Regulation by dietary choline of hepatic fatty acid synthetase in the rat

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Abstract  Fatty acid synthetase activity was measured in the high-speed supernatant fraction of liver homogenates from rats fed a semisynthetic diet low in lipotropic factors. If choline was omitted from the diet, a significant increase of fatty acid synthetase activity was observed after two feedings of the deficient diet. Compared with controls, the increase of fatty acid synthetase activity was of a magnitude that could account for the amount of triglyceride accumulating in the hepatic floating lipid fraction. Gas-liquid chromatographic analysis of the floating lipid triglycerides showed an increased content of palmitic acid due to choline deficiency; this increase could be predicted from the increased fatty acid synthetase activity and its known characteristic yield of palmitic acid.

Supplementary key words  liver · floating lipid triglycerides · palmitic acid

It has been shown (1, 2) that 4'-phosphopantetheine is a prosthetic group in the multienzyme complex of fatty acid synthetase from the livers of both pigeon and rat. The remarkably high proportion of fatty acid synthetase protein (1.4% of the total liver protein [3]) could thus explain the failure of choline deficiency to cause fatty livers in rats that were at the same time deprived of dietary pantothenic acid (4). The possibility arises that fatty acid synthetase is rate limiting not only in hepatic lipogenesis but also in the increased lipogenesis caused by choline deficiency. Furthermore, an involvement of dietary choline is anticipated in the regulation of fatty acid synthetase activity. We have, therefore, investigated the effects of choline deficiency on hepatic fatty acid synthetase in the rat. Evidence has been obtained that fatty acid synthetase activity is increased in choline deficiency.

METHODS AND MATERIALS

Animals
Male albino rats from Canadian Breeding Laboratories, St. Constant, P. Q., weighing 100 ± 10 g were housed in individual cages with raised bottom screens. Commercial chow (Maple Leaf Mills Ltd., Toronto) and water were offered ad lib.

Diets and dietary treatment
After the rats had reached a body weight of 140 ± 10 g on the commercial diet, feeding was continued with a semisynthetic diet, low in lipotropic factors, consisting of 60% sucrose, 20% protein (6% casein, 7% soya protein, and 7% protein as alcohol-extracted peanut meal), 9% Primex, 1% corn oil, and further vitamins and minerals as described in an earlier publication (5). In the diet used for the choline-supplemented control rats, choline chloride was added (0.4% of the diet), and the sucrose content was reduced correspondingly. The animals were fed at 4 p.m.; the food trays were removed at 9 a.m. on the following day. Food consumption was calculated as the amount of food offered less the food spilled and left in the food trays. The data of both food consumption and increase in body weight served as reliable guides in judging the health of the rats. These data are not reported since they were identical with earlier published data from experiments in which the same type of rats and diets were used (6). The rats were fed the semisynthetic diet supplemented with choline for 8 days, and then the same diet without choline for 1 or more days. In the experiments without adjustment prior to feeding the choline-deficient diet, the control rats continued to receive the choline-supplemented diet. Since it was desirable to complete the enzyme tests on the same day, the animals were killed in groups of four (deficient), and four choline-supplemented rats from the same batch of animals were killed 2 days later. By subjecting twice the number of rats in each group to the dietary treatment, it was possible to use rats whose food intake and body weight were comparable for both control and deficient groups.

Hepatic enzyme extracts and floating lipid fraction
At 9 a.m. the rats were anesthetized with ether and were bled from the abdominal aorta. The livers were excised, blotted, and weighed. The chilled livers were ho-
TABLE 1. Effects of choline deficiency on the activity and yield of hepatic fatty acid synthetase in the rat

<table>
<thead>
<tr>
<th>Control</th>
<th>Days Fed Choline-deficient Diet</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Specific activity of enzyme (nmoles NADPH oxidized min⁻¹ mg protein⁻¹)</td>
<td>17.9 ± 1.1</td>
</tr>
<tr>
<td>Enzyme yield⁶</td>
<td>12.4 ± 0.8</td>
</tr>
<tr>
<td>Floating lipid triglyceride (mg/liver of a 100-g rat)</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>Significance of difference vs. control</td>
<td>e: P &lt; 0.2</td>
</tr>
<tr>
<td>Specific activity of enzyme</td>
<td>f: P &lt; 0.2</td>
</tr>
<tr>
<td>Enzyme yield</td>
<td>h: P &lt; 0.005</td>
</tr>
</tbody>
</table>

After an adjustment period of 8 days on the semisynthetic diet with choline (control), groups of eight rats were fed the same diet without choline for different periods of time as shown in the table. All values are means ± SEM.

* 25°C, pH 7.0.

The enzyme yield represents enzyme units X 10⁻³ in the liver of a rat of 100 g body wt, i.e., cytosol protein/liver X specific activity X 10⁻³.

TABLE 1. Effects of choline deficiency on the activity and yield of hepatic fatty acid synthetase in the rat

The effects of early choline deficiency on the activity and yield of fatty acid synthetase in the liver of the rat are shown in Table 1.

Gas-liquid chromatography

The fatty acid distribution in the floating lipid triglycerides was obtained by gas-liquid chromatographic separation of the methyl esters on a column containing 10% ethylene glycol succinate polyester in an F & M model 402 gas chromatograph equipped with an on-column injection system and a hydrogen flame ionization detector. The analyses were performed isothermally at a column temperature of 180°C and a carrier gas flow rate of 100 ml/min. The percentage composition of the esters and peak ratios were calculated from the weights of the paper carefully cut out to approximate the peak areas. Quantitative results with National Heart Institute fatty acid standards D, E, and F agreed with the stated composition with an error of less than 2% for the major components. The triglyceride composition was determined with approximately 1% solutions of the lipids in chloroform using the conditions described by Kukis and Breckenridge (10). The instrument was a Beckman GC-4 gas chromatograph equipped with a modified on-column injection heater.

Fatty acid synthetase assay

The clear supernatant solution obtained from the liver homogenate, after centrifugation and removal of the floating lipid, served as a source of the enzyme. For quantitative determination we measured the rate of oxidation of NADPH in the presence of malonyl CoA at 25°C in a system described by Bressler and Wakil (11), with the additional precautions recommended by Hsu, Wasson, and Porter (3) concerning the dilution of the enzyme solutions. The protein in the original supernate was determined spectrophotometrically (12); in this case the supernate was diluted 40- to 50-fold with distilled water. The results are reported in enzyme units, i.e., nmoles of NADPH oxidized min⁻¹ at 25°C. Dithiothreitol, NADPH, CoASH, and acetyl CoA were obtained commercially from Boehringer (Mannheim, Germany), P-L Biochemicals (Milwaukee, Wis.), and Sigma Chemical Co. (St. Louis, Mo.). Malonyl CoA was prepared from CoASH and excess S-malonyl-N-octanoyl-2-mercaptoethanolamine by transesterification. The S-malonyl derivative was synthesized from monomarylchloride and N-octanoyl-2-mercaptoethanolamine as described by Eggerer and Lynen (13).

RESULTS

The effects of early choline deficiency on the activity and yield of fatty acid synthetase in the liver of the rat are shown in Table 1.

In livers of rats fed the choline-deficient diet for 2 days,
a significant increase in specific activity was observed. The enzyme yield, i.e., the amount of enzyme expressed in units, in the whole liver likewise showed a significant increase. These results confirmed observations made in preliminary experiments using identical dietary conditions and feeding the test animals a deficient diet for 2 days. In these preliminary experiments, the rate of oxidation of NADPH was measured at 30-sec intervals in place of using a recording spectrophotometer. No further increase in either measurement was observed in animals fed the choline-deficient diet for 4 days. Since it was intended to correlate changes of enzyme activity with those of triglycerides in the liver of the same rat, data on the changes of floating lipid triglycerides were again included in the present investigation, although the effects of choline deficiency on this hepatic lipid fraction have already been documented (6).

In a second series of experiments, the adjustment of the rats to the choline-supplemented semisynthetic diet was omitted. As shown in Table 2, the response of the rats to this dietary treatment differed in several respects from that shown in Table 1, where the rats were adjusted to the choline-supplemented diet before changing to the choline-deficient diet. After two feedings of the semisynthetic diet, the control levels of both fatty acid synthetase activity and floating lipid triglycerides were higher as were the enzyme activities and floating lipid levels of the choline-deficient group. It is of interest to note the considerable difference between the activities of fatty acid synthetase from rats fed the chow diet and those fed the complete semisynthetic diet. The relative C16 content of the floating lipid triglycerides obtained by gas-liquid chromatography increased with increasing amounts of floating lipid and enzyme activity.

### Dietary adjustment

In Table 1, the specific activities of the enzyme and the amount of hepatic floating fat resulting from feeding rats a choline-deficient diet from 1 to 4 days (after a standard adjustment period on the choline-supplemented semisynthetic diet) were compared with these parameters in a control group of rats that were fed the same semisynthetic diet supplemented with choline for 8 days. It was assumed that the base line values shown by the "adjusted" control group did not change after feeding the choline-supplemented diet for longer periods. In view of the considerable effects on hepatic fatty acid synthetase and floating fat when the diet was changed from a commercial formulation to the choline-supplemented semisynthetic diet (Table 2), it was deemed desirable to verify the validity of the base line value used in Table 1. A systematic study was therefore carried out to show the effects of dietary adjustment on hepatic fatty acid synthetase, floating fat, plasma triglyceride, and plasma phospholipid of the same rats. The results are shown in Table 3.

The data indicate that our adjustment of 8 days on the choline-supplemented semisynthetic diet resulted in a base line value which was virtually identical with that shown by the control in Table 1. No change of the base line value was found after 10 days' adjustment. As judged
from the values for "triglyceride" and phospholipid in plasma, adjustment is not complete after 6 feedings because a significant increase of plasma triglyceride and phospholipid occurred after 8 feedings.

**DISCUSSION**

In choline deficiency, the accumulation of triglycerides in the liver of rats is limited to the floating lipid fraction (6). If this accumulation were brought about by an increase of fatty acid synthetase activity as demonstrated in Tables 1 and 2, a quantitative relationship would be anticipated between the increased capacity of the enzyme to generate palmitic acid and the increase of triglyceride that occurs in the hepatic floating lipid. In Table 2, it is shown that the liver of a 100-g rat fed a choline-deficient diet for 2 days accumulated 244 mg more triglyceride than the liver of the choline-supplemented control, i.e., 301 μmoles, assuming a molecular weight of 807 (tripalmitin). In the same group of rats, the hepatic fatty acid synthetase increased by 8000 units above the control value, i.e., 18.3 μmoles/hr at 37°C, using the relationship 14,000 units = 14 μmole of triglyceride. A factor of 1.6 has been applied to convert enzyme activity at 25°C to that at 37°C. The increased enzyme activity would, therefore, be capable of generating the fatty acid in the above 301 μmoles by exercising its activity for 8.2 hr/day. This is in agreement with our observation that triglyceride accumulation in the liver of rats occurs within a period of 10 hr/day (6). The time required to synthesize the triglycerides after 2 and 4 days of feeding the choline-deficient diet (Table 1) by the increased activity of the fatty acid synthetase would be 4.3 and 8.5 hr/day, respectively. When rats were given a single feeding of the choline-supplemented semisynthetic diet (Table 2), there was more triglyceride in the floating fat, and the increased fatty acid synthetase activity could form this increased triglyceride in 1.4 hr. It is recognized that data obtained in vitro were applied to a situation in vivo, and, therefore, favorable as the evaluation appears to be, reservations as to the activity of the enzyme in vivo have to be kept in mind.

It has been demonstrated recently (14) that considerable amounts of fatty acid synthetase are associated with the microsomal membranes. The assessment of total hepatic fatty acid synthetase activity would therefore require the additional determination of the activity of the same enzyme in the microsomal fraction. Possible interferences by dietary or environmental factors with the unknown process of releasing the lipogenic enzymes into the cytosol could thus be obviated. In the rat, triglyceride synthesis from fatty acid is not rate limiting (15), and it has been shown by Chang et al. (16) and confirmed by others (17) that liver and other tissues of the same animal have comparable activities of both acetyl CoA carboxylase and fatty acid synthetase. Thus, it would be expected that in choline

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**TABLE 3. Effects of dietary adjustment on hepatic fatty acid synthetase, enzyme yield, hepatic floating lipid triglycerides, plasma triglyceride, and phospholipid**

<table>
<thead>
<tr>
<th>Commercial Chow</th>
<th>Days Fed Semisynthetic, Choline-supplemented Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity of enzyme</td>
<td>1</td>
</tr>
<tr>
<td>6.1 ± 0.8</td>
<td>(8)</td>
</tr>
<tr>
<td>Enzyme yield</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Floating lipid triglyceride</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>215 ± 14</td>
</tr>
<tr>
<td>Plasma phospholipid</td>
<td>98.8 ± 1.3</td>
</tr>
</tbody>
</table>

Groups of rats were fed a commercial chow diet until they weighed 140 ± 10 g in the groups that were allowed to adjust for 8-10 days and 210 ± 10 g in the groups that were given one and two feedings of the semisynthetic diet. The intermediary group, i.e., 4-6 days of dietary adjustment, was allowed to reach 175 ± 10 g on the chow diet in order to obtain animals of comparable body weight at the time of killing. The results in the table are means ± SEM. The figures in parentheses indicate the number of rats in each group.

*The temperature coefficient of the fatty acid synthetase has been obtained through the kind cooperation of Prof. F. Lynen, Munich, Germany, and is based on measurements with purified fatty acid synthetase from yeast.
deficiency acetyl CoA carboxylase would change in the same direction as fatty acid synthetase. This is compatible with the observation of Chalvardjian (18), who reported increased acetyl CoA carboxylase activity in the liver of rats fed a choline-deficient diet for 3 days. The acetyl CoA carboxylase activity increased significantly from 23 to 36 nmoles of acetyl CoA incorporated. This is a 1.6-fold increase, which is comparable to the increase of fatty acid synthetase activity shown in Table 1. When expressed in the same way, i.e., nmoles acetyl CoA carboxylated/mg protein/hr at 37°C, the enzyme activities observed by Chalvardjian (18) are low compared with those published by Chakrabarty and Levelle (17), i.e., 284 nmoles of acetyl CoA carboxylated. Suboptimal assay conditions, e.g., absence of Sephadex 25 treatment, as pointed out by Chang et al. (16) may be offered as an explanation for Chalvardjian’s (18) low acetyl CoA carboxylase values.

The possibility remains that acetyl CoA carboxylase is not rate limiting in choline-deficient animals and fatty acid synthetase could assume the role of a regulatory enzyme (19). Hence, changes in fatty acid synthetase activity could influence accumulation of hepatic triglycerides.

Whenever the activity of the fatty acid synthetase was found to increase, it was observed that the content of palmitic acid of the triglycerides in the floating lipid had also increased. A relative increase of palmitic acid in the hepatic lipids of choline-deficient rats has been reported by Chalvardjian (20), who interpreted this observation as an impairment of the enzyme systems involved in desaturation. Because of the increase of fatty acid synthetase with the increase of palmitic acid, and because fatty acid synthetase is known to produce in vitro 74–80% palmitic acid in relation to total fatty acids (11, 21), it is conceivable that the increased palmitic acid in floating lipid triglyceride may have originated from the increased activity of the fatty acid synthetase. The effects of dietary factors on the activities of the enzyme systems involved in chain elongation and desaturation in vivo are consistent with the values we have obtained for the palmitic acid content (e.g., 57%) for the newly formed triglycerides after 2 days of choline deficiency. In the absence of any effect of the desaturase, this value would have been anticipated to approach 74%, the value characteristic for palmitic acid formation in vitro by the fatty acid synthetase.

Impairment of lipoprotein synthesis and secretion in choline deficiency has been suggested as a mechanism for the accumulation of triglycerides in the liver of the rat (22, 23). Secretion of very low density lipoproteins decreased to 54% of the control in choline deficiency. This is small compared with the decrease in rats fed orotic acid (24), where secretion of lipoproteins was 10% of the control. Since accumulation of hepatic triglycerides in choline deficiency is not inferior to that shown by livers of rats fed orotic acid, other factors causing fatty infiltration of the liver must be implicated. Increased fatty acid synthetase activity as shown in the present work could conceivably play such a role.

Many investigators (22, 25, 26) have observed that lesser amounts of injected labeled fatty acids appear in the plasma triglycerides of choline-deficient rats. This observation has been interpreted as evidence in favor of impaired hepatic lipoprotein synthesis and secretion. In comparison with the decrease of very low density lipoproteins to 54% of the control (Ref. 22, Table 2), the specific activity decreased to 27% of the control, and in the case of the low density fraction of lipoproteins, which remained unchanged in choline deficiency, the specific activity decreased to 38%. It is possible that, in addition to impaired lipoprotein synthesis and secretion in the liver, the radioactive precursor triglyceride in the microsomal membrane might have been diluted; however, this has not been measured. Increased fatty acid synthetase activity would result in an increased de novo synthesis of fatty acid, which could compete with labeled fatty acid in the active pool of the membrane. Such an additional mechanism could explain the formation of triglyceride and lipoprotein of a specific activity lower than anticipated from impaired hepatic synthesis and secretion alone.

The author is indebted to Dr. A. Kukis for the gas-liquid chromatographic analysis of floating lipid triglycerides and to Dr. F. Lynen, Munich, for a gift of S-malonyl-N-octanoyl-2-mercaptoethanolamine. Thanks are due to Mr. Brian Loptson for his valuable technical assistance.

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REFERENCES