Effect of Hyperketonemia and Hyperlacticacidemia on Symptoms, Cognitive Dysfunction, and Counterregulatory Hormone Responses During Hypoglycemia in Normal Humans

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The brain usually depends almost exclusively on glucose for its energy requirements. During hypoglycemia associated with prolonged fasting or strenuous exercise, circulating ketone-body and lactate levels increase severalfold; in both situations, certain signs and symptoms of hypoglycemia are diminished. Therefore, to test the hypothesis that hyperketonemia or hyperlacticacidemia of the magnitude seen during certain clinical situations can substitute for glucose as an energy source for the brain and alter physiological responses to hypoglycemia, we assessed autonomic and neuroglycopenic symptoms, counterregulatory hormone responses, and cognitive function during standardized insulin-induced hypoglycemia in normal volunteers with and without infusion of β-hydroxybutyrate (BOHB) or lactate designed to reproduce circulating levels of these substrates seen during prolonged fasting and strenuous exercise. Compared with paired control experiments, infusion of BOHB and lactate increased the glycemic threshold (required greater hypoglycemia for initiation) and reduced the magnitude of autonomic and neuroglycopenic symptoms, counterregulatory hormone responses, and cognitive dysfunction (all $P < 0.05$). The hypoglycemic threshold for autonomic symptoms increased from 3.8 ± 0.1 to 3.1 ± 0.2 mmol/l during BOHB infusion and from 3.7 ± 0.1 to 2.8 ± 0.1 mmol/l during lactate infusion, and the threshold for neuroglycopenic symptoms increased from 2.8 ± 0.1 to 2.4 ± 0.1 and 2.3 ± 0.1 mmol/l, respectively. The magnitude for autonomic symptoms decreased from 12 ± 2 and 11 ± 1 to 6 ± 2 and 4 ± 1 during BOHB and lactate infusion, respectively. Neuroglycopenic symptoms decreased from 11 ± 2 to 3 ± 1 during both series of experiments. Infusion of BOHB and lactate-reduced responses for all counterregulatory hormones, the reduction being the greatest for epinephrine (~57 and 73%, during BOHB and lactate infusion, respectively) and least for cortisol (~28 and 29%, respectively). These results indicate that under certain clinical conditions, BOHB and lactate may substitute for glucose as a fuel for the brain and alter physiological responses to hypoglycemia. *Diabetes* 43:1311–1317, 1994

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BOHB, β-hydroxybutyrate; IDDM, insulin-dependent diabetes mellitus.

Because of blood-brain barrier transport restrictions and the limited availability of alternate fuels, glucose is normally the exclusive substrate used to satisfy the brain's energy requirements (1). Moreover, since the brain does not produce glucose and has only minimal glycogen stores, its functional integrity requires a continuous provision of glucose from the circulation (1). Indeed, an acute decrease in arterial glucose concentration from 5.0 to 3.5 mmol/l initiates a characteristic hierarchy of responses aimed at preventing further decreases (2,3).

Under certain circumstances, however, adaptive changes may occur to protect the brain from hypoglycemia. Rats fed a ketogenic diet exhibit less behavioral deterioration during hypoglycemia (4). In humans, fasts of >72 h reduce symptoms, cognitive dysfunction, and counterregulatory hormone responses to acute insulin-induced hypoglycemia (5–7). Similar findings have been observed in humans during prolonged and repetitive episodes of hypoglycemia (8–10). Under these conditions, alternate fuels such as ketone bodies may become available to the brain, and/or blood-brain barrier substrate transport may change. During prolonged starvation in humans, for example, the circulating concentration of ketone bodies increases to an extent that they become the major provider of energy for the brain (11–13). Similarly, prolonged hypoglycemia in rats increases blood-brain glucose transport (14,15).

Regarding the availability of alternate fuels for the brain, during hypoglycemia of several hours' duration as well as during recovery from brief hypoglycemia, the circulating levels of ketone bodies can increase as much as sixfold (16) so that they could become a significant fuel for the brain. However, studies examining the effects of infusions of ketone bodies during hypoglycemia have yielded inconsistent results regarding counterregulatory hormone responses and symptoms (17–21). None have specifically examined cognitive function. To date, there has been little direct support for appreciable use of lactate by the central nervous system during acute hypoglycemia in humans. Animal studies, however, indicate increased uptake of lactate by the brain during hypoglycemia (22,23). During strenuous exercise in humans, plasma lactate levels can reach 10 mmol/l (24,25); under such conditions, plasma glucose levels can decrease <3.0 mmol/l without any apparent symptoms of hypoglycemia (26,27), which suggests possible substitution of lactate for glucose as

fuel for the brain. Therefore, to test the hypothesis that hyperketonemia of the magnitude seen during prolonged hypoglycemia or during recovery from acute hypoglycemia or that hyperlacticacidemia of the magnitude seen during strenuous exercise could provide sufficient fuel for the central nervous system so as to diminish symptoms, cognitive dysfunction, and counterregulatory hormone release during hypoglycemia, we assessed these responses in normal volunteers during the infusion of β-hydroxybutyrate (BOHB) and of lactate.

RESEARCH DESIGN AND METHODS

Informed, written consent was obtained from 13 volunteers (8 men and 5 women) at 34 ± 3 years of age (body mass index: 28 ± 1 kg/m^2). The protocol had been reviewed and approved by the institutional review board.

Each subject was studied at least twice in random order. Data of the control experiments of seven subjects who participated in both lactate and BOHB protocols have been used in previous publications (2,10). Six additional subjects were recruited: three underwent only control and lactate infusion studies, and three underwent only control and BOHB studies. The shortest interval between studies was 2 weeks, and the longest interval between studies was ~1 year. On each occasion, subjects were admitted to the Clinical Research Center the evening before the experiments and were given a standard dinner between 5:30 and 6:30 P.M. (30 kcal/kg, 50% carbohydrate, 35% fat, and 15% protein) and a standard snack (~4 h later) at bedtime (10 kcal/kg, 50% carbohydrate, 35% fat, and 15% protein). Between 7:00 and 7:30 A.M., a hand vein was cannulated retrogradely and maintained in a plexiglass thermoregulated box (70°C) for sampling of arterialized venous blood. After a 60-min equilibration period, a continuous intravenous infusion of insulin was begun (1 mU · kg^{-1} · min^{-1} for 270 min, followed by 2 mU · kg^{-1} · min^{-1} for an additional 60 min). Plasma glucose was clamped by variable glucose infusions at sequential target glucose concentrations of 4.3, 3.7, 3.0, and 2.4 mmol/l. The plasma glucose concentration was allowed to decrease ~0.66 mmol/l over 45 min, and a plateau was maintained for 45 min before the next decrease. At 90 min after the start of the hypoglycemic clamp, a primed (40 μmol · kg^{-1} · min^{-1} during 20 min) continuous (20 μmol · kg^{-1} · min^{-1}) infusion of sodium DL-BOHB (Sigma, St. Louis, MO) on one occasion or Na-L-lactate (50 μmol · kg^{-1} · min^{-1} during 20 min, 30 μmol · kg^{-1} · min^{-1}) (Sigma) was started in random order in a single-blind fashion. Arterialized venous blood samples were drawn every 30 min from 0 to 360 min for determination of plasma insulin, growth hormone, glucagon, cortisol, epinephrine, norepinephrine, BOHB, and lactate.

A semi-quantitative symptom questionnaire was administered every 15 min. Subjects scored each of the following symptoms from 0 (none) to 5 (severe): dizziness, tingling, blurred vision, difficulty in thinking, faintness, anxiety, palpitations, sweating, irritability, hunger, and tremor. The first five symptoms were considered neuroglycopenic, and the other six were considered autonomic. The sum of each of these constituted the symptom score.

In addition, during each of the glycemic plateaus, the following standard cognitive tests were administered (2): Trail Making Part B, Verbal Fluency, Interference Subtest from the Stroop Test, Simple and Choice Visual Reaction Time, Word and Color Subtests from the Stroop Test, Digit Vigilance Test, Trail Making Part A, Verbal Memory Test, and Forward and Backward Digit Span.

Analytical methods. Plasma glucose was measured using a Yellow Springs Instrument glucose analyzer (YSI, Yellow Springs, OH). Plasma counterregulatory hormones were measured by previously described assays (2,3). Plasma BOHB and lactate were determined microfluorometrically (28).

Statistical analysis. Glycemic thresholds for various parameters were considered to be the plasma glucose concentration at which changes in the parameters first exceeded the 95% confidence interval observed for those parameters at the corresponding time point in previously published euglycemic control experiments (2). Results of each cognitive test were transformed to z scores to provide unitless data and were summed (29). Results are given as means ± SE and were evaluated using the paired Student's t test (29). $P < 0.05$ was considered statistically significant.

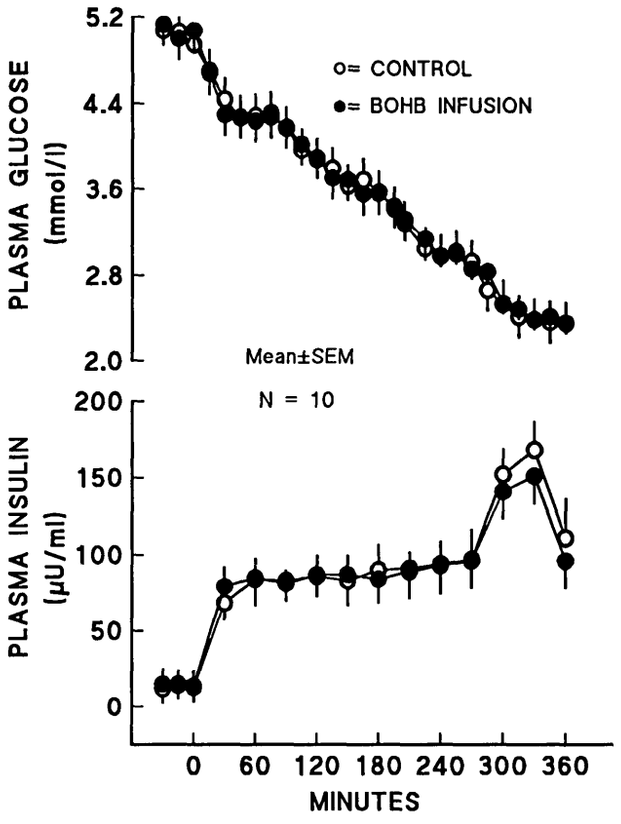

FIG. 1. Plasma glucose and insulin concentrations in control (saline) and BOHB experiments.

RESULTS

Effects of BOHB infusion during hypoglycemia

Plasma glucose, insulin, BOHB, and counterregulatory hormone concentrations. Plasma glucose and insulin levels were comparable throughout in control and BOHB infusion experiments (Fig. 1).

Basal plasma BOHB concentrations were comparable in both experiments (Fig. 2). During the control experiments, plasma BOHB concentrations decreased from a basal level of 113 ± 33 μmol/l to a nadir of 18 ± 4 μmol/l at 150 min and subsequently increased to ~50 μmol/l at the end of the experiment. During the BOHB infusion experiments, plasma BOHB levels initially decreased from a basal level of 105 ± 36 μmol/l to a nadir of 10 ± 5 μmol/l at 90 min just before starting the BOHB infusion. Plasma BOHB subsequently increased to ~1.9 mmol/l within 30 min and averaged ~1.7 mmol/l for the remainder of the experiment (Fig. 2).

Basal plasma counterregulatory hormone concentrations were comparable in both experiments. Infusion of BOHB significantly reduced the responses for all counterregulatory hormones, the reduction being greatest for epinephrine (~57%) and least for cortisol (~28%) (Table 1, Fig. 2). Moreover, initial increases for all counterregulatory hormones began at significantly lower plasma glucose concentrations in the BOHB infusion experiments ($P < 0.05$) (Table 2).

Symptom scores and cognitive tests. Infusion of BOHB reduced both autonomic and neuroglycopenic symptom scores (Fig. 3), and there was less deterioration in cognitive function ($P < 0.05$) (Fig. 4). Moreover, the plasma glucose concentrations at which both groups of symptoms and cognitive dysfunction began were significantly lower than those in the control experiments ($P < 0.05$) (Table 2).

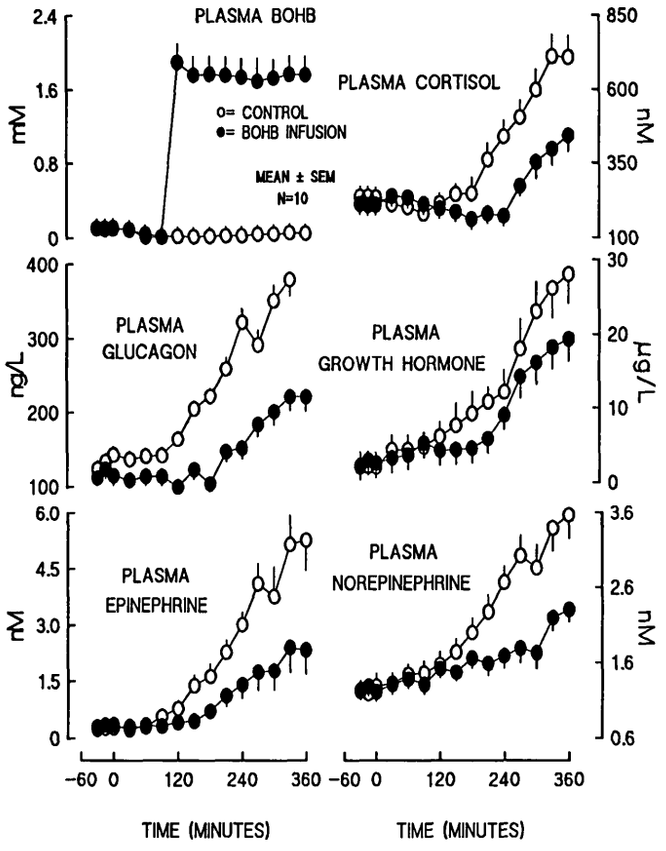

FIG. 2. Plasma BOHB, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol concentrations in control (saline) and BOHB experiments.

Effects of lactate infusion during hypoglycemia

Plasma glucose, insulin, lactate, and counterregulatory hormone concentrations. Plasma glucose and insulin levels were comparable throughout in control and lactate infusion experiments (Fig. 5).

Basal plasma lactate concentrations were comparable in both experiments. During the control experiments, plasma lactate concentrations increased slightly but significantly ($P < 0.03$) from a basal level of 1.2 ± 0.2 to ~1.5 mmol/l toward the end of the experiment. During the lactate infusion experiments, plasma lactate levels increased to levels of ~5.4 mmol/l within 60 min of starting the lactate infusion and remained at that level for the remainder of the experiment (Fig. 6).

Basal plasma counterregulatory hormone concentrations were comparable in both experiments. Infusion of lactate significantly reduced the responses for all counterregulatory hormones, the reductions being greatest for epinephrine (~73%) and least for cortisol (~29%) (Table 3, Fig. 6). Moreover, initial increases for all counterregulatory hormones began at significantly lower plasma glucose concentrations in the lactate infusion experiments ($P < 0.05$) (Table 4).

TABLE 1
Magnitudes of plasma counterregulatory hormone responses, symptoms, and cognitive dysfunction during hypoglycemia

	Control experiments	BOHB infusion
Epinephrine (nmol/l)	6.1 ± 1.1	2.6 ± 0.4*
Norepinephrine (nmol/l)	4.1 ± 0.6	2.5 ± 0.3*
Glucagon (ng/l)	426 ± 58	248 ± 44*
Growth hormone (μg/l)	30 ± 4	20 ± 4*
Cortisol (nmol/l)	748 ± 61	538 ± 55*
Autonomic symptom score	12 ± 2	6 ± 2*
Neuroglycopenic symptom score	11 ± 2	3 ± 1*
Cognitive dysfunction	11 ± 3	3 ± 1*

Data are means ± SE. Cognitive dysfunction data are the sums of z scores. *$P < 0.05$.

TABLE 2
Glycemic thresholds for plasma counterregulatory hormone responses, symptoms, and cognitive dysfunction during hypoglycemia

	Control experiments	BOHB infusion
Epinephrine	3.9 ± 0.1	3.3 ± 0.2*
Norepinephrine	3.7 ± 0.1	3.0 ± 0.1*
Glucagon	3.8 ± 0.2	3.0 ± 0.2*
Growth hormone	3.7 ± 0.2	3.1 ± 0.2*
Cortisol	3.3 ± 0.1	2.8 ± 0.2*
Autonomic symptoms	3.8 ± 0.1	3.1 ± 0.2*
Neuroglycopenic symptoms	2.8 ± 0.1	2.4 ± 0.1*
Cognitive functions	3.0 ± 0.1	2.6 ± 0.1*

Data are means ± SE in mmol/l. *$P < 0.05$.

Symptom scores and cognitive tests. Infusion of lactate reduced both autonomic and neuroglycopenic symptom scores (Fig. 7), and there was less deterioration in cognitive function ($P < 0.05$) (Fig. 8). Moreover, the plasma glucose concentrations at which both groups of symptoms and

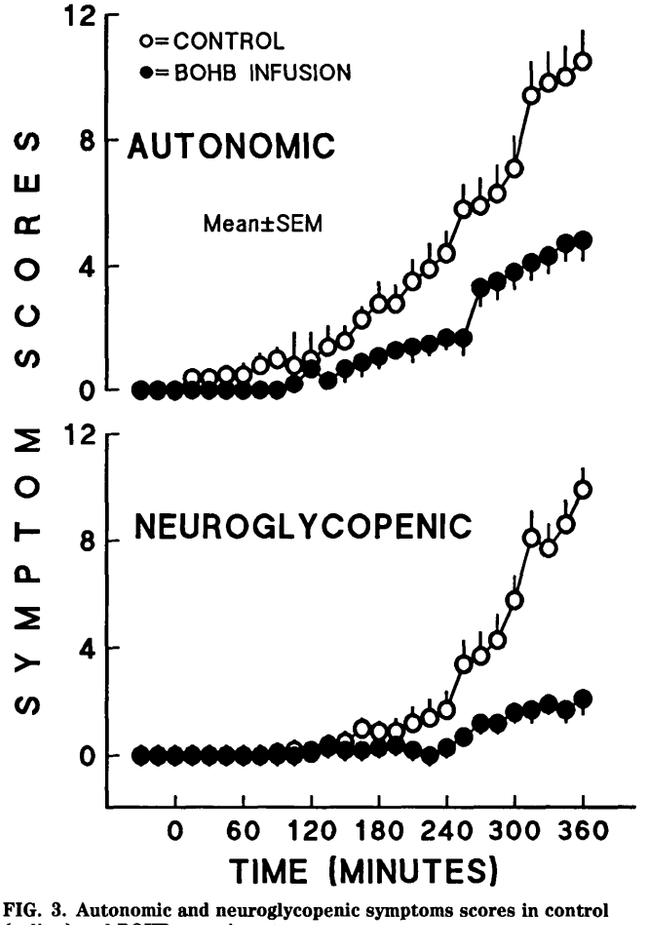

FIG. 3. Autonomic and neuroglycopenic symptoms scores in control (saline) and BOHB experiments.

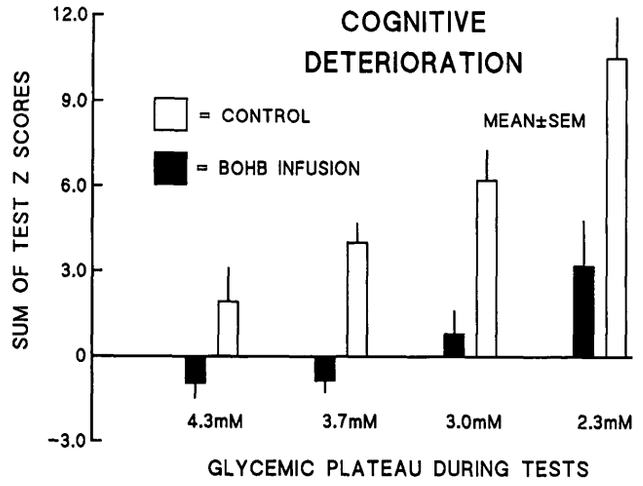

FIG. 4. Changes in sum of *z* scores of cognitive function tests in control (saline) and BOHB experiments.

cognitive dysfunction began were significantly lower than those in the control experiments ($P < 0.05$) (Table 4).

DISCUSSION

The present experiments were undertaken to test the hypothesis that increased availability of ketone bodies and of lactate of the magnitude seen in certain clinical situations could provide sufficient fuel for the central nervous system so as to reduce symptoms, cognitive dysfunction, and counterregulatory hormone release during hypoglycemia. For this purpose, we assessed these parameters in control experiments and in experiments in which BOHB or lactate was

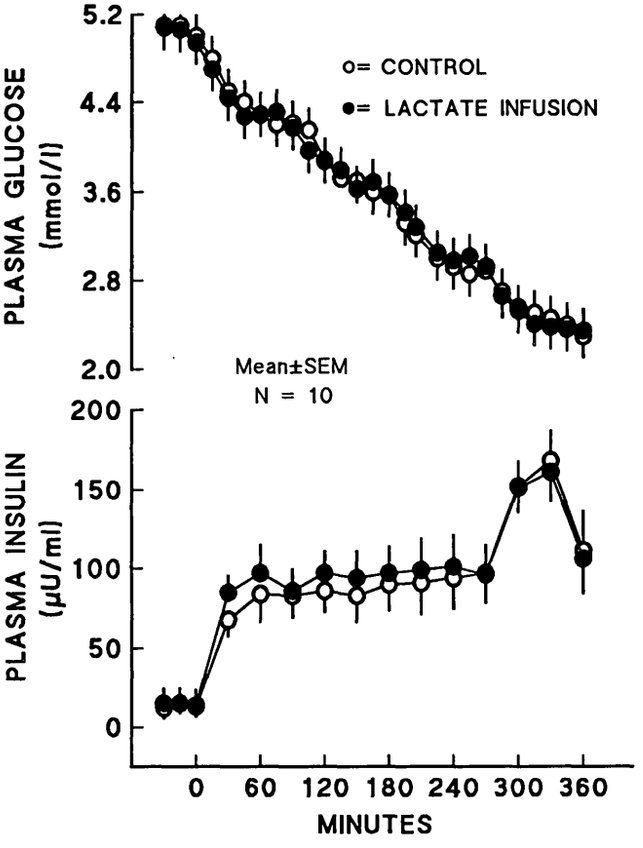

FIG. 5. Plasma glucose and insulin concentrations in control (saline) and in lactate experiments.

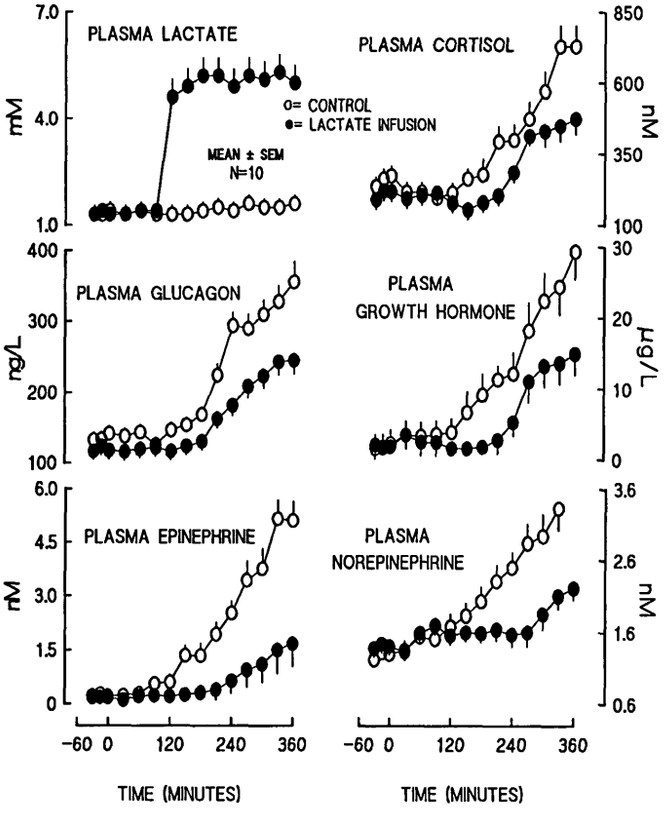

FIG. 6. Plasma lactate, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol concentrations in control (saline) and in lactate experiments.

infused. The infusion rate of BOHB was chosen to simulate circulating ketone-body levels observed during prolonged fasting (11,30) and recovery from hypoglycemia (16). The rate of lactate infusion was chosen to simulate plasma lactate concentrations similar to those observed during moderate exercise (24,25).

Both infusion of BOHB and infusion of lactate reduced counterregulatory hormone responses, autonomic and neuroglycopenic symptoms, and cognitive dysfunction during hypoglycemia. It is difficult to compare responses to each of the infusions because the circulating levels of lactate (~5.4 mmol/l) and BOHB (~1.7 mmol/l) were different. However, considering their different molecular weights and the fact that roughly similar responses were found, one could conclude that BOHB was the more effective agent. Interestingly, a similar pattern in the relative suppression of counterregu-

TABLE 3
Magnitudes of plasma counterregulatory hormone responses, symptoms, and cognitive dysfunction during hypoglycemia

	Control experiments	Lactate infusion
Epinephrine (nmol/l)	6.2 ± 1.0	1.7 ± 0.3*
Norepinephrine (nmol/l)	4.3 ± 0.6	2.7 ± 0.5*
Glucagon (ng/l)	442 ± 68	206 ± 56*
Growth hormone (μg/l)	29 ± 4	16 ± 2*
Cortisol (nmol/l)	776 ± 55	555 ± 80*
Autonomic symptom score	11 ± 1	4 ± 1*
Neuroglycopenic symptom score	11 ± 2	3 ± 1*
Cognitive dysfunction	12 ± 3	3 ± 1*

Data are means ± SE. Cognitive dysfunction data are sums of *z* scores. *$P < 0.05$.

TABLE 4
Glycemic thresholds for plasma counterregulatory hormone responses, symptoms, and cognitive dysfunction during hypoglycemia

	Control experiments	Lactate infusion
Epinephrine	3.9 ± 0.1	3.3 ± 0.1*
Norepinephrine	3.8 ± 0.1	3.0 ± 0.2*
Glucagon	3.7 ± 0.1	2.8 ± 0.4*
Growth hormone	3.7 ± 0.2	2.8 ± 0.1*
Cortisol	3.4 ± 0.1	2.6 ± 0.1*
Autonomic symptoms	3.7 ± 0.1	2.8 ± 0.2*
Neuroglycopenic symptoms	2.8 ± 0.1	2.3 ± 0.1*
Cognitive functions	3.0 ± 0.1	2.6 ± 0.1*

Data are means ± SE in mmol/l. *$P < 0.05$.

latory hormone responses (epinephrine > glucagon > growth hormone > cortisol) was observed with each substrate.

Our results, while confirming some previous studies (17,18,20) regarding the ability of acute hyperketonemia to reduce counterregulatory hormone responses, demonstrate for the first time to our knowledge that acute hyperketonemia not only reduces autonomic and neuroglycopenic symptoms but also reduces cognitive dysfunction during hypoglycemia. Amiel et al. (20) reported in a study similar to ours in six normal volunteers that the maximal responses for all counterregulatory responses (except glucagon) were decreased and that only the glycemic threshold for epinephrine was altered. The discrepancy with our data could be explained by the lower plasma ketone-body levels in Amiel et al.'s study and the fact that, since the changes in counterregulatory hormones other than epinephrine are less than those of epinephrine, a change in their threshold would be more difficult to detect. Another possible explanation is the difference in methods by which the glycemic thresholds were calculated in that study and our own. It should be pointed out that we infused BOHB and did not measure levels of acetoacetate, the other ketone body. This was done because of the greater stability of BOHB. We know of no reason why results would have been different had we infused acetoacetate instead of BOHB.

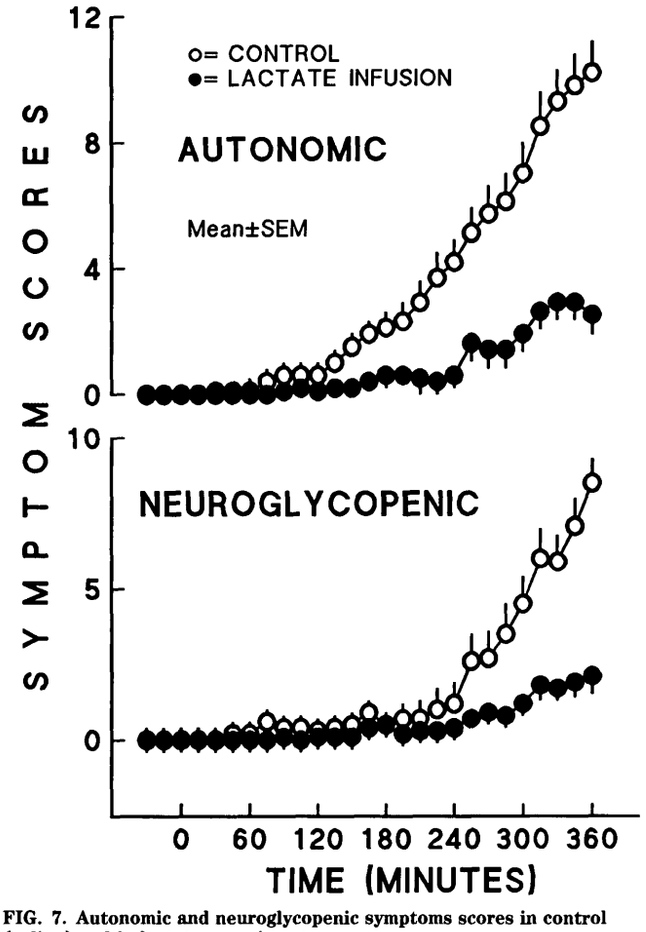

FIG. 7. Autonomic and neuroglycopenic symptoms scores in control (saline) and in lactate experiments.

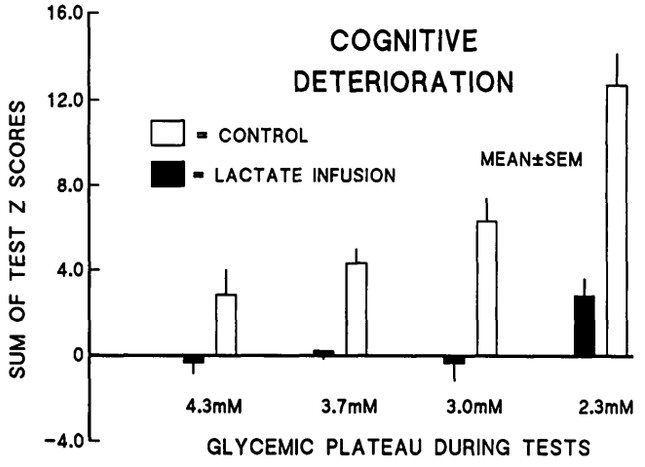

FIG. 8. Changes in sum of z scores of cognitive function tests in control (saline) and in lactate experiments.

Our experiments indicate a similar effect of hyperlacticacidemia on the responses to hypoglycemia. In insulin-dependent diabetes mellitus (IDDM) dogs, Avogaro et al. (22) showed that brain lactate uptake increased severalfold during hypoglycemia. Similarly, Nemoto et al. (23) found evidence that lactate can be an important energy source in adult dogs during hypoglycemia. They measured AV-differences (superiorsagittal sinus) of lactate across the brain of adult dogs and found that, during hyperlacticacidemia (7–8 mmol/l) and hypoglycemia (2 mmol/l), cerebral lactate uptake increased by many times. They concluded that lactate can replace ~25% of the glucose used as a brain substrate during hypoglycemia. The only other study examining whether increased availability of lactate could influence the physiological responses to hypoglycemia in humans, which appeared while our manuscript was under revision (31), found results essentially identical to ours except that neither the threshold nor magnitude of plasma glucagon responses were affected. The reason for this discrepancy is unclear, but twofold greater elevations of plasma lactate were used in our studies.

Our findings have implications regarding brain substrate utilization during strenuous exercise. Coyle et al. (26), however, have reported that during strenuous exercise in which there was a threefold increase in plasma lactate concentrations, hypoglycemia (2.5 mmol/l) did not result in hypoglycemic symptoms (weakness, light-headedness, and nausea). Moreover, at a plasma glucose concentration of 3 mmol/l, the perceived exertion was the same as during euglycemic control studies. Similarly, Felig et al. (27) reported that during strenuous exercise, the plasma glucose concentration in 7 of 19 normal subjects fell below 2.5 mmol/l, and despite

this hypoglycemia, the subjects continued to exercise for 70 min with plasma glucose levels as low as 1.4 mmol/l. Plasma lactate concentrations had increased threefold during this period, and it was noted that the perceived exertion was not increased in subjects who became hypoglycemic. Taken together, these observations and our results suggest that during strenuous exercise, lactate may become an important fuel for the brain. It is also of interest that these conditions are often associated with acidosis, and, therefore, it is possible that acidosis may be an additional factor in brain adaption to use alternative fuels.

It is not clear what role these alternative substrates play in the development of hypoglycemia unawareness and adaptation to hypoglycemia. Many patients with IDDM develop hypoglycemia unawareness and impaired counterregulation after improvement in their metabolic control (32,33) and have increased episodes of hypoglycemia (34). Normally, however, the alternative substrates are not quantitatively important at their plasma levels found in the postabsorptive state (35). Recently, Grill et al. (36) reported basal plasma BOHB concentrations of 1.6 mmol/l in IDDM patients in good control (HbA_{1c} 6.1%). Others have reported increases in plasma lactate during normalization of plasma glucose levels in patients with IDDM (37,38). It could be postulated that repetitive episodes of mild hypoglycemia with concomitant increases in plasma ketone bodies, and possibly lactate, induce changes in blood-brain transport so that ketone bodies and lactate can more readily substitute for glucose during hypoglycemia. This mechanism would lead to diminished counterregulation, reduced awareness of hypoglycemia, and an increased risk for severe hypoglycemia.

In summary, we found that infusion of BOHB or lactate, which increased the circulating levels of these substrates to those found during prolonged fasting and strenuous exercise, reduces awareness of hypoglycemia, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia. These findings suggest that, under certain clinical conditions, these substrates may substitute for glucose as a fuel for the brain and that they may be involved in the pathogenesis of the hypoglycemia unawareness phenomenon found in patients with IDDM.

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