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GLYCOLYTIC AND GLUCONEOGENIC METABOLITES AND ENZYMES IN THE LIVER OF OBESE-HYPERGLYCEMIC MICE (KK) AND ALLOXAN DIABETIC MICE

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ABSTRACT

Hepatic glycolytic and gluconeogenic metabolites and enzymes were assayed in obese-hyperglycemic mice (KK), alloxan diabetic mice, and the control mice. Alloxan diabetic mice showed distinctly decreased and increased concentrations of metabolites in fed and starved conditions respectively. There were no differences between KK and the control in these conditions. But in glucose load tests, KK showed the retention of hepatic metabolites in the metabolic pathway of glycolysis at five positions. They were glucose, fructose-6-phosphate, glyceraldehyde-3-phosphate, 2-phosphoglycerate, and oxaloacetic acid. This phenomenon was also observed in alloxan diabetes. In alloxan diabetes, glucokinase, and pyruvate kinase activities were lowered while glucose-6-phosphatase and fructose-1, 6-diphosphatase activities were higher by 75% and 45%, respectively. On the contrary, KK mice showed higher glucokinase and hexokinase activities than the control by 20% as well as higher glucose-6-phosphatase activity (50% increase). Concentrations of corticosterone in the serum were the same levels in KK and the control while insulin-like activity was higher in KK. NAD^+/NADH ratio in hepatic cytoplasm suggested the increased reaction in the direction of α-glycerophosphate in KK. High level of cytoplasm ATP/AMP ratio was supposed to be the cause of depression of glycolysis. And this might elevate the citrate concentration resulting in increased fatty acid synthesis.

INTRODUCTION

Metabolic disturbances of several strains of hereditary obese-hyperglycemic mice have been reported. American obese-hyperglycemic mice[1], the New Zealand strain of obese mice[2], obese yellow mice[3], F-1 hybrid mice[4], and KK mice[5,6] showed high insulin content of the pancreas[4,7,8,9,10], high insulin-like activity in the serum[8,11,12], decreased glucose oxidation[2,12,13,14,15], and increased lipogenesis[2,3,16,17,18,19]. In Japan, KK mice were intensively investigated by M. Nakamura[20,21,22] and I. Tsuchida[12] and were considered to have some disorders in the sensitivity of peripheria to insulin. But the etiology of hyperglycemia accompanied by obesity still remains uncertain.

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The liver plays a very important role in the maintenance of homeostasis of the mammalian organism. Especially from the viewpoint of the maintenance of high blood glucose level, no one can neglect the liver because that organ is the principal performer of gluconeogenesis since foodstuff come at first to the liver and then go out into the circulation. Recently, it is considered that glycolysis and gluconeogenesis are regulated by various hormones [23) 24) 25) 26) 27) 28) 29)] or by metabolic intermediates[30) 31) 32) 33) 34) 35) 36)], by activating and inactivating, or inducing and suppressing hepatic key enzymes, such as glucokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1, 6-diphosphatase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase.

In this paper, oxidation of glucose and pyruvate and hepatic glycolytic and gluconeogenic intermedites and key or other enzyme activities were measured in KK mice, alloxan diabetic mice, and the control mice in certain conditions. Moreover the role of the liver was discussed.

MATERIALS AND METHODS

Animals

Male KK mice were used as the genetic obese-hyperglycemic group because female KK have not so much obese-hyperglycemic syndromes. KK mice have not any clearly separated lean litter mates, so male C 57 BL or male CF 1 mice are used as the control group. Alloxan was injected intraperitoneally after 24 hours starvation by 35 mg per 100 g body weight to female CF 1 mice. Three or four days later, mice were examined for the alloxan diabetic group.

The mice were maintained at 20°C in alminous cages, 3 to 5 animals in each cage, and fed laboratory chow, OA-2. Unless otherwise noted, animals were killed at 10 a.m.

Chemicals

1-^{14}C glucose and U-^{14}C pyruvic acid (sodium salt) were purchased from The Radio Chemical Center (England). $NADP^+$, NAD^+, ATP, ADP, AMP, and α-ketoglutaric acid were obtained from Sigma Chem. Co. (U.S.A.). NADH, glucose-6-phosphate (G 6 P), glucose-1-phosphate (G 1 P), fructose-1, 6-diphosphate (FDP), 3-phosphoglyceric acid (3PG), 2, 3-diphosphoglyceric acid, phosphoenolpyruvic acid (PEP), malate dehydrogenase, glutamic oxalacetic transaminase, lactate dehydrogenase, pyruvate kinase, enolase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, glycerin-1-phosphate dehydrogenase, triosephosphate isomerase, aldolase, phosphohexoisomerase, phosphoglucomutase and glucose-6-phosphate dehydrogenase were obtained from C. F. Boehringer and Soehne G. m. b. H., Mannheim (West Germany).

Measurement of hepatic intermediates

Mice were killed by cervical fracture. Liver tissue was removed, weighed, and homogenized as soon as possible at 2°C in 3 ml of 6% (v/v) perchloric

acid according to the method of Lardy *et al.*[37]. The liver homogenate was centrifuged at 5,000 × *g* for 15 min at 0°C. The supernatant fraction was adjusted to pH 5-6 using 5% potassium carbonate solution, and the volume of liver extract was adjusted to 5 ml per g of liver by the addition of water.

G6P, G1P, F6P, FDP, dihydroxyacetone phosphate (DHAP), glyceraldehyde-3-phosphate (GAl3P), 3PG, 2-phosphoglycerate (2PG), PEP, pyruvate (PYR), lactate (LAC), oxaloacetate (OAA), and aspartate (ASP) were determined enzymatically as described in Bergmeyer[38]. Liver glycogen was determined by the method of Hassid *et al.*[50]. Blood glucose was determined using glucose oxidase according to Hugget *et al.*[51].

Determination of activities of hepatic enzymes

Liver was homogenized in 10 volumes of ice-cold buffer solution (pH 7.0) of the following composition: 0.15 M KCl; 0.005 M sodium ethylenediaminetetraacetate; 0.005 M $MgCl_2$. After centrifugation of homogenate at 105,000 × *g* for 30 min. at 2°C, the clear yellow supernatant solution was used for spectrophotometric determination of the activities of the following enzymes: hexokinase and glucokinase[40]; gucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase[41]; fructose-1, 6-diphosphatase[42]; glyceraldehyde-3-phosphate dehydrogenase[43]; 3-phosphoglycerate kinase[44], phosphoglyceromutase[45]; and pyruvate kinase and phosphoglucomutase by the modified methods of Bergmeyer[38].

In determination of phosphofructokinase, liver was homogenized in 10 volumes of ice-cold solution (pH 8.2) consisted of 0.15 M trisaminomethane, 0.02 M KH_2PO_4, and 0.001 M sodium ethylenediaminetetraacetate. The supernatant fraction obtained by centrifugation at 105,000 × *g* for 30 min. was used for the enzyme assay according to the modified method of Mansour[46].

Phosphorylase was assayed by the method of Sutherland[47], and glucose-6-phosphatase by the method of Swanson[48].

Measurement of glucose and pyruvate oxidation

1-^{14}C glucose or U-^{14}C pyruvate was injected intraperitoneally to mice and placed in a glass metabolic chamber maintained at 30°C. The expired CO_2 was trapped by 40 ml of the solution of ethanolamine and ethylene glycol monomethyl ether (1 : 2 v/v), and the radioactivity was counted in a liquid scintillation counter (Model GSL-163, Kobe Kogyo Co.) according to Jeffay *et al.*[49]. Liver glycogen was prepared by the method of Somogyi[55] and incorporated radioactivity was measured by the same method described above.

Measurement of hormones in the serum

Insulin-like activity was measured by the oxidation of 1-^{14}C glucose to $^{14}CO_2$ using epididymal fat pads of rats by the method of Renold *et al.*[52]. The serum was collected from 8 to 10 mice.

To determine plasma corticosterone, animals were anesthetized by the injection of sodium nembutal by 5 mg per 100 g body weight. Twenty minutes later the blood was drawn up into a heparinized syringe. The blood was centrifuged at 3,000 r.p.m. for 10 min., and the supernatant fraction was used for the determination according to the modified method of Richard[53].

RESULTS

The typical physiological data of KK mice, the control mice and alloxan diabetic mice of 4–5 month old are shown in Table 1. Under fed conditions, the blood glucose level of KK mice was much higher than that of the control. But the liver glycogen content did not parallel with the blood glucose. After 24 hour starvation, the blood glucose level and the liver glycogen content of KK mice fell to much lower level than those of the control. In alloxan diabetes, the blood glucose level and the liver glycogen content were still high after 24 hour starvation. Livers of KK mice were relatively larger than those of the control.

TABLE 1. Physiological data of mice. The control mice was C 57 BL strain. Animals were all male and 4–5 months old. Number of animals is in parenthesis. Standard errors were calculated from the equation of $\text{S.E.}=\sqrt{\frac{(X-\bar{X})^2}{n(n-1)}}$

Mice	Body weight (fed) (g)	Blood glucose (fed) (mg/dl)	Blood glucose (fast) (mg/dl)	Liver weight (fed) (g)	Liver weight / body weight ($\times 10^2$)	Liver glycogen (fed) (mg/g)	Liver glycogen (fast) (mg/g)
KK[10]	39.7±2.1	233± 24	70± 6	2.46±0.40	6.2±0.7	37.5±7.9	0.7±0.1
Control[4]	31.4±4.0	128± 12	90± 2	1.43±0.26	4.6±0.5	80.7±9.7	0.9±0.1
Alloxan[3]	25.0±2.1	665±203	421±30	1.51±0.13	6.1±0.1	40.8±3.8	25.8±3.9

Since KK mice showed the high glucose level and high serum insulin-like activity at young age, too[12,19], all other experiments were carried out using 2–3 months old mice.

1-^{14}C D-glucose was injected intraperitoneally with unlabelled D-glucose of 2 g per kg body weight to mice starved overnight. Percent radioactivities incorporated into respiratory CO_2 and liver glycogen for 2 hours were measured after injection (Table 2). Decreased oxidation of glucose was observed in KK mice and alloxan diabetic mice. Higher radioactivity was converted into liver glycogen in KK mice compared with the control while a depressed incorporation was detected in alloxan diabetes. This depression of incorporation in alloxan diabetes might depend on the high level of hepatic glycogen content at fasting[54].

To investigate the oxidation of other metabolic intermediate, U-^{14}C pyruvate was injected intraperitoneally to mice starved overnight after loading 2 g

TABLE 2. Oxidation of glucose. 1 μC of 1-^{14}C D-glucose was injected intraperitoneally with glucose of 2 g per kg body weight to mice overnight fasted. After 2 hours of injection the blood glucose and ^{14}C conversion were determined. Animals were male and 3 month old, and number was in parenthesis. The control was CF_1 strain.

Mice	Body weight (g)	Blood glucose (mg/dl)	% Radioactivity in CO_2	% Radioactivity in liver glycogen
KK[3]	30.2±4.1	342±115	20.3±5.8	14.3±2.3
Control[3]	24.6±3.7	116± 7	37.9±3.0	9.7±3.2
Alloxan[2]	19.8±1.0	536± 64	22.9±1.2	0.9±0.1

TABLE 3. Oxidation of pyruvate. 1 μC of U-^{14}C pyruvate was injected intraperitoneally after 30 minutes of glucose of 2 g per kg body weight to mice overnight fasted. After 30 minutes of injection the blood glucose and ^{14}C incorporation were measured. Mice were male and 2 months old. The control was CF_1 strain.

Mice	Body weight (g)	Blood glucose (mg/dl)	% Radioactivity in CO_2	% Radioactivity in liver glycogen
KK[1]	23.4	272	23.2	2.7
Control[2]	24.0±0.9	209±16	20.2±0.3	2.1±0.5
Alloxan[1]	26.0	206	15.5	<0.1

glucose per kg body weight. Thirty minutes later, the radioactivity was measured in respiratory CO_2 and liver glycogen (Table 3). KK mice did not show decreased oxidation from pyruvate to CO_2. This observation differed from that of glucose oxidation. In the case of glucose, higher radioactivity was recovered in liver glycogen of KK mice compared with the control. In alloxan diabetes the oxidation of pyruvate to expired CO_2 was decreased and glyconeogenesis was intensively depressed.

Furthermore, U-^{14}C pyruvate was injected intraperitoneally to well-fed mice, and the recovery of radioactivity in respiratory CO_2 was determined at various time (Fig. 1). In alloxan diabetic mice the oxidation of pyruvate was decreased, but no difference of oxidation was observed between in KK mice and the control mice.

The different behavior of KK mice in the oxidation of glucose and pyruvate suggested the presence of some depression mechanisms between glucose and pyruvate in the metabolic pathway of glycolysis of KK mice.

Table 4 shows hepatic glycolytic or gluconeogenic intermediates in well-fed and 24 hour-fast mice. In livers of well-fed KK mice slightly higher levels of G6P, G1P and F6P were found than those of the control. In starved state, G6P level of KK mice was lower than that of the control. But in alloxan diabetic mice, decreased amounts of hepatic glucose metabolites were

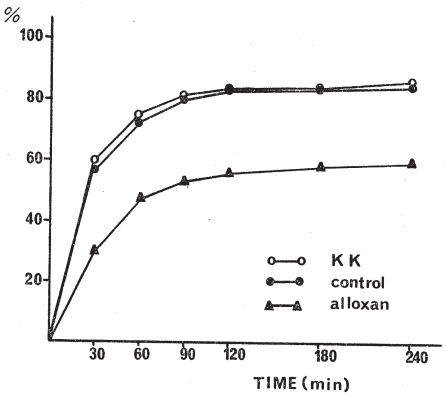

FIG. 1. Recovery of ^{14}C in respiratory CO_2. 1 μC of U-^{14}C pyruvate was injected to male well-fed mice. Control mice were C57BL strain. Animals were 3 months old. Values were given as percent of injected radioactivity.

TABLE 4. Hepatic metabolites were determined in well-fed and 24 hour-fast mice. Values were given in millimicromoles per gram liver (blood glucose in mg/dl, glycogen in mg/g liver). Mice were 2-3 months old, and number was in parenthesis. The control was CF_1 strain.

	Well-fed			24 Hour-fast		
	KK[2]	Control[3]	Alloxan[3]	KK[3]	Control[4]	Alloxan[2]
Glucose	168± 15	140± 19	709±183	70± 4	75± 3	294± 16
G 6 P	390± 28	334± 27	197± 34	19± 5	50±13	255± 12
G 1 P	76± 0	60± 3	16± 4	26± 2	12± 1	22± 8
Glycogen	18± 2	29± 3	41± 4	0.7±0.1	0.9±0.1	26± 4
F 6 P	117± 27	86± 3	38± 6	11± 3	9± 3	74± 2
FDP	39± 4	39± 2	0± 0	6± 1	10± 2	12± 2
DHAP	126± 45	140± 2	36± 10	17± 2	29± 6	45± 12
GAl 3 P	67± 5	84± 10	2± 2	5± 3	1± 1	5± 5
3 PG	312± 2	347± 11	748± 30	295±23	452±23	547± 43
2 PG	26± 5	24± 4	14± 0	2± 2	2± 1	24± 2
PEP	59± 21	43± 5	22± 4	25± 3	6± 2	53± 14
PYR	42± 10	55± 6	27± 3	9± 6	7± 1	43± 7
LAC	5290±330	4250±510	1522±108	1143±29	1238±79	2440±775
OAA	34± 15	34± 7	33± 3	7± 2	11± 3	50± 7
ASP	390± 58	401± 19	301± 13	246±39	218±26	842±428

observed at feeding, and increased metabolites were detected at fasting. Ratios of the amounts of metabolites in starved mice to those in well-fed mice were shown in Table 5. The ratios in alloxan diabetic mice were much larger than either those of KK mice or the control mice. These ratios seemed to indicate the intensity of diabetes.

To examine the simple effect of glucose on hepatic metabolites, 2 g of glucose per kg body weight were given intraperitoneally to KK mice, alloxan diabetic mice and the control mice fasted for 24 hours. One hour later, the amounts of hepatic metabolites were determined as shown in Fig. 2. Data were expressed as percentage of control. KK mice and alloxan diabetic mice were similar in their patterns of metabolites. There were some barriers that

TABLE 5. Ratios of metabolites in starvation to those in feeding were calculated from Table 4

Metabolites	KK	Control	Alloxan
Blood glucose	0.42	0.54	0.41
G 6 P	0.05	0.15	1.29
G 1 P	0.34	0.20	1.37
Glycogen	0.04	0.03	0.63
F 6 P	0.09	0.10	1.96
FDP	0.15	0.26	∞
DHAP	0.13	0.21	1.28
GAl 3 P	0.07	0.01	2.50
3 PG	0.94	1.30	0.73
2 PG	0.08	0.08	1.70
PEP	0.42	0.11	2.41
Pyruvate	0.21	0.13	1.59
Lactate	0.21	0.29	1.60
Oxaloacetate	0.21	0.32	1.51
Aspartate	0.63	0.54	2.80

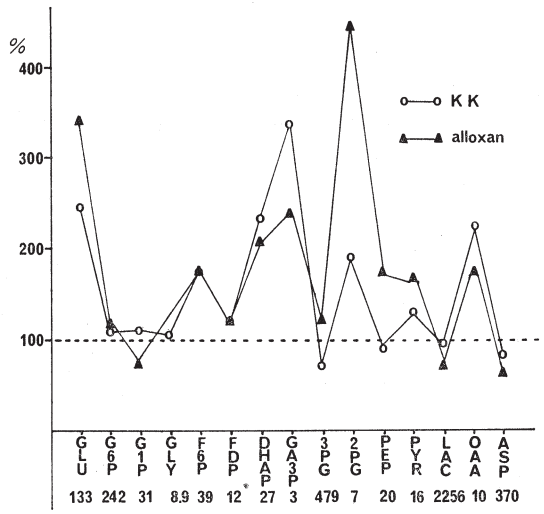

FIG. 2. Hepatic metabolites after 1 hour of glucose load. Glucose was injected 2 g per kg body weight. The experimental curves are given as percent of control value (CF_1 mice). Control values were shown at the bottom of the figure in millimicromoles per g liver. Each value represents the mean value of three experiments. GLU: glucose (mg/dl), GLY: glycogen (mg/g).

prevented the flow of the metabolic pathway causing retentions of glucose, F 6 P, GAl 3 P, 2 PG and OAA. They were at reaction sites of glucokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase or 3-phosphoglycerate kinase, enolase and glutamic oxalacetic transaminase.

To investigate the flow of these accumulated metabolites, concentrations

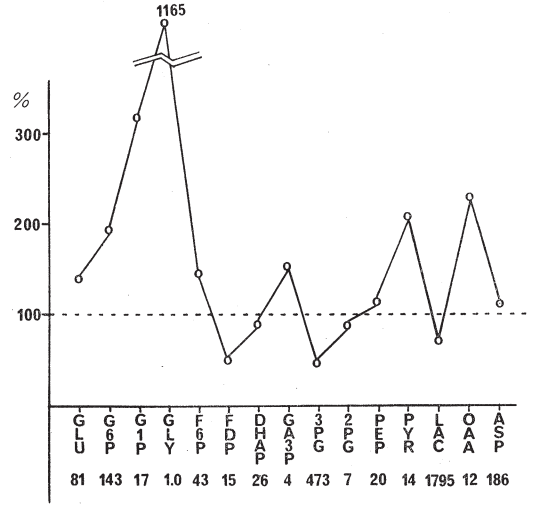

FIG. 3. Hepatic metabolites of KK after 3 hours of glucose load. Data is percent of control (CF_1 strain). Control values were given at the bottom of the figure in millimicromoles per g liver. Other conditions are same to Fig. 2.

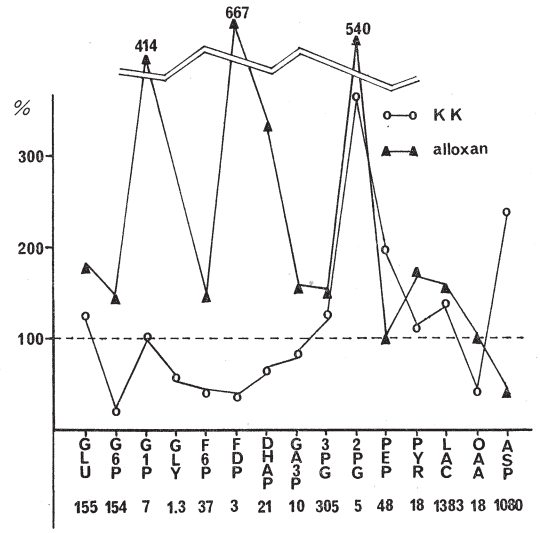

FIG. 4. Hepatic metabolites after 1 hour of DL-alanine load. 2 g DL-alanine per kg body weight were injected intraperitoneally to KK, alloxan diabetes and control (CF_1 mice). Values were given as percent of control. Control values were shown at the bottom of the figure in millimicromoles per g liver. Each value represents the mean value of three animals.

of hepatic metabolites were also determined 3 hours after the glucose load. Amounts of metabolites of KK mice were expressed as percentage of the control mice (Fig. 3). Accumulations of metabolites were still observed after 3 hours at three steps. Those were at the catalyzing sites of phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase or 3-phosphoglycerate kinase, and glutamic oxalacetic transaminase. Increasing accumulation of G 6 P, G 1 P, glycogen and F 6 P suggested the presence of a strong depressing mechanism in glycolysis at the site of phosphofructokinase in the liver of KK mice.

On the other hand, 2 grams of DL-alanine per kg body weight were injected intraperitoneally to mice starved for 24 hours to examine the flow of metabolites in the direction of gluconeogenesis. Concentrations of metabolites were measured 1 hour after the injection and expressed as percentage of control in Fig. 4. Promoted gluconeogenesis was observed in alloxan diabetic mice. In KK mice, however, some barrier was again shown between glyceraldehyde-3-phosphate and 3-phosphoglycerate. But 3 hours later, this retention of metabolites shifted to steps of hexose-phosphates as shown in Fig. 5.

It was interesting and seemed to be paradoxical that delayed gluconeogenesis was found in KK mice, so time courses after alanine load were taken about pyruvate, 3PG, DHAP, G 6 P, glycogen and blood glucose. Depressed gluconeogenesis was observed for 1 hour after the injection in KK

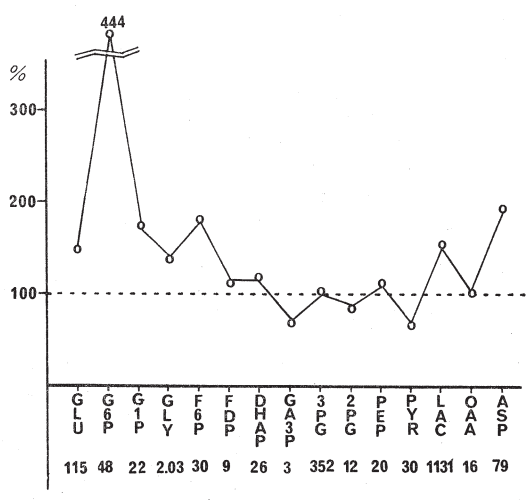

FIG. 5. Hepatic metabolites of KK mice after 3 hour of DL-alanine load. Values were expressed as percent of control CF_1 mice. Other conditions were same as in Fig. 4.

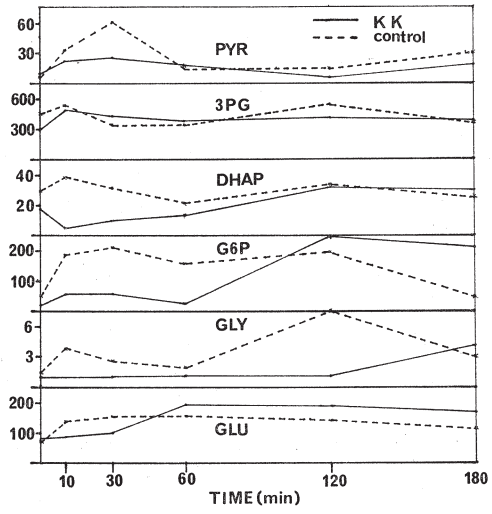

FIG. 6. Time courses of metabolites after injection of DL-alanine. Values were expressed in millimicromoles per g liver. The control was CF_1 mice. Each value represents the mean value of 3 experiments. (GLY: glycogen in mg/g, GLU: blood glucose in mg/dl).

TABLE 6. Hepatic enzyme activities were assayed in fed and 40 hour-starved mice. Activities were given in millimicromoles/min/mg protein. Number of mice is in parenthesis. Control was C 57 BL or CF_1 (*) strain. **; 24 hour starvation

Enzymes	KK		Control		Alloxan fed
	Fed	Fast	Fed	Fast	
Hexokinase	3.0±0.7(8)	2.6±0.7(5)	2.6±0.5(5)	2.4±0.3(3)	2.6(1)
Glucokinase	24.1±1.8(8)	21.6±2.5(5)	21.5±6.0(5)	13.6±0.5(5)	6.4±0.9(4)
Glucose-6-phosphatase	147± 1(2)	162± 6(3)	93± 2(2)	139± 3(3)	172± 7(3)
Phosphorylase	197±14(4)		173±23(4)		
Phosphoglucomutase	279± 8(8)	304±12(5)	331± 6(2)	293±12(3)	265± 1(2)
G 6 P dehydrogenase*	5.9±1.3(4)	6.9±1.2(5)	5.1±1.4(4)	5.4±1.3(3)	6.1±1.2(4)
6 PG dehydrogenase*	9.4±1.0(4)	12.4±0.3(5)	8.3±0.6(4)	10.1±2.1(3)	7.8±0.6(4)
Phosphofructokinase	17.0±0.9(6)		16.5±1.0(6)		16.5±0.8(2)
Fructose-1, 6-diphosphatase	167± 0(2)	157± 5(3)	163± 3(2)	137±10(3)	247± 3(3)
Glyceraldehyde-3 P-dehydrogenase*	1033±49(3)	623±21(3)**	1087±71(3)	723± 7(3)**	
3 P-glycerate kinase*	607±43(3)	627±50(3)**	800±51(3)	630±51(3)**	
Phosphoglycerate mutase*	263±24(3)	367±46(3)**	423±53(3)	287±12(3)**	
Pyruvate kinase	227±10(4)	238±36(5)	224±15(4)	207±11(3)	147± 2(2)

mice (Fig. 6).

To clarify whether such barriers can be due to enzyme activities which are catalyzing these reaction sites, activities of glycolytic and gluconeogenic enzymes of liver were assayed in mice fed *ad libtum* or starved for 40 hours (Table 6). Whereas in alloxan diabetes the activities of glucokinase and pyruvate kinase were decreased, KK mice showed slightly higher activities in glycolytic enzymes, and this tendency was promoted in fasting. But in the direction of glucose formation, activities of glucose-6-phosphatase and phosphorylase of KK mice were higher than those of the control. And alloxan diabetic mice showed higher activities of glucose-6-phosphatase and fructose-1, 6-diphosphatase. Of other reversible enzymes, phosphoglucomutase, 3-phosphoglycerate kinase and phosphoglyceromutase were less active in KK mice under well-fed condition. But these differences were not observed in fasting. Recently, attention are paid to glyceraldehyde-3-phosphate dehydrogenase as a regulatory enzyme which is linked with NAD^+, and starved KK mice showed lower activity of this enzyme.

Since hepatic glucose-6-phosphatase has direct relationship to blood glucose and KK mice show high blood glucose level from young age, the relationship between blood glucose level and hepatic enzyme activities were examined in the developmental course of KK mice as shown in Fig. 7. Data were expressed as percentage of level at 6 months old. Good interrelation was observed among blood glucose, glucose-6-phosphatase and glucokinase while phosphoglucomutase kept the same level of activity.

Gluconeogenic enzymes, such as glucose-6-phosphatase, are known to be

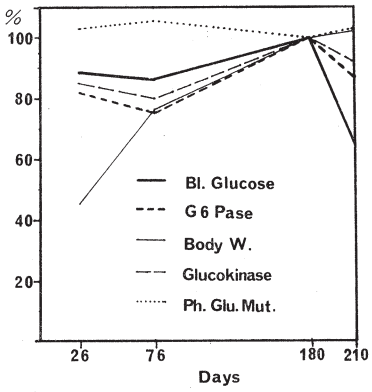

FIG. 7. Developmental alteration of blood glucose, body weight and hepatic enzymes in KK mice. The experimental data were given as percent of the level of 6 months old. Each value represents the mean value of 4 animals.

TABLE 7. Insulin-like activity and corticosterone in the serum. Mice were male, and values represent the mean value of 8 animals. The control was C 57 BL strain.

		KK	Control
Insulin-like activity (units/ml plasma)		4.6×10^{-4}	1.0×10^{-4}
Corticosterone (μg/dl plasma)	fed	10.8±2.1	10.7±3.0
	fast	24.2±0.7	26.8±0.7
Adrenal weight (mg)		3.7±1.1	4.4±0.4
Adrenal weight/body weight		1.0±0.1	1.6±0.2

induced by glucocorticosteroid hormone and suppressed by insulin[23] so corticosterone and insulin-like activity in the plasma were assayed and compared in Table 7. The same levels of corticosterone were detected in both mice, and insulin-like activity of KK mice was much higher than that of the control. These facts seemed to be paradoxical and increased glucose-6-phosphatase with high phosphorylase activity suggested the presence of other regulatory mechanisms.

The lactate dehydrogenase system reflects the NAD^+/NADH ratio in the cytoplasm, and the ratio can be calculated from the concentrations of pyruvate and lactate[56]. Calculated ratios of NAD^+/NADH under several conditions were shown in Table 8. The ratios of the alloxan diabetes were always higher than either those of KK mice or the control mice. This seemed to be due to the decrease of NADH. Although in alanine load tests ratios of KK mice were lower than in the control, in glucose load tests ratios of KK mice became higher than that of the control.

Since concentrations of lactate in livers of KK mice in glucose load tests were lower level, increased NAD^+/NADH ratios in KK mice at glucose load

TABLE 8. Ratio of NAD^+ to NADH in the hepatic cytoplasm. Ratios were calculated from the concentration of lactate and pyruvate according to the equation of Williamson. Values represent the mean value of 3 male mice. Glucose and alanine were given intraperitoneally by 2 g per kg body weight. The control was CF_1 strain.

	Control	KK	Alloxan
Fed	117	71	160
Fast (24 h)	51	71	158
DL-Alanine load			
30 min	306	164	
1 h	117	92	129
2 h	109	60	
3 h	239	99	
D-Glucose load			
30 min	57	134	
1 h	64	87	165
2 h	50	111	
3 h	70	215	

tests were supposed to be caused by the promoted reaction of α-glycerophosphate dehydrogenase catalyzing from dihydroxyacetonephosphate to α-glycerophosphate. In alloxan diabetes the activtiy of α-glycerophosphate dehydrogenase was decreased[57], but in KK mice some unknown regulatory mechanisms might be acting in the direction to α-glycerophosphate by depressing the reaction of glyceraldehyde-3-phosphate dehydrogenase and maybe by promoting the reaction of α-glycerophosphate dehydrogenase. In KK mice, less increase of ratios at alanine loading may be partially due to the depressed reaction of glyceraldehyde-3-phosphate dehydrogenase causing the accumulation of metabolites (in Fig. 4). But there is the possibility that the rate-limiting site of the pathway in alanine load tests is the site of 3-phosphoglycerate kinase coupled by co-factor ATP.

DISCUSSION

From experiments of oxidation of intermediates to CO_2 *in vivo*, depression of glycolysis in KK mice was supposed to be between glucose and pyruvate, while in alloxan diabetes decreased glycolysis were observed before and after pyruvate. And in glucose load tests, remarkable retention of hepatic metabolites were found at rate-limiting sites in KK mice and alloxan diabetic mice. In alloxan diabetes these rate limitation can be well accounted for by levels of enzyme activities, but KK mice showed normal or high activities of glycolytic enzymes by *in vitro* assays. Some other different regulatory mechanisms should be considered in KK mice.

Of these rate-limiting sites, reactions of glucokinase, phosphofructokinase,

and 3-phosphoglycerate kinase were coupled by adenine nucleotides. Recently, it is considered that ATP/AMP ratio rather than the absolute concentration of ATP or ATP/ADP ratio in the cytoplasm is more important controlling factor for enzymes involved in carbohydrate metabolism[34]. Phosphofructokinase is specially inhibited by high ATP/AMP ratio[46]. Depressed glycolysis of KK mice at the site of phosphofructokinase may be due to this mechanism. Pyruvatekinase is also thought to be a key enzyme and is inhibited by ATP, too[33], but from the concentration of hepatic metabolites there was no rate limitation.

Delayed gluconeogenesis of KK mice in earlier stage of alanine load suggests the complicated, different from alloxan diabetes, mechanisms of gluconeogenesis in KK mice, and this phenomenon might have relationship to the ATP/AMP ratio at the site of 3-phosphoglycerate kinase.

Furthermore, high ratio of ATP/AMP is considered to increase the level of citrate[58], and citrate does activate acetyl-CoA carboxylase[59] resulting in increased fatty acid synthesis. Besides, it is suggested from the ratio of NAD^+/NADH that the production of α-glycerophosphate is elevated in KK mice, and this product might be used for lipogenesis. These speculations are in agreement with the observation of distinctly increased fatty acid synthesis by about 5 fold in the liver of KK mice[19].

Higher level of glucokinase in KK mice indicates that enough insulin-like activity is acting in the liver as far as the induction of the hepatic enzyme by insulin[25] is concerned. Therefore, increased glucose-6-phosphatase activity of KK mice is not due to the lack of insulin reppression nor due to the indution by corticosterone. In rat, the activity of glucose-6-phosphatase reaches to the level of adult animal in 9 days after birth[60] and glucokinase in 28 days[61]. KK mice showed alterations of hepatic enzyme activity in its developmental course. In this case glucokinase is considered to be reflecting the alteration of the blood glucose level because glucokinase is induced by glucose in the presence of insulin[39]. And blood glucose level may be reflecting the change of the activity of glucose-6-phosphatase. At the age of 76 days old, there were transient depression in the blood glucose level, glucose-6-phosphatase, and glucokinase activities. There may be some regulatory mechanisms to correct the abnormal metabolism in this stage.

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