to enhance sensitivity to capillary blood flow without radiation exposure, to measure differences between hypoglycemia aware (HA) and IAH brain responses to hypoglycemia. 3D pCASL uses radiofrequency pulses to magnetically tag arterial blood, quantitatively measuring cerebral blood flow (CBF) as a sensitive and convenient surrogate marker of brain activity (14).

RESEARCH DESIGN AND METHODS

Participants

Right-handed, nonobese adults with type 1 diabetes were recruited. We excluded individuals with type 2 diabetes; renal impairment (estimated glomerular filtration rate $<$60 mL/min/1.73 m^2); evidence of cardiovascular disease, peripheral vascular disease, or stroke; major psychological diagnoses; previous significant head injury; neurological conditions expected to produce MRI changes; or contraindications to MRI. The protocol was approved by the Dulwich Research Ethics Committee (National Research Ethics Service, London, U.K.), in accordance with the Declaration of Helsinki. Participants gave written informed consent. Participants were classified into HA or IAH groups on the basis of the Gold score, a validated measure of hypoglycemia awareness where a score of 1–2 indicates HA and a score of 4–7 indicates IAH (3). The score was validated by clinical history and retrospectively by absence of symptoms of hypoglycemia during the scans.

Study Protocol

As previously reported (15), consenting, eligible volunteers avoided alcohol, caffeine, and strenuous activity for 48 h before study. Participants were admitted to the King's College Hospital Clinical Research Facility the evening before scanning, after their evening meal. Multiple daily injection participants omitted their evening dose of basal insulin; no participant used an ultra-long-acting basal insulin. Individuals using continuous subcutaneous insulin infusion discontinued basal infusion when starting intravenous (IV) insulin. A variable rate IV insulin infusion was used to maintain blood glucose between 90 and 144 mg/dL (5–8 mmol/L) overnight, with venous sampling every 30–60 min. After 10:00 P.M., participants were only permitted water or preemptive hypoglycemia treatment if blood glucose fell to 81 mg/dL (4.5 mmol/L). The study was rescheduled if blood glucose fell to $<$54 mg/dL (3.0 mmol/L). In the morning, a hyperinsulinemic clamp was commenced for glucose stabilization at least 60 min before the scan, with a target glucose of 90 mg/dL (5 mmol/L). A primed IV insulin infusion (Actrapid; Novo Nordisk, West Sussex, U.K.) replaced the overnight insulin at a maintenance rate of 1.5 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} with a variable rate 20% glucose solution (Baxter, Berkshire, U.K.). An IV cannula was inserted into a left-side dorsal hand vein. A CE-registered, heated thermal pack was applied to warm the hand, arterializing venous blood (16). Blood was obtained every 5 min, and plasma was extracted and glucose measured with a glucose oxidase analyzer (YSI 2300 STAT PLUS; Yellow Springs Instruments, Yellow Springs, OH). Participants were positioned in supine on the scanner table and provided with earplugs and earphones to reduce exposure to the acoustic noise of the scanner. The head was stabilized, and participants were asked to remain as still as possible to avoid artifact. Within the scanner, plasma glucose was held at 90 mg/dL (5 mmol/L) for ~30 min for 3D pCASL scans 1 and 2 (ASL 1 and ASL 2). Thereafter, plasma glucose was lowered over a period of 20 min. Once 47 mg/dL (2.6 mmol/L) was achieved and maintained for ~20 min, 3D pCASL scans 3 and 4 (ASL 3 and ASL 4) were performed. After each ASL scan, samples were collected for counterregulatory hormones, and participants reported, using a button box, autonomic and neuroglycopenic symptoms on a 7-point visual analog scale. Participants rated their hypoglycemic symptoms (1 = not at all, 7 = severely). Seven symptoms were classified as autonomic (anxiety, pounding heart, shaking, tingling, sweating, hunger, and nausea) and four as neuroglycopenic (drowsiness, irritability, visual disturbance, and confusion) (17). Once symptom scoring was complete, the next ASL scan was commenced. Participants were blinded to their plasma glucose level throughout. After completion of the scanning protocol, IV insulin was stopped, and IV glucose and oral carbohydrate were used to restore normoglycemia. After a meal, glucose was stabilized, and participants were provided with support to avoid hypoglycemia for the next 48 h and discharged.

Biochemical Analysis

Epinephrine and norepinephrine were analyzed by high-performance liquid chromatography with electrochemical detection (17). Automated immunoassay was used to analyze cortisol (Centaur XPT; Siemens), growth hormone, and free insulin (IMMULITE 2000 XPi; Siemens).

Statistical Analysis of Nonimaging Data

Statistical analyses were performed using SPSS version 22 software (IBM Corporation). Continuous demographic data were compared using unpaired two-sample Student *t* tests. The χ^2 test was used to compare categorical sex data.

Within-group symptom scores, mean glucose concentrations, and hormonal responses were analyzed using paired Student *t* tests. Unpaired Student *t* tests were used for between-group comparisons. Data are presented as mean ± SD unless otherwise stated. $P < 0.05$ was considered statistically significant.

Power Calculation

A PET study of nine healthy individuals detected a 26% reduction in regional CBF in the hippocampus and a 6.4–7.8% change in CBF in the cortex and brainstem (18). Using pulsed ASL in nine individuals, Page et al. (19) found that regional CBF increased twofold in the hypothalamus (from 22.9 to 44.5 mL/100 g/min) in response to hypoglycemia. In another pulsed ASL fMRI study, Mangia et al. (9) described differences of between 8% and 10% in regional CBF in the thalamus and orbitofrontal cortex (OFC) of 12 individuals without diabetes and 11 with IAH ($P < 0.02$). On the basis of these data, we had 80% power to detect an effect size of 0.6 or a 5% change in regional CBF in 12 patients.

MRI Parameters

MRI images were acquired using a 3T GE Healthcare MR750 scanner (GE Medical Systems, Milwaukee, WI). Radiofrequency was transmitted with the scanner body coil, while signal was received with a 12-channel receive-only head coil. After an initial localizer scan, high-resolution anatomical images were acquired using an adapted 3D T1-weighted magnetization-prepared rapid gradient echo sequence with the following parameters: 1.2-mm isotropic resolution, repetition time of 7.312 ms, echo time of 3.01 ms, and inversion time of 450 ms. CBF maps were acquired using 3D pCASL to determine changes in regional resting perfusion. The sequence used four nonselective radiofrequency pulses for suppression of the static background signal, which increased sensitivity to the labeled arterial blood signal. The sequence used a labeling time of 1.5 s and a postlabeling delay of 1.5 s. Movement correction of the time series of perfusion-weighted difference images was not possible. Four control-label pairs were collected, each within ~45 s. After the postlabeling delay, images were acquired using a multishot, segmented 3D fast spin echo stack-of-spiral sequence with an effective resolution of 2 × 2 × 3 mm. A proton density image was also acquired in the same series to enable the computation of quantitative CBF maps (14). T1-weighted, T2-weighted, and fluid-attenuated inversion recovery scans were reported by a neuroradiologist, and participants were excluded if any pathology was observed.

Statistical Analysis of Neuroimaging Data

CBF maps were analyzed using Statistical Parametric Mapping (SPM) version 12 (University College London, London, U.K.). As part of this process, the maps were transformed to the standard space of the Montreal Neurological Institute using a custom-built software package called Automatic Software for ASL Processing (ASAP) (Department of Neuroimaging, King's College London) (20). A proton density image (acquired within the same 3D pCASL sequence) was coregistered to the T1 image after realigning the origin of both images. The transformation matrix of this coregistration step was then applied to the CBF map, transforming the CBF map to the space of the T1 image. Unified segmentation of the T1 image scan generated a brain-only binary mask. This mask was then multiplied with the CBF map in the space of the T1 image, eliminating extracerebral signal from the CBF map. Normalization of the participant's T1 image and the skull-stripped CBF map was performed using the parameters of the unified segmentation matrices. Finally, spatial smoothing of the normalized individual CBF maps was carried out using an 8-mm Gaussian smoothing kernel.

For each participant, two CBF maps obtained at euglycemia (ASL 1 and ASL 2) were averaged, and two obtained during hypoglycemia (ASL 3 and ASL 4) were averaged. Global CBF change between euglycemia and hypoglycemia was measured from the mean of all gray matter voxels in the brain volume in both conditions. Differences within and between groups (HA and IAH) were compared using a paired and unpaired Student *t* test, respectively. To identify regional CBF change between euglycemia and hypoglycemia, a voxel-wise paired *t* test (within the SPM framework) was performed within each group. Only clusters that remained statistically significant after family-wise error (FWE) correction for multiple comparisons ($P_{FWE} < 0.05$) are reported. Clusters of significant change were determined using the cluster-extent criterion ($P_{FWE} < 0.05$) from an uncorrected voxel-wise cluster-forming threshold of $P < 0.005$. CBF maps from both groups and both states (euglycemia and hypoglycemia) were then analyzed using a 2 × 2 flexible factorial ANOVA model within SPM version 12 to assess the interaction of group and glycemic state across the whole brain. Again, significance was defined as any result on the map that survived FWE correction on the basis of cluster extent ($P_{FWE} < 0.05$) using an uncorrected voxel-wise cluster-forming threshold of $P < 0.005$. Between-group, hypothesis-led analyses of a priori–defined regions of interest (ROIs)—the thalamus, anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), OFC, and hippocampus—were performed. These regions were selected on the basis of a review of published literature establishing key cerebral structures involved in the response to hypoglycemia (9–11,17,18,21–24). ROI masks were created using the Wake Forest University School of Medicine PickAtlas (25).

Statistical analysis was applied to all the voxels within each anatomically defined ROI, thereby applying an adjustment for small volume, or small-volume correction. The small-volume option in SPM was used to apply the same statistical model to all voxels within each ROI. Peak-level significance values were used, the inference of which is derived from a correction for FWE (i.e., multiple comparisons) that includes all the voxels of the ROI. Additional Bonferroni correction for the number of ROIs was applied, giving a critical α of $P < 0.01$. A gray matter mask was used in each analysis, and global CBF was added to each model as a covariate to control for the effect of global perfusion. To demonstrate the amplitude of CBF change across the whole of each ROI, mean CBF values were extracted from all voxels within each ROI using SPM, MATLAB (matrix laboratory), and ASAP (20). Differences within (euglycemia vs. hypoglycemia) and between (HA vs. IAH) groups were compared using a paired and unpaired Student *t* test, respectively.

RESULTS

Participant Characteristics

Fifteen participants with type 1 diabetes and HA and 23 with type 1 diabetes and IAH were recruited. Four potential participants recruited by Gold score and history into the IAH group demonstrated subjective awareness to low plasma glucose (to which they were blind) during the scan and were withdrawn, leaving 19 participants in the IAH group. Retained participants had long diabetes duration (HA 24.0 ± 12.8 years, IAH 22.2 ± 7.2 years, $P = 0.6$) and were also matched for age, sex, BMI, and HbA_{1c} (Table 1). By design, IAH participants had a significantly higher Gold score than HA participants (IAH 5.8 ± 1.3 vs. HA 1.5 ± 0.5, $P < 0.001$). Severe hypoglycemia rate in the 12 months before the study was greater in IAH participants (IAH 2.3 ± 2.8 per year vs. HA 0.2 ± 0.6 per year, $P = 0.009$).

Glucose and Insulin Concentrations

Plasma glucose targets were achieved, with no significant difference between groups ($P = 0.99$) (Fig. 1). Mean glucose concentrations during the euglycemic phase of ASL scan acquisition were 96 ± 6 mg/dL (5.4 ± 0.4 mmol/L) and 97 ± 8 mg/dL (5.4 ± 0.4 mmol/L) for HA and IAH participants, respectively ($P = 0.92$). Corresponding concentrations during the hypoglycemic phase were 47 ± 2 mg/dL (2.6 ± 0.1 mmol/L) and 46 ± 3 mg/dL (2.5 ± 0.2 mmol/L) ($P = 0.24$). Total mean glucose infusion rate was greater in the IAH group, but this did not reach statistical significance (HA 333 mg/kg/min, IAH 412 mg/kg/min, $P = 0.06$). Insulin concentrations were not different between groups throughout.

Symptomatic and Hormonal Responses to Hypoglycemia

Hypoglycemia induced a significant symptom response in HA participants (Fig. 2*A*–*C*), while IAH participants had no change in total or autonomic symptom scores, with a small increase in neuroglycopenic score (8.8 ± 4.1 from 7.4 ± 3.0, $P = 0.03$) (Fig. 2*A*–*C*). Overall, symptomatic responses to hypoglycemia were significantly lower in the IAH group compared with the HA group ($P < 0.001$). A reduced epinephrine response to hypoglycemia was seen in those with IAH (mean rise HA vs. IAH 1.2 ± 0.9 vs. 0.4 ± 0.4 nmol/L, $P = 0.003$) (Fig. 2*D*). Norepinephrine concentrations did not increase significantly in either group (Fig. 2*E*). Cortisol concentrations rose significantly in the HA group ($P = 0.01$), with no significant change in those with IAH ($P = 0.72$) (Fig. 2*F*). Growth hormone increased in both groups ($P < 0.001$), with a smaller response in IAH ($P = 0.04$) (Fig. 2*G*).

Global CBF Responses to Hypoglycemia

Global CBF increased significantly in response to hypoglycemia in both HA [$CBF_{(Hypoglycemia)} - CBF_{(Euglycemia)}$ 2.65 ± 3.7 mL/100 g/min, 6.8 ± 9.2%, $P = 0.01$] and IAH groups (2.62 ± 5.1 mL/100 g/min, 7.8 ± 13.5%, $P = 0.04$) (Fig. 2*H*), with no significant difference between groups ($P = 0.99$).

Regional CBF Responses to Hypoglycemia

Voxel-Wise Within-Group Analyses

HA participants demonstrated a significant increase of CBF in the thalamus, with a reduction of CBF in the left hippocampus and temporal cortex bilaterally, during hypoglycemia ($P_{FWE} < 0.05$, uncorrected voxel-wise cluster-forming threshold of $P < 0.005$) (Fig. 3*A*). IAH participants demonstrated CBF increases in the left OFC and DLPFC, with reductions in the right temporal cortex ($P_{FWE} < 0.05$, uncorrected voxel-wise cluster-forming threshold of $P < 0.005$) (Fig. 3*B*).

Effect of Awareness Status: Between-Group Analysis

Whole-Brain Analysis. Whole-brain ANOVA demonstrated no significant differences between HA and IAH groups after FWE correction for multiple comparisons ($P_{FWE} < 0.05$) at an uncorrected voxel-wise cluster-forming threshold of $P < 0.005$.

ROI Analysis. Testing for differences in the mean CBF signal (i.e., the average of the CBF values for all voxels within each a priori–selected region) in the thalamus, ACC, OFC, DLPFC, and hippocampus demonstrated a significant hypoglycemia-related increase in CBF in the whole thalamus, OFC, and DLPFC in HA participants and the whole thalamus in those with IAH, even after Bonferroni correction for the number of ROIs (critical $\alpha P < 0.01$) (Table 2). No significant differences in whole ROI CBF change [$CBF_{(Hypoglycemia)} - CBF_{(Euglycemia)}$] were observed between the HA and IAH groups (Table 2).

ROI small-volume correction analysis within the SPM framework showed a reduced CBF response within the thalamus ($P_{FWE} = 0.002$; peak voxel coordinates 6, −12, 16; *t* score 4.86), right lateral OFC ($P_{FWE} = 0.002$; peak voxel coordinates 56, 28, −8; *t* score 4.82), and right DLPFC ($P_{FWE} = 0.036$; peak voxel coordinates 58, 34, 18; *t* score 4.15) and a lesser decrease of CBF in the left

Table 1—Participant characteristics

	HA ($n = 15$)	IAH ($n = 19$)	*P* value
Age, years	39.1 ± 13.5	37.3 ± 10.7	0.655
Sex			
Female	9	10	0.738
Male	6	9	
BMI (kg/m^2)	24.7 ± 4.0	24.6 ± 4.6	0.941
HbA_{1c}			
%	7.6 ± 1.0	7.8 ± 1.0	0.625
mmol/mol	59.7 ± 11.2	61.6 ± 10.8	
Type 1 diabetes duration (years)	24.0 ± 12.8	22.2 ± 7.2	0.599
Gold score	1.5 ± 0.5	5.8 ± 1.3	<0.001
Severe hypoglycemia (rate, episodes in year preceding study)	0.2 ± 0.6	2.3 ± 2.8	0.009

Data are mean ± SD or *n*. Gold score is a measure of hypoglycemia awareness whereby a score of 1 or 2 indicates HA and a score of 4–7 indicates IAH.

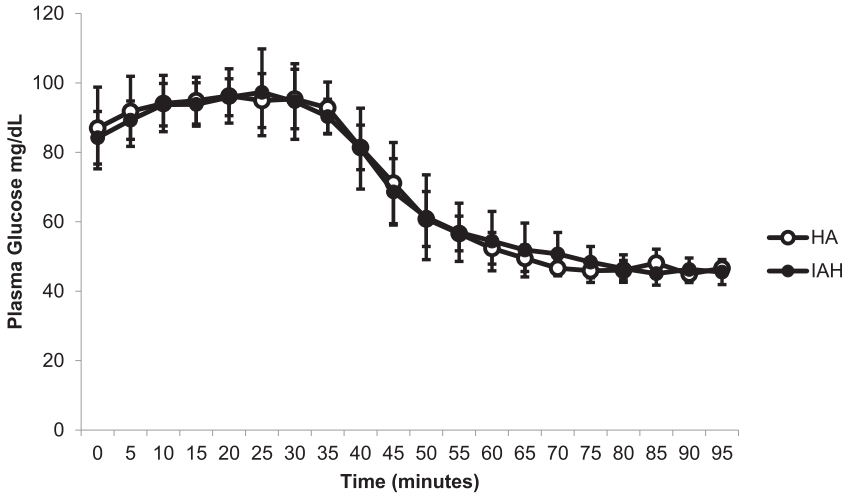

Figure 1—Glucose concentrations, presented as mean ± SD, during hyperinsulinemic euglycemic-hypoglycemic clamp. Between-group differences analyzed by unpaired Student *t* test ($P = 0.99$).

hippocampus ($P_{FWE} = 0.023$; peak voxel coordinates −22, −40, 8; *t* score 3.70) in IAH compared with HA participants. Thalamic and right lateral OFC differences survived Bonferroni correction for the number of ROIs (critical α $P < 0.01$).

CONCLUSIONS

This study set out to evaluate differences in brain responses to hypoglycemia between HA and IAH adults with long-duration type 1 diabetes. As expected, symptom, epinephrine, cortisol, and growth hormone responses were reduced or absent in those with IAH. There was no difference in global CBF response to hypoglycemia; however, key differences were seen in the regional CBF response. IAH participants had a reduced response to hypoglycemia within the thalamus, right OFC, right DLPFC, and hippocampus, areas involved in arousal, decision making, and memory, important factors when avoiding problematic hypoglycemia. Importantly, participants were matched for key variables that might independently affect responses, specifically age, BMI, sex, HbA_{1c}, and duration of diabetes. HA status was based on a clinical score (Gold score [3]) validated by the differences in symptom and epinephrine response to the study hypoglycemia between HA and IAH groups.

An earlier study reported no significant hypoglycemia-induced change in global CBF in seven participants with HA ($P = 0.08$) but an 8% increase in six with IAH ($P < 0.05$) at a plasma glucose nadir of 50 mg/dL (2.8 mmol/L) (10). The authors hypothesized that increased brain blood flow in IAH might account for diminished responses to hypoglycemia. Our data, showing no impact of awareness status on the global CBF response to hypoglycemia, instead suggest that an increase in global CBF is the normal physiological reaction to a reduction of fuel to the brain, regardless of awareness status. The earlier study (10) differs in size and in depth of hypoglycemia, which may contribute to the discrepancy. An increase in global CBF has also been described during hypoxia, another fuel-deficient state (26).

Despite similarity of global CBF, regional CBF responses to hypoglycemia were different between HA and IAH groups in our study. Within our HA group, hypoglycemia resulted in an increase in thalamic CBF, a region involved in sensory and motor signal relay (27), and a reduction in hippocampal and temporal cortex CBF on whole-brain analysis, data consistent with previous reports (10,11,17,24,28). Hippocampal and temporal structures are involved in multiple aspects of memory, classically episodic (personal autobiographical events) and semantic (factual information) memory and, more recently, short-term or working memory (29). Reduction of CBF in these areas during hypoglycemia may be related to impairment in short-term, delayed, and working memory seen in individuals with type 1 diabetes during hypoglycemia (30). Within our IAH group, no significant changes in thalamic perfusion were seen on whole-brain analysis; instead, we observed an increase in CBF in the left OFC and DLPFC, which are involved in executive function, and a decrease in CBF in the right temporal cortex.

Across the whole brain, no significant interaction was seen between group (HA and IAH) and glycemic state ($P_{FWE} < 0.05$, uncorrected voxel-wise cluster-forming threshold of $P < 0.005$). No between-group differences were seen in whole-ROI CBF change [i.e., $CBF_{(Hypoglycemia)} - CBF_{(Euglycemia)}$] when mean CBF signal was extracted from all voxels within each ROI. Differences were observed on small-volume correction ROI analysis within the SPM framework, a more sensitive method for detecting subtle differences within large, complex structures. Our ROIs were selected a priori, each representing functional associations that are important factors in hypoglycemia awareness, arousal and sensory transmission (thalamus), autonomic function (ACC), reward and decision making (DLPFC and OFC), and memory (hippocampus). All five regions have been commonly observed in PET and fMRI studies as responding to hypoglycemia (9–11,17,18,21–24,28). Small-volume correction revealed CBF increases within the thalamus, right lateral OFC, and right DLPFC seen in HA were significantly less in IAH, and the fall in hippocampal CBF seen in HA was reduced in IAH. The thalamus is involved in arousal, relay of stimuli, attention, and vigilance (31); thus, disruption of thalamic blood flow may contribute to the lack of awareness and responsiveness that characterizes IAH. Our thalamic data are compatible with earlier studies (9,10) and support the hypothesis that thalamic activity may be involved in the symptomatic response to hypoglycemia. In a nondiabetic model of IAH, Arbelaez et al. (32) reported an increase in thalamic activity during hypoglycemia using water PET. Counterregulatory and symptomatic responses were successfully suppressed in their participants, but we and other investigators examined hypoglycemia-experienced adults with type 1 diabetes rather than individuals without diabetes exposed to a single prolonged episode of hypoglycemia. This may explain the different pattern of thalamic activation. Notably,

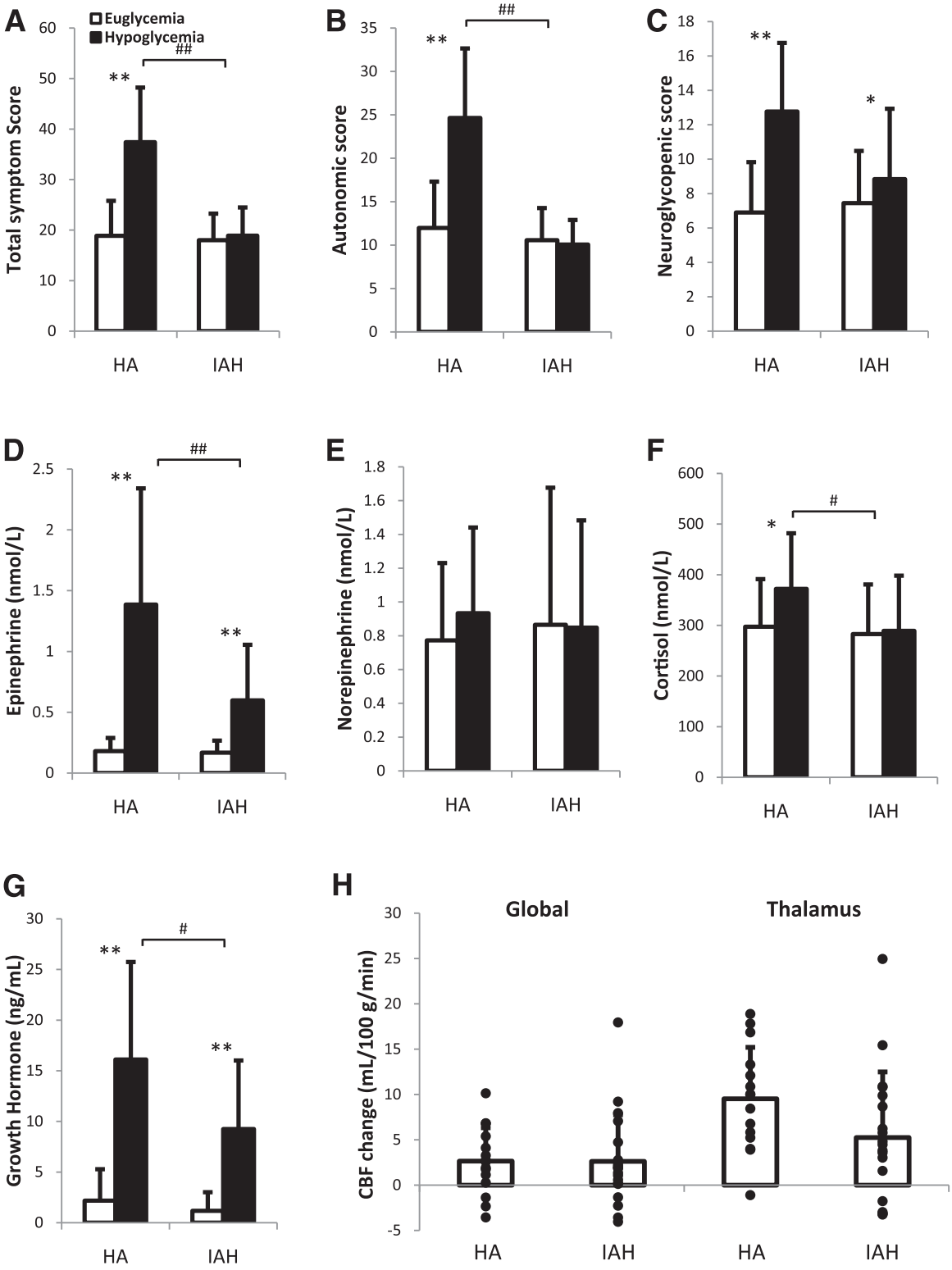

Figure 2—*A*–*G*: Hypoglycemia-induced changes in total symptom score, autonomic symptom score, neuroglycopenic symptom score, epinephrine, norepinephrine, cortisol, and growth hormone. Mean CBF values ± SD are plotted with individual data points. Data are mean ± SD for the euglycemic phase (ASL scans 1 and 2) and hypoglycemic phase (ASL scans 3 and 4). *H*: Global and thalamic hypoglycemia-induced CBF change [$CBF_{(Hypoglycemia)} - CBF_{(Euglycemia)}$]. Mean CBF values were extracted from global gray matter and all voxels from within each ROI using SPM, MATLAB, and ASAP (20). *$P < 0.05$, **$P < 0.005$ for euglycemia vs. hypoglycemia; #$P < 0.05$, ##$P < 0.005$ for HA vs. IAH.

however, these data all provide evidence of thalamic involvement in the cerebral response to hypoglycemia.

HA individuals had greater increases in CBF within the right lateral OFC and right DLPFC, which are involved in decision making, feeding behavior, and reward (33,34), than those with IAH. The HA response to hypoglycemia seen in our

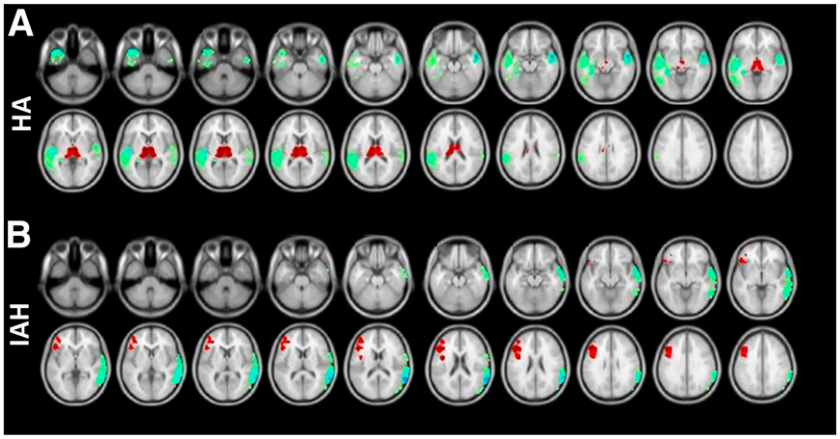

Figure 3—Effect of hypoglycemia on CBF, within-group analysis. *A* and *B*: HA group and IAH group. Statistical parametric maps projected onto brain images show significant rise (red) and significant fall (blue-green) in CBF. A voxel-wise two-sided paired *t* test was performed on each group to identify the effect of hypoglycemia within the group (FWE correction for multiple comparisons [P_{FWE}] < 0.05, uncorrected voxel-wise cluster-forming threshold of $P < 0.005$). Whole-brain ANOVA comparing euglycemia and hypoglycemia between HA and IAH groups demonstrated no significant differences after FWE correction for multiple comparisons ($P_{FWE} < 0.05$) at an uncorrected voxel-wise cluster-forming threshold of $P < 0.005$.

participants was more extensive than that seen by Hwang et al. (12), who described decreased activity in the OFC with no change in the DLPFC. This may reflect our stronger hypoglycemic stimulus of 47 mg/dL (2.6 mmol/L) versus 60 mg/dL (3.3 mmol/L) in the Hwang et al. study. Age and duration of diabetes may also play a role. The HA participants in Hwang et al. were younger than their IAH counterparts, with one-half the duration of diabetes, while our participants were matched for both. Aging suppresses the hormonal and symptomatic responses to hypoglycemia in individuals without diabetes (35). The impact of aging on CBF responses to hypoglycemia has not been studied, but age-related changes in brain vascular reactivity are likely, and an impact of aging on brain responses to other stimuli has been described (36). The most important difference, however, is likely to be the depth of hypoglycemic challenge. Brain responses evolve as hypoglycemia progresses (11,17), and counterregulatory responses can be triggered at concentrations <60 mg/dL in diabetes (37). Clinically, the majority of individuals with type 1 diabetes will experience plasma glucose concentrations <60 mg/dL (3.3 mmol/L) (6). We used moderate rather than mild hypoglycemia (47 mg/dL [2.6 mmol/L]) to investigate the full cerebral and hormonal response to established hypoglycemia. Together, the two data sets may be describing glucose thresholds for the different responses in these groups. Hwang et al. used blood-oxygen-level–dependent fMRI, a technique that generates functional images reflecting dynamic changes in CBF. ASL yields similar results to PET when measuring the cerebral response to hypoglycemia (21); however, blood-oxygen-level–dependent fMRI has been shown to have different sensitivities (38).

Changes in the hypothalamus, a known glucose sensor, were not seen in our study or the study by Hwang et al. (12). This is interesting and may be due to insufficient spatial resolution or sensitivity because the hypothalamus is a small organ. Alternatively, differences in depth and duration of hypoglycemia may be responsible. Hypothalamic blood flow has been shown to increase early in the response to hypoglycemia, even as plasma glucose concentrations fall within the euglycemic range (19,22). We, however, investigated late and sustained hypoglycemia. This hypothesis is compatible with observations by Teh et al. (17) and Hwang et al. that brain responses to hypoglycemia are dynamic. Using water PET, Dunn et al. (11) described a more widespread cortical response to hypoglycemia in nine HA and eight IAH men and identified differences between the two groups in additional regions: ACC, insula, lingual gyrus, and precuneus. Thalamic activation, however, was not reported as different in this single-sex study. In our investigation, both sexes were represented ($n = 34$, female 19 [56%]). Sex affects CBF (39) and hypoglycemia counterregulation (40), which may in part explain the differences between the two studies. Duration of diabetes may also have an impact (41). Although diabetes duration was not significantly different in the PET study, the mean duration of diabetes in the IAH participants was twice that of their

Table 2—Global and a priori–selected ROI absolute CBF values during euglycemia and hypoglycemia in HA and IAH groups extracted from all voxels within global gray matter and whole ROIs

	HA			IAH			
	Euglycemia	Hypoglycemia	*P* value	Euglycemia	Hypoglycemia	*P* value	HA vs. IAH* *P* value
Global and ROI CBF (mL/100 g/min)							
Global	41.9 ± 9.3	44.5 ± 9.3	0.01	41.1 ± 8.4	43.7 ± 7.4	0.04	0.99
Thalamus	49.5 ± 8.8	59.0 ± 11.9	<0.001	46.3 ± 7.5	51.6 ± 7.4	0.005	0.07
OFC	55.4 ± 13.5	61.8 ± 14.2	0.002	54.5 ± 11.2	58.6 ± 9.9	0.03	0.34
DLPFC	49.8 ± 13.8	55.4 ± 12.7	0.009	46.8 ± 11.8	50.9 ± 9.3	0.03	0.55
Hippocampus	48.6 ± 7.0	46.7 ± 7.0	0.02	48.9 ± 7.3	49.5 ± 7.6	0.66	0.14
ACC	56.3 ± 9.3	59.1 ± 10.5	0.04	58.7 ± 9.3	60.7 ± 7.5	0.19	0.67

Data are mean ± SD. No significant global or regional CBF differences between HA and IAH groups at baseline euglycemia ($P > 0.05$). Mean signal across whole brain and whole ROI extracted using SPM, MATLAB, and ASAP (20). For ROI data, critical α of $P < 0.01$ applied after Bonferroni correction for five ROIs. *$CBF_{(Hypoglycemia)} - CBF_{(Euglycemia)}$.

HA counterparts. Most importantly, however, Dunn et al. reported response to mean plasma glucose nadirs of 40 ± 5 mg/dL (2.2 ± 0.3 mmol/L) in those with HA and 41 ± 4 mg/dL (2.3 ± 0.2 mmol/L) in those with IAH, while we attained 47 ± 2 mg/dL (2.6 ± 0.1 mmol/L) and 46 ± 3 mg/dL (2.5 ± 0.2 mmol/L), respectively. The depth of hypoglycemia in the PET study may explain why there was a more extensive cortical response and why the thalamus was activated in both HA and IAH.

Together, Dunn et al. (11), the current study, and Hwang et al. (12) have demonstrated regional CBF changes at various stages of hypoglycemia. Reduced activity was seen in the right lateral OFC in IAH compared with HA during hypoglycemia. A meta-analysis of OFC function showed that while the medial OFC is involved in monitoring reward value, lateral regions are implicated in the evaluation of negative consequences, potentially enabling a change in behavior (34). This is consistent with data demonstrating that those with IAH have difficulty modifying their behavior to avoid or prevent hypoglycemia (3,7). Hypoglycemia-induced left prefrontal activation was seen within our IAH group, while right prefrontal activation was significantly less in those with IAH than with HA. Prefrontal function lateralization has been described by Bechara et al. (42). Using a delay task to assess working memory, they reported that participants with a right DLPFC lesion showed a deficit in working memory, while those with left lesions showed no such impairment. Other studies have shown greater activation of the right DLPFC during similar tasks (43), suggesting that the right prefrontal cortex may be preferentially recruited for higher functions, such as working memory. Attenuated activity in the right OFC and DLPFC, seen in IAH, may contribute to the differences in behavioral response to hypoglycemia between HA and IAH groups. Reduction of hippocampal CBF seen in individuals with HA and control subjects without diabetes (17,24,28) was diminished in those with IAH, another departure from what may be classified as the typical response to hypoglycemia. Although this difference did not survive Bonferroni correction, Dunn et al. reported a similar pattern of diminished deactivation within the temporal cortex of individuals with IAH, a region also involved in memory.

A limitation of this study is the lack of a euglycemic arm to control for time in the scanner. This is in keeping with other similar studies and reduces participant burden, complexity, and cost (9,10). Quantification of regional CBF is a surrogate marker of neuronal activity, relying on the well-established phenomenon of neurovascular coupling, whereby changes in neuronal activity are linked to the regulation of arteriolar diameter and, hence, magnitude of local CBF (44). We maximized the relevance of our CBF measurements, with respect to neurovascular coupling, by using a postlabeling delay (1,500–1,800 ms) long enough to ensure that the signal from the labeled arterial water resides in arteriolar and capillary territories rather than in the macroscopic vasculature. Our ASL pulse sequence also used background suppression pulses to minimize the influence of other confounding factors arising from the static signal as well as to provide reduced sensitivity to motion-induced artifact (14). Nonneuronal sources of CBF changes were largely accounted for by using the mean global CBF as a covariate because these sources tend to be ubiquitous rather than regional. A further limitation of this study is that not all potentially relevant regions were included in our ROI analysis. We opted for five ROIs reflecting particular aspects of the cerebral hypoglycemic response of interest. Importantly, limiting our a priori analysis to five regions reduced the impact of correcting for multiple comparisons. In this type of study, sample size is usually a limitation, although to our knowledge, our experiment includes the largest cohort of participants with IAH to date. Significant changes have been described in smaller, similar studies (9). As Hwang et al. (12) suggested, previous calculations of sample size have not been based on complex and relatively uncommon conditions, such as IAH in type 1 diabetes. Finally, we should comment on our participant selection. Four potential participants in our IAH group were excluded from the study because of subjective awareness of hypoglycemia during the scans at low glucose concentrations. While history and scoring systems such as the Gold score are generally used to define awareness status, IAH is not a fixed state because awareness can be restored by hypoglycemia avoidance (45). We may speculate that such a change occurred in the four excluded participants between recruitment and study.

Our data suggest that in addition to impaired counterregulation and reduced or absent symptomatic awareness, IAH consists of altered thalamic and prefrontal cortex activity during hypoglycemia. Distinctive changes in blood flow in regions known to be involved in arousal, decision making, and reward may leave individuals with IAH unable to recognize, reason, and deal with hypoglycemia effectively or respond definitively to awareness restoration programs. Further work is required to ascertain whether these adaptations in the brain can be altered when hypoglycemia awareness is successfully restored.

Acknowledgments. The authors thank the participants; the clinical research staff at the National Institute for Health Research and Wellcome Trust King's Clinical Research Facility Louisa Green and John Lord Villajin; the laboratory staff at Viapath, King's College Hospital, Tracy Dew, Gemma Cross, Andrew Given, and Joseph Molloy; and the radiographers and administrative staff at the Centre for Neuroimaging Sciences, King's College London.

Funding. This study was funded by Diabetes UK (13/0004653).

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

Author Contributions. M.N. recruited the participants. M.N., S.A.A., O.O., S.M.C., I.A.M., F.O.Z., and P.C. reviewed and edited the manuscript. M.N., S.A.A., O.O., F.O.Z., and P.C. contributed to the conception and design of the research and to interpretation of the data. M.N., S.A.A., F.O.Z., and P.C. wrote the manuscript. M.N., O.O., and F.O.Z. analyzed the neuroimaging data. M.N. and M.L.B. collated and analyzed study data. M.N., M.L.B., B.M.W., A.P., and P.C. performed the study. S.M.C. and I.A.M. analyzed the catecholamine data. P.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 77th Scientific Sessions of the American Diabetes Association, San Diego, CA, 9–13 June 2017.

References

1. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with type 1 diabetes. Diabet Med 2008;25:501–504

2. Choudhary P, Geddes J, Freeman JV, Emery CJ, Heller SR, Frier BM. Frequency of biochemical hypoglycaemia in adults with type 1 diabetes with and without impaired awareness of hypoglycaemia: no identifiable differences using continuous glucose monitoring. Diabet Med 2010;27:666–672

3. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. Diabetes Care 1994;17:697–703
4. Duncan EAS, Fitzpatrick D, Ikegwuonu T, Evans J, Maxwell M. Role and prevalence of impaired awareness of hypoglycaemia in ambulance service attendances to people who have had a severe hypoglycaemic emergency: a mixed-methods study. BMJ Open 2018;8:e019522
5. Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. J Clin Invest 1993;91:819–828
6. UK Hypoglycaemia Study Group. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. Diabetologia 2007;50:1140–1147
7. Smith CB, Choudhary P, Pernet A, Hopkins D, Amiel SA. Hypoglycemia unawareness is associated with reduced adherence to therapeutic decisions in patients with type 1 diabetes: evidence from a clinical audit. Diabetes Care 2009; 32:1196–1198
8. Rogers HA, de Zoysa N, Amiel SA. Patient experience of hypoglycaemia unawareness in type 1 diabetes: are patients appropriately concerned? Diabet Med 2012;29:321–327
9. Mangia S, Tesfaye N, De Martino F, et al. Hypoglycemia-induced increases in thalamic cerebral blood flow are blunted in subjects with type 1 diabetes and hypoglycemia unawareness. J Cereb Blood Flow Metab 2012; 32:2084–2090
10. Wiegers EC, Becker KM, Rooijackers HM, et al. Cerebral blood flow response to hypoglycemia is altered in patients with type 1 diabetes and impaired awareness of hypoglycemia. J Cereb Blood Flow Metab 2017;37:1994–2001
11. Dunn JT, Choudhary P, Teh MM, et al. The impact of hypoglycaemia awareness status on regional brain responses to acute hypoglycaemia in men with type 1 diabetes. Diabetologia 2018; 61:1676–1687
12. Hwang JJ, Parikh L, Lacadie C, et al. Hypoglycemia unawareness in type 1 diabetes suppresses brain responses to hypoglycemia. J Clin Invest 2018;128:1485–1495
13. Neil HA, Gale EA, Hamilton SJ, Lopez-Espinoza I, Kaura R, McCarthy ST. Cerebral blood flow increases during insulin-induced hypoglycaemia in type 1 (insulin-dependent) diabetic patients and control subjects. Diabetologia 1987; 30:305–309
14. Alsop DC, Detre JA, Golay X, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: a consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med 2015;73:102–116
15. Nwokolo M, Amiel SA, O'Daly O, et al. Hypoglycemic thalamic activation in type 1 diabetes is associated with preserved symptoms despite reduced epinephrine. J Cereb Blood Flow Metab. 20 April 2019 [Epub ahead of print]. DOI: 10.1177/271678X19842680
16. Brooks DC, Black PR, Arcangeli MA, Aoki TT, Wilmore DW. The heated dorsal hand vein: an alternative arterial sampling site. JPEN J Parenter Enteral Nutr 1989;13:102–105
17. Teh MM, Dunn JT, Choudhary P, et al. Evolution and resolution of human brain perfusion responses to the stress of induced hypoglycemia. Neuroimage 2010;53:584–592
18. Teves D, Videen TO, Cryer PE, Powers WJ. Activation of human medial prefrontal cortex during autonomic responses to hypoglycemia. Proc Natl Acad Sci U S A 2004;101:6217–6221
19. Page KA, Arora J, Qiu M, Relwani R, Constable RT, Sherwin RS. Small decrements in systemic glucose provoke increases in hypothalamic blood flow prior to the release of counterregulatory hormones. Diabetes 2009;58:448–452
20. Mato Abad V, García-Polo P, O'Daly O, Hernández-Tamames JA, Zelaya F. ASAP (Automatic Software for ASL Processing): a toolbox for processing Arterial Spin Labeling images. Magn Reson Imaging 2016;34:334–344
21. Arbeláez AM, Su Y, Thomas JB, Hauch AC, Hershey T, Ances BM. Comparison of regional cerebral blood flow responses to hypoglycemia using pulsed arterial spin labeling and positron emission tomography. PLoS One 2013;8: e60085
22. 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
23. Arbeláez AM, Rutlin JR, Hershey T, Powers WJ, Videen TO, Cryer PE. Thalamic activation during slightly subphysiological glycemia in humans. Diabetes Care 2012;35:2570–2574
24. Rosenthal JM, Amiel SA, Yágüez L, et al. The effect of acute hypoglycemia on brain function and activation: a functional magnetic resonance imaging study. Diabetes 2001;50:1618–1626
25. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 2003;19:1233–1239
26. Xu F, Liu P, Pascual JM, Xiao G, Lu H. Effect of hypoxia and hyperoxia on cerebral blood flow, blood oxygenation, and oxidative metabolism. J Cereb Blood Flow Metab 2012;32:1909–1918
27. Steriade M, Llinás RR. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 1988;68:649–742
28. Gejl M, Gjedde A, Brock B, et al. Effects of hypoglycaemia on working memory and regional cerebral blood flow in type 1 diabetes: a randomised, crossover trial. Diabetologia 2018;61: 551–561
29. Jeneson A, Squire LR. Working memory, long-term memory, and medial temporal lobe function. Learn Mem 2011;19:15–25
30. Sommerfield AJ, Deary IJ, McAulay V, Frier BM. Short-term, delayed, and working memory are impaired during hypoglycemia in individuals with type 1 diabetes. Diabetes Care 2003;26:390–396
31. Kinomura S, Larsson J, Gulyás B, Roland PE. Activation by attention of the human reticular formation and thalamic intralaminar nuclei. Science 1996;271:512–515
32. Arbelaez AM, Powers WJ, Videen TO, Price JL, Cryer PE. Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition: a mechanism for hypoglycemia-associated autonomic failure. Diabetes 2008;57:470–475
33. Heekeren HR, Marrett S, Ungerleider LG. The neural systems that mediate human perceptual decision making. Nat Rev Neurosci 2008;9:467–479
34. Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol 2004;72:341–372
35. Matyka K, Evans M, Lomas J, Cranston I, Macdonald I, Amiel SA. Altered hierarchy of protective responses against severe hypoglycemia in normal aging in healthy men. Diabetes Care 1997;20:135–141
36. Cheah YS, Lee S, Ashoor G, et al. Ageing diminishes the modulation of human brain responses to visual food cues by meal ingestion. Int J Obes 2014;38:1186–1192
37. 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
38. Stewart SB, Koller JM, Campbell MC, Black KJ. Arterial spin labeling versus BOLD in direct challenge and drug-task interaction pharmacological fMRI. PeerJ 2014;2:e687
39. Rodriguez G, Warkentin S, Risberg J, Rosadini G. Sex differences in regional cerebral blood flow. J Cereb Blood Flow Metab 1988;8:783–789
40. Amiel SA, Maran A, Powrie JK, Umpleby AM, Macdonald IA. Gender differences in counterregulation to hypoglycaemia. Diabetologia 1993; 36:460–464
41. Rodriguez G, Nobili F, Celestino MA, et al. Regional cerebral blood flow and cerebrovascular reactivity in IDDM. Diabetes Care 1993;16: 462–468
42. Bechara A, Damasio H, Tranel D, Anderson SW. Dissociation of working memory from decision making within the human prefrontal cortex. J Neurosci 1998;18:428–437
43. McCarthy G, Blamire AM, Puce A, et al. Functional magnetic resonance imaging of human prefrontal cortex activation during a spatial working memory task. Proc Natl Acad Sci U S A 1994;91:8690–8694
44. Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA. Glial and neuronal control of brain blood flow. Nature 2010;468: 232–243
45. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA. Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. Lancet 1994;344: 283–287