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 \([^{13}\text{C}]\)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 \([^{13}\text{C}]\)glucose to prelabel glycogen, the rate of label wash-out from \([^{13}\text{C}]\)glycogen was higher (0.12 ± 0.05 vs. 0.03 ± 0.06 \(\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}\), means ± 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 \([^{13}\text{C}]\)glucose over 22–54 h after undergoing hyperinsulinemic-euglycemic (glucose concentration 92.4 ± 2.3 mg/dl) and hyperinsulinemic-hypoglycemic (52.0 ± 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 (\(P < 0.01\) 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 \(\mu\text{mol/g}\) (1–4), an amount much higher than brain glucose at euglycemia (1–1.5 \(\mu\text{mol/g}\)) (5). Although brain glycogen content is much lower than liver (200–400 \(\mu\text{mol/g}\)) (6) and muscle (80 \(\mu\text{mol/g}\)) (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, \([^{13}\text{C}]\)glucose is administered intravenously and its incorporation into and wash-out from brain glycogen is tracked (9,10). \([^{13}\text{C}]\)glucose has been the substrate of choice since the NMR signal of \([^{13}\text{C}]\)glucose in glycogen is well resolved from those of free \([^{13}\text{C}]\)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 \(\mu\text{mol/g}\) glucose is stored in the form of glycogen in the awake human brain (4). This is in agreement with a measurement of 5–6 \(\mu\text{mol/g}\) 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\(_{\text{glc}}\)) 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 \(\sim 1–2\%\) of CMR\(_{\text{glc}}\) (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...
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/m², means ± 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 13C-glucose infusion and blood sampling. At 7:00 P.M., a bolus of [1-13C]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). During the hypoglycemic clamps, blood was also sampled every 30 min for the later determination of glucagon, catecholamines, growth hormone, and cortisol. Four [1-13C]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 [1-13C]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 [13C]glycogen were obtained at ~23, 28, 38, and 46 h after the start of the [1-13C]glucose infusion.

Glycogen supercompensation study. Five healthy volunteers (four men and one woman, age 43 ± 14 years, BMI 26 ± 4 kg/m², means ± 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, [1-13C]glucose infusion was started with an initial bolus to rapidly raise blood glucose enrichment. 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 [1-13C]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 [1-13C]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 [13C]glycogen were obtained at ~23, 28, 38, and 46 h after the start of the [1-13C]glucose infusion.

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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 $1H/13C$ 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 $[^{13}C]$glycogen NMR signal localized in a $7 \times 5 \times 6$ cm$^3$ voxel in the occipital lobe was acquired as described previously (4,10). Each spectrum/data point presented here was acquired over 30 min. The amount of $1H$ label in the C1 position of glycogen was quantified by the external reference method (9,10). The $[^{13}C]$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 synthesis ($V_{syn}$) 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 $V_{Glyc} = V_{syn} - V_{phos}$. The cerebral metabolic rate of glucose (CMR$_{glc}$) in the human brain was assumed to be 0.4 $\mu$mol·g$^{-1}$·min$^{-1}$ ($1 \times 24 $ $\mu$mol·g$^{-1}$·h$^{-1}$) (15) and the glucose-6-phosphate concentration 0.1 $\mu$mol/g (14). Sensitivity analysis indicated that the results were not affected over large ranges of both of these variables (18–30 $\mu$mol·g$^{-1}$·h$^{-1}$ for CMR$_{glc}$, and up to 1 $\mu$mol/g for glucose-6-phosphate concentration). Concentration and rate estimates are reported as means±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 during moderate hypoglycemia. Average glycogen integrals, each normalized to first clamp measure, during euglycemia were higher than during hypoglycemia (0.98±0.05 vs. 0.87±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±0.05 $\mu$mol·g$^{-1}$·h$^{-1}$) than during euglycemia (0.03±0.06 $\mu$mol·g$^{-1}$·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 hypoglycemia 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±0.2 $\mu$mol/g and turnover rate ($V_{syn} = V_{phos}$) of 0.18±0.01 $\mu$mol·g$^{-1}$·h$^{-1}$, indicating a turnover time constant of 24 h, in excellent agreement with prior results (4).

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±0.05 vs. 0.87±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±0.05 $\mu$mol·g$^{-1}$·h$^{-1}$) than during euglycemia (0.03±0.06 $\mu$mol·g$^{-1}$·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).

RESULTS

Glycogen utilization study. Brain glycogen of five healthy volunteers was prelabeled via an overnight intravenous infusion of $[^{13}C]$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±9.7 mg/dl and during the euglycemic clamps 95.1±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 hypoglycemic clamps of the other four subjects was 53.3±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±14 vs. 11±48%, $P=0.13$, 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±0.05 vs. 0.87±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±0.05 $\mu$mol·g$^{-1}$·h$^{-1}$) than during euglycemia (0.03±0.06 $\mu$mol·g$^{-1}$·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 hypoglycemia 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±0.2 $\mu$mol/g and turnover rate ($V_{syn} = V_{phos}$) of 0.18±0.01 $\mu$mol·g$^{-1}$·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

| TABLE 1 | Comparison of glucose and glycogen measurements before clamps in the utilization study (means±SD between subjects) |
|---------|------------------------------------------------------------------------------------------------|
| Euglycemia study | Hypoglycemia study | $P$ (paired $t$ test) |
| Mean plasma glucose (mg/dl) | 129±13 | 129±22 | 0.97 |
| Mean isotopic enrichment of plasma glucose during overnight infusion (%) | 83±9 | 78±11 | 0.22 |
| $[^{13}C]$-glycogen at baseline (µmol/g) | 1.2±0.2 | 1.3±0.2 | 0.86 |
| Newly synthesized glycogen at baseline (µmol/g) | 1.4±0.3 | 1.5±0.2 | 0.36 |
| $[^{13}C]$-glycogen at the beginning of clamp (µmol/g)† | 1.4±0.1 | 1.2±0.3 | 0.12 |
| Newly synthesized glycogen at the beginning of clamp (µmol/g)† | 1.7±0.3 | 1.5±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.
glucose isotopic enrichment at the end of the hypoglycemic clamps was 13 ± 1% (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 [13C]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 [1-13C]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 [1-13C]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 13C 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 [13C]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 13C-glucose infusions (Fig. 6, P ≤ 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 13C 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 [13C]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 13C 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 13C label wash-out from prelabeled glycogen during hypoglycemia relative to euglycemia. The 13C 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
levels were equal before the hypoglycemic and euglycemic clamps. We designed the study with a 1- to 2-h [12C]glucose infusion after the 13C to chase the [13C]glucose from blood, such that any [13C]glucose removed from glycogen during the clamps would not be replenished by [13C]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%), which might have even reduced the difference in [13C]glycogen levels between the hypoglycemic and euglycemic clamps. We do not expect this to be a factor because the [13C]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. 4. Glycogen utilization during moderate hypoglycemia in the human brain. A: Proton-decoupled 13C 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 × 5 × 6 cm3) 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.

FIG. 5. Glucose, insulin and 13C 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 [1-13C]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 13C enrichment of plasma glucose in one volunteer. [1-13C]glucose (29% enriched) was administered intravenously for 54 h as also apparent from the rapid drop in isotopic enrichment after this time point.
Our observations demonstrate that the human brain employs mechanisms of hypoglycemia response that are similar to those in the rodent brain (23–26). The [13C]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-phosphate 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 glycolysis rate during hypoglycemia we used the formula d[13C]-glycogen/dt = IE_\text{glyc} × V_{\text{syn}} - IE_{\text{glyc}} × V_{\text{phos}} using the average blood IE_{\text{glyc}} (isotopic enrichment of free glucose) and assuming constant IE_{\text{glyc}} (isotopic enrichment of glycogen) during the 2-h clamps. IE_{\text{glyc}} was ~40% based on the measured [13C]glycogen level relative to total glycogen (4). Because brain glucose isotopic enrichment closely follows the blood glucose isotopic enrichment, IE_{\text{glyc}} was set equal to the average isotopic enrichment measured in the blood during the hypoglycemic clamps, 22%. The net glycogenolysis rate (V_{\text{phos}} - V_{\text{syn}}) could then be estimated by investigating two limiting conditions, with V_{\text{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 glyco-genolysis rate still constitutes a very small fraction (~1%) of CMR_{\text{glc}} (if CMR_{\text{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. 5D). 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 [13C]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 [13C]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 (HAAPF) (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 [13C]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
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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 (~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.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health Grant R01 NS035192 (to E.R.S.). The CMRR is supported by National Center for Research Resources (NCRR) Biotechnology Research Resource Grant P41RR008079 and Neuroscience Center Core Blueprint Award P30NS057091. The GCRC is supported by NCRR Grant M01RR00400.

Parts of this study were presented in abstract form at the Scientific Meeting of the International Society for Magnetic Resonance in Medicine, Berlin, Germany, 19–25 May 2007; at the 67th Scientific Sessions of the American Diabetes Association, Chicago, Illinois, 22–26 June 2007; at the Perugia 2007 Hypoglycemia Symposium, Perugia, Italy, 12–15 May 2007; and at the 69th Scientific Sessions of the American Diabetes Association, New Orleans, Louisiana, 5–9 June 2009.

We thank the nurses and medical assistants of the GCRC for their enthusiastic support of the glucose infusion studies, the staff of the CMRR for maintaining and supporting the NMR system, Gregor Adryan for invaluable help with the NMR coil, and Alexander Shestov, Felipe Barros, and Gerry Dielen for discussions and comments on our work.

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