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-14C] glucose into liver fatty acid in vivo was more than four times higher in KK compared with control mice (CF1), but no significantly increased incorporation was observed into carcass fatty acid. Incorporation of [1-14C] 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 acetyl 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.

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, however, the fundamental mechanism(s) of obesity is still unknown.

KK mouse is an inbred strain originated from Japanese native mouse by Kondo, and has been found to show moderate obesity, polyphagia, polyuria, and high blood glucose levels under condition of ad libitum feeding in spite of an increased insulin content of pancreas and a high level of serum insulin.

Received for publication April 1, 1968.
like activity\textsuperscript{13}. Relative insulin resistance with respect to response of blood glucose level \textit{in vivo} and to glucose uptake in adipose tissue \textit{in vitro} was also reported\textsuperscript{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 \textit{in vivo} and \textit{in vitro}, and acetyl CoA carboxylase, which is considered as the rate limiting step of fatty acid synthesis from acetyl CoA\textsuperscript{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.

\textbf{MATERIALS AND METHODS}

\textit{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.

\textit{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\(^\circ\) and were supplied with food and water \textit{ad libitum}.

\textit{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 \(\times g\) for an hour at 1\(^\circ\), one ml of each supernatant was passed through Sephadex G-25 column (1 cm \(\times\) 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 transferred 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 × 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 spectrophotometrically 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)\(^{20}\), and 200–300 mg of each tissue slice was transferred into 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) which contained 20 mM sodium \(\text{[1-}^{14}\text{C}]\) acetate (2 \(\mu\)c), 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 atmosphere of \(\text{O}_2 + \text{CO}_2\) (95 : 5 v/v) in a shaking water bath (70 strokes/min.). After incubation, 0.4 ml of monoethanolamine + ethylene glycol monomethyl ether \(\text{(1 : 2 v/v)}\)\(^{29}\) was injected into center wells of the vessels to trap \(\text{CO}_2\) and 0.2 ml of 10 n.t HSO\(_4\) 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 transferred 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 \(^{14}\text{CO}_2\) from no-tissue incubation and \(^{14}\text{C}\) 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 HSO\(_4\), 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 transferred 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 Re- nold, 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 atmosphere of \(\text{O}_2 + \text{CO}_2\) (95 : 5 v/v). In each vessel, 11.1 mM of \(\text{[1-}^{14}\text{C}]\) glucose \((0.5 \mu\text{c})\) 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 \((25 \text{ units/mg})\) 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 \mu units/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 ($-20^\circ$). Assay of each sample was duplicated. Glucose was determined with glucose oxidase and peroxidase as described Huggett et al.\textsuperscript{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-diphenyl-oxazole (Dotite DPO) and 80 mg of 1,4-bis (5-phenyloxazolyl) benzen (Dotite POPOP) per liter.\textsuperscript{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 (CF1) 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.

Unlike other strains of obese-hyperglycemic mice\textsuperscript{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.

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

* 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-14C] 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-14C] 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-14C] 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.

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

* Epididymal fat pads.
** Percent incorporated into liver fatty acid or carcass fatty acid per injected 14C dose.
*** Carcass refers to the body with liver removed.
state. Each mouse was given 1 mg of [U-14C] 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 control.

Incorporation into carcass fatty acid from [U-14C] 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 [1-14C] acetate into liver fatty acid in vivo

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

**TABLE 3. Conversion of [1-14C] 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. [1-14C] 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.

<table>
<thead>
<tr>
<th></th>
<th>%* of liver fatty acid</th>
<th>[1-14C] acetate incorporated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d.p.m./total liver fatty acid</td>
<td>d.p.m./100 mg of liver fatty acid</td>
</tr>
<tr>
<td>Control (CF1)</td>
<td>3.8±0.2</td>
<td>16,400±6,000</td>
<td>30,400±6,900</td>
</tr>
<tr>
<td>KK</td>
<td>3.3±0.2</td>
<td>90,100±26,000</td>
<td>184,000±50,800</td>
</tr>
</tbody>
</table>

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

[1-14C] 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-14C] 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 in C57BL/6J-ob, an obese-hyperglycemic strain of mice from the Jackson Laboratory, Bar Harbar, Maine.

**Fatty acid synthesis from [1-14C] 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 CO₂ 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 atmosphere 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 \(\mu\)c), 11.1 mM of glucose and 0.3% of bovine serum albumin. In this experiment, liver slices were obtained from a single animal.

<table>
<thead>
<tr>
<th>Incubation time (hr.)</th>
<th>[1-(^{14})C] acetate converted ((\mu)moles/g of liver)</th>
<th>CO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fatty acid</td>
<td></td>
</tr>
<tr>
<td>Control (CF 1)</td>
<td>KK</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.159</td>
<td>0.418</td>
</tr>
<tr>
<td>2</td>
<td>0.379</td>
<td>0.846</td>
</tr>
<tr>
<td>3</td>
<td>0.464</td>
<td>1.284</td>
</tr>
<tr>
<td></td>
<td>KK</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.93</td>
<td>3.45</td>
</tr>
<tr>
<td>2</td>
<td>4.42</td>
<td>4.53</td>
</tr>
<tr>
<td>3</td>
<td>9.35</td>
<td>8.47</td>
</tr>
</tbody>
</table>

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 until they were sacrificed. Results are given as means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>[1-(^{14})C] acetate converted ((\mu)moles/g of liver/2 hr.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>fatty acid</td>
<td>CO(_2)</td>
</tr>
<tr>
<td>Control (CF 1)</td>
<td>0.245±0.097</td>
<td>5.70±1.31</td>
</tr>
<tr>
<td>KK</td>
<td>0.756±0.295</td>
<td>5.57±0.74</td>
</tr>
</tbody>
</table>

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.
LIPOGENESIS IN OBESE MICE

from citrate in the cytoplasm. And the level of this enzyme is regulated by dietary and hormonal states. 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 (mumoles carboxylated/mg of protein/min.) and total activity (mumoles 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>
<thead>
<tr>
<th>Specific activity</th>
<th>Total activity</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>Control (CF 1)</td>
<td></td>
</tr>
<tr>
<td>2.47±0.94</td>
<td>366±140</td>
</tr>
<tr>
<td>KK</td>
<td></td>
</tr>
<tr>
<td>4.42±0.76</td>
<td>733±198</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Specific activity</th>
<th>Total activity</th>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (CF 1)</td>
<td></td>
</tr>
<tr>
<td>2.30±0.32</td>
<td>360±56</td>
</tr>
<tr>
<td>KK</td>
<td></td>
</tr>
<tr>
<td>2.94±0.41</td>
<td>564±87</td>
</tr>
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</table>

Enzyme activity is expressed as mumoles of NADH oxidized/mg of 105,000 x g supernatant protein/min., or mumoles of NADH oxidized/total liver/min.
agreed with the recent report on C57BL/6J-obese mice\(^9\).

Kornacker reported\(^9\) a high level of citrate-cleavage enzyme in the liver of C57BL/6J-obese mouse. In the present study, as shown in Table 7, total activity (m,\textmu moles 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 (m,\textmu moles 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 C57BL/6J-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 glycerol-

<table>
<thead>
<tr>
<th>TABLE 8. Fatty acid synthesis from [1-(^{14})C] acetate in epididymal adipose tissue <em>in vivo.</em></th>
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<tbody>
<tr>
<td>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 <em>ad libitum</em> until they were sacrificed. Results are given as averages of 4 animals ± S.D.</td>
</tr>
<tr>
<td>[(^{14})C] acetate converted (\textmu moles/g of tissue/2 hr.)</td>
</tr>
<tr>
<td>fatty acid</td>
</tr>
<tr>
<td>Control (CF 1)</td>
</tr>
<tr>
<td>KK</td>
</tr>
</tbody>
</table>
kinase in the white adipose tissue\(^3\). However, the present results support
the suggestion by Shreeve and Jansen that the fundamental metabolic abnor-
mality 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 hyper-
glycemia and hyperinsulinemia may accelerate the glycolytic and lipogenic

<table>
<thead>
<tr>
<th>Exp. series</th>
<th>Serum insulin-like activity ((\mu)units/ml)</th>
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</thead>
<tbody>
<tr>
<td>Control (CF 1)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>136</td>
</tr>
<tr>
<td>KK</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1100</td>
</tr>
<tr>
<td>2</td>
<td>3860</td>
</tr>
</tbody>
</table>

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 \(ad\ libitum\) until their serum was collected. Each result is the value obtained with combined serum from 7–9 mice. See text for experimental details.

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

Seidman, \(et\ al\).\(^{9}\) reported the elevated activities of glucokinase, phospho-
fructokinase, 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\(^5\) 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
\(\alpha\)-glycerophosphate in the liver, and would result in accelerated hepatic lipo-
genesis of this mouse.

Hyperinsulinemia, which is probably induced by hyperglycemia, may ac-
celerate lipogenesis in this manner in the liver and the synthesized fatty acid
may be transferred 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 in-
sulin 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.

ACKNOWLEDGMENTS

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

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