Small Decrements in Systemic Glucose Provoke Increases in Hypothalamic Blood Flow Prior to the Release of Counterregulatory Hormones

Kathleen A. Page,[1] Jagriti Arora,[2] Maolin Qiu,[2] Rachna Relwani,[1] R. Todd Constable,[2] and Robert S. Sherwin[1]

OBJECTIVE—The hypothalamus is the central brain region responsible for sensing and integrating responses to changes in circulating glucose. The aim of this study was to determine the time sequence relationship between hypothalamic activation and the initiation of the counterregulatory hormonal response to small decrements in systemic glucose.

RESEARCH DESIGN AND METHODS—Nine nondiabetic volunteers underwent two hyperinsulinemic clamp sessions in which pulsed arterial spin labeling was used to measure regional cerebral blood flow (CBF) at euglycemia (~95 mg/dl) on one occasion and as glucose levels were declining to a nadir of ~50 mg/dl on another occasion. Plasma glucose and counterregulatory hormones were measured during both study sessions.

RESULTS—CBF to the hypothalamus significantly increased when glucose levels decreased to 77.2 ± 2 mg/dl compared with the euglycemic control session when glucose levels were 95.7 ± 3 mg/dl ($P = 0.0009$). Hypothalamic perfusion was significantly increased before there was a significant elevation in counterregulatory hormones.

CONCLUSIONS—Our data suggest that the hypothalamus is exquisitely sensitive to small decrements in systemic glucose levels in healthy, nondiabetic subjects and that hypothalamic blood flow, and presumably neuronal activity, precedes the rise in counterregulatory hormones seen during hypoglycemia. ***Diabetes*** **58:448–452, 2009**

The brain relies on glucose as its main energy substrate, and small decrements in circulating glucose provoke an elaborate counterregulatory hormonal feedback response (1,2). Activation of the counterregulatory response requires effective detection of a falling glucose level. Although multiple glucose sensors may be involved (3–7), the hypothalamus has emerged as the dominant brain region responsible for sensing and integrating responses to changes in circulating glucose levels (8–12). Although most prior studies have relied on animal models to study the neurophysiological response to changes in glucose, newer imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) provide an in vivo method to study the effect of changes in peripheral glucose levels on human brain activity. Several fMRI studies in humans have demonstrated that a rise in systemic glucose after glucose ingestion leads to an inhibition of hypothalamic activity (13–16). In addition, Musen et al. (17) recently used fMRI based on the blood oxygenation level–dependent (BOLD) contrast mechanism and found that insulin-induced hypoglycemia leads to hypothalamic activation. However, the fMRI-BOLD approach used in that study assesses only relative changes in oxygenated hemoglobin in specific brain regions and does not directly measure tissue perfusion. Magnetic resonance imaging (MRI) pulsed arterial spin labeling (PASL) provides a method for measuring absolute blood flow responses throughout the brain to changes in circulating glucose levels. PASL magnetically tags the arterial blood before entering the brain and then examines the transit time for the tagged blood to reach specific tissues, thereby providing a direct measure of cerebral blood flow (CBF) (18–20). Increased CBF has long been associated with neuronal activation, dating back to the first PET studies of brain function (21).

Some studies, using PET (22), single-photon emission computed tomography (23), high-field magnetic resonance perfusion (24), and continuous arterial spin labeling (25), have shown region-specific increases in brain CBF in response to hypoglycemia. However, none of these studies specifically demonstrated changes in blood flow to the hypothalamus during hypoglycemia. Moreover, in these studies, CBF measurements were performed after hypoglycemic levels had been achieved; thus, none of these studies determined the CBF response to smaller decrements in systemic glucose or the relationship between regional brain activation and the initiation of the counterregulatory hormonal response.

We used PASL to determine the effect of small decrements in circulating glucose on hypothalamic blood flow in healthy volunteers. We performed CBF measurements as glucose levels were declining. This approach allowed us to address the following questions: *1*) Does a decline in plasma glucose provoke hypothalamic activation? *2*) If so, what is the plasma glucose level that correlates with hypothalamic activation? *3*) How does hypothalamic activation temporally relate to the counterregulatory hormone response to hypoglycemia? An understanding of how the hypothalamus, a key brain glucose-sensing region, responds to decrements in circulating glucose levels in healthy humans provides critical information that can be

From the [1]Department of Internal Medicine, Division of Endocrinology, Yale University School of Medicine, New Haven, Connecticut; and the [2]Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut.
Corresponding author: Kathleen A. Page, kathleen.page@yale.edu.
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used to determine how metabolic disorders, such as diabetes, may alter this response.

RESEARCH DESIGN AND METHODS

Nine (eight men, one woman) healthy, nondiabetic subjects participated in this study. Subject participants had a mean (± SD) age of 28 ± 5 and a mean (BMI of 23.6 ± 2. Subjects underwent a screening history, physical examination, and laboratory testing, and only individuals with no history of significant disease, including diabetes, were included in the study. Exclusion criteria also included any contraindications for MRI including pregnancy and metal implants. Before each study session, female subjects were required to have a negative urine pregnancy test. The Yale University School of Medicine Human Investigation Committee approved this study, and all subjects provided informed, written consent before participation in the study.

General experimental protocol

Subjects participated in two study sessions that were separated by a minimum of 7 days. On the morning of the study, an intravenous catheter was inserted into a distal arm or hand vein; this arm was gently heated, allowing for sampling of arterialized venous blood. A second intravenous catheter was established for the administration of insulin and glucose. During the 135-min study sessions, a primed continuous infusion of intravenous insulin at 2 mU · kg^{-1} · min^{-1} was initiated, with a variable infusion of 20% glucose adjusted to achieve euglycemia (plasma glucose ~95 mg/dl) on one occasion and hypoglycemia (plasma glucose ~50 mg/dl) on the other occasion. Regional CBF measurements were performed using PASL at ~30 min after the start of the plasma glucose decline toward hypoglycemic levels and at ~90 min during the euglycemic session. Plasma glucose levels were measured at 5-min intervals, and additional plasma samples were drawn at −20, 0, 30, 60, 120, and 135 min for measurement of insulin, glucagon, catecholamines, cortisol, and growth hormone. C-peptide was measured at −20-, 0-, 30-, and 60-min time points. The two study sessions were carried out in a single-blind fashion, in variable order across subjects.

fMRI imaging protocol

MRI was performed on a 3T Siemens Trio whole-body scanner (Siemens Medical Systems, Erlangen, Germany) with a circularly polarized head coil. PASL using the EPISTAR QUIPSS PASL MRI technique was used to measure CBF. The PASL acquisition parameters were as follows: field of view 256 × 256 mm^2; matrix 60 × 64; bandwidth 2,298 Hz/pixel; slice thickness 6 mm; and interslice spacing 3 mm. Ten anterior commissure/posterior commissure (AC-PC) aligned slices were acquired from inferior to superior in an ascending order. The whole imaging slab was positioned on the upper part of the brain, with the lowest slice passing through AC-PC to acquire the top part of the brain; the imaging slab positioned on the lower part of the brain with the seventh slice from the bottom passing through AC-PC to acquire the bottom part of the brain. Acquisition of each slice took ~54 ms. The repetition time (TR) was 3,000 ms; the echo time (TE) was 26 ms. During each echo planar image acquisition, fat was suppressed and the phase-correction echoes were collected and applied. A bipolar gradient of encoding velocity V_{enc} = 20 mm/s was applied to the imaging slices for intravascular signal suppression.

To quantify regional CBF for both upper and lower parts of the brain, 2 vol 10–proton density–weighted images were acquired with the same perfusion sequence, except for the following changes: TR = 8,000 ms; TD = 0 ms; TI = 7,375 ms; and TE = 26 ms. Mapping for the apparent longitudinal relaxation time T_{lapp} was performed with an ultra-fast Look-Locker echo-planar imaging T1 mapping sequence.

Two additional image acquisitions were acquired to aid in multisubject registration. First, a high-resolution whole-brain T1-weighted three-dimensional image was acquired for each subject using magnetization prepared rapid acquisition with gradient-echo imaging (MPRAGE), with the following settings: 160 sagittal slices with field of view = 256 × 256 mm^2, voxel size 1 × 1 × 1 mm^3, TR = 1,500 ms, TI = 800 ms, TE = 2.83 ms; flip angle 15°, and 1 average. Second, two-dimensional T1-weighted images were acquired during each magnetic resonance session using the same slice positions as the perfusion-weighted images and the following additional settings: field of view = 256 × 256 mm^2, in-plane resolution 1 × 1 mm^2, TR = 300 ms, TE = 3.69 ms, flip angle 60°, and 2 averages.

fMRI imaging analysis

Preprocessing of PASL images. Perfusion-weighted and the proton density–weighted images were motion corrected using the Statistical Parametric Mapping package (SPM99) via a six-parameter rigid-body transformation.

Calculation of absolute CBF. Perfusion-weighted images were obtained by pairwise "surround" subtraction between interleaved label and control pairs (18–20) while the subject was at rest, resulting in a complete perfusion map every 2 TRs, i.e., 4 s in our case. One perfusion-induced volume (ΔM) was calculated by performing the subtraction described in Eq. 1 (below) between the tagged and untagged images. The mean image of the motion-corrected proton density images, M_0*, was also used for CBF mapping. The absolute CBF f (ml · 100 g^{-1} · min^{-1}) was calculated as follws:

$$\Delta M(t) = M^{ctrl}(t) - M^{label}(t) = \frac{2cf(t-\tau_a)M_0^*}{\lambda} e^{-t/T_{1a}} \quad (1)$$

in which λ is the tissue-blood partition coefficient for water;

$$c = \alpha_\pi \frac{1 - e^{-(t-\tau_a)(1/T_{1app} - 1/T_{1a})}}{(t - \tau_a)(1/T_{1app} - 1/T_{1a})} \quad (2)$$

is the correction factor, which accounts for exchange of labeled magnetization from intravascular to extravascular space and clearance of the labeled blood water out of the capillary bed; τ_a is the arterial transit time, which is the time for the labeled blood water to arrive at the capillary bed after labeling; and

$$M_0^* = M_0 e^{-TE/T_2^*} \quad (3)$$

T_{lapp} is the apparent longitudinal relaxation time, which was estimated with an ultrafast Look-Locker echo-planar imaging T1 mapping sequence (26).

Other parameters used in CBF quantification are as follows: T_{1a} = 1,490 ms, λ = 0.9 ml/g, α_π = 0.95, $t - \tau_a$ = 700 ms, and TI = 1,400 ms for the first slice.

The activation maps were obtained by contrasting the average CBF images for two conditions. After M_0*, T_{lapp}, and ΔM have been measured on a per-voxel basis, CBF (f) can then be estimated using Eq. 1.

Multisubject analysis. A standard whole brain template (MNI-1 mm) was used for subject spatial normalization of the individual data. Subject integration and registration were carried out using the BioimageSuite software package (http://www.bioimagesuite.org) (27) for the PASL images under conditions of euglycemia and hypoglycemia. Two transformations were calculated and used in multiple subject integration: *1*) an affine transformation was estimated by coregistering the two-dimensional anatomical image to the high-resolution three-dimensional anatomical image of each individual, and this was then used to transform the individual maps of the resting state CBF to the high-resolution three-dimensional anatomical space of that subject; and *2*) a nonlinear transformation was used to coregister the high-resolution three-dimensional anatomical image of each individual to the brain template, which enabled warping of all the transformed maps of an individual subject from step (1) to a common brain space. Tri-linear interpolation was used for image regridding. The mean, SD, and other statistics were estimated in the common template space on the pooled subject data. In this common reference space, voxel-wise contrasts between conditions (euglycemia and hypoglycemia) were estimated in the common space on the pooled subject data using a t statistic to test the null hypothesis. Region of interest analysis was performed on the hypothalamus.

Laboratory analyses

Plasma glucose was measured by an enzymatic reaction using glucose oxidase (Yellow Springs Instruments, Yellow Springs, OH). Plasma concentrations of insulin and glucagon were measured with the use of double-antibody radioimmunoassay (RIA) kits (Millipore, St. Charles, MO). Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography (ESA, Chelmsford, MA). Plasma growth hormone and cortisol were measured by RIA (Irvine, CA; Diagnostic Products, Los Angeles, CA), and plasma C-peptide was measured by use of double-antibody RIA kits (Diagnostic Products).

RESULTS

Plasma glucose and glucoregulatory hormone concentrations. Plasma glucose levels were not significantly different at baseline before the euglycemic and hypoglycemic clamp sessions (Fig. 1; Table 1). In the euglycemic-hyperinsulinemic session, plasma glucose levels were indistinguishable from baseline values at the time of brain perfusion acquisition. In the hypoglycemic-hyperinsulinemic session, plasma glucose was gradually lowered over 60 min and then was maintained at 52.8 ± 0.6 mg/dl for the remainder of the study (Fig. 1). During the hypoglycemic session, the brain perfusion acquisition measurements were obtained at the time of the slow glucose decline (30 min) when plasma glucose averaged 77.2 ± 2 mg/dl. During the euglycemic session, brain perfusion measurements were performed at ~90 min when plasma glucose averaged 95.7 ± 3 mg/dl. As shown in Fig. 1, steady-state plasma insulin levels during the hypoglycemic and euglycemic sessions were not significantly different (123 ± 9 vs. 124 ± 10 μU/ml, respectively).

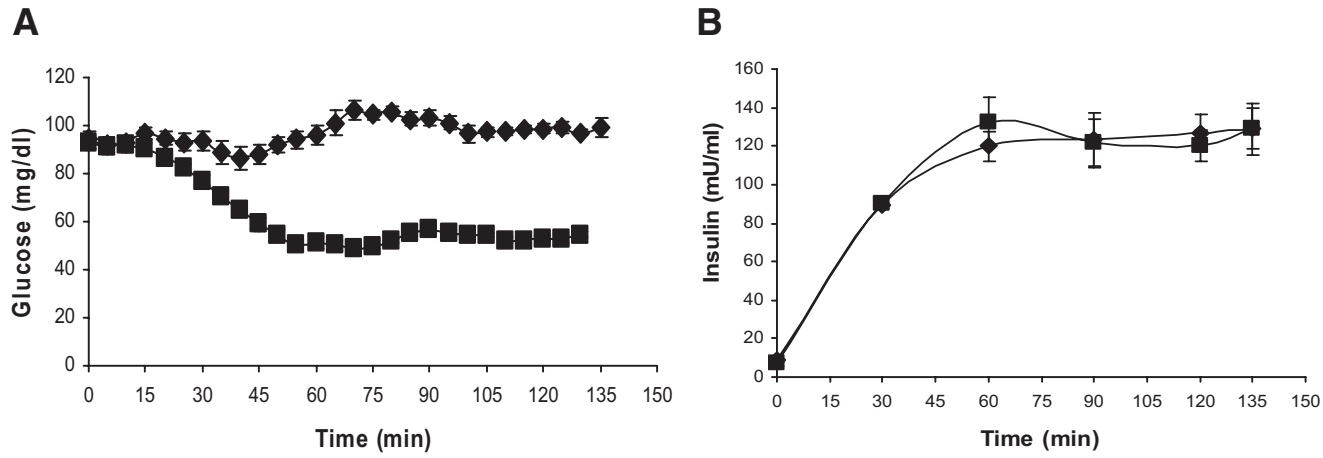

FIG. 1. *A*: **Plasma glucose levels (in mg/dl) obtained during the euglycemic (♦) and hypoglycemic (■) clamp sessions.** *B*: **Insulin levels were raised comparably during euglycemic and hypoglycemic clamp sessions.**

At the time that brain perfusion measurements were obtained, plasma epinephrine, norepinephrine, cortisol, and growth hormone were not altered during the hypoglycemic or euglycemic clamp study, but glucagon was reduced from baseline during the euglycemic session (Fig. 2; Table 1). Subsequently, hypoglycemia provoked an increase when compared with the euglycemic session in plasma epinephrine (56 ± 15 vs. 15 ± 3 pg/ml, $P = 0.02$ at 60 min), glucagon (88 ± 13 vs. 49 ± 7 pg/ml, $P = 0.006$ at 90 min), and growth hormone (14.97 ± 4 vs. 1.91 ± 0.22 ng/ml at 90 min, $P = 0.01$). Small increases in plasma cortisol and norepinephrine were observed at 120 and 135 min, respectively ($P < 0.05$).

In contrast to the counterregulatory hormones, the small decline in plasma glucose at 30 min caused a significant fall in plasma C-peptide concentration from 0.47 ± 0.02 to 0.34 ± 0.02 pmol/ml ($P < 0.001$). There was a further reduction in C-peptide to 0.19 ± 0.02 by 60 min. In contrast, C-peptide did not significantly change during the euglycemic session.

Regional CBF response. Figure 3 shows the whole brain mean difference map for the hypoglycemic compared with the euglycemic session ($P < 0.05$, uncorrected) that was obtained when plasma glucose averaged 77.2 ± 2 mg/dl. This mild decrease in plasma glucose caused a significant increase in hypothalamic blood flow. Region of interest analysis demonstrated that mean hypothalamic perfusion was twofold greater during the hypoglycemic session (44.523 ml · 100 g^{-1} · min^{-1}) than the euglycemic session (21.990 ml · 100 g^{-1} · min^{-1}) ($P = 0.0009$) (Fig. 4). The right anterior cingulate cortex, left caudate, left putamen, left superior temporal gyrus, left inferior frontal gyrus (IFG), and left visual association cortex (Broca's area [BA] 18) also exhibited increased blood flow, whereas the cerebellum, right pars opercularis (BA 44), and right medial frontal gyrus (BA 46) exhibited decreased blood flow during the hypoglycemic session compared with the euglycemic session ($P < 0.05$) (Table 2). We used the orbitofrontal cortex (OFC) as a control region and found no difference in CBF to either the right OFC (hypoglycemia, 16.229 ml · 100 g^{-1} · min^{-1}; euglycemia, 18.509 ml · 100 g^{-1} · min^{-1}, $P = 0.35$) or left OFC (hypoglycemia, 21.439 ml · 100 g^{-1} · min^{-1}; euglycemia, 18.261 ml · 100 g^{-1} · min^{-1}, $P = 0.2$) during the hypoglycemic session when compared with the euglycemic session.

DISCUSSION

We used PASL to quantify hypothalamic perfusion after small decrements in circulating glucose in healthy human volunteers. PASL is an indirect measure of neuronal activity believed to reflect changes in metabolic state because there is a clear relationship between changes in the local rate of oxygen consumption and changes in local tissue blood flow (20). Although previous studies have investigated the effects of hypoglycemia on CBF in nondiabetic subjects, the current study examined the effects of small glucose decrements within the normal range, whereas earlier studies measured CBF after moderate hypoglycemic levels (plasma glucose ≤60 mg/dl) were achieved (22,24,25). As a result, we were able to investigate the time sequence relationship between hypothalamic activation and the initiation of the counterregulatory hormonal response.

We focused our attention on the hypothalamic blood flow response to decrements in systemic glucose based on earlier animal studies demonstrating the importance of hypothalamic glucose-sensing neurons in hypoglycemia detection

TABLE 1
Plasma glucose and counterregulatory hormones at baseline, approximate time of CBF acquisition for hypoglycemic and euglycemic clamp sessions, and peak levels for the hypoglycemic session

	Baseline	Time of CBF acquisition	Peak	Baseline	Time of CBF acquisition
Glucose (mg/dl)	93.1 ± 3	77.2 ± 2*†	—	94.3 ± 4	95.7 ± 3
Epinephrine (pg/ml)	16 ± 4	14 ± 4	178 ± 36*	13 ± 3	14 ± 3
Norepinephrine (pg/ml)	120 ± 13	124 ± 11	169 ± 15*	136 ± 15	123 ± 10
Glucagon (pg/ml)	67 ± 9	65 ± 10†	117 ± 20*	70 ± 7	50 ± 8*
Cortisol (mg/dl)	12.9 ± 1.8	9.9 ± 1.4	16.9 ± 1.5	12.2 ± 1.8	12.1 ± 1.0
Growth hormone (ng/ml)	1.8 ± 0.3	1.7 ± 0.2	21.3 ± 5.4*	3.1 ± 1.0	2.9 ± 0.7

Data are means ± SE. *$P < 0.05$ vs. baseline. †$P < 0.05$ hypoglycemic vs. euglycemic session at time of CBF acquisition.

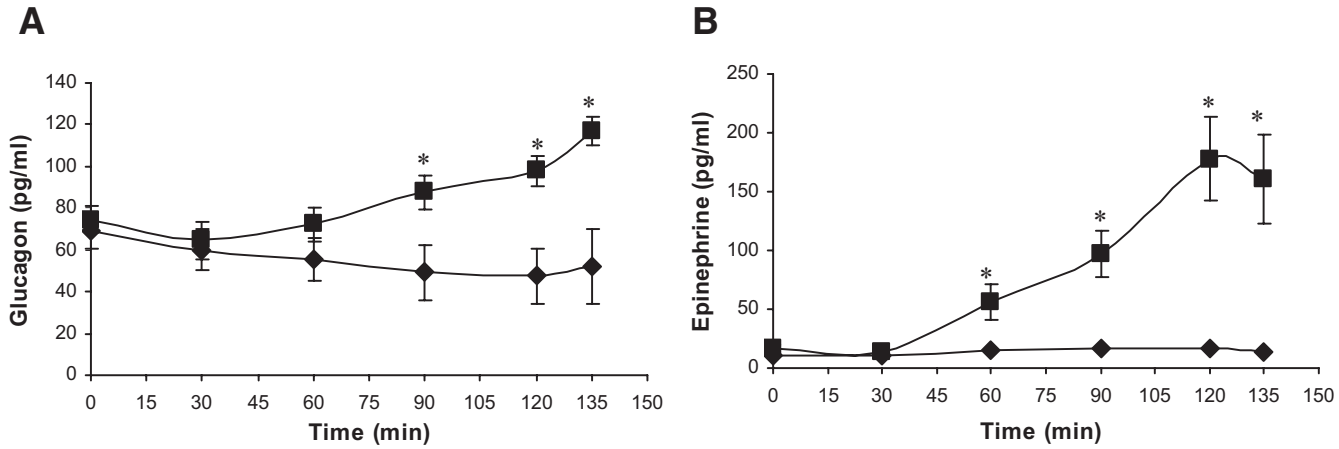

FIG. 2. Plasma epinephrine and glucagon response during hyperinsulinemic-euglycemic (♦) and -hypoglycemic (■) sessions. *$P < 0.05$ hypoglycemic vs. euglycemic session.

and in the activation of counterregulatory hormonal responses (8–11). Moreover, the hypothalamus plays a central role in the regulation of appetite and energy expenditure—responses known to be stimulated by hypoglycemia. Our data are consistent with this hypothesis and with prior studies using fMRI-BOLD demonstrating that the hypothalamus is responsive to changes in systemic glucose levels (13–17). On the other hand, previous studies investigating the effects of hypoglycemia on regional CBF in nondiabetic subjects have not identified an increase in hypothalamic blood flow following insulin-induced hypoglycemia (22,24,25). This may be because the hypothalamus is a very small brain region, making it difficult to detect significant changes in blood flow when compared with larger brain regions. We used PASL, which may have given us greater spatial resolution than other methods for measuring CBF, such as PET. In addition, unlike previous studies, we used region of interest analysis focusing on the hypothalamus, which may also have contributed to some of the differences in regional CBF results in our study.

It is noteworthy that CBF increased in the hypothalamus after a very small reduction in glucose (from 93.1 ± 3 to 77.2 ± 2 mg/dl) when compared with a euglycemic control study. Our data are consistent with those of Musen et al. (17), who used fMRI-BOLD and found a slightly lower glucose threshold (plasma glucose 68 ± 9 mg/dl) for hypothalamic activation in nondiabetic individuals. However, in that study, the relationship between the onset of hypothalamic activation and systemic hormone release was not examined. The current data show that hypothalamic perfusion was significantly increased before glucose levels reached a point at which there was a significant elevation of counterregulatory hormones. Because the acquisition of CBF measurements was limited to only one time point during the euglycemic and hypoglycemic clamp sessions, measurements of regional CBF at multiple time points during euglycemic and hypoglycemic clamp sessions will be required for more precise time course analyses of hypothalamic activation and the counterregulatory hormonal response. A potential limitation of the current study is that CBF measurements were performed at 30 min during the hypoglycemic session and at ~90 min during the euglycemic session. It is noteworthy, however, that plasma insulin levels were higher at the 90-min time point during the euglycemic session when compared with the 30-min time point during the hypoglycemic session (124 ± 10 vs. 90 ± 13 mU/ml). Insulin has known vasoactive effects, and a recent report by Seaquist et al. (28) shows that insulin has direct vasodilatory effects on cerebral vasculature in humans that are independent of effects on neuronal activation. This suggests that higher circulating insulin levels in the euglycemic study session may increase regional CBF, which would be expected to attenuate the results that we observed in our study. Therefore, it is unlikely that the changes in hypothalamic CBF that we observed are due to measurement time differences. As has been previously reported (2), we observed a fall in endogenous insulin secretion, as measured by C-peptide, before there was a detectable rise in counterregulatory hormones. This occurred at plasma glucose levels that were similar to those that stimulated hypothalamic blood flow. Although this finding does not establish a causal relationship between hypothalamic activation and suppression of endogenous insulin secretion, it raises the possibility

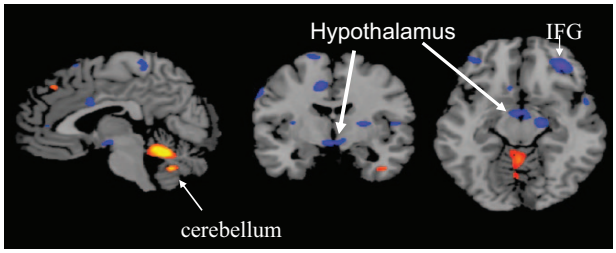

FIG. 3. Saggital (*left*), coronal (*middle*), and axial (*right*) images showing the mean difference (CBF euglycemia − CBF hypoglycemia) map of CBF from nine subjects. Blue represents increased and yellow/orange represents decreased blood flow during the hypoglycemic session relative to the euglycemic session. During hypoglycemia, flow was greater to the hypothalamus and left IFG and less to the cerebellum (other regions are noted in Table 1; $P < 0.05$, uncorrected). (Please see http://dx.doi.org/10.2337/db08-1224 for a high-quality digital representation of this figure.)

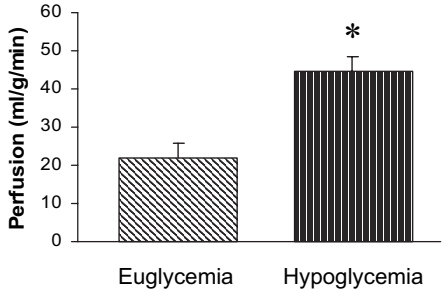

FIG. 4. Hypothalamic perfusion: region of interest analysis showing that blood flow to the hypothalamus was significantly higher during hypoglycemia than euglycemia. *$P = 0.0009$.

TABLE 2
Talairach coordinates for areas showing increased or decreased activation during hypoglycemia relative to euglycemia at a threshold of $P < 0.05$ (uncorrected)

Brain region	BA	Talairach coordinates X Y Z	Response to hypoglycemia
Left hypothalamus		−3/−6/−8	Activation
Right hypothalamus		+3/−6/−8	Activation
Right anterior cingulate cortex	24/32	4/37/4	Activation
Left caudate		−10/11/8	Activation
Left putamen		−23/−8/8	Activation
Left superior temporal gyrus	22	−54/0/−4	Activation
Left pars triangularis	45	−34/24/8	Activation
Left inferior frontal gyrus	47	−35/38/−12	Activation
Left visual association cortex	18	19/−74/28	Activation
Right pars opercularis	44	50/3/20	Deactivation
Cerebellum		2/−48/−16	Deactivation
Right medial frontal gyrus	46	38/40/16	Deactivation

that these may be coordinated events. Whether there is a neural or hormonal cue that may act to coordinate hypothalamic activation and suppression of endogenous insulin secretion as glucose levels are declining is unclear and will require further investigation.

In summary, the present analysis suggests that the hypothalamus, a key central glucose-sensing region, is exquisitely sensitive to small decrements in systemic glucose levels and that hypothalamic blood flow and, presumably, neuronal activity precede the rise in counterregulatory hormones seen during hypoglycemia. These data lay the groundwork for future studies to determine how metabolic disorders, such as diabetes, alter the hypothalamic response to changes in systemic glucose.

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

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