Human Brain Glycogen Metabolism During and After Hypoglycemia

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OBJECTIVE—We tested the hypotheses that human brain glycogen is mobilized during hypoglycemia and its content increases above normal levels ("supercompensates") after hypoglycemia.

RESEARCH DESIGN AND METHODS—We utilized in vivo ^{13}C nuclear magnetic resonance spectroscopy in conjunction with intravenous infusions of [^{13}C]glucose in healthy volunteers to measure brain glycogen metabolism during and after euglycemic and hypoglycemic clamps.

RESULTS—After an overnight intravenous infusion of 99% enriched [1-^{13}C]glucose to prelabel glycogen, the rate of label wash-out from [1-^{13}C]glycogen was higher (0.12 ± 0.05 vs. 0.03 ± 0.06 μmol $\cdot$ g^{-1} $\cdot$ h^{-1}, means $\pm$ SD, $P < 0.02$, $n = 5$) during a 2-h hyperinsulinemic-hypoglycemic clamp (glucose concentration 57.2 ± 9.7 mg/dl) than during a hyperinsulinemic-euglycemic clamp (95.3 ± 3.3 mg/dl), indicating mobilization of glucose units from glycogen during moderate hypoglycemia. Five additional healthy volunteers received intravenous 25–50% enriched [1-^{13}C]glucose over 22–54 h after undergoing hyperinsulinemic-euglycemic (glucose concentration 92.4 ± 2.3 mg/dl) and hyperinsulinemic-hypoglycemic (52.9 ± 4.8 mg/dl) clamps separated by at least 1 month. Levels of newly synthesized glycogen measured from 4 to 80 h were higher after hypoglycemia than after euglycemia ($P \leq 0.01$ for each subject), indicating increased brain glycogen synthesis after moderate hypoglycemia.

CONCLUSIONS—These data indicate that brain glycogen supports energy metabolism when glucose supply from the blood is inadequate and that its levels rebound to levels higher than normal after a single episode of moderate hypoglycemia in humans. *Diabetes* **58:**1978–1985, 2009

Glucose is the primary fuel for the adult brain. During euglycemia and hyperglycemia, the brain receives more glucose from the blood than it utilizes and normal metabolism can be maintained. However, how the energy needs of the brain are met during hypoglycemia has been a matter of debate. Mobilization of glucose stored in the form of glycogen is one potential mechanism that could support brain metabolism when blood glucose is low. Glycogen content of the brain has been measured at 3–10 μmol/g (1–4), an amount much higher than brain glucose at euglycemia (1–1.5 μmol/g) (5). Although brain glycogen content is much lower than liver (200–400 μmol/g) (6) and muscle (80 μmol/g) (7), we have previously estimated that it can augment cerebral energy needs during short periods of glucose deficit in humans (4). In the current study, we addressed this question in normal human volunteers using nuclear magnetic resonance (NMR) methodology first developed in rats (8) and then translated to humans (9,10). With this technique, [^{13}C]glucose is administered intravenously and its incorporation into and wash-out from brain glycogen is tracked (9,10). [1-^{13}C]glucose has been the substrate of choice since the NMR signal of [1-^{13}C]glucose in glycogen is well resolved from those of free [1-^{13}C]glucose and other glucosyl positions. The ^{13}C NMR measurement of brain glycogen was recently validated by comparing glycogen concentrations obtained in vivo in rats to those measured in extracted tissue by a standard biochemical assay (11).

Using ^{13}C NMR, we recently estimated that 3–4 μmol/g glucose is stored in the form of glycogen in the awake human brain (4). This is in agreement with a measurement of 5–6 μmol/g in normal gray and white matter obtained by biopsies during surgery of patients with epilepsy (12) because anesthesia is known to trigger glycogen accumulation (13). Based on these studies, the glycogen content of the brain represents a significant glucose reservoir relative to free glucose. We found that human brain glycogen turns over very slowly relative to the cerebral rate of glucose utilization (CMR$_{glc}$) under normal physiology (4), similar to what has been observed in the rodent brain (1,8,14). Namely, at euglycemia and hyperglycemia, bulk brain glycogen turns over at a rate that is ~1–2% of CMR$_{glc}$ (15–18) in both humans and rodents. Importantly, glycogen synthesis and breakdown rates can be altered by many factors, such as nutrients, neurotransmitters, and hormones, including glucose and insulin (19–22). The low metabolic rate of glycogen under normal physiology, together with the capacity to acutely regulate glycogen synthase and phosphorylase in response to nutritional and hormonal state, indicate that glycogen may serve as an emergency reservoir when glucose supply from the blood is inadequate. Indeed, brain glycogen is mobilized during hypoglycemia in the rodent brain (23–26), but whether a similar event occurs in humans during hypoglycemia is unknown.

In rodents, brain glycogen was observed to rebound to levels higher than normal, a phenomenon termed "supercompensation," after a single hypoglycemic episode (23). This led to the hypothesis that glycogen may be involved in the pathogenesis of hypoglycemia unawareness by supplying extra fuel to the brain during episodes experienced
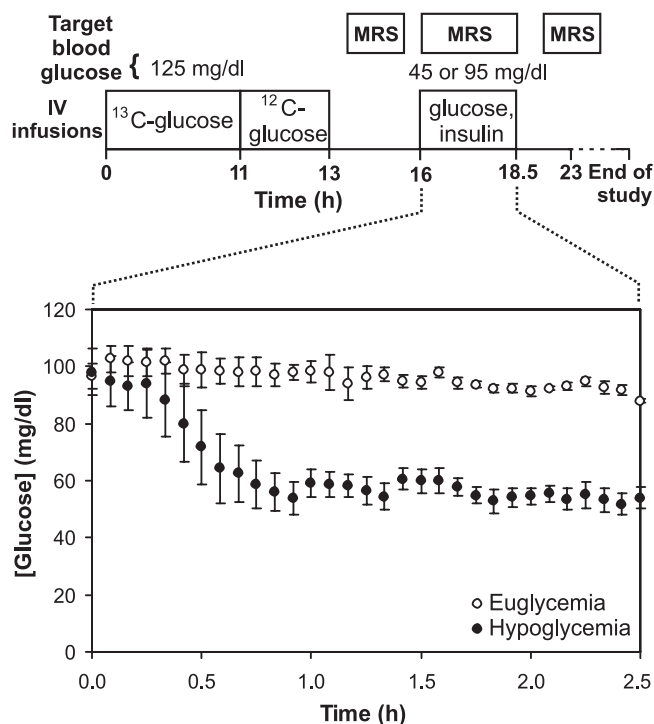
FIG. 1. Experimental protocol of the glycogen utilization study. Average (±SEM) blood glucose levels during the hyperinsulinemic-hypoglycemic and hyperinsulinemic-euglycemic clamps are also shown.

FIG. 2. Experimental protocol of the glycogen supercompensation study. Average (±SEM) blood glucose levels during the hyperinsulinemic-hypoglycemic and hyperinsulinemic-euglycemic clamps are also shown.

soon after the initial hypoglycemia (23,27). Glycogen supercompensation has not yet been studied in the human brain.

The aims of the current study were *1)* to assess glycogen mobilization in the human brain during moderate hypoglycemia and *2)* to determine if the glycogen synthesis rate is increased after a hypoglycemic episode indicating supercompensation in the human brain.

RESEARCH DESIGN AND METHODS

Glycogen utilization study. Five healthy volunteers (four men and one woman age 43 ± 13 years, BMI 25 ± 3 kg/m^2, means $\pm$ SD) on no medications participated in a paired experiment after giving informed consent using procedures (Fig. 1) approved by the University of Minnesota Institutional Review Board. Subjects were studied on two separate occasions separated by at least 1 week, with each subject serving as their own control. Subjects reported to the General Clinical Research Center (GCRC) at 6:00 P.M. after starting a fast at 2:00 P.M. Intravenous catheters were placed antegrade in contralateral arms for [^{13}C]glucose infusion and blood sampling. At 7:00 P.M., a bolus of [1-^{13}C]glucose was given to rapidly raise blood glucose enrichment. A continuous infusion was then given at a variable rate to maintain blood glucose 25% above basal levels to minimize endogenous hepatic glucose production and achieve stable glucose enrichments, because postprandial insulin levels are known to suppress hepatic glucose output (28). Blood glucose was measured on an automatic glucose meter (OneTouch SureStep; Lifescan, Milpitas, CA). Additional samples were collected hourly and immediately frozen for the later determination of isotopic enrichment of plasma glucose by gas chromatography–mass spectroscopy (GC-MS) as described previously (15). A total of 186 g of [1-^{13}C]glucose (Isotec, Miamisburg, OH, and Cambridge Isotope Laboratories, Andover, MA; prepared as 20% weight/volume D-glucose in water with 99% isotopic enrichment) was administered for 11.1 ± 1.2 h to prelabel glycogen. Subjects were then given an unlabeled glucose infusion for 1.6 ± 0.6 h to wash-out [^{13}C]glucose from blood so that [1-^{13}C]glucose removed from glycogen would not be replenished by plasma [1-^{13}C]glucose.

After the wash-out period, subjects were transferred to the Center for Magnetic Resonance Research (CMRR) for a baseline scan, which was omitted in one subject. They were prepared for a 2-h hyperinsulinemic clamp study by retrograde placement of a third intravenous catheter into a foot to provide venous access for arterialized blood sampling (29) while the subject
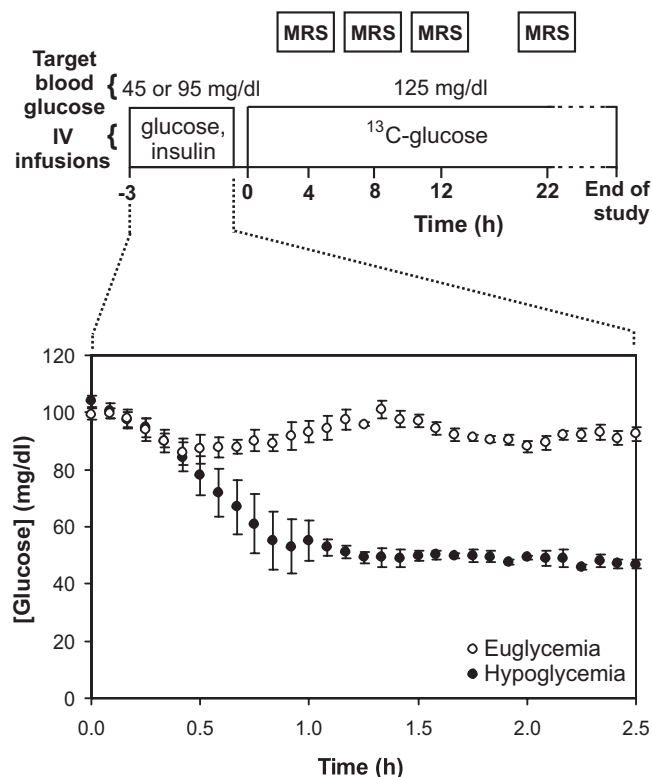
was in the magnet. The two-arm intravenous catheters were used for administration of glucose, insulin (2 mU · kg^{-1} · min^{-1}) and potassium phosphate (4 mEq/h). Glucose (20% dextrose in water) was titrated to achieve target blood glucose of 45 mg/dl (2.5 mmol/l) on one occasion and 95 mg/dl (5.3 mmol/l) on the other occasion, in random order. Blood was obtained every 5 min for immediate measurement of glucose using an autoanalyzer (Analox Instruments, Lunenburg, MA). During the hypoglycemic clamps, blood was also sampled every 30 min for the later determination of glucagon, catecholamines, growth hormone, and cortisol. Four [1-^{13}C]glycogen NMR spectra were acquired using methods described below while blood glucose levels were clamped at the target levels, starting 4.9 ± 1.3 h after the end of the [1-^{13}C]glucose infusion. Subjects were then removed from the magnet, the insulin and potassium infusions were stopped, glucose was administered to bring the blood glucose to 95 mg/dl, and subjects were fed a regular meal. Additional spectroscopic measurements of [^{13}C]glycogen were obtained at $\sim$23, 28, 38, and 46 h after the start of the [1-^{13}C]glucose infusion.

Glycogen supercompensation study. Five healthy volunteers (four men and one woman, age 43 ± 14 years, BMI 26 ± 4 kg/m^2, means $\pm$ SD) on no medications participated in a paired experiment after giving informed consent using procedures (Fig. 2) approved by the University of Minnesota Institutional Review Board. Subjects were studied on two occasions separated by at least 1 month. Subjects reported at 7:00 A.M. to the GCRC in the fasting state. Two intravenous catheters were placed antegrade in contralateral arms for administration of glucose, insulin, and KPhos. Each volunteer underwent a 2-h hyperinsulinemic-euglycemic clamp on one occasion and a hyperinsulinemic-hypoglycemic clamp on the other, in random order, as described above. After the clamp, the insulin and potassium infusions were stopped and glucose was administered to bring the blood glucose to 95 mg/dl. Thirty minutes after the end of the clamp, [1-^{13}C]glucose infusion was started with an initial bolus to rapidly raise blood glucose enrichment. The two-arm intravenous lines were used for [^{13}C]glucose infusion and blood sampling. The infusion rate was adjusted to maintain blood glucose 25% above basal levels. A total of 820–1,420 g of [1-^{13}C]glucose (25–50% isotopic enrichment) was administered over 22–54 h. During the infusion, additional blood samples were collected and frozen for the later determination of isotopic enrichment of plasma glucose by GC-MS. Subjects were transferred to the CMRR to acquire [1-^{13}C]glycogen NMR spectra at $\sim$4, 8, 12, 22, 29, 35, 46, 53, 60, 70, and 80 h after the start of the [1-^{13}C]glucose infusion. During the infusion, subjects

TABLE 1
Comparison of glucose and glycogen measurements before clamps in the utilization study (means $\pm$ SD between subjects)

	Euglycemia study	Hypoglycemia study	P (paired t test)
[Mean plasma glucose] during overnight infusion (mg/dl)	129 $\pm$ 13	129 $\pm$ 22	0.97
Mean isotopic enrichment of plasma glucose during overnight infusion (%)	83 $\pm$ 9	78 $\pm$ 11	0.22
[^{13}C-glycogen] at baseline (μmol/g)	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2	0.86
[Newly synthesized glycogen] at baseline (μmol/g)*	1.4 $\pm$ 0.3	1.5 $\pm$ 0.2	0.36
[^{13}C-glycogen] at the beginning of clamp (μmol/g)†	1.4 $\pm$ 0.1	1.2 $\pm$ 0.3	0.12
[Newly synthesized glycogen] at the beginning of clamp (μmol/g)†	1.7 $\pm$ 0.3	1.5 $\pm$ 0.2	0.24

*Newly synthesized: corrected for plasma glucose isotopic enrichment during infusions. †These are the glycogen levels obtained in the first 30 min of the 2-h clamps.

were fed isocaloric, low-carbohydrate meals designed to minimize the impact of dietary carbohydrate on [^{13}C]glucose enrichment.

NMR spectroscopy. All measurements were performed on a 4-tesla, 90-cm bore magnet (Oxford Magnet Technology, Oxford, U.K.) with an INOVA console (Varian, Palo Alto, CA). Subjects were positioned supine on the patient bed with the occipital lobe just above the ^{1}H/^{13}C surface coil (30). Subjects wore earplugs to minimize exposure to gradient noise and were positioned in the coil holder using cushions to minimize head movement.

The [1-^{13}C]glycogen NMR signal localized in a $7 \times 5 \times 6$ cm^3 voxel in the occipital lobe was acquired as described previously (4,9,10). Each spectrum/data point presented here was acquired over 30 min. The amount of ^{13}C label in the C1 position of glycogen was quantified by the external reference method (9,10). The [1-^{13}C]glycogen concentrations were divided by the plasma glucose isotopic enrichment to correct for differences in isotopic enrichment between subjects and to determine the newly synthesized glycogen concentrations.

Modeling glycogen turnover. A model of glycogen metabolism (4) was fitted to the time courses of [^{13}C]glycogen using the software SAAM II (The SAAM Institute, Seattle, WA). Data from the euglycemic clamp studies of each subject were used for modeling, together with their blood glucose isotopic enrichment time courses as input function. Glycogen synthase (V_{syn}) and phosphorylase (V_{phos}) rates were set to be equal and brain glycogen concentration was set to be constant. Thus, the fitted variables were total glycogen concentration (Glyc) and turnover rate $V_{syn} = V_{phos}$. The cerebral metabolic rate of glucose (CMR$_{glc}$) in the human brain was assumed to be 0.4 μmol $\cdot$ g^{-1} $\cdot$ min^{-1} = 24 μmol $\cdot$ g^{-1} $\cdot$ h^{-1} (15) and the glucose-6-phosphate concentration 0.1 μmol/g (14). Sensitivity analysis indicated that the results were not affected over large ranges of both of these variables (18–30 μmol $\cdot$ g^{-1} $\cdot$ h^{-1} for CMR$_{glc}$ and up to 1 μmol/g for glucose-6-phosphate concentration). Concentration and rate estimates are reported as means $\pm$ SD.

Statistical analysis. In the utilization study, summary statistics and paired t tests were used to compare within-subject differences in plasma glucose, glucose enrichment, and glycogen on hypoglycemic versus euglycemic study days, at each baseline and during clamps. Repeated measures ANOVA was used to compare euglycemic measures to hypoglycemic measures of glycogen, for both within-clamp measures and after-clamp measures. In the supercompensation study, summary statistics and paired t tests were used to compare within-subject differences in plasma glucose, plasma insulin, and the 4-h glycogen measurement on hypoglycemic versus euglycemic study days.

RESULTS

Glycogen utilization study. Brain glycogen of five healthy volunteers was prelabeled via an overnight intravenous infusion of [1-^{13}C]glucose before a euglycemic or hypoglycemic clamp study in the scanner (Fig. 1). Average plasma glucose levels and isotopic enrichments during the overnight infusion were not significantly different for the euglycemia versus hypoglycemia study days, leading to equal glycogen prelabeling before both clamp studies (Table 1). Target levels for blood glucose during the clamps were reached in ~30 min after starting the insulin infusion (Fig. 1). Average blood glucose concentration during the hypoglycemic clamps was 57.2 $\pm$ 9.7 mg/dl and during the euglycemic clamps 95.1 $\pm$ 3.3 mg/dl. The hypoglycemic glucose concentration was slightly above our target level mainly because of one subject who did not require glucose infusion and stayed above 60 mg/dl during the clamp period. Average blood glucose during the hypo-

glycemic clamps of the other four subjects was 53.3 $\pm$ 4.7 mg/dl. Counterregulation during hypoglycemia was demonstrated by measurement of serum glucagon, growth hormone, cortisol, and catecholamines (Fig. 3). The residual plasma glucose enrichment after the chase with [^{12}C]glucose (Fig. 1) during the hypoglycemic clamps tended to be higher than that during the euglycemic clamps (22 $\pm$ 14 vs. 11 $\pm$ 4%, P = 0.13, paired t test) likely because of mobilization of [^{13}C]-labeled hepatic glycogen during hypoglycemia. However, considering the approximately twofold higher glucose concentrations during the euglycemic clamps, the level of cerebral [^{13}C]glucose available for incorporation into glycogen was equal between the hypoglycemic and euglycemic clamps, which was also apparent from the residual glucose peaks in the spectra (Fig. 4A).

The glycogen signal was stable during the euglycemic clamps, while it decreased during the hypoglycemic clamps (Fig. 4A), indicating mobilization of glucose units from glycogen during moderate hypoglycemia. Average glycogen integrals, each normalized to first clamp measure, during euglycemia were higher than during hypoglycemia (0.98 $\pm$ 0.05 vs. 0.87 $\pm$ 0.08, P < 0.0001, repeated measures ANOVA) (Fig. 4B). Glycogen utilization was confirmed by a higher rate at which newly synthesized glycogen levels decreased during hypoglycemia (0.12 $\pm$ 0.05 μmol $\cdot$ g^{-1} $\cdot$ h^{-1}) than during euglycemia (0.03 $\pm$ 0.06 μmol $\cdot$ g^{-1} $\cdot$ h^{-1}, P < 0.02, paired t test). This label wash-out rate during euglycemia was the same as we previously observed after an 11-h prelabeling period (4). To further analyze the consistency between our previous observations during euglycemia and slight hyperglycemia and this study, we fitted a model of glycogen turnover to the time courses of [^{13}C]glycogen obtained during the euglycemia studies of the five volunteers. This resulted in estimates of glycogen content of 4.3 $\pm$ 0.2 μmol/g and turnover rate ($V_{syn} = V_{phos}$) of 0.18 $\pm$ 0.01 μmol $\cdot$ g^{-1} $\cdot$ h^{-1}, indicating a turnover time constant of 24 h, in excellent agreement with prior results (4).

Newly synthesized glycogen levels after the clamp (at 23, 28, 38, and 46 h time points) were not different for euglycemia versus hypoglycemia studies (P = 0.64, repeated measures ANOVA). Note that the "newly synthesized glycogen" levels refer to measured [^{13}C]glycogen levels divided by the isotopic enrichment of plasma glucose during the preclamp infusions; therefore, they do not necessarily reflect new glycogen synthesized after the clamps. Based on the lower [^{13}C]glycogen levels at the end of the hypoglycemic clamps, one might expect the glycogen measurements after hypoglycemia to also be lower than those after euglycemia. However, the average plasma
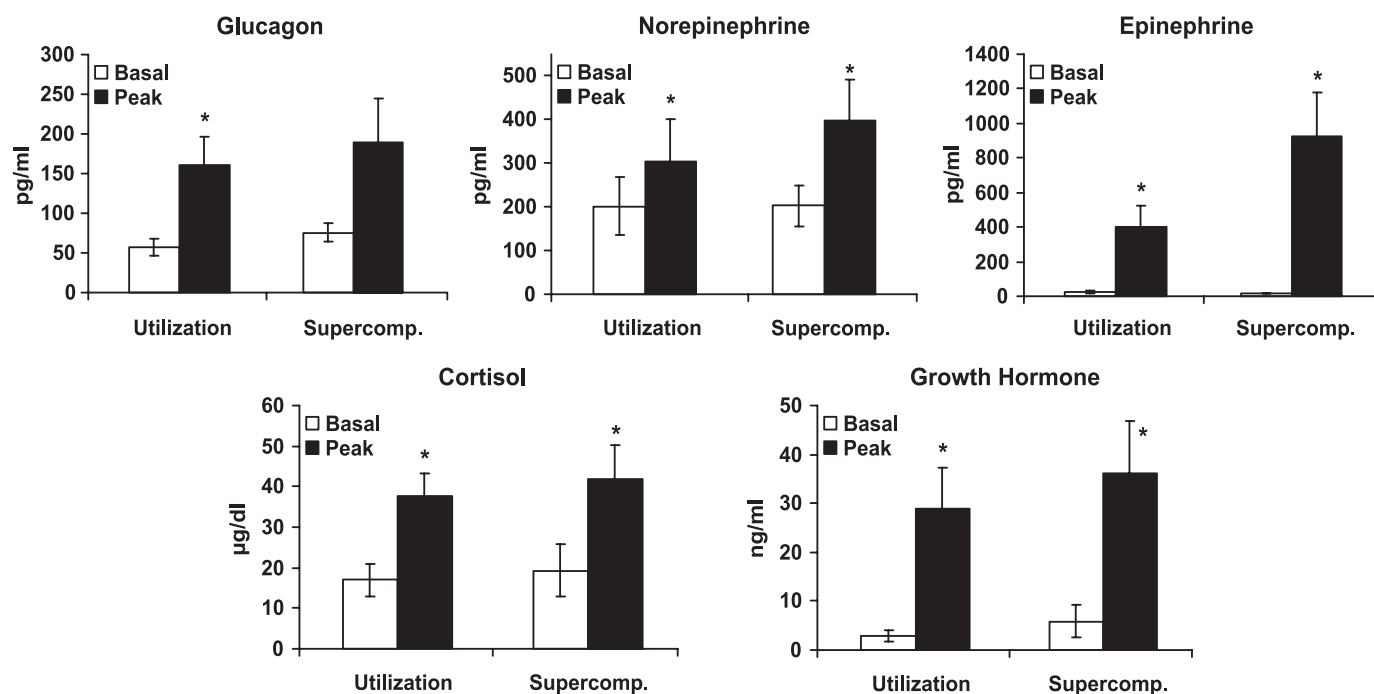
FIG. 3. Counterregulatory hormone response during the hypoglycemic clamps in the glycogen utilization and supercompensation (Supercomp) studies. Basal (5 min and immediately before the clamp) versus peak (maximum observed over the 2-h clamp period) values (average ± SEM) are shown. * $P < 0.05$, paired t test.

glucose isotopic enrichment at the end of the hypoglycemic clamps was 13 ± 11% (as opposed to 2 ± 1% at the end of the euglycemic clamps) and this enriched glucose could have been incorporated into glycogen once blood glucose levels were rescued, thereby equalizing [^{13}C]glycogen levels in the following scans. This effect would have been augmented by glycogen supercompensation after hypoglycemia. Therefore, we investigated if glycogen synthesis was increased after hypoglycemia in the next set of experiments.

Glycogen supercompensation study. In this experiment, label incorporation from intravenous [1-^{13}C]glucose into brain glycogen was measured after a euglycemic or hypoglycemic clamp (Fig. 2). Target levels for blood glucose during the clamps were reached in 40–60 min after starting the insulin infusion (Fig. 2). Average blood glucose concentration was 52.9 ± 4.8 mg/dl (means ± SD between subjects) during the hypoglycemic clamps and 92.4 ± 2.3 mg/dl during the euglycemic clamps. Counterregulation during hypoglycemia was demonstrated by measurement of serum glucagon, growth hormone, cortisol, and catecholamines (Fig. 3). Average plasma glucose levels during the [1-^{13}C]glucose infusion were 115 ± 8 mg/dl and average insulin levels 40 ± 12 mU/l, with no difference between the euglycemia and hypoglycemia studies ($P = 0.38$ for glucose levels and 0.51 for insulin, paired t test) (Fig. 5A and B). Steady ^{13}C isotopic enrichment levels in blood glucose were achieved during the long infusions as demonstrated by data obtained in one subject in Fig. 5C. We fitted a model of glycogen turnover to the time courses of [^{13}C]glycogen obtained during the euglycemia studies of the five volunteers. This resulted in estimates of glycogen content of 3.5 ± 0.1 µmol/g and turnover rate ($V_{syn} = V_{phos}$) of 0.20 ± 0.01 µmol · g^{-1} · h^{-1}, in agreement with the results of the glycogen utilization study and our prior published results for euglycemia and slight hyperglycemia (4). The newly synthesized glycogen

levels were higher after hypoglycemia than after euglycemia across all time points during and after the ^{13}C-glucose infusions (Fig. 6, $P \leq 0.01$ paired t test for each subject separately), indicating increased glycogen synthesis after hypoglycemia. The glycogen synthesis rate can be estimated from the initial rate of label incorporation when ^{13}C enrichment of glycogen is negligible and the labeling kinetics primarily represents synthesis. The first glycogen data point obtained from each volunteer at 4 h was used for this purpose. The synthesis rate of glycogen was 0.25 ± 0.03 µmol · g^{-1} · h^{-1} after euglycemia and 0.32 ± 0.05 µmol · g^{-1} · h^{-1} after hypoglycemia ($P < 0.02$, paired t test). The difference between newly synthesized glycogen levels increased steadily over time during the [^{13}C]glucose infusion (Fig. 6) reaching ~1 µmol/g at 34 h, indicating a net synthesis of ~1 µmol/g glycogen occurred over this time period.

DISCUSSION

Here we present the first evidence for glycogen utilization during, and supercompensation after, moderate hypoglycemia in the healthy human brain. Using ^{13}C NMR, we found that brain glycogen content decreased by ~15% during modest hypoglycemia, whereas it was unchanged under isoinsulinemic euglycemia. Our data also indicate that brain glycogen content increased after a period of modest hypoglycemia but did not change after isoinsulinemic euglycemia in a second group of healthy volunteers.

In the utilization experiment, we detected glycogen mobilization by an increased ^{13}C label wash-out from prelabeled glycogen during hypoglycemia relative to euglycemia. The ^{13}C label was incorporated into glycogen mainly via turnover, as net synthesis does not occur at the euglycemia and slight hyperglycemia we utilized during prelabeling (4). Hence, the total and labeled glycogen
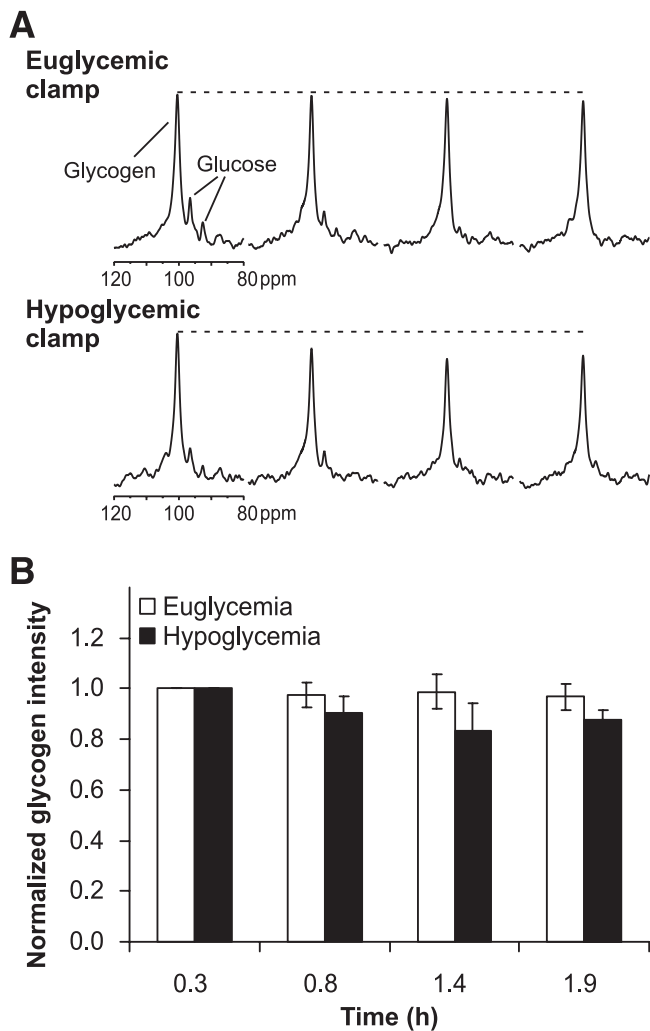
FIG. 4. Glycogen utilization during moderate hypoglycemia in the human brain. *A*: Proton-decoupled ^{13}C NMR spectra acquired over four consecutive 30-min periods during the hypoglycemic and euglycemic clamps of the utilization study. The C1 peak of glycogen at 100.5 ppm and the two C1 glucose peaks originating from α- and β-glucose are marked. Spectra were averaged over the five subjects (4,096 transients per spectrum per subject with a repetition time of 0.45 s) and normalized with respect to the first half-hour spectrum. The volume-of-interest was 210 ml ($7 \times 5 \times 6$ cm^3) in the occipital lobe. *B*: Glycogen integrals over four consecutive 30-min periods normalized to the spectrum acquired during the first 30 min of the clamp. Error bars indicate SD between subjects.

levels were equal before the hypoglycemic and euglycemic clamps. We designed the study with an ~1- to 2-h [^{12}C]glucose infusion after the ^{13}C to chase the [^{13}C]glucose from blood, such that any [^{13}C]glucose removed from glycogen during the clamps would not be replenished by [^{13}C]glucose from the blood, increasing our chances to detect glycogen mobilization. Ideally, one would turn over all glycogen molecules before the clamp and keep the isotopic enrichment of the blood constant and equal to that of glycogen (31) during the clamps such that the glycogenolysis rate would equal the rate of label wash-out from glycogen. However, it takes 3–5 days to turn over the total human brain glycogen pool (4) and it is very difficult to keep blood isotopic enrichments constant during hypoglycemia based on our prior experience, making this experimental design unfeasible in humans. The isotopic enrichment of glucose during hypoglycemia in our studies tended to be higher than during euglycemia (22 vs. 11%),
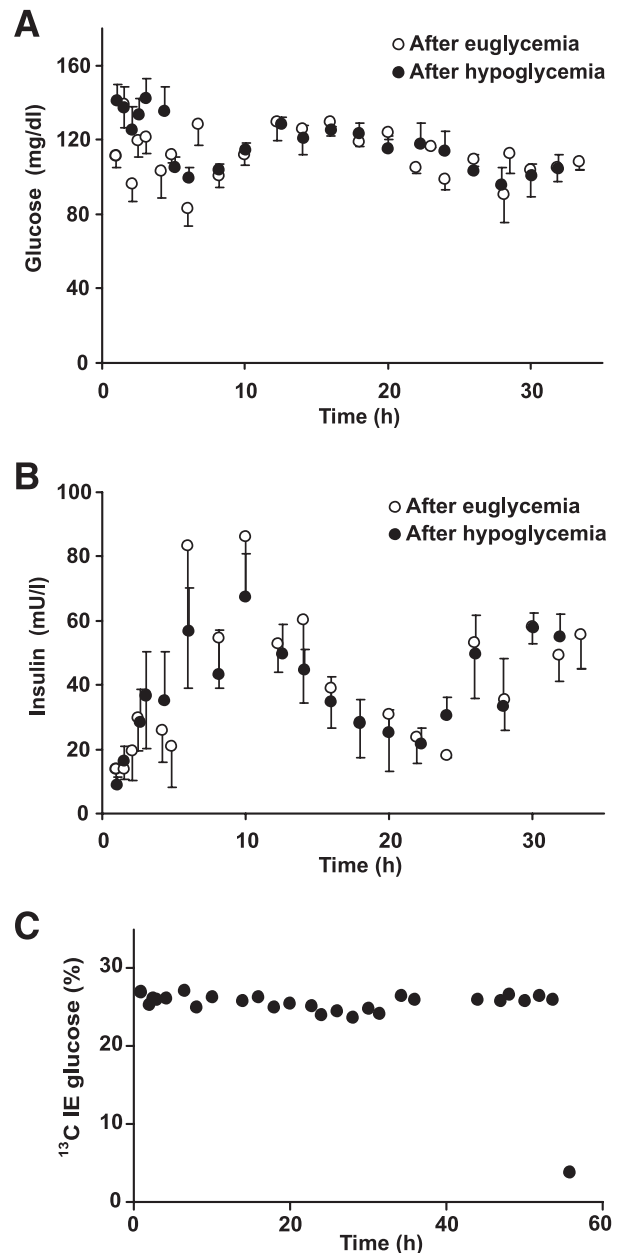
FIG. 5. Glucose, insulin and ^{13}C isotopic enrichment (IE) in the blood of volunteers after the euglycemic and hypoglycemic clamps in the supercompensation study. *A* and *B*: Plasma glucose and insulin levels (average ± SEM) during the [1-^{13}C]glucose infusion are shown for those time points where data are available from two or more subjects. Only one volunteer was infused with glucose longer than 34 h. *C*: Stability of ^{13}C enrichment of plasma glucose in one volunteer. [1-^{13}C]glucose (29% enriched) was administered intravenously for 54 h as also apparent from the rapid drop in isotopic enrichment after this time point.

which might have even reduced the difference in [^{13}C]glycogen levels between the hypoglycemic and euglycemic clamps. We do not expect this to be a factor because the [^{13}C]glucose levels available for incorporation into glycogen were comparable during the two clamps considering the higher glucose levels during euglycemia. In theory, the increased label wash-out from glycogen during hypoglycemia may have been because of increased turnover; however, this possibility is highly unlikely considering the known reciprocal regulation of glycogen synthase and phosphorylase (22).
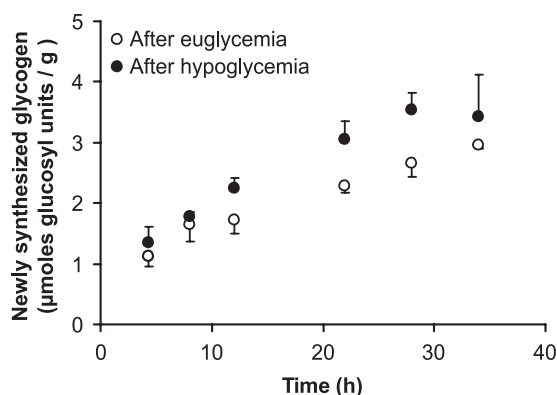
FIG. 6. Glycogen supercompensation after moderate hypoglycemia in the human brain. Newly synthesized glycogen concentrations (average ± SD) during ^{13}C glucose infusions after euglycemic and hypoglycemic clamps are shown for those time points where data are available from two or more subjects.

Our observations demonstrate that the human brain employs mechanisms of hypoglycemia response that are similar to those in the rodent brain (23–26). The [^{13}C]glycogen signal decreases with a rate of ~64%/h in the rat brain at ~1.5 mmol/l blood glucose (23) and ~10%/h in the human brain at ~3 mmol/l blood glucose, indicating a mobilization rate commensurate with the severity of hypoglycemia. The rat study implied that glycogen was not mobilized until brain glucose levels were zero (23); however, in the current study brain glucose was 0.6–0.8 μmol/g based on reported glucose transport parameters for the human brain (5,32). At these glucose levels, hexokinase is 92–94% saturated (K_M = 50 μmol/l), whereas it is 95–97% saturated at euglycemia (1–1.5 μmol/g brain glucose). This slight desaturation of hexokinase may have been enough to trigger glycogen mobilization to supplement the glucose-6-phospate deficit. Alternatively, a more general brain stress response may have been operative, involving the activation of brainstem catecholaminergic neurons, shown to occur with hypoglycemia (33–35). In particular norepinephrine is very effective in increasing glycogen breakdown (22) and may do so in the absence of a significant glucose deficit. Interestingly, after treatment with a glycogen phosphorylase inhibitor to increase brain glycogen content, neuronal function is prolonged during severe hypoglycemia in rats (36), providing further evidence that the brain may rely on glycogen stores to augment reduced energy delivery during hypoglycemia.

We only utilized the data from the euglycemia studies to fit a glycogen metabolic model because the model assumes the data were collected under steady-state conditions, which was not true during hypoglycemia. With the euglycemia data, we obtained glycogen content and turnover values in agreement with our previous findings (4). To roughly estimate the glycogenolysis rate during hypoglycemia we used the formula d[^{13}C-glycogen]/dt = IE$_{glc}$ × V_{syn} − IE$_{glyc}$ × V_{phos}, using the average blood IE$_{glc}$ (isotopic enrichment of free glucose) and assuming constant IE$_{glyc}$ (isotopic enrichment of glycogen) during the 2-h clamps. IE$_{glyc}$ was ~40% based on the measured [^{13}C]glycogen level relative to total glycogen (4). Because brain glucose isotopic enrichment closely follows the blood glucose isotopic enrichment, IE$_{glc}$ was set equal to the average isotopic enrichment measured in the blood during the hypoglycemic clamps, 22%. The net glycogenolysis rate (V_{phos} − V_{syn}) could then be estimated by

investigating two limiting conditions, with V_{syn} set to 0 or to the turnover rate of glycogen, 0.18 μmol · g^{-1} · h^{-1}. This way we estimated a glycogenolysis rate of 0.22–0.30 μmol · g^{-1} · h^{-1}, that is, that 0.4–0.6 μmol/g glycogen was mobilized during the 2-h hypoglycemic clamp. This glycogenolysis rate still constitutes a very small fraction (~1%) of CMR$_{glc}$ (if CMR$_{glc}$ does not change under hypoglycemia) and shows that the blood supplies the majority of glucose utilized by the brain during moderate hypoglycemia. This was the case even during severe hypoglycemia in rats where glycogen was shown to supplement a small glucose deficit (23).

In the supercompensation experiment, we observed a higher synthesis rate for human brain glycogen after hypoglycemia versus euglycemia. This higher rate could not be because of any differences in insulin levels (23,31) as these were the same in the paired studies (Fig. 5B). Clearly, some of this synthesis had to replenish the glycogen mobilized during the 2-h hypoglycemia. However, because a net synthesis of ~1 μmol/g glycogen occurred during 34 h of [^{13}C]glucose infusion and only 0.4–0.6 μmol/g glycogen was mobilized during the prior hypoglycemic clamp, our data indicate that glycogen content was higher after moderate hypoglycemia. It would be ideal to observe [^{13}C]glycogen levels higher than the normal glycogen levels (3–4 μmol/g) to confirm supercompensation; however, this would require even longer experimental periods in humans than the 4 days in this study.

It is unlikely that the glycogen content of the brain can increase many-fold because of the restriction of brain volume within the skull and water retention by glycogen. However, up to fourfold increases above basal brain glycogen content have been observed (37) and can likely be accommodated because of the low glycogen content of the brain. Glycogen supercompensation has been observed after multiple metabolic stressors in the rodent brain, such as hypoxia (38), hypoglycemia (37), ischemia (37), brain injury (39), and sleep deprivation (3), and in other tissues, such as the muscle after exercise (40). Furthermore, supercompensation of muscle glycogen after its depletion with exercise is augmented with repeated bouts of exercise, that is, in exercise-trained rodents and humans (40). Therefore, glycogen supercompensation may be a protective measure taken by the affected tissue in preparation for the next bout of metabolic stress (38).

Our data that indicate supercompensation of human brain glycogen are in agreement with similar NMR studies in rats (23). Furthermore, glycogen supercompensation in the hypothalamus and cortex after recurrent glucopenia was demonstrated recently in a rat model of hypoglycemia-associated autonomic failure (HAAF) (41). Although these observations suggest that glycogen supercompensation may be involved in the development of hypoglycemia unawareness, recent experiments by Herzog et al. (24) failed to demonstrate glycogen supercompensation in the cortex, cerebellum and hypothalamus in awake rats 6 and 24 h after hypoglycemia. They attributed this failure to confirm the prior rat NMR study (23) to anesthesia and severe hyperglycemia levels used for the NMR experiments. However, our current data, that also indicate supercompensation, were acquired with awake humans maintained at mild hyperglycemia (115 mg/dl) during the [^{13}C]glucose infusion after the clamps (Fig. 5A). We suggest that the variability among measurements in animals studied at different time points might be the reason that Herzog et al. did not observe supercompensation after

hypoglycemia in the rat model. In NMR experiments, time courses are monitored in individual subjects, thereby facilitating the observation of treatment effects relative to extraction studies where all data points are obtained from different animals. Indeed, in the Herzog et al. study, even though the cortical glycogen levels were almost doubled in cortex after recurrent versus acute hypoglycemia ($\sim$7 vs. 4 μmol/g), this difference was not statistically significant, likely because of the large variance between animals.

Taken together, our observations demonstrate that brain glycogen is a dynamic source of energy and provide the first support for the hypothesis that brain glycogen may be used to offset the loss of substrate that occurs in humans during hypoglycemia. They further demonstrate increased brain glycogen synthesis after moderate hypoglycemia in humans and indicate glycogen supercompensation. The potential involvement of glycogen in the development of HAAF in humans, and specifically if supercompensated glycogen provides additional substrate to the brain during subsequent hypoglycemic episodes, need to be investigated in future studies.

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REFERENCES

1. Choi IY, Gruetter R. In vivo ^{13}C NMR assessment of brain glycogen concentration and turnover in the awake rat. Neurochem Int 2003;43:317–322
2. Cruz NF, Dienel GA. High glycogen levels in brains of rats with minimal environmental stimuli: implications for metabolic contributions of working astrocytes. J Cereb Blood Flow Metab 2002;22:1476–1489
3. Kong J, Shepel PN, Holden CP, Mackiewicz M, Pack AI, Geiger JD. Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. J Neurosci 2002;22:5581–5587
4. Öz G, Seaquist ER, Kumar A, Criego AB, Benedict LE, Rao JP, Henry PG, Van De Moortele PF, Gruetter R. Human brain glycogen content and metabolism: implications on its role in brain energy metabolism. Am J Physiol Endocrinol Metab 2007;292:E946–E951
5. Gruetter R, Ugurbil K, Seaquist ER. Steady-state cerebral glucose concentrations and transport in the human brain. J Neurochem 1998;70:397–408
6. Roden M, Petersen KF, Shulman GI. Nuclear magnetic resonance studies of hepatic glucose metabolism in humans. Recent Prog Horm Res 2001;56:219–237
7. Krssak M, Petersen KF, Bergeron R, Price T, Laurent D, Rothman DL, Roden M, Shulman GI. Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy study. J Clin Endocrinol Metab 2000;85:748–754
8. Choi IY, Tkáč I, Ugurbil K, Gruetter R. Noninvasive measurements of [1-13C]glycogen concentrations and metabolism in rat brain in vivo. J Neurochem 1999;73:1300–1308
9. Öz G, Henry PG, Seaquist ER, Gruetter R. Direct, noninvasive measurement of brain glycogen metabolism in humans. Neurochem Int 2003;43:323–329
10. Öz G, Henry PG, Tkáč I, Gruetter R. A localization method for the measurement of fast relaxing ^{13}C NMR signals in humans at high magnetic fields. Appl Magn Reson 2005;29:159–169
11. Lei H, Morgenthaler F, Yue T, Gruetter R. Direct validation of in vivo localized ^{13}C MRS measurements of brain glycogen. Magn Reson Med 2007;57:243–248
12. Dalsgaard MK, Madsen FF, Secher NH, Laursen H, Quistorff B. High glycogen levels in the hippocampus of patients with epilepsy. J Cereb Blood Flow Metab 2007;27:1137–1141
13. Brunner EA, Passonneau JV, Molstad C. The effect of volatile anaesthetics on levels of metabolites and on metabolic rate in brain. J Neurochem 1971;18:2301–2316
14. Watanabe H, Passonneau JV. Factors affecting the turnover of cerebral glycogen and limit dextrin in vivo. J Neurochem 1973;20:1543–1554
15. Gruetter R, Seaquist ER, Ugurbil K. A mathematical model of compartmentalized neurotransmitter metabolism in the human brain. Am J Physiol Endocrinol Metab 2001;281:E100–E112
16. Toyama H, Ichise M, Liow JS, Modell KJ, Vines DC, Esaki T, Cook M, Seidel J, Sokoloff L, Green MV, Innis RB. Absolute quantification of regional cerebral glucose utilization in mice by ^{18}F-FDG small animal PET scanning and 2-^{14}C-DG autoradiography. J Nucl Med 2004;45:1398–1405
17. Henry PG, Lebon V, Vaufrey F, Brouillet E, Hantraye P, Bloch G. Decreased TCA cycle rate in the rat brain after acute 3-NP treatment measured by in vivo ^{1}H-{^{13}C} NMR spectroscopy. J Neurochem 2002;82:857–866
18. Öz G, Berkich DA, Henry PG, Xu Y, LaNoue K, Hutson SM, Gruetter R. Neuroglial metabolism in the awake rat brain: CO_2 fixation increases with brain activity. J Neurosci 2004;24:11273–11279
19. Dringen R, Hamprecht B. Glucose, insulin, and insulin-like growth factor I regulate the glycogen content of astroglia-rich primary cultures. J Neurochem 1992;58:511–517
20. Hamai M, Minokoshi Y, Shimazu T. L-Glutamate and insulin enhance glycogen synthesis in cultured astrocytes from the rat brain through different intracellular mechanisms. J Neurochem 1999;73:400–407
21. Magistretti PJ, Morrison JH, Shoemaker WJ, Sapin V, Bloom FE. Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. Proc Natl Acad Sci U S A 1981;78:6535–6539
22. Magistretti PJ, Sorg O, Martin J. Regulation of glycogen metabolism in astrocytes: physiological, pharmacological, and pathological aspects. In *Astrocytes: Pharmacology and Function.* Murphy S, Ed. San Diego, CA, Academic Press, 1993, pp. 243–265
23. Choi IY, Seaquist ER, Gruetter R. Effect of hypoglycemia on brain glycogen metabolism in vivo. J Neurosci Res 2003;72:25–32
24. Herzog RI, Chan O, Yu S, Dziura J, McNay EC, Sherwin RS. Effect of acute and recurrent hypoglycemia on changes in brain glycogen concentration. Endocrinology 2008;149:1499–1504
25. Ratcheson RA, Blank AC, Ferrendelli JA. Regionally selective metabolic effects of hypoglycemia in brain. J Neurochem 1981;36:1952–1958
26. Agardh CD, Folbergrova J, Siesjo BK. Cerebral metabolic changes in profound, insulin-induced hypoglycemia, and in the recovery period following glucose administration. J Neurochem 1978;31:1135–1142
27. Gruetter R. Glycogen: the forgotten cerebral energy store. J Neurosci Res 2003;74:179–183
28. Alzaid AA, Dinneen SF, Turk DJ, Caumo A, Cobelli C, Rizza RA. Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans. J Clin Invest 1994;94:2341–2348
29. Seaquist ER. Comparison of arterialized venous sampling from the hand and foot in the assessment of in vivo glucose metabolism. Metabolism 1997;46:1364–1366
30. Adriany G, Gruetter R. A half-volume coil for efficient proton decoupling in humans at 4 Tesla. J Magn Reson 1997;125:178–184
31. Morgenthaler FD, van Heeswijk RB, Xin L, Laus S, Frenkel H, Lei H, Gruetter R. Non-invasive quantification of brain glycogen absolute concentration. J Neurochem 2008;107:1414–1423
32. de Graaf RA, Pan JW, Telang F, Lee JH, Brown P, Novotny EJ, Hetherington HP, Rothman DL. Differentiation of glucose transport in human brain gray and white matter. J Cereb Blood Flow Metab 2001;21:483–492
33. Morilak DA, Fornal CA, Jacobs BL. Effects of physiological manipulations

on locus coeruleus neuronal activity in freely moving cats. III. Glucoregulatory challenge. Brain Res 1987;422:32–39

34. Lachuer J, Gaillet S, Barbagli B, Buda M, Tappaz M. Differential early time course activation of the brainstem catecholaminergic groups in response to various stresses. Neuroendocrinology 1991;53:589–596

35. Yuan PQ, Yang H. Neuronal activation of brain vagal-regulatory pathways and upper gut enteric plexuses by insulin hypoglycemia. Am J Physiol Endocrinol Metab 2002;283:E436–E448

36. Suh SW, Bergher JP, Anderson CM, Treadway JL, Fosgerau K, Swanson RA. Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*,S*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmet hyl)-propyl]-1H-indole-2-carboxamide). J Pharmacol Exp Ther 2007;321: 45–50

37. Folbergrova J, Katsura KI, Siesjo BK. Glycogen accumulated in the brain following insults is not degraded during a subsequent period of ischemia. J Neurol Sci 1996;137:7–13

38. Brucklacher RM, Vannucci RC, Vannucci SJ. Hypoxic preconditioning increases brain glycogen and delays energy depletion from hypoxia-ischemia in the immature rat. Dev Neurosci 2002;24:411–417

39. Shimizu N, Hamuro Y. Deposition of glycogen and changes in some enzymes in brain wounds. Nature 1958;181:781–782

40. Holloszy JO, Kohrt WM, Hansen PA. The regulation of carbohydrate and fat metabolism during and after exercise. Front Biosci 1998;3:D1011–D1027

41. Alquier T, Kawashima J, Tsuji Y, Kahn BB. Role of hypothalamic adenosine 5′-monophosphate-activated protein kinase in the impaired counterregulatory response induced by repetitive neuroglucopenia. Endocrinology 2007;148:1367–1375