Changes of Glycolytic Enzyme Activities and Plasma Insulin Levels in Diabetic Herbivorous Voles and KK Mice

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ABSTRACT. In the diabetic voles fed with concentrate diet and KK mice, changes in hepatic glycolytic enzyme activities and plasma insulin levels were measured. The hepatic glycolytic enzyme (glucokinase and pyruvate kinase) activities of normal voles and KK mice were about one fourth and one half of that of normal C57BL mice, respectively. Both voles and KK mice had the same characteristic which showed that hepatic glycolytic ability was very low. In the slight diabetic voles, glycolytic enzyme activity increased to twice of normal level in proportion to increasing of plasma insulin level, but in the serious diabetic voles plasma insulin level and hepatic glycolytic enzyme activity decreased considerably. In young KK mice without glycosuria, plasma insulin level was considerably high, over three times higher than normal level of C57BL mice. In old KK mice with glycosuria, although plasma insulin level increased moreover, the hepatic glycolytic enzyme activity did not increase. In conclusion, the diabetes of voles is considered to be classified to insulin dependent type and that of KK mice to non-insulin dependent type. —**KEY WORDS:** diabetes, glycolytic enzyme, herbivorous vole, KK mouse.

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As previously reported, in over 50% of herbivorous voles fed with high carbohydrate and low fiber diet, high concentration of glycosuria was induced [1, 3]. The diabetic voles showed remarkably low plasma insulin levels with the progress of the diabetes, finally falling into complete insulin deficiency in the serious diabetic stage [4]. On the other hand, KK mice is well known as obesity mice with hyperglycemia [12, 14, 19] and some researchers have appeared dealing with various aspects of the diabetic syndrome in KK mice [7, 17, 18]. In the present paper, hepatic glycolytic enzyme activity controlled by insulin [21, 22] and plasma insulin levels were measured in normal and diabetic voles and KK mice, and the difference of type of diabetes between voles and KK mice was studied.

MATERIALS AND METHODS

Animals. Herbivorous voles, *Microtus arvalis* Pallas, KK mice and C57BL/6J mice maintained in our laboratory were used for the present study. Normal voles were fed with pellet for herbivore (ZC: Oriental Yeast Co.) and cubed hay. KK mice and C57BL mice were fed with mouse pellet (CMF: Oriental Yeast Co.). Voles in glycosuric strain [4] were fed with mouse pellet, CMF. The ambient temperature of the animal room was maintained at about 25°C. The lights in the room were on from 7:00 a.m. to 9:00 p.m.

Glucose tolerance test. The glucose tolerance test was performed on voles, KK mice and C57BL mice. Animals were fasted for 18 hr and anaesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 40 mg/g body weight. Glucose in 10% solution (w/v) was loaded by intraperitoneal

injection at a dose of 1 mg/g body weight. Blood samples were taken from the orbital venous plexus in heparinized microcapillary tubes at 0, 30, 60, 90 min after the glucose injection. Changes in levels of blood glucose were measured.

Preparation of liver extracts. Animals were killed by decapitation. Livers were removed, washed and homogenized with four volumes of buffer containing 0.05 M Tris-HCl (pH 7.4), 1 mM ethylenediaminetetraacetate (EDTA) and 5 mM 2-mercaptoethanol in a glass homogenizer [15]. The homogenate was centrifuged at $100,000\times g$ for 30 min at 2°C and the resulting supernatant was used for the assays of glycolytic enzymes, glucokinase and pyruvate kinase, as enzyme extracts.

Glycolytic enzyme assays. Glucokinase activity was determined by the method of Bergmeyer *et al.* [6]. The reaction mixture in a final volume of 2.51 ml containing 40 mM triethanolamine (pH 7.4), 0.91 mM NADP, 0.64 mM ATP, 8.0 mM MgCl_2 and 0.55 U/ml of glucose-6-phosphate dehydrogenase was used. In addition to these ingredients, cuvette A had 50 mM N-acetylglucosamine, cuvette B had 0.5 mM glucose, and cuvette C had 200 mM glucose. The reaction was started by the addition of 0.02 ml freshly prepared enzyme extract. The formation of NADPH was recorded spectrophotometrically at 340 nm for 15 min at 25°C . The difference between cuvette B and A was a measure of hexokinase activity and that between cuvette C and B gave glucokinase activity. The activity of glucokinase is expressed as n moles of NADPH formed per min per mg protein.

The activity of pyruvate kinase was assayed according to the method of Hess and Wieker [9]. The reaction mixture in a final volume of 2 ml containing 0.1 M phosphate buffer (pH 6.0), 5.0 mM phosphoenolpyruvate, 30 mM MgSO_4 , 0.25 mM NADH, 10 mM ADP and 36 U/ml

lactate dehydrogenase was used. The reaction was initiated by the addition of 0.02 ml enzyme extract and proceeded for 10 min at 25°C . The enzymatic activity was determined spectrophotometrically by measuring the rate of disappearance of NADH at 340 nm. The activity of pyruvate kinase is expressed as n moles of NADH degraded per min per mg protein.

Other assays. Protein was determined in the enzyme extract by the method of Lowry *et al.* [16]. Blood samples were taken from jugular vein when animals were killed by decapitation. Plasma insulin level was measured according to the ELISA sandwich method of Arai *et al.* [2]. In this method, anti-bovine insulin prepared in guinea-pig was coated on the microplate and standard curve of insulin level was made by the use of purified swine insulin. The plasma insulin level of each animal was considered to express relative value. Blood glucose level was measured by glucoseoxidase method of Huggett and Nixon [10]. Fresh urine was examined weekly by Tes Tape (Eli Lilly Co.) at 10:00 a.m. Glycosuria was classified to +(0.1%), ++(0.25%) and +++(0.5%) by Tes Tape.

RESULTS

Levels of blood glucose and plasma insulin and hepatic glycolytic enzyme activity in normal voles, KK and C57BL mice are shown in Table 1. Herbivorous voles, 10 to 12 weeks old, fed with high fiber diet, showed low blood glucose level, about 70 mg/dl, and low activity of hepatic glycolytic enzyme, about one fourth of that of C57BL mice. The plasma insulin level of voles was almost same as that of C57BL mice. In young KK mice, 6 to 8 weeks old, glycosuria was not detected. The blood glucose level was about 100 mg/dl. The hepatic glycolytic enzyme activity was considerably low, about one half of that of C57BL mice. However,

Table 1. Levels of blood glucose, plasma insulin and hepatic glycolytic enzyme activities in normal voles, KK and C57BL/6J mice

	Age in weeks	Glycosuria	Blood glucose (mg/dl)	Plasma insulin (μ U/ml)	Hepatic GK	Hepatic PK
					(n moles/mg/min)	
Vole (n=10, ♂ 5, ♀ 5)	10-12	—	72.6 $\pm$ 4.5 ^{a)}	21.8 $\pm$ 4.6	10.2 $\pm$ 3.0	108 $\pm$ 26
KK mice (n=6, ♂ 3, ♀ 3)	6- 8	—	98.0 $\pm$ 12.0	64.0 $\pm$ 18.0	18.6 $\pm$ 6.4	208 $\pm$ 44
C57BL/6J mice (n=8, ♂ 4, ♀ 4)	10-12	—	108.4 $\pm$ 10.6	26.4 $\pm$ 8.0	48.6 $\pm$ 14.4	440 $\pm$ 50

a) Means $\pm$ S.D. Fig. 1. Changes of blood glucose levels (means $\pm$ S.D.) with glucose tolerance test in voles, KK and C57BL/6J mice.

- a. serious diabetic vole (n=6, ♂ 3, ♀ 3),
 b. slight diabetic vole (n=8, ♂ 4, ♀ 4),
 c. normal vole (n=10, ♂ 5, ♀ 5),
 d. diabetic KK mice (n=6, ♂ 4, ♀ 2),
 e. young KK mice (n=6, ♂ 3, ♀ 3),
 f. C57BL/6J mice (n=8, ♂ 4, ♀ 4).

in KK mice, the average plasma insulin level was 64.0 μ U/ml, two or three times higher than that in C57BL mice.

Changes of blood glucose levels with glucose tolerance test in normal voles, KK and C57BL mice are shown in Fig. 1, c, e, f. In both voles and C57BL mice, the blood glucose level increased to the peak level at 30 min after glucose administration and decreased immediately, returning to the fasting level at 90 min after glucose administration. In young KK mice (6 to 8 weeks old), the blood glucose level remained still

Table 2. Appearance ratio of glycosuria in voles and KK mice

Animal ^{a)}	Male	Female
Voles	83/133 ^{b)} (62.4%)	74/120 (61.7%)
KK mice	19/ 60 (31.7%)*	5/ 60 (8.3%)

a) 20-week-old.

b) Number of glycosuric animals/Number of animals examined.

* Significantly ($p<0.05$) high against the value in female in χ^2 -test.

elevated by far above the fasting level even 90 min after glucose administration. The glucose tolerance of KK mice was impaired considerably compared with those of voles and C57BL mice.

In normal voles fed with high fiber diet, glycosuria was seldom detected. In the glycosuric strain of voles fed with mouse pellet, glycosuria began to appear at 5 to 6 weeks of age and its appearance ratio increased over 60% at 20 weeks of age. In the voles, there was no sex difference in the aspect of glycosuria appearance ratio. In young KK mice, 6 to 8 weeks old, glycosuria was not detected. The appearance ratio was 31.7% and 8.3% in male and female KK mice at 20 weeks of age, respectively. That in KK mice was considerably low compared with the voles.

Changes of hepatic enzyme activity, levels

of blood glucose and plasma insulin in the diabetic voles and KK mice are shown in Table 3. The diabetic stages were classified into two stages, slight and serious, according to the results of glucose tolerance test (Fig. 1). In the slight diabetic voles, two or three weeks after onset of glycosuria, blood glucose level rose up to 148.6 mg/dl, twofold higher than normal level and plasma insulin level increased to 70 to 130 μ U/ml. The glucose tolerance declined slightly. The hepatic enzyme activity increased to twice of normal levels. In the serious diabetic voles, glycosuria continued for over 6 weeks, the blood glucose level increased to 258 mg/dl and the plasma insulin level decreased to 7.6 μ U/ml. The glucose tolerance was impaired considerably and blood glucose level was maintained over 300 mg/dl, three times higher than the fasting level, at 90 min after glucose administration. The hepatic glycolytic enzyme activity decreased to below one half of that of normal voles.

On the other hand, in the diabetic KK mice, the blood glucose level rose up to 305 mg/dl. The glucose tolerance of diabetic KK mice was impaired equally with that of young KK mice. The hepatic glycolytic

enzyme activity did not increase significantly although the plasma insulin level was maintained at high level, over 205 μ U/ml.

DISCUSSION

In the diabetic voles, the plasma insulin level changed greatly with the progress of the diabetes and the hepatic glycolytic enzyme activity varied according to changing of plasma insulin level. In the serious diabetic stage, the voles fell into complete insulin deficiency with a decrease in the hepatic glycolytic ability [3, 4]. Therefore, it was considered that the diabetes of herbivorous voles was classified to insulin dependent type (Type I). Many investigators have reported that the diabetic characters of KK mice which resemble those observed in human maturity onset diabetes [11, 19]. In young KK mice without glycosuria, the plasma insulin level was considerably higher than that in C57BL mice or voles, and hyperinsulinemia continued for long period. In the diabetic KK mice, the plasma insulin level increased moreover and high insulin level was maintained. However, glycolytic enzyme activity controlled by insulin [21,

Table 3. Changes of hepatic glycolytic enzyme activities, levels of blood glucose and plasma insulin in the diabetic voles and KK mice

	Glycosuria	Blood glucose (mg/dl)	Plasma insulin (μ U/ml)	Hepatic GK (n moles/mg/min)	Hepatic PK
Diabetic voles					
Slight case (n=8, ♂ 4, ♀ 4)	+ ^{a)}	148.6 $\pm$ 40.4 ^{b)}	102.0 $\pm$ 30.0	27.5 $\pm$ 4.4	260 $\pm$ 45
Serious case (n=6, ♂ 3, ♀ 3)	+++	258.0 $\pm$ 44.0	7.6 $\pm$ 4.0	6.0 $\pm$ 2.8	44 $\pm$ 8
KK mice					
Normal case (n=6, ♂ 3, ♀ 3)	—	98.0 $\pm$ 12.0	64.0 $\pm$ 18.0	18.6 $\pm$ 6.4	208 $\pm$ 44
Diabetic case (n=6, ♂ 4, ♀ 2)	+++	305.0 $\pm$ 35.0	205.6 $\pm$ 50.0	26.8 $\pm$ 9.6	264 $\pm$ 60

a) By Tes Tape; +: 0.1%, ++: 0.5%.

b) Means $\pm$ S.D.

22] did not increase in KK mice. In the diabetic KK mice with continuous glycosuria for over 10 weeks, the plasma insulin level was considerably high. Hyperglycemia was associated with elevation of gluconeogenic enzymes level in obese KK mice and the hyperglycemia was accounted for by diminished glucose metabolism resulting from decreased insulin sensitivity of peripheral tissue [11]. Activated secretion of insulin may cause development of obesity through induction of hepatic enzymes involved in glycolysis, pentose phosphate pathway and lipogenesis, which may result in active lipogenesis from glucose [20]. Disorder of lipid metabolism following obesity was considered to induce glycosuria in KK mice. The diabetes of KK mice was considered to be classified to non-insulin dependent type (Type II).

In ruminants, the hepatic glycolytic enzyme activity was very low [5, 8], and the voles had low activity of hepatic glycolytic enzyme and low renal threshold for glucose as the characteristic of herbivore [4]. So it is considered that the voles degrade less amount of glucose than mice do. The most important factor to induce glycosuria in voles is considered that hepatic glycolytic ability is very low. On the other hand, in KK mice, obesity was induced by feeding with high fat diet [12, 17], or administration of monosodium glutamate [7]. Obesity and aging accelerate onset of diabetes by reduction of insulin sensitivity [13]. Moreover in KK mice, the hepatic glycolytic enzyme activity was considerably low compared with that in C57BL mice. Glucose intolerance of KK mice has been considered to be due to congenital insulin resistance and low activity of hepatic glycolytic enzyme [12, 20].

As a result, both herbivorous voles and KK mice have the same characteristic which shows that hepatic glycolytic ability is lower than that in C57BL mice. However, type of diabetes is different between voles and KK

mice. The diabetes of voles is insulin dependent type and that of KK mice is considered to result in disorder of lipid metabolism following obesity. It is classified to non-insulin dependent type.

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要 約

糖尿病ハタネズミと KK マウスにおける解糖系酵素活性と血漿インスリン値の変動：新井敏郎・望月真理子・大木与志雄（日本獣医畜産大学獣医生理化学教室）——正常ハタネズミ，KK マウスの肝解糖系酵素（グルコキナーゼ，ピルビン酸キナーゼ）活性は，C57BL マウスのそれのそれぞれ $1/4$ ， $1/2$ であった。濃厚飼料を給与した糖尿病ハタネズミでは解糖系酵素活性は，糖尿病初期にはインスリン分泌の増加にともない正常値の 2 倍を示し，末期にはインスリン分泌が著しく低下して欠乏状態に陥り，解糖系酵素活性は正常の $1/2$ 以下になった。尿糖陰性の若齢 KK マウスの血漿インスリン値は C57BL マウスの約 3 倍で，尿糖陽性の老齢マウスのそれは，さらに高かったが，肝解糖系酵素活性は，ほぼ正常値と同等であった。以上の成績から，ハタネズミと KK マウスにおける糖尿病のタイプは著しく異なり，ハタネズミではインスリン依存性，KK マウスではインスリン非依存性と考えられた。