Increased 120 kDa Protein in Liver Cytosol of Genetically Obese Zucker Rats

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Summary
In this study, the differences between the pattern of stained proteins from genetically obese Zucker rats and those from lean Zucker rats were analyzed using SDS-polyacrylamide gel electrophoresis. The level of the 120 kDa protein in the liver cytosol fraction of the obese rats was several times higher than that found in the lean rats. This protein was not present in the mitochondrial fractions of either strain. The level of the 120 kDa protein was decreased drastically during a 72-h fast in the obese Zucker rats. An increase in the level of this protein was induced in the lean Zucker rats through a 48-h fast followed by refeeding with a high-carbohydrate diet. However, when the lean Zucker rats were refed with a high-fat diet following the fast, no significant change was observed in the level of the 120 kDa protein. These observations strongly suggest that the elevated levels of the 120 kDa protein seen in the liver cytosol fraction of obese Zucker rats may be responsible for the observed increases in lipogenesis and pathogenesis of obesity in these rats.

Key Words
obese, Zucker rat, lipid synthesis, lipogenesis, glucose-6-phosphate dehydrogenase, triglyceride

Zucker fatty rats are a strain of genetically obese rats (1, 2) whose obese gene, fa, is transmitted as a Mendelian recessive trait. Zucker rats homozygous for the fa gene exhibit great obesity and share many of the traits associated with obesity in human. Several biochemical and hormonal abnormalities have been reported in this strain (3, 4), but there is still no consensus regarding the precise defect responsible for these abnormalities.

Liver and adipose tissue play a crucial role in lipogenesis and lipid deposition in obese Zucker rats. It has been suggested that lipogenic enzyme activity increases in the liver and adipose tissue (5, 6) with the emergence of hyperinsulinemia, and that lipolytic activity, such as that associated with lipoprotein lipase increases during the first days of birth (7, 8). However, there are no reports that describe the specific regulatory mechanisms of these lipolytic enzymes.

To detect the primary product of the fa gene in the liver of obese Zucker rats,
we examined the effects of fasting and diet changes on proteins of the mitochondria and cytosol using silver staining and SDS-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Animals. Experiment I: Female, genetically obese (fa/fa) and lean (Fa/?) Zucker rats (Charles River Laboratories Inc., U.S.A.) were fed standard laboratory chow ad libitum (Oriental Yeast Co., Tokyo) for 11 weeks after birth. The obese rats and lean rats reached weights of 400–450 and 200–250 g, respectively.

Experiment II: Female obese Zucker rats weighing 300–350 g were deprived of food for 72 h. Female obese Zucker rats were used as controls and were fed standard laboratory chow ad libitum.

Experiment III: Heterozygous male and female Zucker rats (Takeda Pharmaceutical Co., Osaka, Japan) were mated and the inbred male lean offspring (Fa/?)) were fed standard laboratory chow until they reached a weight of 360–420 g. The rats were then deprived of food for 48 h, followed by an ad libitum, 24-h feeding of either a high-carbohydrate (HCHO) diet or a high-fat (HFAT) diet. Table 1 shows the composition of these diets.

The animals used in each experiment were matched according to age. They were kept at 22–24°C in 12-h cycles of light and darkness, and were sacrificed by exsanguination from the abdominal aorta following anesthetization with diethyl ether.

Preparation of the mitochondria and cytosol. Liver mitochondria and cytosol were fractionated according to the method of Schneider and Hogeboom (9). Briefly, the livers were homogenized in a Teflon homogenizer with nine volumes of a 0.25 M sucrose, 5 mM Tris-HCl buffer (pH 7.4), and 0.1 mM EDTA. The homogenate was centrifuged at 700×g for 20 min and the resulting supernatant was then centrifuged at 7,000×g for 20 min. The precipitated fraction was washed with the same buffer as was used in homogenization, and was recovered as the mitochondrial fraction. The supernatant was further centrifuged at 105,000×g for 60 min and the resulting supernatant was recovered as the cytosol fraction.

Table 1. Composition of diets.

| Composition                  | High-carbohydrate diet (%) | High-fat diet (%) |
|------------------------------|----------------------------|------------------|
| Vitamin-free casein¹         | 20                         | 20               |
| Sucrose                      | 10                         | 10               |
| Corn starch                  | 61                         | 35               |
| Soy bean oil                 | 4                          | 30               |
| Mineral mixture²             | 4                          | 4                |
| Vitamin mixture²             | 1                          | 1                |

¹ Casein was supplemented with 0.3% DL-methionine. ² Mineral and vitamin mixtures were according to Harper's composition (23). Choline chloride was added 0.2 ml/100 g of food.

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**SDS-polyacrylamide gel electrophoresis (SDS-PAGE).** SDS-PAGE, using 6% acrylamide or 18% acrylamide, was performed according to the Laemmli method (10). Samples were dissolved in a 10% glycerol, 2.3% SDS, 62 mM Tris-HCl (pH 6.8) solution containing 5% mercaptoethanol. After electrophoresis, the gels were fixed and the bands were visualized using a silver-staining kit (11,12) (Wako Pure Chemicals, Osaka).

**Other procedures.** The activity of glucose-6-phosphate dehydrogenase (G6PDH) was determined according to the method of Kornberg and Horecker (13). The enzyme activity was expressed as an absorbance increment at 340 nm/min/mg of protein basis. Protein concentration was determined according to the method of Lowry et al. (14). Serum triglyceride concentration was determined using an enzyme assay kit (15) (Wako Pure Chemicals Co.).

**RESULTS**

The number and size of the proteins in the mitochondrial and cytosol fractions from the liver of lean and obese Zucker rats were estimated using SDS-PAGE (Experiment 1, Fig. 1). Using 18% acrylamide in SDS-PAGE, no conspicuous differences were found in the bands of low molecular weight between the lean and obese Zucker rats (data not shown). However, using 6% acrylamide in SDS-PAGE, the cytosol fraction showed two bands that were more pronounced in obese Zucker rats, corresponding to molecular weights of 120 kDa and >250 kDa. Additionally, a band at 150 kDa was more pronounced in lean rats than in obese rats.

![Fig. 1. Comparison of the results from the SDS-polyacrylamide gel electrophoresis of the mitochondrial and cytosol fractions from the livers of lean and obese Zucker rats. All rats were fed standard chow ad libitum for 11 weeks. 1 and 2, mitochondrial fractions (20 µg protein); 3-6, cytosol fractions (8 µg protein); 1, 3, and 5, lean Zucker rats; 2, 4, and 6, obese Zucker rats. Long arrows indicate standard proteins: myosin (200 kDa) and E. Coli β-galactosidase (116 kDa). Short arrows indicate the 120 kDa protein.

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The density of the cytosol solution was adjusted to 1.21 g/ml with KBr and centrifuged at 105,000 \( \times g \) for 48 h. Then the floated, lipoprotein-like particles were analyzed using a 6% gel in SDS-PAGE. The molecular weight of the major silver-stained bands of these particles in the cytosol of obese and lean Zucker rats corresponded to 150 kDa and > 250 kDa (data not shown).

Next, the effect of starvation on the appearance of the 120 kDa band in the cytosol of obese Zucker rats was investigated (Experiment 2). Other investigators have reported that when food was withheld from obese Zucker rats for 72 h, the activity of G6PDH in the liver cytosol decreased to 48% of that in rats fed a commercial chow (6). We found that under fasting conditions, the 120 kDa band drastically decreased as compared with that in well-fed obese Zucker rats (Fig. 2). The amount of the 120 kDa protein in fasting obese Zucker rats was nearly equal to that in lean Zucker rats fed ad libitum.

Next, lean Zucker rats were fed either a high-carbohydrate (HCHO) diet or a high-fat (HFAT) diet following a 48-h fast (Table 2 and Fig. 3, Experiment 3), and the associated changes in 120 kDa levels were noted. The rats refed with the HFAT diet had the same levels of G6PDH activity and the 120 kDa protein in their liver cytosol as did the rats fed with standard chow. However, the amount of the 150 kDa protein, which is most likely the lipoprotein-like particle, increased drastically following refeeding with an HFAT diet compared with refeeding with an HCHO diet.

The refeeding with an HCHO diet increased the intensity of the 120 kDa and > 250 kDa bands several fold over that of the rats refed with an HFAT diet or those fed with standard laboratory chow. Additionally, the refeeding with an HCHO diet increased both the activity of G6PDH in the liver cytosol and the levels of other proteins.
Table 2. Glucose-6-phosphate dehydrogenase (G6PDH) activity in liver cytosol and serum triglyceride concentration of lean Zucker rats refed a high-carbohydrate diet (HCHO) and a high-fat diet (HFAT).

| Group                          | Feeding | Fast-refeeding |
|--------------------------------|---------|---------------|
|                                | Control | HCHO          |
| Liver cytosol G6PDH (units/mL) | 0.14±0.01 | 0.22±0.01<sup>1</sup> | 0.12±0.01 |
| Serum triglycerides (mg/dl)    | 58±9    | 143±17<sup>1</sup>  | 70±20    |

The enzyme activity is expressed as an absorbance increment at 340 nm/min/mg of protein basis. Values are M±SD from triplicate analyses. <sup>1</sup>Significantly different from the means of the control group: p < 0.05. Control, rats fed a standard laboratory chow ad libitum; HCHO or HFAT, rats refed an HCHO or an HFAT diet after a 48-h fast.

DISCUSSION

When used in combination with 6% acrylamide in SDS-PAGE, silver staining enabled us to detect a 120 kDa protein which was not visible with the less protein-sensitive Coomassie Brilliant Blue R staining (<sup>11,12</sup>). Although no quantitative analysis was performed, a several-fold increase in the level of the 120 kDa protein was detected in the liver cytosol fraction of obese Zucker rats compared with lean Zucker rats.

Our results corresponded to the previous reports that the de novo synthesis of
fatty acid is increased by an HCHO diet as compared with an HFAT diet (6). When refed an HCHO diet, the lean Zucker rats also showed a two-fold increase in the activity of G6PDH in the liver and elevated levels of serum triglyceride. There was also a drastic increase in the amount of the 120kDa protein, and >250kDa of lipoprotein-like particles in the liver following the HCHO diet, and no such increase was seen with the HFAT diet. Similar increases were observed in Wistar strain rats undergoing the same fasting-refeeding procedure (data not shown). These results suggest that the increased levels of the 120kDa protein accompanied by increased lipid synthesis may play an important role in the regulation of lipid metabolism in the liver.

It is unknown whether the 120kDa protein has been previously identified. Its molecular weight does not match any reported enzyme of the lipogenic pathway, such as the malic enzyme (16), fatty acid synthetase (17), hepatic lipase (18), lipoprotein lipase (19), acetyl-CoA carboxylase (20), or G6PDH (21), and no sequence data is yet available for this protein. It has recently been suggested (22) that the specific repression of two peptides (23 and 24kDa) in adipose tissue, concomitant with the emergence of increased fat storage capacity in 7-day-old, pre-obese rats, might play a crucial role in the evolution of this genetic disorder. However, the repression of these two peptides in adipose tissue was transient, and the 120kDa protein in the liver cytosol of obese Zucker rats described here was chronically increased in all ages: 9, 10, 12, 22, 23, 40, 60, 66, and 100 weeks (data not shown).

If the 120kDa protein is derived from the fa gene, or from some other gene linked to the fa gene, some differences might be expected in the concentration of the 120kDa protein between the two different genotypes of lean Zucker rats (Fa/Fa and Fa/fa). However, we detected no significant differences between these genotypes using silver staining and SDS-PAGE analysis (data not shown).

Future investigation will focus on the separation, purification, and quantitative analysis by immunoassay of this 120kDa protein.

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