Vol. 120, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1984 Pages 103-108

DECREASES IN HEPATIC FRUCTOSE-2,6-BISPHOSPHATE LEVEL AND FRUCTOSE-6-PHOSPHATE,2-KINASE ACTIVITY IN DIABETIC MICE: A CLOSE RELATIONSHIP TO THE DEVELOPMENT OF KETOSIS

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Received February 29, 1984

Hyperglycemic mice with streptozotocin diabetes were divided into two groups according to the presence or absence of ketosis. No difference in blood glucose level between two groups was observed in this experiment. However, hepatic fructose-2,6-P_2 level and fructose-6-P,2-kinase activity were decreased only in ketotic diabetic mice. Similar decreases in those indices were observed in 48-h starved normal mice. In ketotic diabetes, insulinization for 24 h was required to normalize fructose-2,6-P_2 level and fructose-6-P,2-kinase activity, while glucose administration normalized altered fructose-2,6-P_2 metabolism in starvation only in 30 min. Hepatic cyclic AMP was increased neither in ketotic nor in non-ketotic diabetic mice. These results indicate that the decrease in hepatic fructose-2,6-P_2 level in diabetes is apparently related to the occurrence of ketosis, but not to hyperglycemia. The mechanisms of the decrease in fructose-6-P,2-kinase activity in ketotic diabetes and starvation are discussed.

It has been reported that the level of hepatic fructose-2,6-P_2, a potent activator of phosphofructokinase (1,2), and the activity of its synthesizing enzyme, fructose-6-P,2-kinase (ATP:D-fructose-6-phosphate 2-phosphotransferase) (3,4) are decreased in the rat with experimental diabetes (5,6). Since fructose-2,6-P_2 inhibits fructose-1,6-bisphosphatase (7,8), a decrease in hepatic fructose-2,6-P_2 may promote gluconeogenesis as well as inhibit glycolysis. It is generally considered that increased hepatic gluconeogenesis contributes at least in part to the development of hyperglycemia in diabetes. On the other hand, the inhibition of hepatic glycolysis may subsequently lead to the increased ketogenesis (9,10). However, it remains to be clarified whether the decrease in hepatic fructose-2,6-P_2 concentration is more responsible for hyperglycemia or hyperketonemia in diabetes. In the present study, we analyzed alterations in hepatic fructose-2,6-P_2 level and fructose-6-P,2-kinase activity in diabetic mice in relation to hyperglycemia or

0006-291X/84 $1.50

hyperketonemia comparing with those in starvation which exhibits hormonal imbalance of hypoinsulinemia and hyperglucagonemia (11) similar to diabetes (12), and tried to clarify a regulatory role of fructose-2,6-P_2.

Materials and Methods

Chemicals: Streptozotocin and rabbit muscle phosphofructokinase were purchased from Sigma. All other enzymes were obtained from Boehringer Mannheim. Monocomponent insulin was the product of Novo Industri A/S. Synthetic fructose-2,6-P_2 was a gift from Dr. T. Fukui, Institute of Scientific and Industrial Research, Osaka University.

Treatment of animals: Streptozotocin (100-150 mg/kg body wt) was injected into a tail vein of male Scl:ICR mice (20-25 g) starved overnight. Mice injected with streptozotocin were judged to be diabetic several days later, if blood glucose level after an overnight fast exceeded 200 mg/dl and if random blood glucose level exceeded 400 mg/dl. Blood samples were taken from the retro-orbital sinus. The development of ketonemia roughly depended on the dose of streptozotocin. Plasma ketone bodies were determined semiquantitatively by nitroprusside reaction using a Ketostix® (Miles Laboratories Inc., Elkhart, IN); acetoacetate above 1 mM is detectable by this method. Diabetic mice were sacrificed within 2 weeks after streptozotocin injection. Normal and diabetic mice were used for experiments following 2-h starvation unless otherwise stated.

Preparation of liver extract and assays for fructose-2,6-P_2 and fructose-6-P,2-kinase: Livers were removed from mice under pentobarbital anesthesia, homogenized in 3 volumes of ice-cold 50 mM Tris-P (pH 8.0) containing 100 mM NaF and 1 mM EDTA and centrifuged at 15,000 x g for 30 min at 4 C. The supernatant solution was used for fructose-2,6-P_2 assay by the method of Uyeda et al. (13). Fructose-6-P,2-kinase activity was measured according to Richards et al. (14); supernatant solution of liver homogenate was incubated in the presence of 5 mM ATP and 1 mM fructose-6-P for 20 min at 30 C. Fructose-2,6-P_2 present in the incubation mixture was then measured. One unit of activity is defined as the amount of the enzyme that catalyzes the formation of 1 μmol of fructose-2,6-P_2 per min.

Other methods: Hepatic cyclic AMP was assayed by the method of Steiner et al. (15). Plasma glucose was determined by the glucose oxidase method.

Results

Hepatic fructose-2,6-P_2 levels in ketotic diabetic mice were markedly decreased without exception (1.2 ± 0.2 nmol/g liver, mean ± S.E.), while the levels in non-ketotic diabetic mice (13.6 ± 2.1) were nearly equal to those in normal mice (15.1 ± 1.3) (Fig. 1). Blood glucose and hepatic cyclic AMP levels are depicted in Table 1. There was no significant difference in blood glucose levels between ketotic and non-ketotic diabetic mice in this experiment. Hepatic cyclic AMP level in either diabetic group was not significantly altered.

The activity of hepatic fructose-6-P,2-kinase in ketotic diabetic mice, 0.68 ± 0.13 mU/g liver (n=5), was significantly lower than that of 1.62 ± 0.15 (n=7) in normal mice ($p < 0.001$), while the activity of 1.85 ± 0.20 (n=4) in non-ketotic diabetic mice was not significantly different from the normal

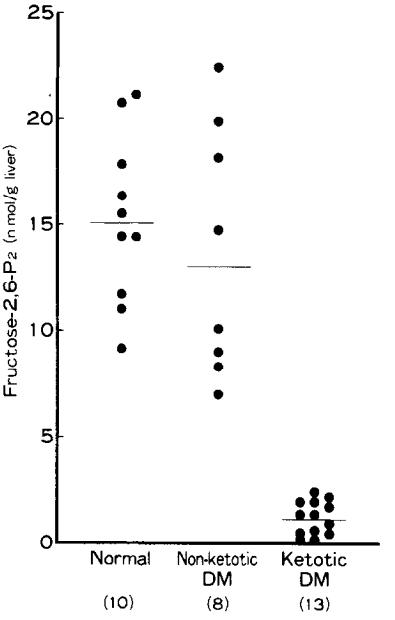

Figure 1. Hepatic fructose-2,6-P_2 concentrations in normal mice and diabetic mice (DM) with and without ketosis. The number of animals is given in parentheses.

value. Fructose-2,6-P_2 levels and fructose-6-P,2-kinase activities in ketotic diabetes treated with insulin are shown in Table 2. Although normoglycemia below 200 mg/dl was attained 1 h after intraperitoneal injection of short-acting insulin (10 units/kg body wt), either fructose-2,6-P_2 level or fructose-6-P,2-kinase activity was not increased. Both of these were normalized after 24-h insulinization with short- and intermediate-acting insulin preparations.

When normal mice were starved for 48 h, both fructose-2,6-P_2 level and fructose-6-P,2-kinase activity were significantly decreased (Experiment 1 in Table 3). Altered fructose-2,6-P_2 level and fructose-6-P,2-kinase activity in

Table 1. Blood glucose levels and hepatic cyclic AMP concentrations in normal and streptozotocin diabetic mice

		Blood glucose (mg/dl)	Cyclic AMP (nmol/g liver)
Normal (fed)	(6)	206 ± 21	0.16 ± 0.01
Ketotic diabetes	(5)	583 ± 53**	0.19 ± 0.03
Non-ketotic diabetes	(5)	668 ± 46**	0.20 ± 0.04

Values are expressed as means ± S.E.
* $p < 0.05$ and ** $p < 0.001$ versus Normal (fed).

Table 2. Effect of insulin treatment on hepatic fructose-2,6-bisphosphate concentration and fructose-6-phosphate,2-kinase activity in ketotic diabetic mice

	Fructose-2,6-P_2 (nmol/g liver)		Fructose-6-P,2-kinase (mU/g liver)	
Untreated diabetes	1.7 ± 0.3	(6)	0.68 ± 0.13	(5)
Insulin-treated, 1 h	3.5 ± 0.9	(3)	0.65 ± 0.23	(4)
Insulin-treated, 24 h	11.7 ± 2.3**	(5)	1.33 ± 0.17*	(3)

Values are means ± S.E.
* $p < 0.05$ and ** $p < 0.005$ versus Untreated diabetes.

20-h starved mice were entirely normalized 30 min after intraperitoneal administration of glucose (Experiment 2 in Table 3).

Discussion

Although decreases in hepatic fructose-2,6-P_2 level and fructose-6-P,2-kinase activity in experimental diabetic rats have been reported (5,6), results in the present study denote that diabetes does not necessarily induce the alteration in hepatic fructose-2,6-P_2 metabolism in mice. The decrease in hepatic fructose-6-P,2-kinase activity, resulting in the fall in fructose-2,6-P_2 level (Table 2), occurred only in ketotic diabetes, while either fructose-2,6-P_2 level or fructose-6-P,2-kinase activity in the liver was not decreased in non-ketotic diabetic mice (Fig. 1). The severity of hyperglycemia in ketotic diabetes was similar to that in non-ketotic diabetes in the present

Table 3. Effect of starvation and glucose administration on hepatic fructose-2,6-bisphosphate level and fructose-6-phosphate, 2-kinase activity in mice

	Fructose-2,6-P_2 (nmol/g liver)		Fructose-6-P,2-kinase (mU/g liver)	
Experiment 1				
Normal (fed)	11.2 ± 3.7	(3)	1.39 ± 0.11	(3)
Starved 24 h	1.6 ± 0.9	(3)		
48 h	0.5 ± 0.1*	(3)	0.86 ± 0.16*	(3)
72 h	0.6 ± 0.1*	(3)		
Experiment 2				
Normal (fed)	14.4 ± 1.4	(3)	1.48 ± 0.14	(4)
Starved 20 h	6.1 ± 0.7*	(2)	0.80 ± 0.07**	(4)
Starved 20 h + Glucose §	16.9 ± 1.4	(2)	1.71 ± 0.16	(4)

Values are means ± S.E. § 30 min after intraperitoneal glucose injection. * $p < 0.05$ and ** $p < 0.01$ versus Normal (fed).

study (Table 1). These results imply that the decrease in hepatic fructose-2,6-P_2 is closely related to the development of ketosis but not necessarily to hyperglycemia in diabetic mice. The decrease in hepatic fructose-2,6-P_2 could be a trigger of enhanced ketogenesis through the inhibition of glycolysis which leads to a decreased supply of oxaloacetic acid or α-glycerophosphate to be coupled with increased acetyl-CoA or fatty acyl-CoAs in diabetes. Hepatic fructose-2,6-P_2 could thus play a key role in the transition of the liver from an organ of carbohydrate utilization to one of fatty acid oxidation and ketone body production.

Insulin has been shown to oppose glucagon action in vitro on fructose-2,6-P_2 metabolism in isolated hepatocytes in a short time (16). However, the normalization of altered fructose-2,6-P_2 metabolism by insulin treatment of diabetic mice needed relatively a long period (Table 2). In addition, hepatic cyclic AMP level in ketotic diabetes was not increased. These two findings in the present study suggest that the decrease in fructose-6-P,2-kinase activity in ketotic mice would not be the result of the rapid inactivation of this enzyme by cyclic AMP-dependent protein kinase (17,18). A turnover of fructose-6-P,2-kinase may be possibly altered in ketotic diabetes.

A decrease in hepatic fructose-2,6-P_2 level in starvation has been reported to be recovered after 24-h refeeding (5). However, normalization of fructose-2,6-P_2 level and its synthesizing enzyme activity by glucose administration required only 30 min in the present study. This result suggests two possible mechanisms of the glucose effect on fructose-6-P,2-kinase. First, hyperglycemia after glucose administration may lower glucagon level and raise insulin level in portal blood. These hormonal changes would result in the rapid increase in hepatic fructose-6-P,2-kinase activity. Second, glucose itself may activate fructose-6-P,2-kinase, as reported in isolated hepatocytes from fasted rats or in hepatocytes incubated with glucagon (19). The present observation that fructose-2,6-P_2 was not decreased in non-ketotic diabetes might be explained by the glucose activation of fructose-6-P,2-kinase due to intrahepatic high glucose level.

Acknowledgements

The authors are grateful to Dr. E. Furuya and Dr. M. Yokoyama (Department of Molecular Physiological Chemistry, Osaka University Medical School) for their encouraging advice.

This study was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (1982-1983) and a research grant from the Muscular Dystrophy Association of U.S.A. (1982-1983).

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