Glucose-Associated Alterations in Ischemic Brain Metabolism of Neonatal Piglets

Abbot R. Laptook, MD; Ron J.T. Corbett, PhD; Orlando Arencibia-Mireles, MS; and Joan Ruley, MS

Background and Purpose: During global brain ischemia or hypoxia-ischemia in adults, hyperglycemia is deleterious to the brain. In contrast, similar adverse effects have not been found in neonatal animals. This investigation examined neonatal piglets to determine if there were specific alterations of ischemic brain metabolism associated with different systemic glucose concentrations and to potentially clarify the effects of hyperglycemia during ischemia in neonates.

Methods: Two groups of animals (n=12 in each group) were studied during partial ischemia to compare the effects of hyperglycemia (plasma glucose concentration, 258±97 mg% [mean±SD]) with modest hypoglycemia (plasma glucose concentration, 62 ±23 mg%). A broad spectrum of cerebral blood flow reduction was achieved by combining inflation of a cervical pressure cuff with varying degrees of hemorrhagic hypotension. High-energy phosphorylated metabolites, intracellular pH, and cerebral blood flow were simultaneously measured using a magnetic resonance spectroscopic technique. Brain metabolic variables (β-ATP, inorganic phosphorus, phosphocreatine, intracellular pH) were plotted as a function of blood flow reduction during partial ischemia for each group.

Results: During ischemia values of cerebral blood flow were comparably distributed between groups and ranged from 15% to 110% of those of control. At a given reduction of cerebral blood flow, hyperglycemic piglets maintained a higher concentration of β-ATP (p=0.011) and had a smaller increase in inorganic phosphorus (p<0.001). At cerebral blood flow <50% of control, the intracellular pH of piglets with modest hypoglycemia during partial ischemia was never reduced to <6.46, whereas intracellular pH fell as low as 5.97 for hyperglycemic animals.

Conclusions: ATP preservation may account for the differing effects of glucose during ischemia in neonates compared with adults, provided that the accentuated brain acidosis is not deleterious to neonatal brain tissue. (Stroke 1992;23:1504-1511)

KEY WORDS • cerebral ischemia • cerebral metabolism • glucose • nuclear magnetic resonance • pigs

In adults infusion of glucose or feeding results in excessive brain acidosis and lactate accumulation during ischemia,1,2 which is associated with adverse clinical neurological status,3 brain metabolism,4 and neuropathological5 outcomes after ischemia. Indeed, insulin administration or fasting before brain ischemia results in less brain acidosis and lactate accumulation and a more favorable outcome.3 In contrast, a deleterious effect of excessive substrate before ischemia has not been noted for newborn animals. For example, increasing carbohydrate stores of young rats via glucose administration led to a longer interval of gasping movements of the head after decapitation.6 Glucose pretreatment of newborn mice prolonged survival during anoxia.7 In 7-day postnatal rats treated with either glucose or saline before hypoxia-ischemia, there were no differences detected in neuropathological damage.8 Thus, the effects of systemic glucose concentration during ischemia or hypoxia-ischemia differ between neonates and adults.

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We have previously examined the relation between plasma glucose concentration and ischemic brain metabolism in newborn piglets using phosphorus-31 magnetic resonance spectroscopy (31P MRS).9 During partial brain ischemia, hyperglycemia was associated with increased brain acidosis. However, hyperglycemia was also associated with better maintenance of high-energy phosphorylated metabolites as evidenced by less of an increase in the hydrolytic product of ATP, inorganic phosphorus (Pi), compared with hypoglycemic animals. These observations suggest that the lack of detrimental effects and/or beneficial effects of substrate during neonatal ischemia may be attributable to a glucose-associated ATP preservation. This conclusion remains tenuous since cerebral blood flow (CBF) was not measured, and comparable reductions in CBF were assumed based on similar changes in perfusion pressure.
Thus, it is conceivable that brain metabolic effects attributable to glucose may reflect differences in CBF during ischemia. The purpose of the present investigation is to determine if elevated plasma glucose concentration is associated with brain ATP preservation at known reductions in CBF.

Materials and Methods

This investigation was approved by the Institutional Review Board for Animal Research at the University of Texas Southwestern Medical Center. Twelve piglets (age, 7 ± 4 [mean ± SD] days; weight, 1.4 ± 0.5 kg) were reared with the sow until the morning of the study and were used to examine brain ischemia with hyperglycemia. To compare lower values of plasma glucose concentration during ischemia, another 12 piglets (age, 9 ± 4 days; weight, 1.6 ± 0.5 kg) were removed from the sow and fasted for 24–48 hours with free access to water. Surgical preparation of neonatal piglets consisted of premedication with ketamine (10 mg/kg i.m.) and inhalation of 70% N2O and 30% O2 to facilitate placement of a 3.5-mm endotracheal tube via tracheostomy. Mechanical ventilation was initiated, and using local anesthesia (1% xylocaine) intravascular catheters were positioned in the distal aorta and inferior vena cava, and two catheters were placed in the left common carotid artery, one directed caudad and the other cephalad. The left external carotid artery was ligated, the scalp was retracted, and a blood pressure cuff was positioned around the neck. It has been established previously that unilateral common carotid artery catheterization and ligation have no effect on blood flow to either side of piglet brain over a wide range of cerebral perfusion rates.10 Animals were immobilized with d-tubocurarine chloride, and analgesia was provided by intravenous nubain (0.1 mg/kg). Piglets were wrapped in a heating blanket to maintain rectal temperature at 38.5°C, positioned supine with the head resting on two rectangular concentric radiofrequency coils (outer coil, 5 × 4 cm; inner coil, 4.5 × 3.5 cm), and placed in a 40-cm-diameter-bore Oxford superconducting magnet (General Electric, CSI system) operating at 4.7 T. A 90-minute stabilization period started after positioning in the magnet.

After stabilization, baseline magnetic resonance (MR) and physiological measurements were determined for all animals during a control period. Brain ischemia was then achieved by inflation of a cervical pressure cuff to 300 mm Hg combined with varying degrees of hemorrhagic hypotension (5–35 ml/kg whole blood removed) to induce a spectrum of blood flow reduction. Once the desired mean arterial pressure (MAP) was achieved, ischemia was maintained for 10 minutes and MR and physiological measurements were acquired over the last 5 minutes. An intravenous glucose infusion (10% dextrose in water) was administered during ischemia to the 12 piglets reared with the sow to induce hyperglycemia; they will be referred to as “fed” animals. The 12 fasted piglets were given insulin (2 units/kg i.v.) after control measurements and observed for 40–60 minutes until the plasma glucose concentration was reduced; they will be referred to as “fasted” animals. Physiological and MR measurements were repeated during hypoglycemia for fasted animals. Brain ischemia was then induced in an identical fashion as described above with physiological and MR measurements obtained during the last 5 minutes of ischemia. During ischemia fasted piglets were infused with NaCl in a volume and osmolarity (0.3 M) similar to those of the glucose infusion. This experimental design was used to study two groups of piglets with distinct plasma glucose concentration during brain ischemia. We have not studied a group of piglets with normoglycemia since prior investigation in this laboratory demonstrated that it is exceedingly difficult to rapidly regulate plasma glucose concentration to normoglycemic levels when piglets are subjected to hemorrhagic hypotension.9 During each experiment the ventilator rate was adjusted to maintain arterial isocapnia.

Blood samples were obtained from the carotid artery (directed caudad) for blood gases, pH, O2 content, and plasma concentrations of glucose and lactic acid as previously described.9 Determination of CBF and phosphorylated metabolites coincided with blood sampling. To measure CBF, deuterium (2H) MRS was used to measure 2H2O clearance from tissues.11 We have previously demonstrated a linear correlation between CBF measured using microspheres and 2H MRS for CBF between 0–200 ml·min⁻¹·100 g⁻¹ as determined by microspheres.12 Furthermore 2H MRS can be combined with 31P MRS to simultaneously measure CBF and phosphorylated metabolites from the same volume of tissue.12 We have recently applied this technique to investigate the effects of repeated intervals of brain ischemia; the results of the 12 fed piglets represent a portion of this work.13 The two concentric radiofrequency coils were tuned to 80 (31P) and 31 (2H) MHz. For 31P, a 200-μsec excitation pulse, 256-msec acquisition time, 4,000–Hz sweep width, and 2,048 data points per free induction decay (FID) were used. For 2H, a 170-μsec excitation pulse, 64-msec acquisition time, 2,000–Hz sweep width, and 256 data points per FID were used. Phosphorus-31 and 2H transients were acquired and saved alternately into individual blocks of computer memory, and the total time to obtain 100 pairs of 31P and 2H FIDs was 5 minutes (delay time between individual 31P and 2H excitation pulses was 3 seconds). For each CBF determination, four pairs of 31P and 2H FIDs were collected to assess the 2H MR background, and a 1-ml bolus of 2H2O reconstituted as 0.9% NaCl followed by 0.6 ml 0.9% NaCl flush was administered to the brain via the catheter placed in the common carotid artery directed cephalad. The clearance rate of 2H2O from brain was determined by following the decrease in 2H MR signal, recorded at 3-second intervals, alternately with 31P MR data collection. For each series of 2H MR spectra, the area–time data sets were analyzed to calculate the exponential rate constant (k) for clearance. The CBF (ml·100 g⁻¹·min⁻¹) was calculated by applying the central volume principal14 CBF=100Akb, where λ, the partition coefficient, was previously determined to equal 0.85±0.07 ml/g for neonatal piglets.12

Phosphorus-31 data were processed by applying left shift removal of the first three data points in the accumulated FID, exponential multiplication corresponding to 20-Hz line broadening, Fourier transformation, and baseline straightening using an interpolation routine. The data analysis package nmr1 (New Methods Research Inc., Syracuse, N.Y.) was used to analyze 31P.
MRS by peak height analysis. Intracellular pH (pHi) was calculated from the chemical shift of the Pi resonance peak relative to the phosphocreatine (PCr) peak using the following equation\textsuperscript{15}:

\[pHi=6.683+\log_{10}\left(\frac{X-3.153}{5.728-X}\right)\]

where X refers to the chemical shift of the Pi peak. Based on work with phantoms in this laboratory, we estimate that the majority of MRS signal is derived from the first 1 cm of cerebral cortex underlying the coil. This represents a composite of gray and white matter from the frontal, parietal, and occipital cerebral cortex.

Systemic physiological and biochemical results at control and during ischemia were compared between the two study groups using independent samples t tests. A paired t test or Wilcoxon-Mann-Whitney rank test was used to examine the effects of insulin before ischemia on systemic and brain variables in fasted animals. Linear regression was used to determine the relation between MAP and CBF (expressed as a percentage of control) during ischemia; combining results of inflation of a cervical pressure cuff and hemorrhagic hypotension. The efficacy of this method to induce a spectrum of partial ischemia was demonstrated by the regression between MAP and plasma lactate concentration were unchanged from control, but arterial pH decreased (7.29±0.05, p<0.001) and Paco2 rose (41.2±2.6 mm Hg, p<0.001) compared with control. These results suggest increased CO\textsubscript{2} production from tissue utilization of glucose since respirator settings, plasma lactate concentration, and calculated bicarbonate concentration were unchanged. Compared with control, hypoglycemia was associated with a significant elevation (36%) in CBF (49±13 ml · 100 g\textsuperscript{-1} · min\textsuperscript{-1}, p<0.001) but an unchanged pH (7.03±0.08) and no alteration in the peak heights of the β-ATP and PCr resonance peaks. Hypoglycemia was associated with a modestly elevated Pi resonance peak (25±26%, p<0.05, Wilcoxon-Mann-Whitney rank test) compared with control.

Partial brain ischemia was achieved by a combination of inflation of a cervical pressure cuff and hemorrhagic hypotension. The efficacy of this method to induce a spectrum of partial ischemia was demonstrated by the regression between MAP and CBF (expressed as a percentage of control) during ischemia; combining re-

### Results

Results of systemic physiological and biochemical variables for both groups of piglets are listed in Table 1. At control, both fasted and fed groups had the same MAP, pH, blood gases, and plasma lactate concentration. Fasted piglets before induction of hypoglycemia had a significantly elevated hematocrit and thus a higher O\textsubscript{2} content. The latter group differences are unexplained. The difference in plasma glucose concentration between groups at control reflects the interval of fasting for one group of animals. CBF, O\textsubscript{2} delivery, and brain metabolic variables for both groups at control are listed in Table 2. CBF was higher in the fed animals (p=0.08) and presumably reflects the group differences in hematocrit and O\textsubscript{2} content. Accordingly calculated O\textsubscript{2} delivery (product of CBF and O\textsubscript{2} content) was similar between groups. To compare brain metabolism of the groups at control, ratios rather than absolute resonance peak heights were examined due to variation in the scaling of spectra obtained from different animals. The groups were comparable for PCr/β-ATP and Pi/β-ATP in addition to pH.

Before ischemia, hypoglycemia was achieved in fasted animals by administration of insulin (2 units/kg i.v.). Forty minutes after insulin injection the plasma glucose concentration was reduced to 33±12 mg% (range, 13–56 mg%; p<0.001 versus control). During hypoglycemia MAP and plasma lactate concentration were unchanged from control, but arterial pH decreased (7.29±0.05, p<0.001) and Paco2 rose (41.2±2.6 mm Hg, p<0.001) compared with control. These results suggest increased CO\textsubscript{2} production from tissue utilization of glucose since respirator settings, plasma lactate concentration, and calculated bicarbonate concentration were unchanged. Compared with control, hypoglycemia was associated with a significant elevation (36%) in CBF (49±13 ml · 100 g\textsuperscript{-1} · min\textsuperscript{-1}, p<0.001) but an unchanged pH (7.03±0.08) and no alteration in the peak heights of the β-ATP and PCr resonance peaks. Hypoglycemia was associated with a modestly elevated Pi resonance peak (25±26%, p<0.05, Wilcoxon-Mann-Whitney rank test) compared with control.

### Table 1. Systemic Physiological and Biochemical Results in Fed and Fasted Piglets During Control and Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Fasted</th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>87±10</td>
<td>90±18</td>
<td>53±24</td>
<td>60±35</td>
</tr>
<tr>
<td>pH</td>
<td>7.37±0.04</td>
<td>7.36±0.05</td>
<td>7.34±0.03</td>
<td>7.26±0.08*</td>
</tr>
<tr>
<td>PaO\textsubscript{2} (mm Hg)</td>
<td>150±31</td>
<td>151±36</td>
<td>134±44</td>
<td>131±46</td>
</tr>
<tr>
<td>PaCO\textsubscript{2} (mm Hg)</td>
<td>36.9±3.1</td>
<td>36.1±2.8</td>
<td>35.8±4.7</td>
<td>39.3±7.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>30±6</td>
<td>39±5t</td>
<td>27±3</td>
<td>37±6†</td>
</tr>
<tr>
<td>O\textsubscript{2} content (vol%)</td>
<td>11.4±2.7</td>
<td>15.9±1.9†</td>
<td>10.1±1.4</td>
<td>14.2±1.8†</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>134±38</td>
<td>102±19*</td>
<td>258±97</td>
<td>62±23†</td>
</tr>
<tr>
<td>Lactic acid (mM)</td>
<td>1.6±0.4</td>
<td>1.8±0.7</td>
<td>3.5±2.0</td>
<td>3.2±1.1</td>
</tr>
</tbody>
</table>

Values are mean±SD. Comparisons are between groups during control and during ischemia. *p<0.025; †p<0.001.

### Table 2. Cerebral Blood Flow, O\textsubscript{2} Delivery, and Brain Metabolic Variables in Fed and Fasted Piglets During Control

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow (ml · 100 g\textsuperscript{-1} · min\textsuperscript{-1})</td>
<td>44±11</td>
<td>36±10</td>
</tr>
<tr>
<td>O\textsubscript{2} delivery (mmol · 100 g\textsuperscript{-1} · min\textsuperscript{-1})</td>
<td>0.22±0.05</td>
<td>0.25±0.06</td>
</tr>
<tr>
<td>Intracellular pH</td>
<td>6.97±0.04</td>
<td>7.00±0.04</td>
</tr>
<tr>
<td>Phosphocreatine/β-ATP</td>
<td>1.34±0.21</td>
<td>1.47±0.39</td>
</tr>
<tr>
<td>Inorganic phosphorus/β-ATP</td>
<td>0.67±0.10</td>
<td>0.58±0.16</td>
</tr>
</tbody>
</table>

Values are mean±SD. No significant differences between groups were observed.
FIGURE 1. Representative in vivo phosphorus-31 and deuterium magnetic resonance spectroscopy (MRS) from brain during control (top panel) and partial ischemia (bottom panel). Horizontal axis of $^{31}$P spectra is chemical shift in parts per million, and vertical dimension of both $^{31}$P and $^2$H results represents relative peak intensity. Seven $^{31}$P resonance peaks are present at control and are identified from left to right as follows: phosphomonoester, inorganic phosphorus, phosphodiester, phosphocreatine, and $\gamma$, $\alpha$, and $\beta$ peaks of nucleotide triphosphate. Typical peak height signal-to-noise ratio for $^1$-ATP at control was 10 to 1. The $^2$H MRS results represent decrease in $^2$H$_2$O MRS peak intensity (expressed as relative scale) measured over time after intracarotid injection of 1 ml $^2$H$_2$O reconstituted as normal saline.

suits for both groups gave a linear correlation ($r=0.84, p<0.001, n=24$) and was described by the regression line $\text{CBF}=10.4+0.73\ MAP$. Thus, when the cervical cuff was inflated graded reductions in CBF were directly correlated with the extent of MAP decrease. During ischemia differences persisted between the two groups in hematocrit and O$_2$ content, and the desired differences in plasma glucose concentrations were achieved (Table 1). An example of interleaved $^2$H and $^{31}$P MRS during control and ischemia acquired from one piglet is illustrated in Figure 1. Changes induced by ischemia were reduction in the resonance peak heights of ATP and PCr, an increase in the Pi resonance peak height, and a narrowing of the chemical shift difference between the Pi and PCr resonance peaks. The change in peak heights of the $^2$H resonance peaks during ischemia was slower compared with control, indicating a reduced CBF.

The effect of plasma glucose concentration on the relation between CBF and brain metabolism during ischemia is plotted in Figures 2 and 3. For this analysis CBF and brain metabolic variables ($\beta$-ATP, Pi, PCr) were expressed as percentage of control values. The method used to induce partial brain ischemia resulted in a spectrum of reduced CBF to as low as 15% of control. Data from both groups appear to be comparably distributed over the range of reduced CBF. As indicated in Table 3, group comparisons for $\beta$-ATP and Pi are not significant. However, CBF is a significant covariate for $\beta$-ATP ($p<0.001$) and Pi ($p<0.001$). When group means are adjusted for CBF as a covariate there are significant differences between groups for $\beta$-ATP ($p=0.011$) and Pi ($p<0.001$). Thus, at a given reduction of CBF, hyperglycemic piglets maintain a higher concentration of $\beta$-ATP and have a smaller increase in Pi. A group difference was not present for PCr ($p=0.66$) after covariate adjustment. When the effect of plasma glucose concentration was examined with respect to pH$_i$, significant differences between groups were not present ($p=0.10$). This occurred in spite of the fact that pH$_i$ was never reduced to <6.46 for hypoglycemic piglets, whereas pH$_i$ fell as low as 5.97 for hyperglycemic piglets. The absence of a statistical effect of glucose on pH$_i$ reflects the fact that prominent reductions in pH$_i$ did not occur until CBF was reduced to <50% of control. During ischemia, CBF reduction to values <50% of control was the same in the two groups (fed, $n=6$, 31±9% versus fasted, $n=6$, 29±14% of control). Values of pH$_i$ for this CBF reduction were 6.30±0.22...
and 6.51±0.06 in fed and fasted animals, respectively (p=0.051). Since group differences in hematocrit and O$_2$ content were present, the effect of plasma glucose concentration on the relation between cerebral O$_2$ delivery and brain metabolism ($\beta$-ATP, Pi) was also examined (Figure 4). After adjusting for the covariate O$_2$ delivery (Table 3), group differences for $\beta$-ATP (p=0.031) and Pi (p=0.003) were present. Thus, hyperglycemic piglets maintained a higher concentration of $\beta$-ATP and had a smaller increase in Pi at given reductions in cerebral O$_2$ delivery. Since O$_2$ delivery is derived from the product of CBF and O$_2$ content, these group differences are not unexpected.

**Discussion**

The results of this investigation indicated that over a broad range of reduced CBF in neonatal piglets, hyperglycemia was associated with ATP preservation when compared with modest hypoglycemia. Accordingly, the hydrolytic product of ATP catabolism, Pi, was increased to a smaller extent in hyperglycemic compared with hypoglycemic animals. These results suggest that during partial brain ischemia in neonates an elevated plasma glucose concentration may be beneficial for the maintenance of high-energy phosphorylated metabolites.

**Table 3. $\beta$-ATP and Inorganic Phosphorus During Ischemia Compared Between Fed and Fasted Piglets With and Without Adjustment for the Covariates Cerebral Blood Flow and O$_2$ Delivery**

<table>
<thead>
<tr>
<th>Observed mean</th>
<th>Cerebral blood flow as covariate</th>
<th>O$_2$ delivery as covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>% $\beta$-ATP*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>76.6±27.2 NS</td>
<td>79.4±15.2 $p=0.011$</td>
</tr>
<tr>
<td>Fasted</td>
<td>64.7±31.5 NS</td>
<td>61.9±15.2 $p=0.003$</td>
</tr>
<tr>
<td>% Inorganic phosphorus*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>224.5±133.4 NS</td>
<td>208.9±77.3 $p&lt;0.001$</td>
</tr>
<tr>
<td>Fasted</td>
<td>338.5±186 NS</td>
<td>354.1±77.3 $p&lt;0.001$</td>
</tr>
</tbody>
</table>

* $\beta$-ATP and inorganic phosphorus are expressed as a percentage of control.
The role of systemic substrate concentration in modulating the vulnerability of fetal, newborn, and adult brain to anoxia, hypoxia, and ischemia has been of keen interest because of potential implications for clinical management. A number of in vivo studies in neonatal animals have examined the influence of altered systemic substrate concentration on brain metabolism and suggested that glucose administration was associated with beneficial effects. Pretreatment of neonatal mice and rats with glucose prolonged survival during anoxia and extended the time to the onset of terminal gasping.6,7,19 Glucose pretreatment increased brain glucose concentration but did not alter the rate of use of brain high-energy phosphorylated compounds during anoxia, and it was associated with higher brain ATP concentration over the first 4 minutes of anoxia compared with NaCl-treated litters.7 Conversely, Vannucci et al20 reported a shortened anoxic survival for hypoglycemic rat pups associated with a more rapid decline in brain ATP concentration compared with normoglycemic animals. Although these reports support a critical role for the systemic glucose concentration during anoxia, none report CBF or systemic hemodynamics and thus do not distinguish alterations in brain metabolism and/or changes in cardiovascular integrity. The influence of systemic glucose concentration has been examined during neonatal hypoxia-ischemia and ischemia.21-23 In 7-day postnatal rats unilateral common carotid artery ligation with hypoxia resulted in a 75% reduction in ipsilateral CBF.21 Pretreatment with glucose or NaCl in this model revealed similar rates of cerebral glucose utilization and comparable reductions of ATP concentration.22 It has been suggested that the lack of ATP preservation during hyperglycemia in this model may reflect low activity of the glucose phosphorylating enzyme hexokinase.22,24 Whether similar maturational changes in hexokinase occur in piglets remains unknown. In lambs changes in plasma glucose concentration during preexisting ischemia had no effect on brain ATP concentration.25

In contrast to these prior reports, this is the first investigation to examine the effects of plasma glucose concentration on ischemic brain metabolism with simultaneous measurement of CBF. Thus, concerns over the comparability of the groups regarding the extent of brain ischemia have been obviated. Furthermore, a method of inducing brain ischemia has been used that provided a spectrum of CBF reduction and allowed determination of whether glucose-associated effects were present within a narrow or broad range of CBF reduction. As indicated by the control values of CBF, 3H MRS–derived measurements are lower than CBF measured using microspheres, as previously reported in this laboratory.21 Potential reasons for this discrepancy have been discussed previously.12 Furthermore, we have rigorously compared CBF measured using 2H MRS and microspheres and demonstrated a highly significant linear correlation between the two methods for microsphere CBF of <200 ml·100 g⁻¹·min⁻¹.12 Thus, 3H MRS can be used to quantify relative changes in CBF. It is apparent that the fasted animals experienced a longer interval between control and ischemia measurements to provide the necessary time to lower the plasma glucose concentration. We do not consider these different time intervals and thus different lengths of exposure to altered plasma glucose concentration of critical importance because our objective was to study different plasma glucose concentrations during ischemia. This is supported by the essentially unchanged brain energy state during hypoglycemia without ischemia in this study, and during hyperglycemia without ischemia (authors’ unpublished observations).

The observed glucose-associated ATP preservation may provide a rationale for maintaining elevated blood glucose concentrations during intervals of ischemia and hypoxia-ischemia. ATP is the critical regulator of cell function, and reduction in ATP concentration during ischemia and hypoxia-ischemia has been linked to a number of important mechanisms (cellular acid–base status, excitotoxins, free radical generation) that initiate a cascade of cellular events thought to be critical for the pathogenesis of brain injury.26,27 However, during ischemia hyperglycemia compared with modest hypoglycemia was associated with greater generation of ATP through anaerobic metabolism, which in turn was coupled to ATP hydrolysis and H⁺ production.28 Therefore, brain acidosis was exacerbated by hyperglycemia. Given the potential deleterious effects of profound brain acidosis as documented in adults,29 a firm conclusion cannot be reached regarding benefit of hyperglycemia during ischemia because the relative impact of changes in brain ATP concentration and pHᵢ on the development of neonatal brain damage remains to be determined. Furthermore, hypoglycemia in this investigation was achieved by a combination of fasting and insulin administration. Recent results in 7-day-old rat pups suggest that there are important differences in neuro-pathological outcome of hypoglycemic animals during hypoxia-ischemia depending on whether the hypoglycemia was insulin induced or secondary to fasting.30 Fasted animals showed a reduction in hypoxic–ischemic brain damage compared with insulin-treated animals, which presumably reflects the ability of perinatal brain to use alternate substrates such as ketone bodies, which were increased in fasted animals.

The observed substrate-associated ATP preservation may contribute to the differing effects of glucose during ischemia or hypoxia-ischemia in neonates compared with adults. Moreover, there is recent evidence that glucose supplementation after hypoxia-ischemia in neonatal rat pups is beneficial in limiting the extent of neuro-pathological changes.31 However, the dichotomy concerning effects of glucose on neonatal and adult brain may not be as clear as previously thought. Increasing numbers of reports have demonstrated that the effects of glucose on adult brain may vary depending on specific experimental conditions. For example, hyperglycemia worsened the outcome of focal thrombotic infarction when the ischemic area received collateral supply but was not detrimental when the infarct was produced by occlusion of an end field arterial bed.22,23 The effects of hyperglycemia on the evolution of ischemic brain damage may thus be critically dependent on the location of ischemic tissue, the extent of collateral circulation, and the timing of glucose administration. It is unclear whether these observations are of relevance to models of global neonatal brain ischemia as used in this study. There are significant regional differences in blood flow reduction even in our model of global ischemia.34 Further investigation will be
necessary to determine if neonates have any topographical characteristics of ischemic brain that parallel focal disease in adults.

This investigation also examined the effects of systemic hypoglycemia on CBF and brain energy metabolism. The increase in CBF during hypoglycemia before ischemia has not been a consistent result in other newborn animals.25,26 Hypoglycemia has been associated with an increased CBF in newborn puppies.27 In a preterm human neonate an elevated CBF during hypoglycemia has been suggested to facilitate coupling metabolic demands and substrate delivery.28 However, in newborn puppies the increase in CBF during hypoglycemia contributed little to the maintenance of glucose utilization by the brain.29 We did not measure the uptake of cerebral substrates and are unable to address this latter issue. Phosphorus-31 MRS demonstrated that cerebral high-energy phosphorylated compounds (ATP, PCr) and pH were unchanged during hypoglycemia and were in agreement with prior observations of newborn puppies.30 The increase in Pi during hypoglycemia remains unexplained in the presence of an unchanged PCr, pH, and ATP concentration. Interestingly, a similar small elevation in Pi was noted in adult rat brain studied with 31P MRS during insulin-induced hypoglycemia.41 In contrast, adult animals such as rats had more striking alterations in brain energy state at similar reductions of plasma glucose concentration.41,42 As discussed by Vannucci et al,33 these developmental differences presumably reflect a greater propensity to use alternate cerebral metabolic fuels in the neonatal period when brain metabolic requirements are less than those of adults.

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**Editorial Comment**

The animal investigation by Laptook et al adds additional information to an increasing body of knowledge that glucose supplementation before or during cerebral hypoxia-ischemia is protective to the perinatal brain (for review, see Reference 1). In the study of Laptook et al, newborn piglets were rendered either hyperglycemic with intravenous glucose or mildly hypoglycemic by fasting and with intravenous insulin, during which cerebral ischemia was produced by cervical compression and partial exsanguination. Despite similar cerebral blood flow reductions in the two groups, cerebral ATP and inorganic phosphorus measured with 31P magnetic resonance spectroscopy were better preserved in the hyperglycemic piglets. The findings are in direct contrast to those in adult experimental animals, in which hyperglycemic cerebral ischemia leads to progressive tissue energy failure and consequently greater brain damage than occurs during normoglycemic cerebral ischemia.2-4 The age-specific paradox regarding the effect of glucose on cerebral metabolism and ultimate neuropathologic outcome arising from cerebral ischemia relates to major differences in the transport of glucose into and metabolism by brain. In the fetus and newborn animal, the blood–brain barrier is immature, such that the transport of glucose becomes rate limiting for anaerobic metabolism during hypoxia or ischemia. As a result, lactic acid rarely reaches the levels seen in adult animals, whose brains are rendered similarly ischemic.1 Accordingly, the injury sustained by the immature brain is more the consequence of glucose deprivation than tissue lactacidosis, which accounts for the observation that glucose supplementation ameliorates perinatal hypoxic-ischemic brain damage.5-7 Glucose as a neuroprotective substrate should be considered as a part of the management strategy of the human fetus and newborn infant suffering hypoxia-ischemia.

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