Nagoya J. med. Sci. 32: 129-141, 1969.

STUDIES ON LIPOGENESIS IN HEREDITARY OBESE-HYPERGLYCEMIC MICE (KK STRAIN)

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ABSTRACT

Fatty acid synthesis in KK mouse, which exhibits a hereditary obese-hyperglycemic syndrome, was studied. Incorporation of [U-^{14}C] glucose into liver fatty acid *in vivo* was more than four times higher in KK compared with control mice (CF 1), but no significantly increased incorporation was observed into carcass fatty acid. Incorporation of [1-^{14}C] acetate into liver fatty acid *in vivo* was also elevated (5 fold) in KK mice. Fatty acid synthesis from acetate by liver slices of KK mice was about three times higher than that of control. However, the conversion of acetate into fatty acid in adipose tissues from KK mice *in vitro* was lower than that of control. Level of Liver acetyI CoA carboxylase was twice as high in KK, but with the activity of liver citrate-cleavage enzyme, no difference was observed between KK and control mouse. Mechanisms of hyperlipogenesis in the liver of KK mouse were discussed in relation to hyperinsulinemia and hyperglycemia.

INTRODUCTION

Recently the study of regulatory mechanisms of lipogenesis has been intensively investigated.

The mechanisms by which fatty acid synthesis is impaired in tissues of starved or insulin depleted animals, and the means by which high carbohydrate diet and insulin administration accelerate the lipogenesis have been extensively studied[1].

As well as these physiological states, investigation of the hereditary obese-hyperglycemic syndrome of mice has been a quite interesting. Much informations about the obese mice have been reported[2)-9)], however, the fundamental mechanism (s) of obesity is still unknown.

KK mouse is an inbred strain[10] originated from Japanese native mouse by Kondo, and has been found to show moderate obesity, pelyphagia, polyuria, and high blood glucose levels under condition of *ad libitum* feeding in spite of an increased insulin content of pancreas[11) 12)] and a high level of serum insulin-

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Received for publication April 1, 1968.

like activity[13]. Relative insulin resistance with respect to response of blood glucose level *in vivo* and to glucose uptake in adipose tissue *in vitro* was also reported[13].

In the present paper, fatty acid synthesis in KK mouse was studied. Results obtained in this study indicate that hyper-lipogenesis is more pronounced in the liver than the extra-hepatic tissue of KK mouse. Higher capacity of fatty acid synthesis of the liver was observed *in vivo* and *in vitro*, and acetyl CoA carboxylase, which is considered as the rate limiting step of fatty acid synthesis from acetyl CoA[14], was a high level in the liver of KK mouse. Enhanced hepatic lipogenesis is probably due in part to hyperinsulinemia and hyperglycemia of this mouse.

MATERIALS AND METHODS

Chemicals

CoA and GSH (reduced glutathione) were supplied from Sigma Chemical Co. NADH and ATP were obtained from C. F. Boehringer and Soehne G. m. b. H., and crystalline bovine serum albumin from Nutritional Biochemical Corp. [U-^{14}C] glucose (5.0 mc/mM), sodium [1-^{14}C] acetate (44.4 mc/mM) and $NaH^{14}CO_3$ (10.0 mc/mM) were supplied from Daiichi Pure Chemicals Co., Ltd. (Japan) and [1-^{14}C] glucose (39.7 mc/mM) from Radiochemical Center (England). 2, 5-diphenyl-oxazole (Dotite DPO) and 1, 4-bis (5-phenyloxazolyl) benzene (Dotite POPOP) were supplied from Wako Pure Chemical Industries, Ltd. Malate dehydrogenase (EC 1. 1. 1. 37), glucose oxidase (EC 1. 1. 3. 4) and peroxidase (EC 1. 11. 1. 7) were obtained from C. F. Boehringer and Soehne G. m. b. H., and crystalline bovine insulin from Novo Industri A/S, Copenhagen, Denmark. All chemicals except listed above were supplied from Katayama Chemicals Co., and were an analytical grade.

Animals

In the present study, CF 1 mice were used as the control of KK mice. Both strains of the mice were bred in our laboratory and male mice of an appropriate age were used. All animals were fed an ordinary commercial chow (OA-2, obtained from Nihon Kurea Company), which had following percentage composition: crude protein 26.5; crude fat 3.5; crude fiber 3.5; N-free soluble substances 53.5. They were housed in an air-conditioned room maintained at 20–24° and were supplied with food and water *ad libitum*.

Assay of liver acetyl CoA carboxylase

The liver of each mouse was homogenized in a solution (25% w/v) consisting of 0.15 M KCl, 0.05 M tris and 0.1 mM EDTA (adjusted to pH 7.2 with HCl). After centrifugation at 105,000 × *g* for an hour at 1°, one ml of each supernatant was passed through Sephadex G-25 column (1 cm × 30 cm) in a

cold room at 2°C. This filtrated extract was used for the assay.

Activity of acetyl CoA carboxylase was measured by the fixation of [^{14}C] bicarbonate in the presence of acetyl CoA as described by Chang *et al.*[8]. The extract (2–3 mg of protein) was preincubated at 37°C for 30 min. in a medium containing 60 mM tris-HCl pH 7.0, 8 mM $MgCl_2$, 3 mM GSH, 0.1 mM EDTA, bovine serum albumin (0.6 mg/ml) and 5 mM potassium citrate with a final volume of 0.75 ml. Reaction was then started by the addition of 0.25 ml of a solution containing the above concentrations of tris-HCl buffer pH 7.0, $MgCl_2$, GSH, EDTA, bovine serum albumin, potassium citrate, as well as acetyl CoA (0.2 μmole), ATP (2 μmole) and $KH^{14}CO_3$ (10 μmoles, 2 μc). After 5 min. at 37°C, the reaction was stopped by the addition of 0.2 ml of 6 N HCl and 0.5 ml of centrifuged supernatant was transfered to a 20-ml scintillation counting bottle. After drying at 45°C, the scintillation medium was added and radioactivity was counted.

Acetyl CoA was prepared from CoA by acetylation with acetic anhydride as described by Simon *et al.*[15].

Assay of liver citrate-cleavage enzyme

Liver homogenates (20% w/v) in 0.15 M KCl containing 0.005 M EDTA, 0.005 M $MgCl_2$ and 0.1 M β-mercaptoethanol were centrifuged at 105,000 $\times$ *g* for an hour. The supernatants were used for the assay. All preparative procedures were carried out in a cold room at 2°C.

Activity of citrate-cleavage enzyme was measured spectrophotometorically by the coupled enzyme assay procedure according to Srere[16]. Oxaloacetate formation from citrate, CoA and ATP was measured as the oxidation of NADH by coupling with malate dehydrogenase. The final concentrations of reaction medium (3 ml of volume) were as follows: tris (adjusted pH 7.3 with 2 N HCl), 100 mM; $MgCl_2$, 10 mM; potassium citrate, 20 mM; β-mercaptoethanol, 3.3 mM; CoA, 0.2 mM; NADH, 0.1 mM; ATP (adjusted pH 7.3), 5 mM; excess of malate dehydrogenase; and liver extract (2–3 mg of protein). Reaction was run at 16°C for 5 min. Protein concentration was determined with biuret method[17].

Procedures of the isotope study in vivo

Each mouse was given 1 mg of [U-^{14}C] glucose (1.78 μc) or 1 mg of sodium [1-^{14}C] acetate (3.3 μc) with 0.3 ml of water intraperioneally. In both cases, food was removed from the cages at the time of injection. After a time as described in Tables, mice were killed by decapitation. Livers were removed, weighed, placed in 2 ml of 30% KOH, and heated in a boiling-water bath for an hour, then 2 ml of 95% ethanol was added and the extracts were refluxed for additional 3 hrs. The remainder of the carcass from each mouse was chopped to small pieces and saponified in 30% KOH in a boiling-water bath. After an hour, aliquot of this extract was taken and equal volume of 95% ethanol was added, then the saponification was continued for three hours.

Procedures of the liver slice study

Liver slices were prepared as described by Elliott[18] and rinsed in Krebs-Ringer bicarbonate buffer (pH 7.4)[19], and 200–300 mg of each tissue slice was transfered into 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) which contained 20 mM sodium [1-^{14}C] acetate (2 μc), 11.1 mM glucose and 0.3% of bovine serum albumin. Incubation was carried out in Warburg vessels at 30°C for 2 hrs. except as noted in Table 4, in an atomosphere of $O_2 + CO_2$ (95 : 5 v/v) in a shaking water bath (70 strokes/min.). After incubation, 0.4 ml of monoethanolamine + ethylene glycol monomethyl ether (1 : 2 v/v)[20] was injected into center wells of the vessels to trap CO_2 and 0.2 ml of 10 N H_2SO_4 was added to the medium of each vessel. The vessels were well mixed and placed overnight at room temperature. Then the liver slices were removed from the medium, rinsed in water and 5% acetic acid, placed in 1 ml of 30% KOH and saponified in a same manner as described above. Monoethanolamine solutions were transfered to each scintillation counting bottle by washing twice with scintillation medium and radioactivities were determined as described in counting procedures. Appropriate corrections were done by the counts of $^{14}CO_2$ from no-tissue incubation and ^{14}C in fatty acid from no-incubation tissue.

Extraction of fatty acid

Fatty acid was extracted with the procedure described by Entenman[21]. From the saponified alkaline solutions, non-saponifiable lipid fractions were removed with petroleum ether (5–10 volumes per volume of alkaline solution). After acidification with 10 N H_2SO_4, fatty acid was extracted twice with 5–10 volumes of petroleum ether. In the case of liver slice experiment, this ether extract was washed twice with 5% acetic acid. Aliquot of the combined ether extract was transfered to a scintillation bottle and after evaporation to dryness and addition of the scintillation medium, radioactivity was measured. Fatty acid was determined colorimetrically[22] with another aliquot.

Assay of serum insulin-like activity

Serum insulin-like activity was measured with a procedure described Renold, *et al.*[23].

About 100 mg of distal parts of epididymal adipose tissues from male albino rats, weighing between 200 and 250 g, were incubated in 2 ml of twice diluted mice serum with Krebs-Ringer bicarbonate buffer pH 7.4 at 37°C for 2 hrs. in an atomosphere of $O_2 + CO_2$ (95 : 5 v/v). In each vessel, 11.1 mM of [1-^{14}C] glucose (0.5 μc) was added.

In one series of assay, six pieces of adipose tissue, obtained from a single rat were used. Four pieces were used for standard insulin assay and the remaining two slices for sample assay. Crystalline bovine insulin (25 units/mg) was diluted with bicarbonate buffer containing 0.2% zelatin. In the case of

standard insulin assay, 2 ml of Krebs-Ringer bicarbonate buffer containing 0.2 % zelatin and above concentration of [1-^{14}C] glucose was used as a medium. After incubation, carbon dioxide was trapped and radioactivity was measured as described above. A linear response to increasing doses of insulin was obtained over 1,000 μunits/ml, when the dose-response relationship was expressed as the logarithms of the d.p.m. per 100 mg of rat adipose tissue and the logarithms of the insulin concentrations.

After the aliquot was taken for measuring the glucose concentration, each sample of serum from 7-9 mice was stocked overnight in the deep freezer (−20°). Assay of each sample was duplicated. Glucose was determined with glucose oxidase and peroxidase as described Huggett *et al.*[24].

Counting procedures

All radioactivities were counted with a liquid scintillation spectrometer (Model GSL-163, Kobe Kogyo, Kobe, Japan).

The scintillation medium used was a toluen: ethylene glycol monomethyl ether (methyl cellosolve) solution (5 : 1 v/v) containing 4.0 g of 2, 5-diphenyloxazole (Dotite DPO) and 80 mg of 1, 4-bis (5-phenyloxazolyl) benzen (Dotite POPOP) per liter[20].

The counting efficiencies as determined by internal standardization, were 60-80%, and all counts were corrected to 100% efficiency of these bases.

RESULTS

Average growth curves of obese (KK) and control (CF 1) mice are shown in Fig. 1. Moderately increased body weight was noticed in KK mouse compared with control after two months of age. At four months they weighed about 40 g and 30 g in KK and control respectively, and a further increase of body weight of KK was not observed over a year.

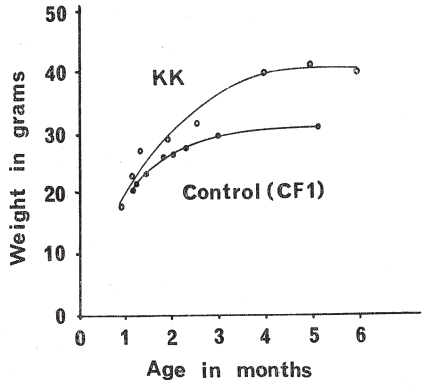

FIG. 1. Average growth curves. Each point indicates average of 7-10 mice.

Unlike other strains of obese-hyperglycemic mice[25,26], KK mice are not so obese, and the heaviest body weight observed in this laboratory was 45 g in male.

With an increase of body weight, marked hyperglycemia was seen in the feeding state. As shown in Table 1, blood glucose levels of *ad libitum* feeding condition in five month-old KK were more than two hundred mg per dl, and glucosuria was often observed in such a case. However, the blood glucose

TABLE 1. Blood glucose levels and fatty acid contents in liver and in carcass

Breeding conditions and analytical details are given in the text. Both strains of mice were approximately 5 months of age. Results are given as means ±S.D.

	Body wt. (g)	Blood* glucose (mg/dl)	Wt. of liver (g)	% of fatty acid**	
				in liver	in carcass
Control (CF 1)	31.6±1.6 (3)***	122±12 (3)	1.50±0.10 (3)	3.7±0.4 (3)	5.1±0.4 (3)
KK	39.7±2.1 (10)	233±24 (10)	2.46±0.40 (10)	5.6±0.3 (3)	33.6±2.4 (3)

* Blood glucose levels under condition of *ad libitum* feeding.
** Weight (g) of total fatty acid (after saponification) / 100 g fresh wt. of liver or/100 g body wt.
*** Number of animals

value was decreased to a normal level after overnight fasting. After one year of age, hyperglycemia was not so prominent.

Fatty acid content of the liver was slightly higher in KK compared with control, but no pathological accumulation of fatty acid was noticed.

Carcass fatty acid content of KK, on the other hand, was quite high and constituted about one third (33.6%) of body weight. From this, it was calculated that the most parts of increased body weight was the deposition of fatty acid in the extra-hepatic tissue.

Conversion of [U-^{14}C] glucose into liver and carcass fatty acid in vivo

Fatty acid synthesis from glucose *in vivo* was carried out under feeding

TABLE 2. Conversion of [U-^{14}C] glucose into liver and carcass fatty acid

Experimental details are given in the text. Both groups of mice, four/group, approximately 2 months of age, were fed *ad libitum*. One mg of [U-^{14}C] glucose (1.78 μc) per mouse was given intraperitoneally and killed 90 min. later. The food was removed from the cages at the time of dosing. Results are given as means ± S.D.

	Body wt. (g)	Wt. of liver (g)	Wt. of 2 pads* (mg)	% dose** in liver fatty acid	% dose** in carcass*** fatty acid
Control (CF 1)	32.7±1.2	1.42±0.12	257±121	0.0159±0.0130	1.51±0.58
KK	29.7±1.1	1.31±0.09	621±37	0.0747±0.0175	1.96±0.19

* Epididymal fat pads.
** Percent incorporated into liver fatty acid or carcass fatty acid per injected ^{14}C dose.
*** Carcass refers to the body with liver removed.

state. Each mouse was given 1 mg of [U.^{14}C] glucose intraperitoneally and incorporation into liver and carcass fatty acid after 90 min. was shown in Table 2. Mice used in this experiment were approximately 2 months of age and with respect to body weight, no difference was observed between KK and control. However, the weight of epidymal fat pads of KK was twice heavier than that of conrol.

Incorporation into carcass fatty acid from [U-^{14}C] glucose was not significantly increased in KK mice. On the other hand, incorporation into liver fatty acid was more than 4 times higher than control.

Conversion [1-^{14}C] acetate into liver fatty acid in vivo

Each mouse, approx. 2 months of age, was given 1 mg of sodium [1-^{14}C] acetate intraperitoneally and incorporation into liver fatty acid after 60 min. was shown in Table 3.

TABLE 3. Conversion of [1-^{14}C] acetate into liver fatty acid

Experimental details are given in the text. Each mouse, approximately 2 months of age, was given 1 mg of sod. [1-^{14}C] acetate (3.3 μc) intraperitoneally and killed 60 min. later. The food was removed from the cages for this interval. Results are given as means of four animals ± S. D.

	% * of liver fatty acid	[1-^{14}C] acetate incorporated	
		d.p.m./total liver fatty acid	d.p.m./100 mg of liver fatty acid
Control (CF 1)	3.8±0.2	16,400±6,000	30,400±6,900
KK	3.3±0.2	90,100±26,000	184,000±50,800

* Weight (g) of total fatty acid (after saponification)/100 g fresh weight of liver.

[1-^{14}C] acetate incorporations in liver fatty acid per total liver and per 100 mg of liver fatty acid were higher in KK with same extent as observed in the case of [U-^{14}C] glucose.

From these results, it was noted that the hyperlipogenesis in KK was prominent in the liver rather than in the tissue of the carcass. Similar results were reported[6) 7)] in C 57 BL/6 J-ob, a obese-hyperglycemic strain of mice from the Jackson Laboratory, Bar Harbar, Maine.

Fatty acid synthesis from [1-^{14}C] acetate by liver slice

Liver slices from 2 months old mice were incubated in a manner as described in materials and methods section.

As shown in Table 4, acetate conversion to fatty acid and oxidation to CO_2 by liver slices from both strains of mice were proceeding continuously throughout 3 hr. incubation period.

TABLE 4. Effect of incubation time on fatty acid synthesis and oxidation to carbon dioxide from [1-^{14}C] acetate by liver slice

Experimental details are given in the text. Liver slices (200~300 mg of wet wt./each vessel) were incubated at 30° in an atomosphere of O_2+CO_2 (95 : 5 v/v). Incubation medium was 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) which contained 20 mM of [1-^{14}C] acetate (2 μc), 11.1 mM of glucose and 0.3% of bovine serum albumin. In this experiment, liver slices were obtained from a single animal.

Incubation time (hr.)	[1-^{14}C] acetate converted (μmoles/g of liver)			
	fatty acid		CO_2	
	Control (CF 1)	KK	Control (CF 1)	KK
1	0.159	0.418	2.93	3.45
2	0.379	0.846	4.42	4.53
3	0.464	1.284	9.35	8.47

The rates of incorporation of acetate into fatty acid and oxidation to CO_2 were shown in Table 5. Fatty acid synthesis from acetate by liver slice was 3 times higher in KK compared with control, whereas the oxidation to CO_2 was to the same extent in KK and control.

TABLE 5. Fatty acid synthesis from [1-^{14}C] acetate by liver slice

Experimental procedures are identical with Table 4., and described in the text. Liver slices of each mouse (four/group) were incubated for 2 hr. at 30° in a shaking water bath. All animals were fed *ad libitum* untill they were sacrificed. Results are given as means ± S. D.

	[1-^{14}C] acetate converted (μmoles/g of liver/2 hr.)	
	fatty acid	CO_2
Control (CF 1)	0.245±0.097	5.70±1.31
KK	0.756±0.295	5.57±0.74

These data indicated that the acetate activation step had the same capacity in the liver slices from both strains of mice, and enhanced acetate conversion to fatty acid in KK was due to the high capacity of fatty acid synthesizing process from acetyl CoA.

Liver acetyl CoA carboxylase and citrate cleavage enzyme

Fatty acid synthesis from acetyl CoA is catalysed by acetyl CoA carboxylase and fatty acid synthetase[27]. It has now noted that this system is regulated at the level of acetyl CoA carboxylase[14]. On the other hand, citrate-cleavage enzyme has also been considered to have an important role in fatty acid synthesis[28]. Extra-mitochondrial acetyl CoA is supplied by this enzyme

from citrate in the cytoplasma[29]. And the level of this enzyme is regulated by dietary and hormonal states[30]. From these respects, the levels of acetyl CoA carboxylase and citrate-cleavage enzyme in the liver of KK and control mouse were measured.

As shown in Table 6, acetyl CoA carboxylase activity was higher in the liver from KK mouse. When activity is expressed as specific activity (mμmoles carboxylated/mg of protein/min.) and total activity (mμmoles carboxylated/total liver/min.), acetyl CoA carboxylase in the liver from KK was 1.8 and 2.0 times higher than that of control respectively. These results essentially

TABLE 6. Liver acetyl CoA carboxylase activity

Experimental details are given in the text. Sephadex G-25 filtrated liver extract from each mouse was preincubated at 37° for 30 min. with citrate. Carboxylation was then measured by the fixation of [^{14}C] bicarbonate in the presence of acetyl CoA. Final incubation medium (1 ml of volume) contained 60 mM tris-HCl buffer pH 7.0, 8 mM $MgCl_2$, 3 mM GSH, 0.1 mM EDTA, bovine serum albumin (0.6 mg), 5 mM pot. citrate, 2 mM ATP, 0.2 mM acetyl CoA, 10 mM (2 μc) $KH^{14}CO_3$ and liver extract. Results are given as means of four animals ± S. D.

	Specific activity mμmoles/min./mg protein	Total activity mμmoles/min./total liver
Control (CF 1)	2.47±0.94	366±140
KK	4.42±0.76	733±198

Enzyme activity is expressed as mμmoles of acetyl CoA carboxylated/mg of Sephadex G 25 filtrated 105,000 × g supernatant protein/min., or mμmoles of acetyl CoA carboxylated/total liver/min.

TABLE 7. Liver citrate-cleavage enzyme activity

Experimental details are given in the text. Enzyme activity was measured by the coupled enzyme assay procedure according to Srere[16]. The final concentrations of reaction medium were: tris (adjusted to pH 7.3 with 2 N HCl), 100 mM; pot. citrate, 20 mM; β-mercaptoethanol, 3.3 mM; CoA, 0.2 mM; NADH, 0.1 mM; ATP (adjusted to pH 7.3), 5 mM; excess of malate dehydrogenase; liver extract. Reaction was run at 16°. Results are given as averages of four animals ± S. D.

	Specific activity mμmoles/min./mg protein	Total activity mμmoles/min./total liver
Control (CF 1)	2.30±0.32	360±56
KK	2.94±0.41	564±87

Enzyme activity is expressed as mμmoles of NADH oxidized/mg of 105,000 × g supernatant protein/min., or mμmoles of NADH oxidized/total liver/min.

agreed with the recent report on C 57 BL/6 J-obese mice[8].

Kornacker reported[4] a high level of citrate-cleavage enzyme in the liver of C 57 BL/6 J-obese mouse. In the present study, as shown in Table 7, total activity (mμmoles of NADH oxidized/total live/min.) of this enzyme in the liver from KK was slightly (1.6 fold) higher than that from control. However, no significant difference was seen when expressed as specific activity (mμmoles of NADH oxidized/mg of protein/min.).

This enzyme was greatly affected with feeding states[31] and strongly induced by high carbohydrate diet[30]. The different results may be obtained as reported on C 57 BL/6 J-obese mice with an appropriate diet in this KK mice.

DISCUSSION

Shreeve[6] and Jansen[7] have indicated that hyperlipogenesis is more prominent in the liver than in the extra-hepatic tissue of the hereditary obese-hyperglycemic mouse. Similar tendency was observed in the present study on KK strain of the mice which was originated by Kondo and exhibits a hereditary obese-hyperglycemic syndrome. Incorporations of [^{14}C] glucose and [^{14}C] acetate into liver fatty acid *in vivo* were greatly increased, but the incorporation of [^{14}C] glucose into carcass fatty acid was not significantly higher than that of control.

Elevated capacity of hepatic lipogenesis was also observed in liver slice experiment. However, in contrast to the report by Mayer's group[32], lipogenic capacity in adipose tissues from KK mice *in vitro* was lower than that of control (Table 8). Similar results were reported by Hellman, *et al.*[33].

Mayer has postulated that the primary inborn error of hereditary obese-hyperglycemic mice is the lack of the normally present repressor to glycero-

TABLE 8. Fatty acid synthesis from [1-^{14}C] acetate in epididymal adipose tissue *in vivo*.

Experimental details are identical with the liver slice experiment. About 100 mg of epididymal adipose tissues from 2 month-old mice, were incubated with a same manner as described in procedures of the liver slice study. Incubation medium (2 ml of Krebs-Ringer bicarbonate buffer pH 7.4) contained 20 mM of sod. [1-^{14}C] acetate, 11.1 mM of glucose and 0.3% of bovine serum albumin. All animals were fed *ad libitum* untill they were sacrificed. Results are given as averages of 4 animals $\pm$ S. D.

	[1-^{14}C] acetate converted (μmoles/g of tissue/2 hr.)	
	fatty acid	CO_2
Control (CF 1)	2.24$\pm$0.41	2.42$\pm$0.16
KK	0.95$\pm$0.24	1.05$\pm$0.34

kinase in the white adipose tissue[3) 5)]. However, the present results support the suggestion by Shreeve and Jansen that the fundamental metabolic abnormality is expressed more pronounced in the liver than in the adipose tissue.

In this strain of obese mice, obesity was not so prominent at 2 months of age, but the considerable high levels of blood glucose (150–180 mg/dl) and serum insulin-like activity (Table 9) were observed at this age. These hyperglycemia and hyperinsulinemia may accelerate the glycolytic and lipogenic

TABLE 9. Serum insulin-like activity

Serum insulin-like activity was measured with a procedure described by Renold *et al*[23)]. Both strains of mice, approximately 2 months of age, were fed *ad libitum* untill their serum was collected. Each result is the value obtained with combined serum from 7–9 mice. See text for experimental details.

	Exp. series	Serum insulin-like activity (μunits/ml)
Control (CF 1)	1	90
	2	136
KK	1	1100
	2	3860

pathway in the liver and some enzymes concerned with these systems may be induced.

Seidman, *et al.*[9)] reported the elevated activities of glucokinase, phosphofructokinase, and pyruvate kinase as well as gluconeogenic enzymes in the liver of the obese mouse. High levels of citrate-cleavage enzyme[4)], and both acetyl CoA carboxylase and fatty acid synthetase[8)] were also reported in the liver of the obese mouse.

In the present study, elevated level of acetyl CoA carboxylase was observed, but no increased activity of citrate-cleavage enzyme was noticed in the liver of KK mouse in our feeding condition.

It is also apparent that abundant glucose and elevated activity of glycolysis would increase the rates of formation of acetyl CoA, reduced coenzyme and α-glycerophosphate in the liver, and would result in accelerated hepatic lipogenesis of this mouse.

Hyperinsulinemia, which is probably induced by hyperglycemia, may accelerate lipogenesis in this manner in the liver and the synthesized fatty acid may be transfered to the extra-hepatic tissue.

On the other hand, adipose tissue from KK mouse has lower capacity of glucose oxidation and does not seem to respond fully to circulating insulin. With this abnormality of adipose tissue and with polyphagia and decreased motility of this mouse, hyperglycemia may be promoted, and with further insulin output from pancreas, hyperlipogenesis may be observed in the liver of

the hereditary obese-hyperglycemic mouse. However, the primary cause of this abnormality is not clear.

ACKNOWLEDGMNET

The author wishes to express his gratitude to Prof. Yahito Kotake and Dr. Yasuhiko Kawamura for their valuable suggestion.

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