Effect of Dietary Fat on Lipid Secretion and Ketone Body Production in Rat Liver

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(Received July 4, 1983)

Summary

Perfused livers from rats fed different fats were used to examine the effect of dietary fat on the rate of hepatic lipoprotein synthesis and fatty acid metabolism. Dietary fats (10% level) used were safflower oil (18:2), camellia oil (18:1) and tristearin (18:0). The results were compared with those obtained from rats fed on a low-fat diet (1% safflower oil). When no oleate substrate was infused, triglyceride secretion rates were approximately the same among the different fat groups. Infusion of the fatty acid substrate increased the secretion of triglyceride to a similar extent in 10% fat groups while the magnitude of increase was more pronounced in the low-fat group. The magnitude of increases in ketone body production due to fatty acid supply was dependent either on the amount or the type of fat; the smallest on a low-fat diet and the greatest on a camellia oil diet. Rates of secretion cholesterol and phospholipid were modified by neither the amount nor the type of fat used. It was suggested that dietary fat may alter the rate of fatty acid catabolism without modifying lipoprotein synthesis. Safflower oil distinctly modified the fatty acid profile of triglyceride secreted. Polyunsaturated fat may exert its potent hypocholesterolemic effect through the modification of the composition of lipoprotein secreted.

Key Words: perfused liver, triglyceride secretion, cholesterol secretion, phospholipid secretion, ketone body production, dietary fat

Hepatic synthesis of lipoproteins can be regulated by changes in the amount and type of dietary carbohydrate and protein consumed (1, 2). The type of dietary fat used is considered to be one of the most crucial dietary factors that affect blood lipid levels. However, only a limited amount of information as to the effect of dietary fat on the synthesis of hepatic lipoprotein is currently available. Kalopissis et al. reported that the addition of lard to the diet decreased very-low-density

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lipoprotein secretion in Triton WR 1339-treated rats (3) as well as in isolated rat hepatocytes (4) concomitant to changes in the metabolism of free fatty acid. Types of fat other than lard were not tested in their studies. Nicolosi et al. demonstrated that the degree of unsaturation of dietary fat is a factor modulating the rate of serum triglyceride accumulation after Triton WR 1339 injection in the gerbil (5) and monkey (6), possibly indicating an alteration of hepatic lipoprotein synthesis by the type of dietary fat concerned. To obtain more direct information, different types of dietary fat were tested as a possible determinant to regulate the secretion of lipid in perfused rat liver, in the present study.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (Kyudo Co., Kumamoto) weighing 170–230 g were fed on purified diets containing different fats at the 10% level. The fats employed were safflower oil, camellia oil and tristearin. The main constituents of these fats as determined by gas-liquid chromatography were linoleic (78.2%), oleic (80.2%) and stearic acid (86.1%), respectively. To avert essential fatty acid deficiency on feeding tristearin, one-tenth of the fat was replaced by safflower oil. Livers from rats fed on a low-fat diet (1% safflower oil) diet were also perfused for comparison. The composition of the basal diet, expressed as weight percent, was as follows: vitamin-free casein, 20; mineral mixture (Oriental Yeast Co., Tokyo), 4; vitamin mixture (Oriental Yeast Co., Tokyo), 1; choline chloride, 0.15; cellulose powder, 4; fat, 10; and sucrose, to 100. Tristearin, solid at room temperature, was ground to a fine powder before being introduced to the diet. Mineral and vitamin mixtures used were according to Harper (7).

Liver perfusion. After 12–16 days of administering respective diets, livers were isolated under Nembutal anesthesia and perfused by recirculating Krebs-Henseleit buffer (pH 7.4) at 37°C containing 1.5% bovine serum albumin, 25% washed bovine erythrocytes and 0.1% glucose, at the rate of 20 ml/min in either the presence or absence of fatty acid substrate, for 4 h. Provision of the fatty acid substrate was made as follows: at the beginning of recirculation, 5 ml of 20 mM sodium oleate (100 µmol) was added and the same solution was continuously infused at the rate of 5 ml/h. At 1 h intervals, about 20 ml of the perfusate was removed for analysis of ketone bodies and lipids.

Analysis of lipids. Lipids in the perfusates and post-perfused livers were extracted and purified (8) and analyzed for triglyceride (9), cholesterol (10) and phospholipid (11). Triglyceride in the perfusate and liver obtained from 4 h perfusion was separated by thin-layer chromatography on silica gel G (9). The fatty acid composition of triglyceride was determined by gas-liquid chromatography (12).

Analysis of ketone bodies. The liver perfusate was deproteinized with 30% perchloric acid and then neutralized with 3 M K₂CO₃ (13). Acetoacetate and β-hydroxybutyrate were assayed enzymatically in the deproteinized sample (9).

Materials. Materials were obtained from the following sources: glycerokinase,
glycero-3-phosphate dehydrogenase and \( \beta \)-hydroxybutyrate dehydrogenase from Boehringer Mannheim GmbH, Mannheim; cholesterol oxidase from Toyobo Co., Osaka; NAD and NADH from Oriental Yeast Co., Osaka; ATP from Kyowa Hakko Co., Tokyo; bovine serum albumin (fraction V) from Sigma Chemical Co., St. Louis, MO.

RESULTS

Secretion of lipids by perfused rat liver

Different types of fat had no effect on the growth or the food intake of rats. As circulating free fatty acid is presumed to be an important source of lipoproteins secreted by the liver (3, 4, 9, 10, 14), livers from rats fed different fats were perfused either in the presence or in the absence of fatty acid. Perfusion of oleate increased the secretion of triglyceride (Fig. 1) and cholesterol (Fig. 2), but not phospholipid.

Fig. 1. Triglyceride secretion in perfused liver of rats fed different amounts and types of fat. Livers from rats fed on a low-fat diet (1% safflower oil, A) or diets containing 10% of safflower oil (B), camellia oil (C) or tristearin (D) for 12–16 days were perfused either in the absence (○) or the presence (●) of the oleate substrate. Number of livers perfused in each group was as follows: low-fat diet, 4 and 5; safflower oil, 5 and 5; camellia oil, 4 and 6; tristearin, 3 and 5, in the absence and presence of the oleate substrate, respectively. Each point represents the mean ± SE.

* Significantly different from rats fed on a low-fat diet.
Fig. 2. Cholesterol secretion in perfused liver of rats fed different amounts and types of fat. Livers from rats fed on a low-fat diet (1% safflower oil, A) or diets containing 10% of safflower oil (B), camellia oil (C) and tristearin (D) for 12–16 days were perfused either in the absence (○) or the presence (●) of the oleate substrate. Number of livers perfused in each group of rats was the same as described in the legend to Fig. 1. Each point represents the mean ± SE.

(Fig. 3). As Fig. 1 shows, triglyceride secretion was approximately the same among the groups of rats when no oleate was provided. On providing oleate, a two-fold increase in triglyceride secretion was demonstrated in rats fed on a low-fat diet while the magnitude of the increase was rather moderate in rats fed on 10% fat diets, consequently resulting in a 20% reduction of triglyceride secretion.

Cholesterol and phospholipid secretion was not altered by the amount or the type of dietary fat under both conditions, i.e. the absence or presence of the oleate substrate (Figs. 2 and 3).

Ketone body production in perfused rat liver

As shown in Fig. 4, there was no detectable difference in ketone body production among various groups in the absence of oleate. The fatty acid substrate greatly enhanced the rate of ketone body production in rats fed on 10% fat diets and among the different fat groups, the ketogenic rate being the highest in those fed camellia oil. The enhancement of ketogenesis was also noticed in rats fed on a low-fat diet, though to a considerably lesser extent than in other groups.

Lipid contents in post-perfused rat liver

After 4 h perfusion, liver lipids were extracted and assayed for triglyceride,
cholesterol and phospholipid. As shown in Table 1, the triglyceride content was highest in rats fed tristearin and lowest in those fed safflower oil in the case where fatty acid was not perfused. However, no such difference was observed in the presence of oleate substrate. Cholesterol content tended to be higher in rats fed camellia oil compared with other groups. No significant difference was found in the phospholipid content between both perfusion systems.

**Fatty acid composition of perfusate and liver triglyceride**

The fatty acid composition of perfusate triglyceride obtained at the end of the perfusion period and in post-perfused liver is presented in Table 2. When the oleate substrate was not perfused, the major constituent fatty acids in the perfusate and liver triglyceride in each group, except for the 10% safflower oil group, were palmitate and oleate. In rats fed safflower oil, a considerable amount of linoleate was also found in the triglyceride fraction. Though perfusion of the oleate substrate increased the percentage of oleate in perfusate-triglyceride in each group, rats fed
Fig. 4. Ketone body production in perfused liver of rats fed different amounts and types of fat. Livers from rats fed on a low-fat diet (1% safflower oil, A) or diets containing 10% of safflower oil (B), camellia oil (C) and tristearin (D) for 12–16 days were perfused either in the absence (○) or the presence (●) of the oleate substrate. Number of livers perfused in each group of rats was the same as described in the legend to Fig. 1. Each point represents the mean ± SE. * Significantly different from rats fed on a low-fat diet at \( p < 0.05 \). + Significantly different from rats fed on a camellia oil diet at \( p < 0.05 \).

Table 1. Lipid contents in post-perfused rat liver.

| Dietary fats          | Triglyceride (µmol/g) | Cholesterol (µmol/g) | Phospholipid (µmol/g) |
|-----------------------|-----------------------|----------------------|-----------------------|
| Without oleate substrate |                       |                      |                       |
| Low fat (4)\(^1\)     | 10.7 ± 1.3\(^{ab}\)   | 5.08 ± 0.24          | 30.7 ± 1.2            |
| Safflower oil (5)      | 8.6 ± 1.6\(^b\)       | 5.62 ± 0.43          | 30.4 ± 2.0            |
| Camellia oil (4)       | 11.4 ± 0.5\(^b\)      | 6.23 ± 0.27          | 32.8 ± 0.5            |
| Tristearin (3)         | 15.0 ± 0.4\(^c\)      | 5.75 ± 0.32          | 32.3 ± 0.2            |
| With oleate substrate  |                       |                      |                       |
| Low fat (5)            | 10.5 ± 0.4            | 5.42 ± 0.22          | 33.0 ± 1.6            |
| Safflower oil (5)      | 11.0 ± 1.6            | 5.49 ± 0.32          | 31.2 ± 0.9            |
| Camellia oil (6)       | 12.0 ± 1.2            | 6.40 ± 0.19          | 34.3 ± 0.9            |
| Tristearin (5)         | 9.3 ± 1.7             | 5.47 ± 0.20          | 35.3 ± 1.2            |

Each value represents the mean ± SE. \(^1\) Number of rats. Values in columns not sharing a common superscript letter are significantly different at \( p < 0.05 \).

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Table 2. Fatty acid composition of triglyceride in perfusate and liver.

| Dietary fats          | 16:0 | 16:1 | Fatty acids | 18:0 | 18:1 | 18:2 |
|-----------------------|------|------|-------------|------|------|------|
|                       |      |      | (weight %)  |      |      |      |
| Perfusate             |      |      |             |      |      |      |
| Without oleate substrate |      |      |             |      |      |      |
| Low fat (4)           | 27.5 ± 0.2 | 9.1 ± 0.8 \textsuperscript{a} | 2.0 ± 0.2 | 55.6 ± 0.9 \textsuperscript{b} | 2.2 ± 0.4 \textsuperscript{b} |
| Safflower oil (5)     | 28.9 ± 1.8 | 5.6 ± 0.9 \textsuperscript{b} | 2.2 ± 0.3 | 33.7 ± 1.1 \textsuperscript{a} | 20.9 ± 1.4 \textsuperscript{a} |
| Camellia oil (4)      | 26.4 ± 0.6 | 6.1 ± 0.5 \textsuperscript{b} | 1.8 ± 0.1 | 57.6 ± 0.5 \textsuperscript{b} | 3.5 ± 0.1 \textsuperscript{b} |
| Tristearin (3)        | 29.1 ± 1.3 | 7.4 ± 0.5 \textsuperscript{ab} | 2.4 ± 0.3 | 55.8 ± 1.0 \textsuperscript{b} | 2.1 ± 0.4 \textsuperscript{b} |
| With oleate substrate |      |      |             |      |      |      |
| Low fat (5)           | 16.9 ± 0.7 | 6.2 ± 0.5 \textsuperscript{ac} | 1.6 ± 0.1 | 68.2 ± 1.3 \textsuperscript{a} | 2.8 ± 0.4 \textsuperscript{b} |
| Safflower oil (5)     | 15.4 ± 0.7 | 3.3 ± 0.6 \textsuperscript{b} | 2.0 ± 0.2 | 56.7 ± 1.0 \textsuperscript{b} | 17.6 ± 1.6 \textsuperscript{a} |
| Camellia oil (6)      | 14.5 ± 0.3 | 4.2 ± 0.3 \textsuperscript{bc} | 1.7 ± 0.2 | 73.3 ± 0.4 \textsuperscript{a} | 3.5 ± 0.2 \textsuperscript{b} |
| Tristearin (5)        | 17.8 ± 0.9 | 5.0 ± 0.4 \textsuperscript{c} | 1.9 ± 0.2 | 70.2 ± 0.7 \textsuperscript{b} | 2.2 ± 0.5 \textsuperscript{b} |
| Post-perfused liver   |      |      |             |      |      |      |
| Without oleate substrate |      |      |             |      |      |      |
| Low fat (4)           | 34.7 ± 3.0 | 8.3 ± 1.5 \textsuperscript{a} | 2.9 ± 0.3 | 50.3 ± 4.3 \textsuperscript{b} | 1.2 ± 0.4 \textsuperscript{b} |
| Safflower oil (5)     | 34.3 ± 0.9 | 3.6 ± 1.1 \textsuperscript{a} | 3.2 ± 0.3 | 27.3 ± 1.4 \textsuperscript{a} | 27.9 ± 2.7 \textsuperscript{a} |
| Camellia oil (4)      | 32.1 ± 1.3 | 6.0 ± 0.4 \textsuperscript{ab} | 2.4 ± 0.1 | 54.8 ± 1.5 \textsuperscript{b} | 2.3 ± 0.3 \textsuperscript{b} |
| Tristearin (3)        | 37.2 ± 0.4 | 8.2 ± 0.5 \textsuperscript{b} | 3.2 ± 0.5 | 48.3 ± 0.6 \textsuperscript{a} | 1.0 ± 0.2 \textsuperscript{b} |
| With oleate substrate |      |      |             |      |      |      |
| Low fat (5)           | 32.8 ± 1.7 | 8.4 ± 0.9 \textsuperscript{a} | 2.8 ± 0.3 \textsuperscript{ab} | 52.4 ± 2.3 \textsuperscript{b} | 2.0 ± 0.9 \textsuperscript{b} |
| Safflower oil (5)     | 29.6 ± 1.6 | 3.3 ± 0.4 \textsuperscript{b} | 3.4 ± 0.4 \textsuperscript{b} | 36.0 ± 3.8 \textsuperscript{a} | 25.8 ± 4.8 \textsuperscript{a} |
| Camellia oil (6)      | 29.3 ± 3.1 | 5.7 ± 1.1 \textsuperscript{cd} | 2.2 ± 0.1 \textsuperscript{a} | 59.7 ± 4.0 \textsuperscript{b} | 1.9 ± 0.2 \textsuperscript{b} |
| Tristearin (5)        | 30.2 ± 1.5 | 6.1 ± 0.5 \textsuperscript{ad} | 3.5 ± 0.2 \textsuperscript{b} | 58.3 ± 1.8 \textsuperscript{b} | 1.1 ± 0.3 \textsuperscript{b} |

Each value represents the mean ± SE. \textsuperscript{1} Number of rats. Values in columns not sharing a common superscript letter are significantly different at $p < 0.05$.

10% safflower oil still retained a considerable amount of linoleate. The fatty acid composition of hepatic triglyceride was less affected by the fatty acid supply.

DISCUSSION

Dietary fat, varying both in amount and nature, affects many aspects of lipid metabolism in liver. However, little is known about the regulation of hepatic lipoprotein synthesis by dietary fat. In our present study, 10% fat diets compared to a low-fat diet caused a 20% decrease in triglyceride secretion in the perfused rat liver when the fatty acid substrate was supplied. No such difference was, however, demonstrated in cholesterol and phospholipid secretion among the groups. In addition, rates of triglyceride secretion were independent of the sources of dietary fat, suggesting that dietary fat per se but not type may be a possible determinant for lipoprotein secretion in perfused rat liver. In this context, Kalopissis et al. found in
rats fed on a 30% lard diet a more profound decrease (about 40%) in very-low-density lipoprotein-triglyceride secretion after Triton treatment (3), as well as in isolated hepatocytes (4). The extent of the decrease may therefore be proportional to the amount of the fat in the diet.

The rate of hepatic triglyceride secretion is largely dependent on the availability of fatty acid for triglyceride synthesis. Fatty acid supplied exogenously is demonstrated to be actively incorporated into hepatic triglyceride and secreted as a component of very-low-density lipoprotein (4, 9, 10). However, the role of fatty acid synthesized de novo within the liver as the precursor of very-low-density lipoprotein-triglyceride is not precisely known. Though Brunengraber et al. (15) have demonstrated incorporation of tritium water into perfusate lipids, the quantitative significance of newly synthesized fatty acid relative to fatty acid from other sources remains to be established. Formerly, we suggested that newly synthesized fatty acid is only a minor source of very-low-density lipoprotein-triglyceride (14). This suggestion is further supported from the present observation of the lack of alteration in triglyceride secretion caused by dietary fat in the absence of fatty acid substrate in spite of marked changes in fatty acid synthesis due to differences in the amount and type of fat (16).

On the other hand, we were able to demonstrate the reduction of triglyceride secretion and enhancement of ketogenesis in 10% fat-fed rats compared to those fed a low-fat diet in the case where fatty acid was infused into the perfusate. The rate of fatty acid oxidation is a critical determinant in the regulation of hepatic triglyceride secretion (9). The enhanced capability to oxidize fatty acid in 10% fat-fed rats as reflected by the increase in ketone body production was presumed to divert exogenous fatty acid to the oxidation pathway and in turn to reduce hepatic triglyceride synthesis and secretion. Kalopissis et al. came to a similar conclusion to account for the reduction of very-low-density lipoprotein secretion in the liver of rats fed on a high-fat diet (3, 4). However, the failure to demonstrate alteration of triglyceride secretion among rats fed different fats in spite of detectable changes in ketone body production in these animals can not be explained by the assumption mentioned above. An alternative mechanism might exist for the regulation of triglyceride secretion by dietary fat.

The hypocholesterolemic action of polyunsaturated fat has long been demonstrated both in humans and in experimental animals. Differences in type of dietary fat affect numerous aspects of cholesterol metabolism including synthesis (12), catabolism (17), excretion (18, 19) and intertissue distribution (20). Lipoprotein catabolism also seems to be affected by the type of fat ingested (21). However, the exact mechanism underlying the hypocholesterolemic action of polyunsaturated fat has yet to be determined. Nicolosi et al. demonstrated the alteration of lipoprotein synthesis by dietary fats with different degrees of unsaturation in the Triton-treated gerbil (5) and monkey (6), which indicated a close relationship between the rate of lipoprotein synthesis and plasma cholesterol levels in these animals. However, in our present study, we were not able to demonstrate
any detectable difference due to the type of dietary fat in cholesterol or triglyceride and phospholipid secretion in perfused rat liver. Thus, it is not plausible that the alteration of lipoprotein synthesis is a factor to account for the cholesterol-lowering effect of polyunsaturated fat, at least in the rat. Dietary fat not only affects the composition and fatty acid profile of the lipid moiety but also alters the composition of the apoprotein moiety of circulating lipoprotein (22, 23). In fact, in the present study dietary safflower oil distinctly modified the fatty acid composition of perfusate triglyceride. Changes in the fatty acid profile of the lipid moiety of lipoprotein may modify its own metabolism and in turn affect the blood cholesterol level.

This study was supported in part by Grant-in-Aid for Scientific Research (No. 57560089 to TI) from the Ministry of Education, Science and Culture of Japan.

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