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Effects of hyperglycemia and effects of ketosis on cerebral perfusion, cerebral water distribution, and cerebral metabolism.

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Diabetic ketoacidosis (DKA) may cause brain injuries in children. The mechanisms responsible are difficult to elucidate because DKA involves multiple metabolic derangements. We aimed to determine the independent effects of hyperglycemia and ketosis on cerebral metabolism, blood flow, and water distribution. We used magnetic resonance spectroscopy to measure ratios of cerebral metabolites (ATP to inorganic phosphate [Pi], phosphocreatine [PCr] to Pi, N-acetyl aspartate [NAA] to creatine [Cr], and lactate to Cr) and diffusion-weighted imaging and perfusion-weighted imaging to assess cerebral water distribution (apparent diffusion coefficient [ADC] values) and cerebral blood flow (CBF) in three groups of juvenile rats (hyperglycemic, ketogenic, and normal control). ATP-to-Pi ratio was reduced in both hyperglycemic and ketogenic rats in comparison with controls. PCr-to-Pi ratio was reduced in the ketotic group, and there was a trend toward reduction in the hyperglycemic group. No significant differences were observed in NAA-to-Cr or lactate-to-Cr ratio. Cortical ADC was reduced in both groups (indicating brain cell swelling). Cortical CBF was also reduced in both groups. We conclude that both hyperglycemia and ketosis independently cause reductions in cerebral high-energy phosphates, CBF, and cortical ADC values. These effects may play a role in the pathophysiology of DKA-related brain injury. Diabetes 61:1831–1837, 2012

Cerebral injury caused by diabetic ketoacidosis (DKA) is an important complication of type 1 diabetes in children. Approximately 1% of children with DKA develop severe, life-threatening cerebral edema and cerebral injury (1). Many more children with DKA, however, develop subtle cerebral edema, which can be demonstrated using magnetic resonance diffusion-weighted imaging (2). Magnetic resonance spectroscopy (MRS) studies in these children show metabolic changes consistent with cerebral injury (3). Recent data also suggest that memory deficits are common in children with diabetes who have experienced DKA, suggesting that subtle cerebral injury may occur frequently (4).

Despite the growing evidence that DKA frequently results in subtle brain injury in children, relatively little is understood about the pathophysiology of brain injury in this setting. Interestingly, magnetic resonance data from animal and human studies demonstrate that DKA is associated with initial cytotoxic cerebral edema, progressing to vasogenic edema during treatment with insulin and saline (2,5,6). In addition, DKA has been shown to be associated with diminished cerebral blood flow (CBF) in animal models (5,6). These findings are similar to those observed in hypoxic/ischemic brain injury. Although some decline in CBF likely results from hypocapnia and circulatory volume depletion during DKA, it is not clear that these disturbances alone are sufficient to cause cerebral injury. Whether other aspects of DKA contribute to cerebral injury by augmenting the reduction in CBF or by compromising cerebral metabolism in other ways is therefore important to investigate. In the current study, we aimed to determine the effects of hypoinsulinemic hyperglycemia and hyperinsulinemic ketosis on CBF, cerebral water distribution, and cerebral metabolism. We hypothesized that effects of one or both of these conditions might alter CBF and/or cerebral metabolism. If our hypothesis is correct, one or both of these conditions might predispose the brain to injury when other conditions (such as severe hypocapnia and/or circulatory volume depletion) are superimposed.

RESEARCH DESIGN AND METHODS

Hyperglycemia model. Juvenile rats (4–6 weeks of age) were administered an intraperitoneal injection of streptozotocin (100 mg/kg for rats <100 g; 130 mg/kg for rats >100 g). Rats were given unlimited access to water with 10% dextrose (D10 W; Fisher Scientific, Santa Clara, CA) in the first 24-h period after streptozotocin injection to prevent hypoglycemia and were subsequently allowed unlimited access to tap water and standard rat chow. Twenty-four hours after streptozotocin injection, rats were treated with subcutaneous insulin (Novolin 70/30 insulin: 3 units daily for rats <100 g, 4 units daily for rats 100–200 g, and 5 units daily for rats >200 g) for a period of 5 days to allow for resolution of any nonpancreatic toxicities of streptozotocin. Urine glucose and ketocids (acetoacetate) were measured daily using Multistix urinalysis strips (Bayer, Santa Clara, CA) up to and including the day of imaging. Animals developing abnormal urine acetocacetate concentrations (above “trace” levels on urinalysis strips: 0.5 mmol/L) at any time prior to imaging were not used for the hyperglycemia model. Serum insulin concentrations were measured by radioimmunoassay (Millipore Rat Insulin RIA; Millipore, Billerica, MA). One day prior to magnetic resonance studies, rats received no insulin treatment and drinking water was changed to D10 W to promote hyperglycemia. Both this protocol and the protocol for the ketosis model (see below) were conducted in accordance with the Animal Use and Care Guidelines issued by the National Institutes of Health using protocols approved by the animal use and care committee at the University of California, Davis.

Ketosis model. As in the hyperglycemia model, juvenile rats (4–6 weeks) were used for this protocol. Rats were given a diet consisting of 50% standard rat chow (LabDiet 5001; Commercial Chow, Richmond, IN) and 50% high-fat diet (category no. D12492, OpenSource Diets, 60% fat; Research Diets) for a period of 5 days. For an additional 5 days, rats consumed only the high-fat diet. Twelve hours prior to imaging, rats were fasted and allowed access only to tap water. Using this protocol, we were able to reliably generate ketosis (β-hydroxybutyrate [βOH] concentration >1.0 mmol/L) measured using Precision Xtra blood ketone test (Abbott Laboratories, Abbott Park, IL). Rats that failed to develop the previously specified level of ketosis were not used in the study.
Diffusion-weighted imaging and perfusion-weighted imaging procedures. Rats in both groups were anesthetized using Na pentobarbital (65 mg/kg i.p.), and then the left femoral vein and artery were cannulated with PE-50 polyethylene tubing as previously described (7). The femoral vein cannula was used for additional anesthesia as needed. The femoral artery cannula was used for blood sampling. A heating pad with circulating water (Gaymar, Orchard Park, NY) was used to maintain body temperature at 36.8–37.0°C throughout surgery and magnetic resonance studies. Rats were also subjected to tracheal intubation and ventilated (Harvard Small Animal Ventilator, Holliston, MA) throughout surgery and magnetic resonance studies. Ventilation was done to offset the tendency toward respiratory depression in the anesthetized rats and to assure similarity to previous studies of rats with DKA conducted by our group. Blood samples were taken for analysis of Pco2 and pH immediately after intubation and the respiratory rate and tidal volume adjusted to maintain the Pco2 level within the normal range. Magnetic resonance diffusion-weighted spin echo images were acquired using a 7-Tesla Bruker Biospec MRS/magnetic resonance imaging (MRI) system as previously described (7). Apparent diffusion coefficient (ADC) values (10−6 cm2/s) were determined from 6 × 4 pixel regions of interest for eight brain regions (six cortex and two striatum) using Paravision 4.0 software with four gradient strengths of 5–95 mT/m. In each rat, CBF (milliliters per 100 grams per second) was also determined, using perfusion-weighted imaging analysis with continuous arterial spin labeling (ASL) and a standard Bruker PERFPACK2 protocol (Bruker, Billerica, MA). ASL data were acquired using the same field of view and slice thickness as used for diffusion-weighted spin echo images, and the ASL regions of interest were chosen from the 128 × 128 matrix so as to measure CBF and ADC on the same voxels (7.5). Images from perfusion-weighted imaging were acquired in 4.37 min using echo time (TE)/repetition time (TR) 12.77 ms/1,023 ms with a 1-s Adiabatic-Fast-Passage labeling pulse in the presence of a 10 mT/m gradient to obtain inversion ±3.515 Hz (±2 cm) from the isocenter (also slice center) for control and labeled images, respectively. T1 maps for the same voxels were also acquired from selected rats in each treatment group to correct CBF measurements for possible location- and treatment-dependent variations in T1.

MRS procedures. MRS measurements were performed in anesthetized rats in a horizontal bore magnet (Oxford Instruments, Oxford, U.K.) using a two-channel Biospec system (Bruker Biospin, Billerica, MA) running Paravision software. A double-tuned 1H/Letz coil (Doty Scientific, Columbia, SC) was used, with X is tunable for 23 Na or 31P. Field homogeneity was optimized by localized shimming on 1H over a 9 × 9 × 9 mm voxel of interest (VOI). The VOI was positioned inside the brain and selected to encompass as much of the cortex as possible. After shimming, 31P and 1H MRS data were acquired.

**Hyperglycemia and ketosis models.** Using the procedures described, substantial hyperglycemia was generated in the hyperglycemic rat model, with mean blood glucose concentrations of ~28 mmol/L (Tables 1 and 2). Daily urine ketone testing after administration of streptozotocin confirmed absence of ketosis in the hyperglycemic group. Of note, some rats in the hyperglycemic group had slightly decreased serum bicarbonate concentrations consistent with mild renal tubular acidosis (4 of 16 total rats used in the hyperglycemia studies; serum bicarbonate concentration range 16–19 mmol/L). This effect was attributed to renal toxicity of streptozotocin (11). Comparison of magnetic resonance measures in hyperglycemic rats with and without mild renal tubular acidosis (RTA), however, revealed no significant effect of mild RTA on the measures of interest (data not shown); therefore, results from all hyperglycemic rats were included in the data reported.

In the ketogenic group, blood ketone concentrations achieved were within a range consistent with moderate ketosis in children with diabetes (Tables 1 and 2). Ketotic rats had normal blood pH and Pco2 (factors that could independently affect CBF). Serum insulin concentrations in both the hyperglycemic group (0.13 ± 0.11 ng/mL, n = 6) and the ketogenic group (1.16 ± 0.44 ng/mL, n = 6) were significantly lower than in the control group (2.52 ± 1.13 ng/mL, n = 11; P < 0.01 for hyperglycemia vs. control, P = 0.015 for ketosis vs. control, P = 0.12 for hyperglycemia vs. ketosis).

Cerebral blood flow. In both the hyperglycemic group and the ketogenic group, there were significant reductions in CBF in the cerebral cortex in comparison with the control group (Fig. 1A). In the striatum, there was a trend toward reduction in CBF in both groups in comparison with controls, but these differences were just short of statistical significance.

Cerebral water distribution. Using diffusion-weighted imaging, we observed a significant reduction in ADC in

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**TABLE 1**

| Value                        | Hyperglycemia | Ketosis | Control | P     |
|------------------------------|---------------|---------|---------|-------|
| n                            | 9             | 10      |         |       |
| Glucose (mmol/L)             | 30.0 (7.8)    | 6.1 (1.2) | 7.9 (1.9) | <0.001 hyperglycemia vs. control, 0.71 ketosis vs. control |
| βOHb (mmol/L)                | —             | 2.1 (0.9) |         |       |
| pH                           | 7.37 (0.07)   | 7.38 (0.04) | 7.42 (0.05) | 0.08 hyperglycemia vs. control, 0.19 ketosis vs. control |
| Pco2 (mmHg)                  | 36 (7)        | 37 (4)  | 38 (6)  | 0.58 hyperglycemia vs. control, 0.97 ketosis vs. control |
| Serum sodium (mmol/L)        | 142 (6)       | 140 (4) | 141 (4) | 0.76 hyperglycemia vs. control, 0.98 ketosis vs. control |
| Serum chloride (mmol/L)      | 109 (7)       | 107 (3) | 107 (4) | 0.53 hyperglycemia vs. control, 1.0 ketosis vs. control |
| Serum bicarbonate (mmol/L)   | 21 (3)        | 24 (2)  | 27 (3)  | <0.01 hyperglycemia vs. control, 0.12 ketosis vs. control |

Data are means (SD) unless otherwise indicated.
TABLE 2
Biochemical values in hyperglycemic and ketotic rats versus control rats used for MRS studies

|                      | Hyperglycemia | Ketosis | Control | P               |
|----------------------|---------------|---------|---------|-----------------|
| Glucose (mmol/L)     | 26.6 (7.4)    | 6.6 (1.1)| 7.8 (1.5)| <0.001 hyperglycemia vs. control, < 0.001 ketosis vs. control |
| βOHb (mmol/L)        | —             | 2.0 (0.6)| —       | —               |
| pH                   | 7.36 (0.08)   | 7.39 (0.06)| 7.42 (0.06)| 0.25 hyperglycemia vs. control, 0.65 ketosis vs. control |
| Pco2 (mmHg)          | 41 (4)        | 39 (6)  | 41 (5)  | 0.95 hyperglycemia vs. control, 0.89 ketosis vs. control |
| Serum sodium (mmol/L)| 142 (6)       | 142 (2) | 139 (4) | 0.45 hyperglycemia vs. control, 0.61 ketosis vs. control |
| Serum chloride (mmol/L) | 108 (7)     | 107 (2) | 106 (4) | 0.78 hyperglycemia vs. control, 0.91 ketosis vs. control |
| Serum bicarbonate (mmol/L) | 24 (3)      | 24 (2)  | 27 (3)  | 0.17 hyperglycemia vs. control, 0.17 ketosis vs. control |

Data are means (SD) unless otherwise indicated.

the cerebral cortex, indicating cytotoxic cerebral edema, in both the hyperglycemic group and the ketotic group in comparison with the control group (Fig. 1B). In the striatum, the differences in ADC in comparison with control values were significant only in the hyperglycemic group.

**Cerebral metabolism.** Using phosphorus MRS, we detected significant reductions in brain ratios of PCr to Pi in the ketotic group and a trend toward reduction in the hyperglycemic group in comparison with controls (Fig. 2). Reductions in brain ATP-to-Pi ratios were significant in both the hyperglycemic group and the ketotic group in comparison with controls. Brain intracellular pH was not significantly different among the three groups. Using proton MRS, we did not detect any significant differences among the groups in brain ratios of NAA to Cr or any differences in brain lactate levels.

**DISCUSSION**

Cerebral injury caused by DKA shares several common features with hypoxic-ischemic brain injury. Early descriptions of postmortem findings in children who died of DKA-related brain injury showed features suggestive of ischemia (12,13). More recent studies demonstrate that DKA results in cytotoxic cerebral edema (cell swelling) progressing to vasogenic cerebral edema (increased extracellular fluid) during treatment with insulin and saline, a pattern similar to that observed in ischemia-reperfusion injury (2,7). Furthermore, brain NAA-to-Cr ratios are diminished in DKA and levels of high-energy phosphates are reduced, similar to findings in hypoxic-ischemic brain injury (5,14,15).

We have previously demonstrated that CBF is reduced in DKA, but the cause of this reduction is not fully understood (6). Cerebral vasoconstriction induced by hypoxia and intravascular volume depletion may account for some reduction in CBF, but the degree of cerebral injury that occurs in at least some children is greater than might be expected to result from these conditions alone. It is possible that other conditions present during DKA might contribute to reducing CBF. In addition, the decrease in CBF observed during DKA is modest in comparison with that typically observed in stroke or other hypoxic-ischemic conditions, raising the question of whether some other aspect of DKA might augment the severity of injury caused by moderate CBF reductions. In the current study, we demonstrate that both hyperglycemia and ketosis are associated with reductions in cortical CBF. In addition, both conditions were associated with reduced brain levels of high-energy phosphates and reduced ADC values that could result from disturbances in membrane ion homeostatic mechanisms. These data suggest that hyperglycemia and/or ketosis may increase the vulnerability of the brain to injury.

Substantial data from previous studies in both humans and animal models demonstrate an association between hyperglycemia and worsening of hypoxic-ischemic brain injury (16–20). In animal studies of stroke, hyperglycemia is associated with increases in infarct volume, edema formation, neutrophil deposition in ischemic tissue, and lactate accumulation (19,21–23). Hyperglycemia is associated with impaired vascular endothelial function resulting in decreased vasodilation (24), as well as with decreased erythrocyte deformability and alterations in blood flow properties that might contribute to ischemia (25,26). Reduced CBF during hyperglycemia has also previously been reported in rats (27). Several studies suggest an association between hyperglycemia and increased morbidity and mortality from ischemic stroke (28–30), and some studies suggest that hyperglycemia may directly cause neuronal injury.

In rats, hyperglycemia (streptozotocin-induced diabetes) has been found to result in alterations in neuronal structure and impaired memory (31,32).

The effects of ketosis in the setting of brain injury have been studied less extensively than the effects of hyperglycemia. In one study, acetoacetate was found to increase production of the vasoconstrictor endothelin-1 in brain microvascular endothelial cells (33). In addition, elevated concentrations of acetoacetate have been found to increase erythrocyte viscosity (34). In apparent contrast to these findings, however, some studies in rodents suggest a neuroprotective effect of ketosis in the setting of hypoxic-ischemic injury or traumatic injury (35–37). The mechanism for this apparent protective effect has been proposed to be related to diminished glycolytically-derived lactate production as well as decreased free radical generation (36). Conversely, studies of long-term effects of ketogenic diets suggest possible detrimental effects on memory and other cognitive functions in both rats and humans (38,39). Thus, the effects of ketosis are unclear and may vary in different physiologic settings. Our results demonstrate that ketosis (induced by a ketogenic diet) reduces CBF and decreases brain levels of high-energy phosphates, which would appear to suggest adverse consequences. In apparent contrast, a study of ketone infusion in adult rats found an increase in CBF—a finding one might not predict from our results (40). The reason for the variation in results between those data and our data is unclear. The ketone infusion model induces metabolic alkalosis, and it is possible that alterations in acid-base balance may have affected CBF. Differences in insulin
A cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) in hyperglycemic and ketotic rats compared to control rats. 

**A** CBF in hyperglycemic and ketotic rats compared to control rats measured using arterial spin labeling (means ± SE for 10 ketotic, 10 control, and 9 hyperglycemic rats). Cortex: \( P = 0.03 \) hyperglycemic vs. control, \( P = 0.02 \) ketotic vs. control. Striatum: \( P = 0.06 \) hyperglycemic vs. control, \( P = 0.07 \) ketotic vs. control. B: Apparent diffusion coefficients in hyperglycemic rats and ketotic rats compared to control rats (means ± SE for 10 ketotic and 10 control rats and 12 hyperglycemic rats). Cortex: \( P = 0.004 \) hyperglycemic vs. control, \( P = 0.012 \) ketotic vs. control. Striatum: \( P = 0.019 \) hyperglycemic vs. control, \( P = 0.46 \) ketotic vs. control.

Conclusions: Differences in cerebral blood flow and diffusion coefficients between hyperglycemic and ketotic rats compared to control rats. The current study has some limitations. The level of ketosis achieved in the ketotic rats was lower than typically observed in children with DKA. Although greater ketosis could have been achieved with an intravenous ketone infusion, the fluid volume required for this infusion would cause changes in circulatory volume and thereby confounding effects on measures of CBF. In addition, higher ketone concentrations could result in metabolic acidosis with compensatory hypocapnia causing alterations in CBF. We therefore opted to evaluate more moderate ketosis and to use dietary manipulations to avoid altering circulatory volume. It is possible that the effects of ketosis might have been greater if higher concentrations of ketone bodies could have been achieved. In addition, several rats in the hyperglycemic group had mild metabolic acidosis, likely reflecting renal tubular acidosis resulting from nephrotoxic effects of streptozotocin treatment (11). We did not, however, detect any significant differences in magnetic resonance measures in hyperglycemic rats with or without RTA, and the degree of acidosis in all rats was mild and unlikely to cause any substantial physiological alterations.

In summary, our results suggest that hyperglycemia and ketosis both result in declines in CBF, reductions in cerebral high-energy phosphate concentrations, and brain cell swelling. The reductions in CBF resulting from hyperglycemia and/or ketosis help to explain the occurrence of cerebral hypoperfusion during DKA that appears to be greater than would be expected to result from hypocapnia and circulatory volume depletion alone. Furthermore, the apparently adverse effects of these conditions on cerebral metabolism might cause the brain to be more vulnerable to injury during modest hypoperfusion associated with DKA. Finally, the similarity in cerebral effects of hyperglycemia and ketosis raises the intriguing possibility that low insulin concentrations might be a predicating factor common to
FIG. 2. Cerebral metabolites in hyperglycemic rats and ketotic rats in comparison with control rats measured using proton and phosphorus MRS (means ± SE; n = 7 for all groups). A: PCr-to-Pi ratio. B: ATP-to-Pi ratio. C: Intracellular (Int) pH. D: Lactate (Lac)-to-Cr ratio. E: NAA-to-Cr ratio.
both protocols or conditions. This hypothesis will require further investigation in future studies.

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N.G. planned and supervised studies, analyzed data, wrote the manuscript, and secured funding for studies. C.N. conducted studies, assisted with data entry and analysis, reviewed and revised the manuscript, and contributed to discussion. S.A. supervised and assisted with technical aspects of studies related to MRI, assisted with data interpretation, reviewed and revised the manuscript, and contributed to discussion. N.Y. conducted studies, assisted with data entry and analysis, reviewed and revised the manuscript, and contributed to discussion. A.T. conducted studies, assisted with data entry, reviewed and revised the manuscript, and contributed to discussion. M.O. planned and supervised studies, assisted with data analysis and interpretation, and reviewed and revised the manuscript. N.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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