EFFECTS OF DIETARY FATS ON THE ACTIVITY OF 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE AND STEROL SYNTHESIS IN THE LIVER OF FASTED-REFED RATS

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Summary The time course of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity and lipogenesis and cholesterogenesis from 1-14C-acetate and 2-14C-mevalonate was examined in the liver of rats refed diets containing different fats at the 10% level after 48 hr fasting. Fasting caused a profound depression of the reductase activity and sterol and fatty acid synthesis. In rats refed for 30 hr, the activity of HMG-CoA reductase was restored to about one-half of the level observed in pre-fasting rats, irrespective of the type of dietary fats. When safflower oil and trilaurin were dietary fats, the activity remained this level until 78 hr, then declined, whereas with tristearin, activity progressively increased until 78 hr. On refeeding for 174 to 222 hr, the reductase activity was significantly higher in the tristearin than in the trilaurin group. Similar patterns were demonstrated in cholesterogenesis either from acetate or mevalonate, though extents of activation after refeeding were markedly different in these precursors. Dietary fat dependent changes in the content of hepatic cholesterol and in the concentration of plasma cholesterol were also observed.

It has been indicated that dietary fat plays an important role in the regulation of the hepatic cholesterogenesis and the activity of HMG-CoA reductase [EC 1.1.1.34] (1–5). Addition of corn oil to the fat-free purified diet stimulates the liver reductase of the rat in proportion to the amount added (2). Also, responses of the enzyme to dietary fats vary according to differences in the type of fats (4, 5). We have demonstrated that the lower the degree of unsaturation and the longer the chain length in the acyl molecules, the higher the enzyme activity the fat produces in rat liver (5).

Refeeding after fasting causes a characteristic response to hepatic cholesteroge-
When a fat-free diet was given to the rat that had been fasted for 2 days, hepatic cholesterogenesis rapidly increased, reached its peak 3 days after and then declined to the fasting level after 5 to 6 days (1, 6). A similar pattern was observed in mice refed a low fat diet (7). Rats refed a fat-containing, commercial non-purified diet did not show such responses, but the activity of HMG-CoA reductase continued to increase and was restored to the level of non-starved rats after 5 to 6 days (1). In mice refed a high corn oil diet for three days, the activation of hepatic sterol synthesis from orally administered U-14C-glucose that was observed on refeeding a low fat diet was largely suppressed (7). Further information as to the effect of dietary fat on hepatic sterol synthesis in fasted-refed animals is not available. In the present study, we examined the effect of different fats on the time course of changes in the activity of HMG-CoA reductase and sterol synthesis in the liver of fasted-refed rats.

MATERIALS AND METHODS

**Materials.** DL-3-14C-HMG (specific activity 22.8 mCi/mM) and sodium 1-14C-acetate (specific activity 2.5 mCi/mM) were obtained from New England Nuclear Corp., Boston. DL-2-14C-Mevalonic acid dibenzylethylene diamine salt (specific activity 36 mCi/mM) was purchased from the Radiochemical Centre, Amersham. DL-3-14C-HMG-CoA (5,000 dpm/nmole) was prepared as described elsewhere (5, 8).

**Animals and diets.** Male Wistar rats obtained from Kyudo Co., Kumamoto, were housed individually in an air conditioned room (22-25°C), and the light was artificially controlled (6 a.m. to 6 p.m.). The rats were maintained on a commercial non-purified diet (Type NMF, Oriental Yeast Co., Ltd., Tokyo) at least for a week before initiation of experiments. Rats were fasted for 48 hr then refed purified diets containing 10% fats ad libitum until sacrifice by decapitation. Fasting and refeeding were started at 6 p.m. and rats were killed at midnight (11:30 p.m. to 0:30 a.m.). Control animals (not fasted or refed) were also killed in each experiment. The composition of diets for refed (in %) was vitamin-free casein, 20; mineral mixture, 4; vitamin mixture, 1; choline chloride, 0.15; cellulose powder, 4; fat (safflower oil, trilaurin or tristearin), 10 and sucrose to 100. Vitamin and mineral mixtures were according to Harper (9). Body and liver weights of rats at time of sacrifice are summarized in Table 1.

**Assay for microsomal HMG-CoA reductase.** The preparation of hepatic microsomes and assay for its enzyme activity were carried out as described previously (5, 8) except 10 mM dithiothreitol was used in place of 20 mM 2-mercaptoethanol in experiment 2.

**Assay for the conversion of 1-14C-acetate and 2-14C-mevalonate to lipid components.** The method of Slakey et al. (6) was slightly modified. The liver was homogenized in 5 volumes of 250 mM sucrose containing 100 mM potassium phosphate buffer (pH 7.4), 30 mM nicotinamide, 4 mM MgCl₂ and 0.12 mM EDTA. The homogenate was centrifuged at 2,500 x g for 30 min and the supernatant was
Table 1. Body and liver weights of rats at time of sacrifice.

<table>
<thead>
<tr>
<th>Groups (No. of rats)</th>
<th>Body weight (g)</th>
<th>Liver weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
</tr>
<tr>
<td>Control (6)</td>
<td>Safflower oil</td>
<td>Trilaurin</td>
</tr>
<tr>
<td>0(3)</td>
<td>255 ± 5</td>
<td>231 ± 5</td>
</tr>
<tr>
<td>6(3)</td>
<td>194 ± 7</td>
<td>164 ± 1</td>
</tr>
<tr>
<td>30(3)</td>
<td>212 ± 5</td>
<td>189 ± 2</td>
</tr>
<tr>
<td>78(3)</td>
<td>223 ± 4</td>
<td>185 ± 1</td>
</tr>
<tr>
<td>126(3)</td>
<td>222 ± 7</td>
<td>210 ± 7</td>
</tr>
<tr>
<td>174(3)</td>
<td>—</td>
<td>206 ± 3</td>
</tr>
<tr>
<td>222(3)</td>
<td>—</td>
<td>215 ± 5</td>
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* Values represent the mean ± SEM.
used as the enzyme source. The incubation system contained in 1.2 ml 2 μmoles NAD, 2 μmoles NADP, 20 μmoles glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase, 3 μmoles ATP, 0.1 μmole CoA and 1 ml of the enzyme solution. The mixture was preincubated at 37°C for 10 min. The reaction was started on addition of the substrates (1.5 μmoles 1-14C-acetate, 1 μCi or 1 μmole 2-14C-mevalonate, 0.05 μCi) and continued at 37°C for 120 min. The mixture was saponified and nonsaponifiable lipids and fatty acids were extracted as described elsewhere (5, 10). When the acetate was the substrate, sterols were isolated from nonsaponifiable lipids as digitonide (5), and when the mevalonate was the substrate the separation of sterols was carried out by thin-layer chromatography using silica gel G (11). The radioactivities were counted in a toluene scintillation fluid (2 g of 2,5-diphenyloxazole and 50 mg of 1,4-di-2-(5-phenyloxazolyl)-benzene per liter of toluene) using an Intertechnique model SL-32 scintillation spectrometer. The radioactivity was corrected for quenching by an external standard.

**Lipid analysis.** Plasma and liver lipids were analyzed as described previously (5, 12).

**RESULTS**

**Effects of refeeding safflower oil (experiment 1)**

Figure 1 shows the time course of changes in hepatic sterol and fatty acid synthesis in rats refed a diet containing 10% safflower oil. The animals were fasted for 48 hr, then refed the diet ad libitum for 6, 30, 78 or 126 hr. As the diet was withdrawn at 6 p.m. and rats were killed at 0 a.m., the values at 0 hr (initiation of refeeding) in each figure and table correspond to those at 54 hr-fasting. Since fasting rats for 24 hr results in profound decreases in hepatic reductase activity and sterol synthesis and no additional depression is observed after 48 hr (6), the steady state is maintained in respect to sterol synthesis during the course of 24 to 48 hr fasting. Thus, it is most likely that additional fasting for 6 hr (totally for 54 hr) exerts no demonstrable effect on sterol synthesis, in comparison with the case of fasting for 48 hr. From these consideration and mainly to avert confusion, the fasting period was tentatively represented as 48 hr, since refed rats were all fasted for 48 hr. Experiments were done in two separate days, and in each time three rats were killed as the control (non-fasted). Because of the considerable enlargement of the liver after refeeding (Table 1), activities were calculated in terms of total activity (nmoles mevalonate formed or substrate incorporated per hr per liver per 100 g body weight) and expressed as per cent of the corresponding control animals.

The reductase activity and the incorporation of labels into lipid components were all markedly depressed after fasting. Until 30 hr of refeeding, the activity of HMG-CoA reductase increased to about one-half of the level observed in the control rats, and remained at this level until refeeding for 78 hr. After refeeding for 126 hr, the activity tended to decline slightly (Fig. 1A).
Fig. 1. Effect of fasting and refeeding safflower oil on 3-hydroxy-3-methylglutaryl-CoA reductase activity and fatty acid synthesis in rat liver. Rats were fasted for 48 hr, then refed a purified diet containing 10% of safflower oil for 6, 30, 48 and 126 hr. Activities were calculated in terms of total activities and expressed as per cent of the control animals (not fasted, corresponding to the value at -48 hr). Each point represents the mean ± SEM of 3 rats except for 6 rats in the controls. HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA, NSL: nonsaponifiable lipids and FA: fatty acids.

The time course of the rate of acetate incorporation into nonsaponifiable lipids and sterols showed the similar pattern until 78 hr, though the extent of activation in the latter appeared at any time lower compared with that in the former. At 126 hr, the decline in the incorporation of acetate into nonsaponifiable lipids and sterols was more profound than that in the reductase activity (Fig. 1B).

Changes in sterol synthesis from mevalonate also resembled those observed with acetate. Again the extent of activation was at any time higher in nonsaponifiable lipids than in sterols. The magnitude of activation at 30 and 78 hr was higher than that with acetate (Fig. 1C).

The rate of fatty acid synthesis from acetate depicted a symmetrical pattern with a very high level at 78 hr (Fig. 1D).

The slight increase, having a peak value at 78 hr, in the content of hepatic cholesterol was found in refed rats and it was almost entirely attributed to the rise in the esterified sterol (Fig. 2A). The time course of the change in the concentration of plasma cholesterol also showed a characteristic pattern, after refeeding the value continued to increase until 78 hr and then declined (Fig. 2B). A similar pattern was observed with the content of hepatic triglyceride, whereas there was no demonstrable change in the content of phospholipids during refeeding (Fig. 2C).
Fig. 2. Effect of fasting and refeeding safflower oil on the content of hepatic cholesterol (A), the concentration of plasma cholesterol (B) and the content of hepatic triglyceride and phospholipids (C). Each point represents the mean ± SEM of 3 rats, except for 6 rats in the controls. F Chol: free cholesterol, E Chol: esterified cholesterol, TG: triglyceride and PL: phospholipids.

Effects of refeeding trilaurin or tristearin (experiment 2)
Rats were treated similarly as in experiment 1, except refeeding was continued for 222 hr. The experiment was again done in two separate days. Among various fats tested, tristearin and trilaurin, when given to rats for 2 to 4 weeks without prior fasting, have been shown to produce the highest and the lowest HMG-CoA reductase activity, respectively (5). Figure 3 summarizes the time course of changes in enzyme activities of refeed rats.
In rats refed tristearin, the activity of HMG-CoA reductase rapidly increased and reached the peak at 78 hr. The pattern observed in rats refed trilaurin was also similar but the magnitude of the elevation was approximately one-half that observed on tristearin and consequently, the values were comparable with those observed on safflower oil. Rats refed tristearin showed at any time higher enzyme activity compared with those fed trilaurin and significant differences were found at 30, 174 and 222 hr. Differences between groups became greater with the duration of feeding periods and thus, the value with tristearin at 222 hr was 4 times higher than that with trilaurin (Fig. 3A).

Similar patterns were observed in the time course of the incorporation rates of acetate or mevalonate into nonsaponifiable lipids and sterols. In agreement with the result of experiment 1, the magnitude of activation was considerably higher with mevalonate than with acetate. Also, extents of activation were higher in nonsaponifiable lipids than in sterols. Significant differences between two groups were observed in the later phase of refeeding (Fig. 3B and 3C).
Changes in dietary fat dependent responses in incorporation of acetate into fatty acid were also observed, though the extent of the elevation due to refeeding was markedly higher than that demonstrated in sterol synthesis. The activation rate appeared considerably lower in saturated fats than in unsaturated fat. This was particularly marked with trilaurin (Fig. 3C).

As Fig. 4 illustrates, the content of hepatic free cholesterol in both groups of rats showed the same pattern, and the response resembled that observed with
safflower oil (Figs. 2A and 4A). In contrast, the profile of the content of esterified cholesterol was very characteristic. Although relatively large variations of the values did not permit significant differences, the observed pattern between two groups seemed quite different. Thus, in rats refed trilaurin the content continued to increase until 78 hr and only slightly declined thereafter. On refeeding tristearin, the peak value was observed at 126 hr and the content steeply decreased thereafter. The amounts accumulated at 78 hr were about two times as much as that on safflower oil (Figs. 2A and 4A). Thirty hours after refeeding trilaurin, the plasma cholesterol level was higher than that of the control (non-fasted) or fasted rats, and remained at this level throughout experiments. While in rats refed tristearin, the increase was first found at 78 hr (Fig. 4B). The content of hepatic triglyceride in both groups continued to increase and attained the peak at 78 hr or at 126 hr with tristearin or trilaurin, respectively, and then declined. The amount accumulated at the peak appeared approximately the same with a corresponding value for rats refed safflower oil (Figs. 2C and 4C).

**DISCUSSION**

Though there are some data involved in the effect of a fat-free diet on hepatic cholesterogenesis in fasted-refed rats (1, 6), information on the role of dietary fats in the regulation of hepatic cholesterogenesis seems scanty. CRAIG et al. (1) showed that the response in hepatic cholesterogenesis was remarkably different between rats refed a fat-free diet and a fat-containing non-purified diet. In the former, the activity of HMG-CoA reductase and sterol synthesis continued to increase until 3 days, reached to the levels comparable with that of pre-fasting and then rapidly declined to very low levels after 5 to 6 days (1, 6). Whereas in rats refed the non-purified diet, the activity continuously increased and restored to pre-fasting levels after 5 to 6 days (1). These data suggest an unique role of dietary fats in the regulation of hepatic cholesterogenesis in fasted-refed rats. However, we should take into consideration that these authors compared the difference in the effect of purified and non-purified diets. It is most likely that these comparisons do not reveal which dietary component is responsible for causing modifications, since diet compositions are completely different from each other. From the studies reported herein, the activity of reductase and sterol synthesis either from acetate or mevalonate had the peak value at 30 or 78 hr and decreased thereafter (Figs. 1 and 3). Thus, the depression of hepatic cholesterogenesis in the later phase, for example 126 hr after, may not be a phenomenon unique to refeeding a fat-free diet but that common to refeeding purified diets. REISER et al. (13) have reported that feeding a purified diet modulates the developmental pattern of the reductase as compared in rats fed a non-purified diet (13, 14). Also, rats fed purified diets have been found to have lower enzyme activity in comparison with those fed non-purified diets (15, 16). Dietary fats are believed to be a factor not responsible for causing these alterations (15).
context, the effect of dietary fats on cholesterogenesis in fasted-refed rats should be compared by using purified diets, such as those used in the present study.

Our previous study (5), in which rats were fed purified diets for 2 to 4 weeks, confirmed that the type of dietary fats as well as the fat per se play an important role in the regulation of hepatic HMG-CoA reductase. The present study also provides the additional evidence that the reductase and sterol synthesis are influenced by the type of dietary fats even in the case of fasting-refeeding. The time course of sterol synthesis in rats refed tristearin was markedly different from that in rats refed trilaurin or safflower oil (Figs. 1 and 3). Effects of dietary fats on the reductase as well as fatty acid synthesis seemed much clear in the later phase of refeeding. Previous experiments (5) have shown that feeding safflower oil for 2 to 4 weeks produced about 2 fold higher and 2 to 3 fold lower enzyme activity in comparison with feeding trilaurin and tristearin, respectively. Also, the enzyme activity was 5 fold higher in rats fed tristearin than in those fed trilaurin. In the present experiment, such marked differences were not observed among the groups at 30 hr. However, the difference observed in rats refed trilaurin or tristearin in the later phase was comparable with the result of previous study (5), indicating the response at the early stage is fat-independent. The activity of fatty acid synthetase (17) and stearoyl-CoA desaturase (18) in the early stage of refeeding has recently been found to be also fat-independent.

Under physiological conditions, HMG-CoA reductase is in general considered to be a rate-limiting step in cholesterol synthesis of tissues (14). In addition prolonged ingestion of cholesterol is reported to depress the activity of cholesterogenetic pathways after mevalonate (14). HMG-CoA reductase and HMG-CoA synthase appear to respond similarly (14, 19). In the present study, the incorporation of acetate and mevalonate into nonsaponifiable lipids or sterols paralleled each other, though extents of responses were rather different. The response patterns resembled those of the reductase activity (Figs. 1 and 3). The regulation of post mevalonate pathways in sterol synthesis by dietary fats has already been described (5). Rats refed trilaurin showed lower incorporation of mevalonate into nonsaponifiable lipids and sterols in the later phase of refeeding in comparison with the animals refed tristearin (Fig. 3C). These results clearly indicate that the reductase is not the only enzyme that is affected by dietary manipulations. Since our results only represent the enzyme activities in vitro, the situation in vivo remains to be elucidated. However, a sharp depression observed between at 78 and 126 hr in sterogenesis either from acetate or mevalonate, but not in the reductase activity, in rats refed safflower oil (Fig. 1) indicates that post mevalonate pathways may also play an important role in determining the rate of sterol synthesis under certain condition, at least in vitro.

Characteristic changes in the content of hepatic cholesterol as well as in the concentration of plasma cholesterol were observed in refed rats (Figs. 2 and 4). Hepatic cholesterol accumulation after refeeding a purified diet containing 1% corn oil has been also observed in mice (7). Inclusion of a large amount of corn oil to the
diet (20%) suppressed the incorporation of orally administered U-14C-glucose into hepatic sterols and at the same time diminished augmented hepatic cholesterol accumulation observed in mice refed a low fat diet (7). In the present study, though the pattern of the time course of sterol synthesis in rats refed safflower oil resembled that in rats refed trilaurin (Figs. 1 and 3), the amount of cholesterol accumulated in the liver was much less in the former (Figs. 2A and 4A). The pattern observed with rats fed tristearin was considerably different from that of the animals refed other fats (Figs. 2A and 4A). The type of dietary fats also modified differently the concentration of plasma cholesterol in refed rats (Figs. 2B and 4B). This observation possibly predicts that not only the sterol synthesis but also the other aspect of cholesterol metabolism are influenced differently as dietary fats differed.

Apparently, more work is needed to elucidate the role of different dietary fats in the regulation of hepatic cholesterogenesis in fasted-refed rats. Such studies are in progress in our laboratory.

REFERENCES


13) REISER, R., HENDERSON, G. R., and O'BRIEN, B. C. (1977): Persistence of dietary suppression of 3-


