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# Metabolism of Glucose, Glycogen, and High-energy Phosphates during Complete Cerebral Ischemia

## A Comparison of Normoglycemic, Chronically Hyperglycemic Diabetic, and Acutely Hyperglycemic Nondiabetic Rats

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***Background:*** **Increases in brain glucose will worsen outcome after global cerebral ischemia, and some experimental evidence suggests that the duration of hyperglycemia also may influence outcome. Different types of hyperglycemia were studied to identify metabolic differences that might account for alterations in postischemic outcome.**

***Methods:*** **Ninety pentobarbital-anesthetized Sprague-Dawley rats were divided into three groups: normoglycemic nondiabetic rats (N) (n = 30), chronically hyperglycemic diabetic rats (HD) (n = 30), and acutely hyperglycemic, glucose-infused nondiabetic rats (HN) (n = 30). These groups were further subdivided into groups of six rats each that received 0, 2.5, 5, 10, or 15 min of complete cerebral ischemia (potassium chloride—induced cardiac arrest). Brains were excised after 10-kW focused microwave radiation and metabolites were measured using enzymatic fluorometric techniques.**

***Results:*** **At all study intervals, plasma glucose concentrations in HD and HN were fourfold greater than in N. Before ischemia, brain glucose concentrations in all groups were proportional to plasma glucose concentrations; however, brain glycogen concentrations did not differ among groups. After the onset of ischemia, there was an immediate diminution of brain glucose, glycogen, adenosine triphosphate (ATP), and phosphocreatine that in all cases was most pronounced during the initial 2.5 min of ischemia. Consumption of carbohydrate stores and lactate production were greater in HD and HN than in N. HD had lesser preischemic ATP concentrations and energy charges relative to N and HN ($P < 0.05$), perhaps reflecting their disease state; however, at 2.5 min of ischemia, the relationship of ATP concentrations and energy charges was HN > HD > N ($P < 0.05$ among all). In all groups, ATP and phosphocreatine were more than 96% depleted by 10 min of ischemia. With few exceptions (ATP concentrations and energy charges before ischemia and at 2.5 min, and lactate concentration in HD < HN at 15 min), there were no measured metabolic differences between HD and HN.**

***Conclusions:*** **In these studies, the duration of hyperglycemia did not affect intraischemic carbohydrate consumption. At short durations of ischemia (2.5 min), both HD and HN groups had greater intraischemic ATP concentrations and energy charges than N; however, at longer durations of ischemia (>5.0 min), high-energy phosphate depletion was similarly severe in all groups. These studies suggest that energy failure is not the origin of worse postischemic neurologic injury in hyperglycemic subjects, nor does energy failure readily explain reported differences between acutely and chronically hyperglycemic subjects exposed to global cerebral ischemia. (Key words: Brain: cerebral metabolism; global cerebral ischemia. Diabetes mellitus: hyperglycemia.)**

IT IS a well recognized clinical and laboratory phenomenon that increases in blood and brain glucose concentrations will worsen neurologic injury after global cerebral ischemia.[1] The proposed mechanism is that glucose, originating as either free brain glucose or glycogen, is anaerobically metabolized, resulting in an intracellular lactic acidosis, and this acidosis adversely affects cellular metabolism.[2–4] Based on this mechanism, numerous reports have attempted to correlate postischemic outcome with concentrations of brain lactate (a marker for intracellular acidosis) and have determined that intraischemic brain lactate concentrations > 16–20 μmol/g are associated with enhanced postischemic injury.[5–7]

Most studies have demonstrated an exacerbation of ischemic neurologic injury in subjects in whom hyperglycemia resulted from exogenous glucose administration or from stress.[4,8,9] Based on extrapolations from these data, it has been theorized that hyperglycemia also contributes to the worse outcome that has been reported after brain ischemia in diabetic subjects.[5,8,10,11] Data from humans having focal ischemia[12]

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and from laboratory animals having global[13] or focal[14] ischemia further suggest that for a given degree of hyperglycemia and a given ischemic insult, chronically hyperglycemic (*e.g.*, diabetic) subjects may experience outcomes different from those of acutely hyperglycemic subjects. The metabolic basis for such an effect is not clear; however, one possibility is that intraischemic carbohydrate metabolism and production of high-energy metabolites differ between acutely and chronically hyperglycemic subjects. These issues were addressed by our study.

The current study in rats evaluated the effect of increases in brain glucose concentrations (in both diabetic and nondiabetic rats) on intraischemic consumption of brain glucose, glycogen, and high-energy phosphates (adenosine triphosphate [ATP], adenosine diphosphate [ADP], adenosine monophosphate [AMP], and phosphocreatine [PCr]), and on intraischemic accumulation of lactate. We tested the hypotheses that during complete cerebral ischemia, (1) the chronically hyperglycemic, diabetic brain metabolizes carbohydrate differently than the acutely hyperglycemic, nondiabetic brain, and (2) when compared to hyperglycemic nondiabetic brains, the diabetic brain protects itself from the harmful effects of lactic acidosis during ischemia and this is manifested as a beneficial energy status.

## Materials and Methods

This protocol, conducted in 90 Sprague-Dawley rats, was reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation. Rats were fed Lab Diet Laboratory Rodent Diet 5001 (PMI Feeds, St. Louis, MO). Two weeks before the study, diabetes mellitus was induced in 30 rats with intraperitoneal streptozotocin 65 mg/kg.[10,15] No rats received insulin before or during the study. The presence of hyperglycemia was confirmed 48–72 h after streptozotocin administration by obtaining a sample of capillary blood from the rat's tail. All rats were fasted for 10–12 h before the study but had free access to water. The rats were weighed and then anesthetized in an induction box with 3–4% halothane in oxygen. After tracheostomy and tracheal intubation, the lungs were mechanically ventilated with a rodent ventilator (7025, Ugo Basile, Varese, Italy). Pancuronium 0.5 mg was given intramuscularly to provide muscle paralysis, and administration was repeated hourly. Anesthesia was maintained with 1.3% inspired halothane in 40% oxygen (balance nitrogen) for the remainder of the preparatory period. The inspired oxygen and halothane concentrations were measured with a laser-type gas analyzer (RASCAL, Albion Instruments, Salt Lake City, UT).

Temperature was measured with a needle thermistor inserted beneath the temporalis muscle (73A, Yellow Springs Instruments, Yellow Springs, OH) and was maintained with a heating lamp and a 1,200-W standard hair dryer. The femoral artery was cannulated with a polyethylene catheter (PE-50) for blood sampling and measurement of mean arterial pressure. A second cannula (PE-50) was inserted *via* the femoral vein into the common iliac vein for the administration of fluids and drugs. Arterial blood gases were measured with electrodes at 37°C (Instrumentation Laboratories, Lexington, MA). Blood glucose, plasma glucose, and plasma lactate were measured using a glucose and lactate analyzer (23A, Yellow Springs). This device has a glucose detection range of 0–28 μmol/ml (0–500 mg/dl) and a sensitivity of 0.1 μmol/dl.[15] The lead II electrocardiogram was monitored continuously, beginning just before tracheal intubation (78B, Grass Instrument, Quincy, MA). The continuous electroencephalogram was monitored from biparietal subperiosteal needles and was amplified and recorded using a polygraph and strip recorder (78B, Grass).

After the preparatory period the halothane was discontinued. Immediately thereafter, rats were given pentobarbital 4–6 mg intravenously followed by pentobarbital in 2-mg increments, as needed, to maintain a synchronized, continuous electroencephalographic pattern consistent with a surgical plane of anesthesia.[16]

Once stable barbiturate anesthesia had been achieved and the halothane had been eliminated, 15-min stabilization periods were allowed, during which the anesthetic depth, ventilation, and oxygenation were adjusted until the changes resulted in data within predetermined criteria: arterial carbon dioxide tension 36–40 mmHg, arterial oxygen tension 125–175 mmHg, mean arterial pressure greater than 60 mmHg, and temperature 36.5–37.5°C. Inclusion into the diabetic or nondiabetic groups was based on the following criteria: diabetic rats were required to have a blood glucose concentration greater than 200 mg/dl, and nondiabetic rats were required to have a blood glucose concentration greater than 60 but less than 120 mg/dl before any intervention.

Rats were divided into three groups based on the type of hyperglycemia and fluid infusion: (1) normoglycemic nondiabetic rats (N) (n = 30) given a saline

infusion, (2) hyperglycemic diabetic rats (HD) (n = 30) given a saline infusion, and (3) hyperglycemic, nondiabetic rats (HN) (n = 30) given a glucose infusion.

The initial studies were performed in 6 N, 6 HD, and 6 HN rats who were not exposed to an ischemic insult. After obtaining control measurements, N and HD received a continuous intravenous infusion of 0.9% normal saline at a rate of 2.0 ml/h. HN received the same infusion of saline to which was added 50% dextrose to produce a final glucose delivery of 1.5–2.0 $g \cdot kg^{-1} \cdot h^{-1}$. In HN, the goal was to increase blood glucose to values similar to those in HD, over a 60 min period. Attempts were made to achieve similar durations of fluid infusions in all groups.

Once the desired study conditions were met, the rats were killed by subjecting the brain to 10-kW focused microwave radiation for 1.05 s (Metabolic Vivostat, Cober Electronics, Stamford, CT). Described by Guidotti *et al.*,[17] microwave radiation halts brain metabolism almost instantaneously by rapidly increasing temperature. The microwave power and time settings used in our study were derived in pilot studies conducted in consultation with factory representatives and other laboratory researchers with experience in microwave technology.‡ Specifically, we identified a dose of radiation within a range that produced minimal tissue dehydration, yet resulted in optimal concentrations of brain metabolites. Stability of brain metabolite concentrations, when comparing values obtained immediately after radiation *versus* after 30 min of "incubation" at room temperature, was interpreted as evidence of complete denaturation of metabolic enzymes.

Immediately after irradiating the study subjects, the brain was left enclosed in the cranium for 180 s, then it was rapidly removed, sealed inside a 4-cm–diameter × 4-cm–deep polyethylene container, and flash-chilled by immersion in a dry ice–alcohol bath for 1 min. Next, it was transferred to a −70°C freezer until it was prepared for metabolic analysis.

The remaining 72 rats were treated in a similar fashion, except that they were subjected to cerebral ischemia of 2.5, 5, 10, or 15 min (n = 6 per group per duration of ischemia), without reperfusion. An intravenous bolus of saturated potassium chloride 1.0 ml was used to produce circulatory arrest (within 2–3 s). The onset of cerebral ischemia was defined as the appearance of a pulseless arterial pressure tracing and was confirmed by an isoelectric encephalogram. In all rats, fluid infusions were discontinued at the onset of ischemia.

Microwave-irradiated cortex was removed using fine dissection techniques under a dissecting microscope in a −10°C environment. The brain was sliced coronally into anterior and posterior halves, and, from these, cortex was dissected for subsequent metabolic analysis. Each metabolite and carbohydrate measurement was determined from the average of the two samples. Brain concentrations of glucose, lactate, and high-energy phosphates (ATP, ADP, AMP, and PCr) were measured by previously described enzymatic fluorometric techniques.[18,19] These methods have been used extensively in our laboratory.[15,20] The energy charge (EC) of the adenylate pool, a measure of the availability of high-energy phosphate bonds, was calculated as EC = ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]).[21]

Brain glycogen content was estimated from a 0.1-g aliquot of tissue using a modification of methods described by Passonneau and Lauderdale[22] and Swanson *et al.*[23] In brief, brain tissue was homogenized in 0.03 N HCl and heated for 10 min at 100°C. A portion of the homogenate was subjected to glucose analysis, and the remaining portion was incubated with amyloglucosidase (Sigma Chemical, St. Louis, MO), which hydrolyzes the glycogen into glycosyl units. Glycogen content (expressed as glycosyl units) was estimated as the difference between glucose concentrations of hydrolyzed and nonhydrolyzed homogenates. This method was validated in our laboratory by analyzing samples that contained known quantities of oyster glycogen (Sigma Chemical).

Data among groups N, HD, and HN, as well as data among ischemic durations (*e.g.*, no ischemia *vs.* 2.5, 5, 10, and 15 min), were compared by two-way analysis of variance that considered only the main effects of group and time. *Post hoc* comparisons were made with F tests. A probability $< 0.05$ was considered statistically significant. All data are reported as means ± standard deviations.

## Results

### *Systemic Physiologic Data*

Groups were well matched for blood gases, *p*H, mean arterial pressure, and temperature both before fluid infusions and at the completion of the 60 min infusion

‡ Todd MM, Pelligrino DA: Personal communications. 1992.

**Table 1. Physiologic Variables, Blood Gases, and Plasma Glucose and Lactate Concentrations at the End of a 60-min Infusion Period and Immediately before Ischemia in Normoglycemic Nondiabetic Rats (N), Hyperglycemic Diabetic Rats (HD), and Hyperglycemic Nondiabetic Rats (HN)**

| Group | $Pa_{O_2}$ (mmHg) | $Pa_{CO_2}$ (mmHg) | pH | MAP (mmHg) | Cranial Temperature (°C) | Plasma Glucose (μmol/ml) | Plasma Lactate (μmol/ml) |
|---|---|---|---|---|---|---|---|
| No ischemia | | | | | | | |
| N | 149 ± 8 | 37 ± 1 | 7.35 ± 0.03 | 138 ± 12 | 37.1 ± 0.3 | 5.38 ± 0.41 | 0.5 ± 0.1 |
| HD | 152 ± 12 | 38 ± 2 | 7.41 ± 0.06† | 128 ± 15 | 37.0 ± 0.1 | 22.66 ± 2.57† | 0.7 ± 0.3 |
| HN | 151 ± 8 | 38 ± 1 | 7.37 ± 0.03 | 139 ± 11 | 37.0 ± 0.2 | 22.28 ± 3.41† | 1.5 ± 0.4†‡ |
| 2.5-min ischemia | | | | | | | |
| N | 159 ± 10 | 39 ± 1* | 7.37 ± 0.03 | 133 ± 12 | 37.0 ± 0.0 | 5.40 ± 0.99 | 0.6 ± 0.1 |
| HD | 146 ± 17 | 38 ± 1 | 7.41 ± 0.04 | 128 ± 15 | 37.0 ± 0.0 | 22.09 ± 2.99† | 0.7 ± 0.2 |
| HN | 156 ± 4 | 39 ± 1 | 7.39 ± 0.03 | 133 ± 19 | 37.0 ± 0.0 | 19.95 ± 1.57† | 1.3 ± 0.6†‡ |
| 5-min ischemia | | | | | | | |
| N | 148 ± 13 | 38 ± 2 | 7.36 ± 0.03 | 133 ± 8 | 37.0 ± 0.2 | 5.09 ± 0.73 | 0.6 ± 0.1 |
| HD | 149 ± 15 | 39 ± 1 | 7.39 ± 0.04 | 128 ± 12 | 37.0 ± 0.0 | 21.64 ± 2.98† | 0.7 ± 0.2 |
| HN | 157 ± 14 | 39 ± 1 | 7.36 ± 0.03 | 123 ± 13 | 37.0 ± 0.2 | 21.81 ± 3.28† | 1.3 ± 0.5†‡ |
| 10-min ischemia | | | | | | | |
| N | 155 ± 8 | 38 ± 1 | 7.35 ± 0.02 | 138 ± 12 | 37.0 ± 0.3 | 5.70 ± 0.91 | 0.5 ± 0.1 |
| HD | 147 ± 11 | 38 ± 1 | 7.38 ± 0.04 | 131 ± 15 | 37.1 ± 0.2 | 22.48 ± 4.58† | 0.6 ± 0.2 |
| HN | 155 ± 7 | 38 ± 2 | 7.37 ± 0.03 | 134 ± 18 | 37.1 ± 0.3 | 20.51 ± 3.42† | 1.5 ± 0.7†‡ |
| 15-min ischemia | | | | | | | |
| N | 155 ± 11 | 38 ± 1 | 7.35 ± 0.04 | 133 ± 10 | 37.0 ± 0.2 | 5.23 ± 1.24 | 0.6 ± 0.2 |
| HD | 148 ± 15 | 39 ± 1 | 7.42 ± 0.05† | 132 ± 5 | 37.0 ± 0.0 | 22.89 ± 3.40† | 0.7 ± 0.1 |
| HN | 142 ± 11 | 38 ± 2 | 7.39 ± 0.03 | 141 ± 13 | 37.0 ± 0.0 | 22.52 ± 3.50† | 1.6 ± 0.2†‡ |

MAP = mean arterial pressure.

* $P < 0.05$ *versus* no ischemia in same group.

† $P < 0.05$ *versus* N at same time peirod.

‡ $P < 0.05$ between HD and HN at same time period.

and stabilization periods (postinfusion data tabulated in table 1). Before fluid infusion, diabetic rats had fourfold greater plasma glucose concentrations than nondiabetic rats (data not tabulated). The weights of HD averaged about 15–20 g less than those of N and HN. This was due to a weight loss in HD in the 2 weeks after streptozotocin injection. Similar weight losses have been observed in previous studies from our laboratory.[15],§ Preischemic plasma glucose concentrations in the HN groups were similar to those of the corresponding HD groups (table 1).

*Brain Glucose, Glycogen, and Lactate*

Throughout the study, when comparing HD and HN, there were no significant differences in brain glucose, glycogen, or lactate concentrations, with one exception: at 15 min, brain lactate in HD was less than that in HN (fig. 1).

§ Lanier WL, Hofer RE: Unpublished data. 1990.

Brain glucose concentrations are listed in table 2. As expected, brain glucose concentrations were greater in HD and HN than in N at all intervals. During ischemia, brain glucose concentrations in N decreased to approximately 0.2–0.3 μmol/g at 2.5 min, and remained at this concentration at longer periods of ischemia. In contrast, in HD and HN, brain glucose concentrations were 2.27 ± 1.66 and 1.52 ± 0.50 μmol/g, respectively, at 2.5 min of ischemia, and continued to decline during longer ischemic durations (table 2). Furthermore, brain glucose concentrations in HD and HN at 15 min of ischemia were numerically greater than in N at 2.5 min of ischemia.

Before ischemia, brain glycogen concentrations did not differ among groups (table 2). During ischemia, brain glycogen concentrations significantly decreased in all groups. Although brain glycogen concentrations were more severely diminished in N at all time intervals (tables 2 and 3), glycogen in N significantly differed from both HD and HN only at 5 min of ischemia. Between 10 and 15 min of ischemia, glycogen concen-

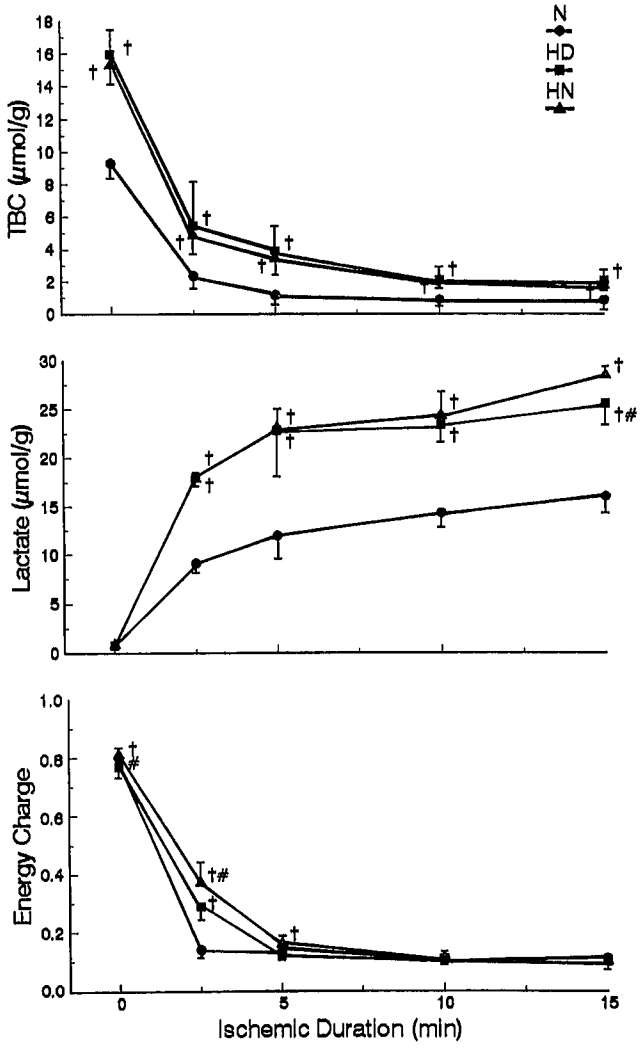


**Fig. 1. The effect of complete cerebral ischemia on (*A*) total brain carbohydrate (TBC) concentration (defined as the sum of brain glucose and glycogen concentrations [glucose equivalents]), (*B*) brain lactate concentration, and (*C*) the energy charge (EC) of the adenylate pool. Data were compared among groups of normoglycemic nondiabetic rats (N), hyperglycemic diabetic rats (HD), and hyperglycemic nondiabetic rats (HN). Each dot = mean value for six rats; vertical bars = 1 SD. †$P < 0.05$ *versus* N at same time period; #$P < 0.05$ between HD and HN at same time period. At all durations of ischemia, all groups had TBC, lactate, and EC values significantly different ($P < 0.05$) from their corresponding nonischemic control groups.**

trations had achieved near plateau values of approximately 0.6 μmol/g in N and 1.0 μmol/g in HD and HN.

Total brain carbohydrate (TBC) stores, defined as the sum of glucose and glycogen concentrations, are presented in figure 1. Although preischemic plasma and brain glucose concentrations in HD and HN were almost fourfold greater than in N, TBC concentrations in HD and HN were less than twice that of N. During ischemia, there was a greater rate of TBC consumption in HD and HN than in N. Furthermore, the rate of consumption (identified as the slope of the lines in fig. 1) differed most among groups during the earlier phases of ischemia. Between 10 and 15 min of ischemia, very little TBC was consumed in either N, HD, or HN. However, residual TBC stores observed at 10 and 15 min of ischemia in HD and HN were similar to stores in N at 2.5 min of ischemia, and twofold greater than N stores of TBC at 10 and 15 min.

The contribution of glycogen to TBC stores, and intraischemic carbohydrate consumption, differed greatly between HD and HN *versus* N (table 3). In N, before ischemia, 74 ± 10% of TBC originated as glycogen. During ischemia, brain glucose concentrations decreased rapidly, and glycogen concentrations also decreased. During all ischemic intervals studied, the majority of TBC consumption in N (68–74%) originated as glycogen, and by 15 min of ischemia, 91% of baseline glycogen stores had been consumed. In contrast, in HD and HN, before ischemia, 40 ± 6% and 42 ± 3%, respectively, of total carbohydrate stores originated as glycogen. With the induction of ischemia, HD and HN consumed large quantities of brain glucose (table 3). Although more than 83 and 86% of baseline glycogen stores were eventually mobilized in HD and HN, respectively, glycogen contributed only 30–41% of TBC consumption during any ischemic measurement interval in these two groups.

Brain lactate concentrations increased in all groups immediately upon initiation of ischemia, and the greatest incremental increase in lactate occurred during the initial 2.5 min of ischemia (fig. 1). At all ischemic periods, HD and HN had greater concentrations of lactate than did N.

### *Brain High-energy Phosphates*

Brain concentrations of high-energy phosphates and ECs are presented in table 2 and figure 1. There were no differences in preischemic high-energy phosphates or ECs between N and HN. However, preischemic ATP concentrations and ECs in HD were slightly less than, but significantly different from, those of the other two groups. During ischemia, all groups experienced a rapid depletion of high-energy phosphates, such that, after 5 min of ischemia, ATP and PCr were less than 5% of control values, and ECs were less than 15% of

**Table 2. Brain Glucose, Glycogen, and High-energy Phosphate Concentrations during Progressively Increasing Durations of Brain Ischemia in Normoglycemic Nondiabetic Rats (N), Hyperglycemic Diabetic Rats (HD), and Hyperglycemic Nondiabetic rats (HN)**

| Group | Glucose (μmol/g) | Glycogen (μmol/g) | PCr (μmol/g) | ATP (μmol/g) | ADP (μmol/g) | AMP (μmol/g) |
|---|---|---|---|---|---|---|
| No ischemia | | | | | | |
| N | 2.42 ± 0.28 | 6.88 ± 0.90 | 4.49 ± 0.30 | 2.27 ± 0.16 | 1.05 ± 0.05 | 0.17 ± 0.08 |
| HD | 9.54 ± 1.04† | 6.42 ± 0.93 | 4.66 ± 0.67 | 2.03 ± 0.11† | 1.11 ± 0.08 | 0.23 ± 0.08 |
| HN | 8.84 ± 0.81† | 6.46 ± 0.44 | 5.06 ± 0.49 | 2.30 ± 0.14‡ | 1.00 ± 0.16 | 0.18 ± 0.07 |
| 2.5-min ischemia | | | | | | |
| N | 0.22 ± 0.08* | 2.11 ± 0.60* | 0.14 ± 0.10* | 0.06 ± 0.02* | 0.67 ± 0.05* | 2.18 ± 0.18* |
| HD | 2.27 ± 1.66*† | 3.17 ± 1.24* | 0.07 ± 0.06* | 0.34 ± 0.10*† | 1.12 ± 0.12† | 1.61 ± 0.17*† |
| HN | 1.52 ± 0.50*† | 3.33 ± 0.63* | 0.08 ± 0.04* | 0.59 ± 0.30*†‡ | 1.11 ± 0.10*† | 1.38 ± 0.17*†‡ |
| 5-min ischemia | | | | | | |
| N | 0.28 ± 0.06* | 0.88 ± 0.66* | 0.08 ± 0.04* | 0.04 ± 0.02* | 0.54 ± 0.06* | 1.92 ± 0.09* |
| HD | 1.33 ± 0.47*† | 2.57 ± 1.31*† | 0.07 ± 0.06* | 0.07 ± 0.04* | 0.63 ± 0.07* | 1.97 ± 0.11* |
| HN | 1.14 ± 0.21*† | 2.26 ± 0.94*† | 0.10 ± 0.03* | 0.10 ± 0.03*† | 0.75 ± 0.14*†‡ | 2.08 ± 0.13* |
| 10-min ischemia | | | | | | |
| N | 0.26 ± 0.08* | 0.62 ± 0.38* | 0.10 ± 0.11* | 0.04 ± 0.02* | 0.41 ± 0.07* | 1.69 ± 0.11* |
| HD | 1.18 ± 0.36*† | 0.93 ± 0.58* | 0.04 ± 0.03* | 0.04 ± 0.01* | 0.44 ± 0.06* | 1.82 ± 0.12* |
| HN | 0.93 ± 0.23*† | 1.18 ± 0.50* | 0.12 ± 0.08* | 0.05 ± 0.04* | 0.45 ± 0.06* | 1.92 ± 0.15*† |
| 15-min ischemia | | | | | | |
| N | 0.24 ± 0.10* | 0.59 ± 0.52* | 0.06 ± 0.11* | 0.01 ± 0.01* | 0.39 ± 0.06* | 1.51 ± 0.27* |
| HD | 0.93 ± 0.23*† | 1.08 ± 0.59* | 0.05 ± 0.07* | 0.02 ± 0.02* | 0.38 ± 0.03* | 1.70 ± 0.14* |
| HN | 0.81 ± 0.19*† | 0.92 ± 0.23* | 0.05 ± 0.07* | 0.02 ± 0.01* | 0.40 ± 0.02* | 1.85 ± 0.22* |

PCr = phosphocreatine; ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate.

* $P < 0.05$ *versus* no ischemia in same group.

† $P < 0.05$ *versus* N at same time period.

‡ $P < 0.05$ between HD and HN at some time period.

control values. However, the pattern of deterioration of adenosine nucleotides differed among groups. At 2.5 min of ischemia, the relationship of ATP concentrations and ECs among the groups was HN > HD > N ($P < 0.05$ among all). At 5 min of ischemia, the relationship became HN > HD = N ($P < 0.05$ for HN *vs.* N), while at 10 and 15 min the relationship was HN = HD = N. PCr concentrations did not differ among groups at baseline or at any ischemic interval.

With the exceptions in ATP and EC noted before ischemia and at 2.5 min of ischemia, high-energy phosphate concentrations and ECs did not significantly differ between HD and HN.

## Discussion

Diabetic humans have a greater incidence of ischemic neurologic deficits than do those who are not diabetic, and once they experience cerebral ischemia, diabetic humans have a worse outcome.[5,8,11,24] Although this phenomenon may be related to a variety of factors (*e.g.*, diabetes-induced differences in blood viscosity,[25] vessel reactivity,[26] the severity of arteriosclerosis,[27] or collateral blood flow to areas of ischemia[10,28]), the phenomenon may also be influenced by alterations in blood and brain glucose concentrations. The latter issue was evaluated by our study.

Increases in brain glucose exacerbate postischemic neurologic injury.[1,9] It has been postulated that the phenomenon is due in part to a toxic effect of lactic acid on the cell.[29] Lactic acid is produced from the anaerobic metabolism of glucose, and there is experimental evidence to suggest that the hydrogen ions resulting from this process initiate cascades of metabolic events that are injurious to the cell.[2-4] For example, several studies have reported that lactic acid is toxic to cellular energy metabolism.[4,30,31] This has been attributed in part to a suppression of glycolytic enzymes by hydrogen ions, particularly suppression of the rate-limiting enzyme and control point in glycolysis, phosphofructokinase.[32,33] It is this detrimental effect of ischemia, or, alternatively, its metabolic products, on further energy metabolism that is thought to contribute

**Table 3. Consumption of Brain Tissue Carbohydrates (Glucose and Glycogen), and Lactate Production with Progressively Longer Periods of Ischemia in Normoglycemic Nondiabetic Rats (N), Hyperglycemic Diabetic Rats (HD), and Hyperglycemic Nondiabetic Rats (HN)**

| Group | Glucose Consumption (μmol/g) | Glycogen Consumption (μmol/g) | TBC Consumption (μmol/g) | Glycogen Consumption (% of TBC consumption) | Lactate Production (μmol/g) |
|---|---|---|---|---|---|
| 2.5-min | | | | | |
| N | 2.20 ± 0.24 | 4.77 ± 0.86 | 6.97 ± 0.81 | 68 ± 5 | 8.42 ± 0.74 |
| HD | 7.27 ± 0.89* | 3.25 ± 1.60* | 10.52 ± 2.17* | 31 ± 9* | 17.26 ± 0.99* |
| HN | 7.32 ± 0.77* | 3.13 ± 0.80* | 10.45 ± 1.35* | 30 ± 5* | 17.11 ± 0.64* |
| 5-min | | | | | |
| N | 2.14 ± 0.23 | 6.00 ± 0.97 | 8.14 ± 0.83 | 74 ± 4 | 11.24 ± 2.33 |
| HD | 8.21 ± 0.93* | 3.85 ± 1.61* | 12.06 ± 1.59* | 32 ± 10* | 22.15 ± 4.95* |
| HN | 7.70 ± 0.81* | 4.20 ± 0.87* | 11.90 ± 1.61* | 35 ± 3* | 22.30 ± 2.00* |
| 10-min | | | | | |
| N | 2.16 ± 0.26 | 6.26 ± 1.07 | 8.42 ± 1.02 | 74 ± 4 | 13.63 ± 1.37 |
| HD | 8.36 ± 0.93* | 5.49 ± 1.17 | 13.85 ± 1.53* | 40 ± 6* | 22.71 ± 1.96* |
| HN | 7.91 ± 0.84* | 5.28 ± 0.54 | 13.19 ± 1.09* | 40 ± 3* | 23.58 ± 2.53* |
| 15-min | | | | | |
| N | 2.18 ± 0.27 | 6.29 ± 1.04 | 8.47 ± 1.04 | 74 ± 4 | 15.35 ± 1.69 |
| HD | 8.61 ± 1.16* | 5.34 ± 1.21 | 13.95 ± 1.65* | 38 ± 6* | 24.92 ± 3.45* |
| HN | 8.03 ± 0.77* | 5.54 ± 0.43 | 13.57 ± 1.12* | 41 ± 2* | 27.66 ± 0.89* |

Reported values were calculated as follows: change (*i.e.*, consumption or production) = concentration at a given time point minus concentration in "no ischemia" group.

TBC = total brain carbohydrate [equal to sum of glucose and glycogen concentrations (glucose equivalents)].

* $P < 0.05$ *versus* N at same time period. (There were no significant differences between HD and HN at same time period.)

to the brain's remarkable susceptibility to ischemic injury.

The effect of increases in brain glucose on periischemic brain energy reserves has been evaluated by previous studies in nondiabetic subjects, with conflicting results. Studies that employed models of complete[30,31,34] or incomplete[30,31,35,36] global cerebral ischemia reported that hyperglycemia was associated with (1) no effect[31,34,35,36] or a mild detrimental effect[4] on brain energy stores during ischemia and (2) no effect[31,34,35] or a detrimental effect[4,30,31] on brain energy stores during recirculation.

In contrast, other investigators have reported that hyperglycemia in nondiabetic subjects has a potentially beneficial effect on periischemic cerebral energy stores. Ekholm *et al.*, using a cardiac arrest model, reported that intraischemic cerebral ATP depletion was accelerated in hypoglycemic rats (compared with normoglycemic rats) and retarded in hyperglycemic rats.[37,38] Hoffman *et al.* used a rat model of unilateral carotid artery occlusion with hypotension and reported that hyperglycemia had a beneficial effect on brain PCr and ATP concentrations after 30 min of ischemia when compared to fasted controls.[39] Finally, Hsu *et al.*, using a rat model of near-complete forebrain ischemia, demonstrated preservation of intraischemic brain ATP concentrations and ECs in glucose-infused, hyperglycemic rats, relative to normoglycemic rats. However, no differences between groups were observed during 60 min of reperfusion.[40]

The above-cited studies reported widely differing effects of acute hyperglycemia on brain concentrations of high-energy metabolites during and after brain ischemia. These discrepancies may result from a variety of factors, including the ischemic model, the presence or absence of a reperfusion period, and the severity of the ischemic insult (*i.e.*, an insult that can be survived *vs.* one that cannot be survived). An additional limitation of the collective studies of global ischemia is that none evaluated diabetic subjects.

The reason for selectively studying diabetic subjects is as follows. Recent studies have suggested that the relation between glucose and postischemic neurologic outcome differs between acutely hyperglycemic subjects and chronically hyperglycemic (*e.g.*, diabetic) subjects. Warner *et al.* subjected rats to transient near-complete forebrain ischemia and evaluated the effect of alterations in blood glucose on outcome.[13] They found that both hyperglycemic diabetic (5–7 days) and hyperglycemic nondiabetic rats had worse outcomes

than did normoglycemic rats (*i.e.,* both nondiabetic and insulin-treated diabetic rats). However, despite equal degrees of hyperglycemia in diabetic and glucose-loaded nondiabetic rats, the diabetic rats had better outcomes.

Studies of focal cerebral ischemia also have reported an apparent effect of the duration of hyperglycemia on outcome, but with conflicting results. In a rat model of permanent right middle cerebral artery occlusion, followed in 4 days by brain excision, Nedergaard and Diemer reported similar volumes of infarction in hyperglycemic diabetic (2 days after streptozotocin injection) and normoglycemic rats.[14] However, long-term diabetic rats (4 months after streptozotocin injection) had significantly larger volumes of infarction compared to normoglycemic rats and diabetic rats 2 days after streptozotocin administration. In contrast, Woo *et al.* compared outcome immediately and 3 months after stroke in 304 patients.[12] They reported that outcome in hyperglycemic diabetic patients was better than in acutely hyperglycemic, nondiabetic patients.

These studies suggest that the effect of increased glucose on the ischemic brain may be influenced, in part, by the duration of the hyperglycemic state. The current study evaluated this issue, using a model of complete brain ischemia, and high-energy phosphates as a marker of ischemic metabolic changes. We could find few significant differences in HN rats *versus* HD rats. Of these, brain ATP concentrations and ECs at 2.5 min of ischemia in HD were intermediate between the values in N and HN. However, these differences did not conform to a pattern (*i.e.,* better energy preservation) intuitively associated with a better postischemic outcome.[41] The pattern of similarly preserved intraischemic EC in both diabetic and glucose-infused hyperglycemic subjects (within the same model) is consistent with the results of Folbergrová *et al.,* who used a model of focal ischemia to compare acute glucose-infused rats with rats having streptozotocin-induced diabetes of 2-day duration.[42]

Our observation of better energy preservation in hyperglycemic subjects was confirmed in a subsequent study§ in which we employed the same model of near-complete forebrain ischemia as Warner *et al.*[13] This research evaluated hyperglycemic diabetic rats, normoglycemic insulin-treated diabetic rats, and normoglycemic nondiabetic rats. During transient ischemia, brain energy stores were better preserved in hyperglycemic diabetic rats than in the two normoglycemic groups. Collectively, these studies suggest that after global brain ischemia, the worse outcome in hyperglycemic diabetic subjects is not due to energy failure. Furthermore, if chronically hyperglycemic diabetic and acutely hyperglycemic nondiabetic subjects truly differ in postischemic neurologic outcome (as previously suggested in the studies of Warner *et al.,*[13] Nedergaard and Diemer,[14] and Woo *et al.*[12]), the possibility of a mildly beneficial effect[12,13] in diabetic subjects also is not related to better energy preservation.

If (1) brain ATP concentrations are better preserved in hyperglycemic subjects exposed to a clinically relevant ischemic insult and (2) hyperglycemia increases the duration of ischemia that the brain can tolerate before it undergoes terminal depolarization,[37,43] then (3) why do hyperglycemic subjects have a worse postischemic outcome than normoglycemic subjects? One possibility is that enhanced adenosine production in normoglycemic subjects, resulting from extensive hydrolysis of ATP, may contribute to an improved postischemic outcome.[40,44]

In addition to measuring energy metabolism, our study examined the pattern of carbohydrate metabolism, and the relationship of glucose and glycogen metabolism to lactate production. We discovered that higher levels of glucose were metabolized in preference to glycogen in HD and HN, as expected from known characteristics of the enzymes involved.[45] The heavy reliance on glycogen as a substrate in N explains the poor correlation between preischemic brain glucose concentrations (glucose concentrations in N were 25–27% of concentrations in HD and HN) and intraischemic lactate accumulation (lactate accumulation in N was 55–62% that of HD and HN).

In interpreting our data, it should be noted that baseline values for EC were less,[34,46,47] and concentrations of glycogen were greater,[23,48,49] than those of previous reports. The lesser ECs (0.77–0.81) were probably related to the use of microwave radiation of the brain. Both before and after the current study, our laboratory performed studies in rats in whom the brains were excised after *in situ* freezing.§‖ When data from nondiabetic, nonischemic rats in those studies were compared to data from a comparable group in the current study, we found that microwave radiation resulted in no change in brain lactate or the sum of adenine nucleotide concentrations. However, EC was 11–14% less

‖ Wagner SR, Hofer RE: Unpublished data. 1993.

in microwave-irradiated brains than in brains frozen *in situ*. Our experience is similar to that of other laboratories that have employed both microwave and *in situ* freezing techniques.# The reason for these discrepancies may relate to a small shift in the balance among ATP, ADP, and AMP during the brief instant that metabolism is being inactivated. Specifically, rapid cooling may alter the enzymatic activity in a manner that yields a better preservation (or perhaps an artificial elevation) of high-energy phosphate bonds. In contrast, heat denaturation may have the opposite effect of freezing (partial dephosphorylation during enzyme denaturation).#

The baseline glycogen concentrations in our study (*e.g.*, 6.88 ± 0.37 μmol/g in nonischemic N), were greater than those traditionally reported.[23,45,48,49] These differences probably did not result from microwave radiation because, using *in situ* freezing methods, we have measured glycogen concentrations of 5.90 ± 0.54–6.91 ± 0.53 μmol/g in nonischemic, nondiabetic rats.§‖ It is also unlikely that the large glycogen concentrations in these studies were the result of an analytic error, because the analytic technique relies simply on the measurement of brain glucose before and after the enzymatic hydrolysis of glycogen. The faulty hydrolysis of glycogen would result in an underestimation of concentrations, not the large concentrations we reported. A more likely explanation is that the baseline concentrations of glycogen in our rats were influenced by their dietary history.[48] It should be noted that the glycogen concentrations reported in the current study were subsequently validated by brain lactate concentrations. Based on stoichiometric calculations, the anaerobic metabolism of each molecule of glucose, originating as either free glucose or glycogen, should result in two molecules of lactate.[5] In our study, at 15 min of ischemia (a period in which there should have been a relative equilibrium between TBC consumption and lactate production) the ratios of lactate produced to TBC consumed were as follows: N = 1.81, HD = 1.79, HN = 2.04 (calculated from data in table 3). Thus, the available evidence suggests that our determinations of brain glycogen concentration are valid. However, our data further point out the controversy regarding the magnitude of brain glycogen concentrations and its clinical importance.[45]

In summary, the current study evaluated the effect of complete cerebral ischemia on the metabolism of glucose, glycogen, and high-energy phosphates in N, HD, and HN rats. These studies emphasize the importance of glycogen as an energy source in the ischemic brain and support the hypothesis that the extent of intraischemic brain glycogen metabolism is determined, in part, by brain glucose concentrations. In hyperglycemic rats, regardless of whether the hyperglycemia was chronic (as in HD) or acute (as in HN), energy failure was not the origin of a worsened outcome when compared to normoglycemic rats. While these studies do not elucidate the mechanism of the possible differences in postischemic outcome observed in equally hyperglycemic diabetic and nondiabetic subjects,[12–14] they imply that these differences were not caused by alterations in carbohydrate handling, and probably were not influenced to any great degree by differences in energy metabolism.

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