Dietary Nonprotein Calories and Cerebral Infarction Size in Rats
Claudia Robertson, MD; J. Clay Goodman, MD; Robert G. Grossman, MD; Monica Claypool; and Anne White

Background and Purpose: Conventional diets may cause hyperglycemia in patients with neurological injuries. The purpose of this study was to examine the effect on the severity of cerebral infarction of replacing carbohydrates as the primary dietary source of nonprotein calories.

Methods: Sixty-nine Long-Evans rats were either fasted for 24 hours, fed isocaloric amounts of a control diet containing 51.5% of the calories as carbohydrates, or fed one of five experimental diets before middle cerebral artery occlusion for 45 minutes. In the experimental diets, 60% of the carbohydrate calories were replaced with one or more of the following substrates: 1,3-butanediol, triacetin, tributyrin, and long- and medium-chain triglycerides.

Results: The plasma glucose concentration in the fasted animals was 6.4±1.1 mmol/ml. In the animals receiving the control diet, which contained the greatest number of carbohydrate calories, plasma glucose was 9.1±1.4 mmol/ml. The 1,3-butanediol diet resulted in an intermediate plasma glucose concentration averaging 7.8±1.3 mmol/ml. Plasma β-hydroxybutyrate levels were elevated in the fasted group and with the 1,3-butanediol diet. Plasma acetate levels were increased with the diets supplemented with triacetin. The smallest infarct volume (53±43 mm³) was found in the fasted group and the largest (162±56 mm³) in the control diet group. Infarct volumes that were significantly smaller were found with the 1,3-butanediol diet (98±41 mm³) and with the triacetin/tributyrin diet (105±53 mm³). The volume of the infarct was directly related to the plasma glucose concentration before ischemia (n=69, r=0.47, p<0.01), but not to plasma lactate, ketone body, or acetate levels.

Conclusions: It may be possible to develop a diet for patients with neurological injuries using noncarbohydrate calorie sources, such as 1,3-butanediol, triacetin, or tributyrin, that would supply systemic caloric and protein requirements without the adverse effect of conventional diets. (Stroke 1992;23:564-568)

KEY WORDS • cerebral infarction • hyperglycemia • ketone bodies • rats

Conventional diets, which use carbohydrates as the primary source of nonprotein calories, commonly cause hyperglycemia in patients with neurological injuries. Although the diets do not contain glucose, carbohydrates absorbed by the small intestine are readily metabolized to glucose by the liver. The glucose is delivered to other tissues by the circulation. Hyperglycemia, however, has a detrimental effect on neurological recovery after central nervous system ischemia.1-3

Although insulin has been used in experimental studies to lower blood glucose concentration and the severity of cerebral and spinal cord infarction,4-6 it would be difficult to consistently maintain blood glucose near fasting levels in unstable, acutely injured patients who may also be insulin resistant. A more practical approach may be to provide a source of nonprotein calories that is less likely to be metabolized to glucose than are carbohydrates. Previous studies have suggested that alimentation with nonglycolytic energy substrates, such as ketone bodies and short-chain fatty acids, may have a less detrimental effect on neurological recovery from ischemia than does alimentation with glucose.7-8 1,3-Butanediol, which is converted by the liver to β-hydroxybutyrate, has been the most intensively studied alternate substrate and has been shown to have protective effects during cerebral hypoxia-ischemia.9,10 The purpose of this study was to evaluate the effect on ischemia-induced infarction of alimentation with five experimental diets in which a major portion of the carbohydrate calories were replaced with alternate sources of energy. Occlusion of the middle cerebral artery in the rat was chosen for this study because the size of the cortical infarction produced by a temporary occlusion of 45 minutes has been found to be dependent on the rat's nutritional status. A small infarction or no infarction occurs in the fasted rat, whereas an infarction of significant size occurs in the fed or glucose-infused rat.11,12

Materials and Methods
Sixty-nine Long-Evans rats weighing 300±25 gm were used in this study. Animal care and experimental procedures followed the National Institutes of Health.
**Table 1. Contents of the Six Experimental Diets**

<table>
<thead>
<tr>
<th>Component</th>
<th>Diet 1 (normal control)</th>
<th>Diet 2 (1,3-butanediol)</th>
<th>Diet 3 (triacetin/tributyrin)</th>
<th>Diet 4 (triacetin)</th>
<th>Diet 5 (tributyrin)</th>
<th>Diet 6 (high fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
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<tr>
<td>Carbohydrates</td>
<td>51.5</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Fat</td>
<td>31.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Long-chain triglycerides</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>62</td>
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<tr>
<td>Medium-chain triglycerides</td>
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<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Triacetin</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tributyrin</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>1,3-Butanediol</td>
<td>0</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are given as percentage of calories.

Guide for the Care and Use of Laboratory Animals, and was approved by the local institutional animal research committee.

The rats were randomly assigned to one of seven treatment groups using a blocked design. The investigators performing the studies were blinded to the experimental group. Six of the groups were fed one of the experimental diets shown in Table 1 for 12 hours before the ischemia study. A seventh group (n=15) was fasted for 24 hours before the ischemia study. The normal control diet (diet 1, n=15) was similar to commercially available nasogastric feedings, with 51.5% of the calories contributed by carbohydrates and 17% by protein. The experimental diets all had the same protein—carbohydrate base, with 17% protein calories and 21% carbohydrate calories. Diets 2–5 contained 30% long-chain and medium-chain triglycerides, with the remaining 32% of the calories either 1,3-butanediol (diet 2, n=8), triacetin and tributyrin (diet 3, n=8), triacetin (diet 4, n=8), or tributyrin (diet 5, n=8). The caloric density of all of the diets was 1.5 kcal/ml. In diet 6 (n=7), the remainder of the calories were contributed by long-chain triglycerides. The diets were provided by Ross Laboratories, Cleveland, Ohio.

On the day before the ischemia study, the rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (5 mg/kg i.p.). A polyethylene tube (PE 20) was tunneled under the skin from the right nostril to the midsagittal region of the back. The end of the tube was inserted through the right nostril and the tip positioned in the stomach. The tube was then securely sutured in place. After the animals were fully awake from the anesthesia, the assigned diet was started as a continuous nasogastric infusion at 462 kJ (110 kcal)/(kg body wt)^0.75/day, a rate that would replace 100% of their caloric expenditure over 24 hours. In preliminary studies, it was determined that plasma glucose concentration stayed within 3 hours after infusion was started. The animals were fed for 12 hours before the ischemia study to assure steady-state conditions.

On the day of the ischemia study, the rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (5 mg/kg i.p.). The rectal temperature was monitored continuously and maintained at 37.5±0.5°C with a heating pad placed under the animal and controlled by a rectal thermistor. The right femoral artery was cannulated for monitoring arterial blood pressure and heart rate and for obtaining blood samples for glucose and blood gases. A ventral midline cervical incision was made, and both common carotid arteries were carefully isolated. Care was taken to avoid injury to nerves adjacent to the arteries.

A 1.5-cm scalp incision was made at the midpoint between the right eye and ear. The temporalis muscle was separated in the plane of its fiber bundles and retracted to expose the zygoma and squamosal bone. Using microsurgical technique, a burr hole 2 mm in diameter was made with a dental drill 1 mm rostral to the anterior junction of the zygoma and squamosal bone. Care was taken to avoid thermal or physical injury to the cortex during preparation of the burr hole. The dura was carefully pierced with a #11 scalpel blade, exposing the middle cerebral artery.

Immediately before producing the experimental ischemia, a 1-ml blood sample was obtained through the arterial catheter for measurement of arterial blood gases, plasma glucose, lactate, ketone bodies, acetate, and butyrate concentrations. The blood pressure and rectal temperature were recorded immediately before the ischemia. To minimize the risk of aspiration during the ischemia period, the nasogastric feedings were stopped just before occlusion of the middle cerebral artery.

The middle cerebral artery was temporarily occluded by slipping a curved 100-μm diameter microvascular needle under the artery and gently lifting the vessel. Complete occlusion of the vessel was visually confirmed. At the same time both common carotid arteries were clipped with atrumatic aneurysm clips. After 45 minutes had elapsed, the clips and the needle were removed, and reperfusion was observed in all animals. The arterial catheter was removed, all surgical wounds were sutured, and the animals were allowed to awaken from anesthesia.

The infarct volume in the right middle cerebral artery territory was measured morphometrically using 2,3,5-triphenyltetrazolium chloride (TTC). In previous studies with this model, the size of the cortical infarction in the MCA distribution progressively increased up to 6 hours after ischemia, and remained unchanged from 6 to 72 hours after ischemia. Twenty-four hours after the ischemia period, the rats were deeply anesthetized with ketamine and xylazine and were perfused through the left ventricle with 200 ml 0.9% saline. The brain was removed, cooled in iced saline for 5 minutes, and dissected in the coronal plane at 2-mm intervals using a brain slicer. The brain slices were incubated in 2% TTC in phosphate-buffered saline at 37°C and then stored in...
TABLE 2. Preischemic Physiological Variables of Fasted Rats and Six Diet Groups  

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. animals</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td></td>
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<tr>
<td>Blood pressure</td>
<td>87±3</td>
<td>80±4</td>
<td>79±4</td>
<td>83±4</td>
<td>85±5</td>
<td>81±4</td>
<td>85±5</td>
<td>0.72</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>37.2±0.1</td>
<td>0.31</td>
</tr>
<tr>
<td>Arterial PaO₂</td>
<td>131±14</td>
<td>116±14</td>
<td>100±19</td>
<td>103±19</td>
<td>154±19</td>
<td>148±19</td>
<td>88±20</td>
<td>0.12</td>
</tr>
<tr>
<td>Arterial PaCO₂</td>
<td>49±2</td>
<td>55±2</td>
<td>54±3</td>
<td>53±3</td>
<td>51±3</td>
<td>54±3</td>
<td>56±3</td>
<td>0.45</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.31±0.01</td>
<td>7.32±0.01</td>
<td>7.32±0.02</td>
<td>7.33±0.02</td>
<td>7.35±0.02</td>
<td>7.30±0.02</td>
<td>7.34±0.02</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Values are mean±SD. Probability values determined by analysis of variance.

10% neutral-buffered formalin for morphometric studies. The cross-sectional area of infarction on both the anterior and posterior surfaces of each of eight brain slices was measured using a computerized image analysis system. The total infarct volume was derived from the sum of the average infarct volume from each slice.

Arterial blood gases were measured on a Corning 170 blood gas analyzer. Plasma glucose and lactate concentrations were measured on a YSI analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). Plasma β-hydroxybutyrate and butyrate were determined by high-performance liquid chromatography (HPLC) using a modification of the method described by Chong et al. Plasma samples were diluted 1:1 with 0.01 M dibasic phosphate buffer, pH 8, and centrifugally ultrafiltered (Centrifree; Amicon, Danvers, Mass.). The filtrate was applied to methanol-conditioned Sep-Pak Light C-18 cartridges (Waters, Milford, Mass.), and organic acids were eluted with phosphate buffer. Analysis of the organic acids using HPLC was performed at ambient temperature by injecting 100 µl eluate onto an Aminex column (Bio-Rad, Richmond, Calif.). The mobile phase (0.0025 M sulfuric acid) was delivered isocratically at 0.6 ml/min and detection was performed at 210 nm (ISCO, Lincoln, Neb.). Quantification of peak areas was performed using MAXIMA 820 chromatography software (Dynamic Solutions, Milford, Mass.), and peaks were identified by retention time. The sensitivity of the method was dependent on the ultraviolet absorbance of the individual compounds; therefore, the limit of detectability for the ketones was 0.08 mmol/l, whereas that for poorly absorbing butyrate was 0.2 mmol/l. Plasma acetoacetate and acetate were measured enzymatically using commercially available reagents (Boehringer Mannheim). The lower limit of detectability with this method was 0.010 mmol/l.

All summary data are expressed as mean±SD. The physiological parameters, the plasma concentrations of the energy substrates, and the 24-hour infarct volume were compared by analysis of variance and the Waller-Duncan K ratio t test to adjust for multiple comparisons. The relation of the plasma concentrations of the energy substrates to the infarct volume were also examined by linear regression analysis.

Results

All of the experimental diets were well tolerated during the 12-hour feeding period before the ischemia. No adverse effects such as diarrhea or change in normal activity were observed, and there were no preischemic differences in mean blood pressure, arterial blood gases, or rectal temperature among the experimental groups that could be attributed to the diets (Table 2). Blood pressure and rectal temperature were continuously monitored throughout the ischemia and early reperfusion periods, with no significant difference in any of the experimental groups. Arterial blood gas measurements were not repeated during ischemia or early reperfusion, therefore differences among the groups after the preischemic determination cannot be excluded.

Plasma glucose concentration, however, was significantly altered by the diets (Table 3). The glucose concentration before ischemia in the fastest animals was the lowest; the glucose concentration with the normal control diet (diet 1), which contained the most carbohydrate calories, averaged 9.1±1.4 µmol/ml. The pre-

Table 3. Arterial Concentrations of Energy Substrates (µmol/ml) Before Ischemia in Fasted Rats and Six Diet Groups

<table>
<thead>
<tr>
<th>No. rats</th>
<th>Fasted group (normal control)</th>
<th>Diet 1 (1,3-butanediol)</th>
<th>Diet 2 (triacetin/tributyrin)</th>
<th>Diet 3 (tributyrin)</th>
<th>Diet 4 (tributyrin)</th>
<th>Diet 5 (tributyrin)</th>
<th>Diet 6 (high fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of calories as carbohydrates</td>
<td>51.5</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.4±1.1</td>
<td>9.1±1.4*</td>
<td>7.8±1.3*†</td>
<td>8.8±1.2*</td>
<td>10.1±1.9*</td>
<td>9.4±1.4*</td>
<td>8.7±0.8*</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.5±0.2</td>
<td>0.7±0.2*</td>
<td>0.7±0.1*</td>
<td>0.6±0.2</td>
<td>0.7±0.3</td>
<td>0.5±0.2†</td>
<td>0.5±0.1†</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>0.91±0.62</td>
<td>0.46±0.48*</td>
<td>1.3±0.77†</td>
<td>0.42±0.30</td>
<td>0.44±0.44</td>
<td>0.48±0.28</td>
<td>0.23±0.23†</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>0.10±0.13</td>
<td>0.16±0.11</td>
<td>0.85±0.54††</td>
<td>0.15±0.22</td>
<td>0.15±0.10</td>
<td>0.14±0.14</td>
<td>0.14±0.14</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.06±0.03</td>
<td>0.03±0.03*</td>
<td>0.04±0.03</td>
<td>0.23±0.33†</td>
<td>0.21±0.17†</td>
<td>0.04±0.02</td>
<td>0.04±0.03</td>
</tr>
</tbody>
</table>

Values are mean±SD. *p<0.05 different from fasted rats. †p<0.05 different from diet 1 rats.
ischemic glucose concentration with the 1,3-butanediol was intermediate. The preischemic plasma glucose concentration with diets 3–6 was not significantly different from the normal control diet.

The plasma concentrations of the other energy substrates examined were also significantly altered by the diets (Table 3). The plasma level of the ketone bodies β-hydroxybutyrate and acetoacetate were elevated in the fasted group and in the 1,3-butanediol (diet 2) group. Acetate was elevated in the two groups that received triacetin (diets 3 and 4). Butyrate was not detectable in any of the plasma samples, even in the groups fed tributyrin (diets 3 and 5).

The volume of the infarcted tissue was significantly related to the diet (Figure 1). The smallest infarcts were obtained in the fasted group. The largest infarcts were seen in the normal control diet (diet 1). Of the experimental diets, the smallest infarct volumes occurred in the 1,3-butanediol (diet 2) group and in the triacetin/tributyrin (diet 3) group.

The volume of the infarct was directly related to the preischemic plasma glucose concentration (n = 69, r = 0.47, p < 0.01) (Figure 2). There was no relation between the infarct volume and plasma lactate, ketone body, or acetate levels.

**Discussion**

Nutritional support of the patient with a neurological injury is a complex problem. Patients with a severe head injury are hypermetabolic and catabolic, and they require early and intensive nutritional support to minimize malnutrition-related complications. Nutritional support may, however, adversely affect neurological recovery. Experimental studies have shown that hyperglycemia caused by glucose infusion or the postprandial state worsens neurological recovery from cerebral and spinal cord ischemia. The mechanism of this detrimental effect is not completely understood; however, hyperglycemia has been associated with increased accumulation of lactic acid in many studies. A diet that would supply protein and caloric needs without adversely affecting neurological recovery would have widespread use in patients with central nervous system ischemia or trauma.

1,3-Butanediol, an alcohol converted to β-hydroxybutyrate by the liver, was chosen as one source of nonprotein calories for these studies. Intravenous infusion of 1,3-butanediol in doses up to 47 mmol/kg is well tolerated in experimental animals. Although intake of large doses of 1,3-butanediol has been demonstrated in...
animal studies to cause intoxication, no significant toxicity has been found with chronic administration of lower doses. Studies in normal adults have shown that supplying 1,3-butanediol as 10% of the total caloric intake results in reduced nitrogen loss, decreased blood glucose, and increased hydroxybutyrate concentration. Experimental studies in models of cerebral hypoxia-ischemia have demonstrated a reduced central nervous system lactic acidosis and significant protective effects when 1,3-butanediol is administered intravenously before the hypoxic-ischemic event. It is not clear whether the protective effect is due to the 1,3-butanediol or to the ketone body metabolites.

The short-chain fatty acids acetate and butyrate were chosen as the other nonprotein calorie source in this study. The liver uses these short-chain fatty acids for long-chain fatty acid synthesis and can convert butyrate and acetate into ketone bodies. In addition, these short-chain fatty acids can be metabolized to CO2 through the tricarboxylic acid cycle. Experimental studies in hypermetabolic animals with femoral fractures have demonstrated that acetate, supplied by infusion of monoaetin, is metabolized as efficiently as glucose and has the advantage of not producing hyperglycemia. The effect of short-chain fatty acids on neurological recovery from ischemia have not been previously well studied, but one report suggested that triacetin given intravenously before spinal cord ischemia did not alter outcome. The present study demonstrates that not only the nutritional state (fasted versus fed) but also the content of the diet can alter recovery from cerebral ischemia. The source of the nonprotein calories can alter the blood glucose concentration and the size of the resulting cerebral infarction. It may be possible to develop a diet using nonglycolytic caloric sources, such as 1,3-butanediol, triacetin, or tributyrin, to replace systemic calorie intake results in reduced nitrogen loss, decreased blood glucose concentration and the size of the resulting cerebral ischemia in the rat. Local cerebral blood flow and glucose utilization. Stroke 1980;11:347–354.

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Dietary nonprotein calories and cerebral infarction size in rats.
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