Protective Effects of Ketogenic Diets on Signs of Hypoglycemia

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SUMMARY

The effects of diet-induced ketosis on the signs of hypoglycemia were investigated. Lard, medium chain triglycerides, or 1,3-butylene glycol comprised 43 per cent of the diet fed to mice. The diet containing lard or medium chain triglycerides greatly protected the animals from the manifestations of acute insulin-induced hypoglycemia. Furthermore, both diets protected the animals from the effects of repeated insulin injections (every eight hours) for 10 days. In contrast, 1,3-butylene glycol had no protective effects. These experiments suggest that ketogenic diets may be of value in the treatment of recurrent hypoglycemic conditions. DIABETES 27:1087-91, November, 1978.

Hypoglycemia produces a metabolic encephalopathy because of diminished availability of substrate for the brain. For many years it was believed that glucose was the sole metabolic fuel for the brain.[1] However, Owen et al.[2] found that extended fasting by obese subjects induced tolerance to hypoglycemia and was associated with increased cerebral uptake of β-hydroxybutyrate and acetoacetate. Subsequent investigations have confirmed the fact that the brain can utilize β-hydroxybutyrate and acetoacetate as fuels.[3]

Although the elevation of blood β-hydroxybutyrate and acetoacetate concentrations produced by fasting has been shown to prevent the signs and symptoms of hypoglycemia in human beings[2,4] and animals,[5] prolonged fasting is not a practical treatment for recurrent hypoglycemic conditions.[6] The aim of the present investigation was to determine whether ketosis induced by a high-fat diet would alleviate the manifestations of acute and chronic hypoglycemia.

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METHODS

Sprague-Dawley Swiss albino female mice (22 to 28 gm.) were used for all experiments. Table 1 lists the composition of the control and high-fat diets. The high-fat diet contained a large quantity of lard and was composed of 23 per cent protein, 27 per cent carbohydrate, 1.7 per cent fiber, and 48 per cent fat. The control diet contained 4 per cent fat and 55 per cent carbohydrate. Because addition of fat-soluble vitamins to the diet was found to accelerate the development of rancidity, a mixture of β-carotene, calciferol, α-tocopherol, and vitamin K, calculated to meet requirements, was directly administered orally to each animal every other day. Ketosis was also induced by feeding the mice Purina Mouse Chow (containing 20 per cent protein, 4 per cent fat, and 25 per cent carbohydrate) and substituting an emulsion of medium chain triglycerides (MCT, Mead Johnson) for the drinking water. The MCT emulsion consisted of 60 per cent MCT, 39 per cent water, and 1 per cent gelatin. This mixture was homogenized with a two-stage Manton-Gaulin model 125 homogenizer at 2,500 psi and 500 psi, pasteurized at 71° C. for 30 minutes (to prevent bacterial growth during storage), and stored at 3° C. Ketosis was also induced by substituting a 20 per cent aqueous solution of 1,3-butylene glycol (Celanese) for the drinking water. Crystalline zinc insulin (Eli Lilly, Indianapolis) or NPH U40 insulin was administered subcutaneously with 50-μl. Hamilton microsyringes. Signs of hypoglycemia were characterized by catatonia, lethargy, coma, tonic or chronic seizures, localized twitching, or uncoordinated activity.

For the studies of chronic hypoglycemia, three groups, each consisting of 20 mice, were investigated: group I was fed the control diet and received no insulin; group II was fed the high fat diet and received no insulin; group III was fed the high fat diet. On the

TABLE 1

Composition of control and high-fat diet

	Control diet (gm./kg.)	High-fat diet (gm./kg.)
Whole yellow corn	153.6	44
Whole oats	290.0	86
Soybean oil meal	290.0	215
Casein	61.0	129
Sucrose	131.4	—
Lard	22.2	430
Corn oil	2.9	47.8
Mineral mix*	26.1	25.8
Phosphate mix*	8.7	8.6
Vitamin mix*	1.0	1.0
Sodium chloride	9.7	9.7
Choline chloride	3.9	3.9

*H. DeLucca, personal communication.

fifth day a series of insulin injections consisting of 20 U. NPH insulin per kilogram of body weight every eight hours was instituted.

Animals were killed by decapitation, and the blood was heparinized and chilled; the red cells were sedimented by centrifugation. The plasma was analyzed for glucose by the glucose oxidase (Glucostat, Worthington) method.[7] Plasma was deproteinized with 10 per cent perchloric acid, and the neutralized protein-free supernatant was analyzed for lactate, pyruvate, β-hydroxybutyrate, and acetoacetate using nicotinamide adenine dinucleotide (NAD)–linked enzyme assays.[8] During the chronic hypoglycemia studies, blood was obtained from the tail vein for glucose analysis using Dextrostix (Ames) and an Ames reflectance meter. Urine acetoacetate concentrations were estimated with Ketostix (Ames).

Statistical analysis was performed using Student's *t*-test.

RESULTS

Effects of the High-fat Diet on Plasma and Urine Metabolite Concentrations (Table 2)

Table 2 demonstrates the effects of the high-fat diet (table 1) on plasma metabolites after one week. The glucose concentration of the animals fed the high-fat ketogenic diet was not significantly affected. In contrast, β-hydroxybutyrate was elevated 13-fold. The acetoacetate concentration was increased, but this was not significant. Therefore, the β-hydroxybutyrate-acetoacetate ratio was significantly increased from 2.9 to 18.5. The concentration of lactate was significantly reduced by the high-fat diet while the pyruvate concentration was unchanged. Urine ketone bodies ranged from 0 to 1^+.

Effects of Acute Insulin Administration on Signs of Hypoglycemia (Figure 1)

Figure 1 depicts the acute effects of insulin-induced hypoglycemia and compares the mice fed the high-fat diet with the control group. When 4 U. of crystalline zinc insulin per kilogram of body weight was administered, 85 per cent of the control animals developed signs of hypoglycemia within 100 minutes. Only 10 per cent of the mice fed the high-fat diet exhibited signs when administered 4 U. of insulin per kilogram of body weight. At 6, 8, and 10 U. of insulin per kilogram of body weight, 90 to 100 per cent of the control animals manifested signs of hypoglycemia while 50 per cent of the ketotic mice were affected. Furthermore, the animals fed the high-fat diet exhibited signs of hypoglycemia consistently at a later time than the control group.

Effect of Acute Insulin Administration on Plasma Glucose (Figure 2)

To determine if the protective effect of the high-fat diet was due to elevation of blood glucose, plasma glucose was measured. Figure 2 demonstrates that at 4 U. of insulin per kilogram of body weight, there was no significant difference in the plasma glucose concentration between control and experimental groups. Furthermore, 30 minutes after receiving 6 U. of insulin per kilogram, the animals fed the high-fat diet had a significantly lower plasma glucose than the controls. These results indicate that the protective effect of the high-fat diet is not related to alterations of blood glucose and is probably due to provision of

TABLE 2

Effects of the high-fat diet on plasma metabolites

	Control diet	Ketogenic diet
Glucose	7.2 ± 0.2 (n = 22)	6.8 ± 0.3 (n = 8)
β-hydroxybutyrate	0.023 ± 0.011 (n = 23)	0.296 ± 0.068 (n = 8)
Acetoacetate	0.008 ± 0.002 (n = 22)	0.016 ± 0.008 (n = 8)
Total "ketone bodies"	0.032 ± 0.116 (n = 22)	0.315 ± 0.074† (n = 8)
β-hydroxybutyrate/ acetoacetate	2.9 ± 1.4 (n = 23)	18.5 ± 5.1† (n = 8)
Lactate	6.0 ± 0.4 (n = 21)	4.0 ± 0.2* (n = 8)
Pyruvate	0.13 ± 0.012 (n = 22)	0.10 ± 0.004 (n = 8)
Lactate/pyruvate	47.1 ± 2.9 (n = 20)	39.41 ± 1.81* (n = 8)

Values are expressed as means ± S.E.M. All data except ratios are millimolar.

Raised symbols indicate statistical significance between ketogenic diet and control diet: *$P < 0.05$, †$P < 0.01$.

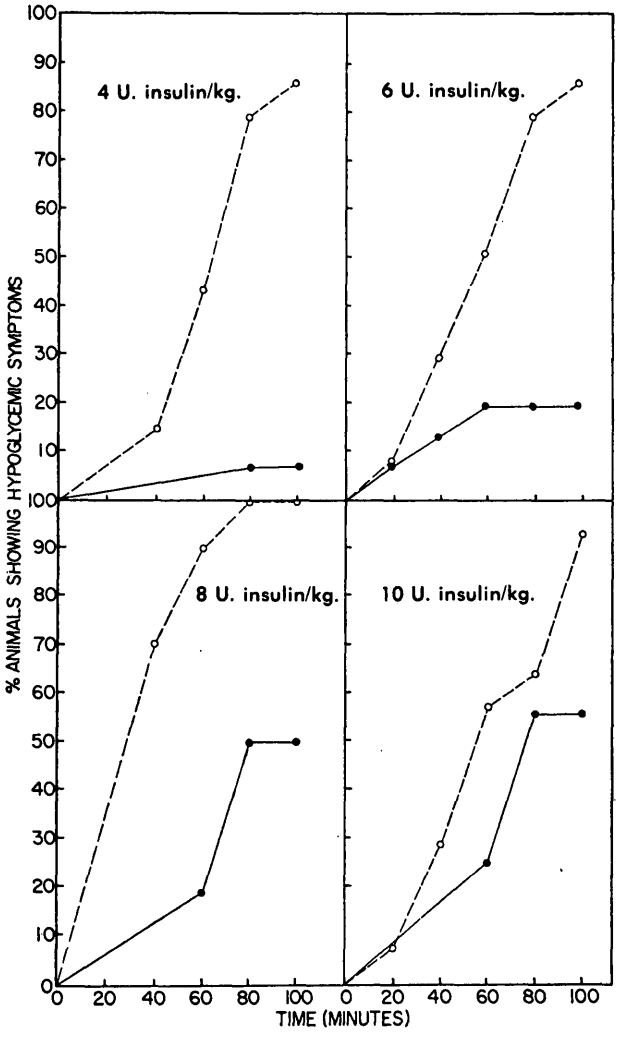


FIG. 1. Effects of acute administration of crystalline zinc insulin and ketosis on signs of hypoglycemia. Twenty control and 20 ketotic mice were studied at each concentration of insulin. The results for 20 control mice are shown by open circles; the results for 20 mice fed the high-fat diet are shown by closed circles.

β-hydroxybutyrate and acetoacetate for utilization by the brain.

Time Course of the Protective Effect of the High-fat Diet

In order to determine the time course of the effects of the high-fat diet, a series of acute experiments was performed with 6 U. of insulin per kilogram of body weight. This dose produces signs of hypoglycemia within 100 minutes in more than 80 per cent of mice fed the control diet (figure 1). After being fed the high-fat diet for 24 hours, the incidence of hypoglycemic manifestations was the same as in controls. After 48 hours of being fed the diet, the incidence of hypoglycemic manifestations after acute insulin administration was reduced to 20 per cent, achieving the maximal protection of the diet.

Effects of the High-fat Diet on the Response to Chronic Hypoglycemia

To determine if the high-fat diet protected against the chronic effects of hypoglycemia, three groups of mice were investigated. Figure 3 illustrates the weights of the three groups of animals receiving the high-fat diet. Groups II and III gained weight more rapidly than did the controls. In addition, the initiation of repeated insulin injections on day 5 (group III) caused an increase in body weight. The animals receiving repeated insulin injections had blood glucose concentrations (just before insulin injection) ranging from 5 to 20 mg. per deciliter. Three animals died during the first four days of insulin injections and four more animals died by the tenth day.

Protective Effects of Dietary Medium Chain Triglycerides and 1,3-Butylene Glycol

Because of the mortality associated with repeated insulin injections to animals fed the high-fat diet, an attempt was made to induce ketosis by administering an MCT emulsion instead of the drinking water. Twenty mice were fed Purina Mouse Chow supplemented with tap water. After three days, the MCT-emulsion was substituted for the drinking water. Within one day, the animals developed 2^+ to 4^+ ketonuria. Administration of 20 U. of NPH insulin every eight hours for two weeks was associated with an overall mortality of 10 per cent; this was a significantly ($P < 0.01$) lower mortality than was associated with the high-fat diet.

The administration of 1,3-butylene glycol has been previously associated with systemic ketosis.[9] The protective effects of this substance were investigated by substituting a 20 per cent aqueous solution for the drinking water of mice fed Purina Mouse Chow. Within a day, all mice showed 4^+ ketonuria. However, after five days of this treatment no protective effects against the signs of acute insulin (8 U. of crystalline zinc insulin per kilogram of body weight) hypoglycemia could be detected.

DISCUSSION

The primary purpose of this investigation was to determine whether ketosis, induced by a high-fat intake, could protect against the effects of hypoglycemia. Both the lard-containing high-fat diet and the emulsion of medium chain triglycerides clearly protected against the effects of acute insulin-induced hypoglycemia. When chronic hypoglycemia was pro-

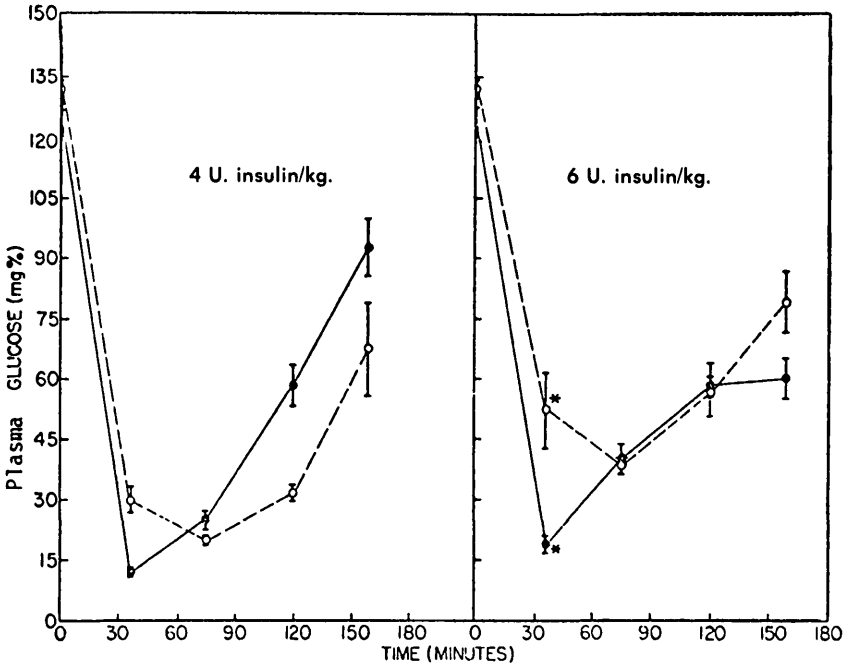


FIGURE 2

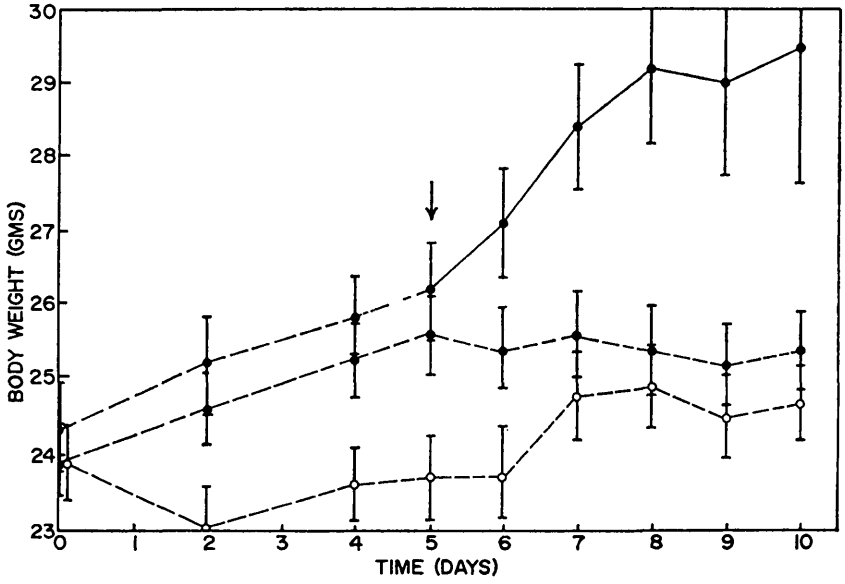
Effects of insulin and ketosis on plasma glucose concentrations. Thirty control (open circles) and 30 ketotic (closed circles) mice were studied at each concentration of insulin. Five mice from each group were killed at 40-minute intervals. The data are expressed as the means ± S.E.M. At 6 U. of insulin per kilogram of body weight there was a significant ($P < 0.05$) difference between the control and experimental plasma glucose concentrations.

duced by repeated injections of long-acting insulin, the high fat was associated with considerable mortality; this appeared to be in part increased by the irritating properties of the dietary mixture, which seemed to induce infections of the skin and mucous membranes. The use of an MCT emulsion in place of the drinking water was not associated with any obvious complications. Animals rendered ketotic by this approach tolerated chronic hypoglycemia well, and the mortality was relatively low. Although 1,3-butylene glycol was associated with ketonuria, it provided no protection against hypoglycemia. We have no explanation for this finding.

The present experiments demonstrate that ketotic animals tolerate chronic hypoglycemia for at least two weeks. Several tissues of the body have been thought to depend upon glycolysis as a major source of energy; these include red cells (which do not have the enzymes of oxidative metabolism), kidney medulla, and intestinal mucosa (which are relatively anaerobic). The model of chronic ketosis and hypoglycemia may provide an approach for the investigation of the

FIGURE 3

The effects of ketosis and chronic hypoglycemia on body weight. Data are expressed as means ± S.E.M. Group I (n = 20), fed control diet, is shown by open circles. Groups II (n = 20) and III (n = 20), fed the ketogenic diet, are shown by the closed circles connected with dotted lines. On day 5, group III was treated with 20 U. NPH insulin every eight hours (solid line). After day 7, the mean body weight of group III was significantly ($P < 0.05$) greater than that of groups I or II.



physiologic importance of glycolysis in these and other tissues.

The most direct clinical implications of the present results concern the treatment of hypoglycemia. Tumors of the pancreatic islet cells are associated with frequent and repeated bouts of extreme hypoglycemia. Current therapy includes frequent carbohydrate ingestion or inhibition of insulin production by the tumor.[6] Perhaps diet-induced ketosis, by feeding medium chain tryglycerides, might aid in the alleviation of this troublesome clinical problem. Similarly, recurrent attacks of hypoglycemia due to other causes might also respond to this approach.

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REFERENCES

[1]Kety, S. S.: Metabolism of the nervous system. Richter, D., Editor. London, Pergamon Press, 1957, p. 221.

[2]Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., and Cahill, G. F., Jr.: Brain metabolism during fasting. J. Clin. Invest. *46*:1589, 1967.

[3]Sokoloff, L.: Metabolism of ketone bodies by the brain. Annu. Rev. Med. *24*:271, 1973.

[4]Drenick, E. F., Alvarez, L. C., Tamasi, G. C., and Brickman, A. S.: Resistance to symptomatic insulin reactions after fasting. J. Clin. Invest. *51*:2757, 1972.

[5]Tamasi, G. C., and Drenick, E. J.: Resistance to insulin convulsions in fasted mice. Endocrinology *92*:1277, 1973.

[6]Bondy, P. K., and Felig, P.: *In* Duncan's Diseases of Metabolism. Bondy, P. K., and Rosenberg, L. E., Editors. Philadelphia, W. B. Saunders, 1974, p. 305.

[7]Washka, M. E., and Rice, E. W.: Determination of glucose by an improved "Glucostat" procedure. Clin. Chem. 7:542, 1961.

[8]Bergmeyer, H.: Methods of Enzymatic Analyses. Second edition. New York, Academic Press, 1965.

[9]Kies, C., Tobin, R. B., Fox, H. M., and Mehlman, M. A.: Utilization of 1,3-butanediol and nonspecific nitrogen in human adults. J. Nutr. *103*:1155, 1973.