Effect of Dietary Soybean Phospholipid and Fats Differing in the Degree of Unsaturation on Fatty Acid Synthesis and Oxidation in Rat Liver

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Summary The activities of enzymes in fatty acid oxidation and synthesis in the liver of rats fed soybean phospholipids and soybean oil corresponding to the dietary levels of 3% fatty acid added to the diets containing a saturated fat (coconut oil) and a polyunsaturated fat (safflower oil) at the amounts corresponding to 12% fatty acid levels were compared. Soybean phospholipid compared with soybean oil added to both coconut and safflower oil diets significantly reduced the activities of enzymes in fatty acid synthesis (fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme). However, there were no significant differences in the activities of enzymes in fatty acid oxidation (carnitine palmitoyltransferase, acyl-CoA dehydrogenase and acyl-CoA oxidase) between the groups of rats fed soybean phospholipid and soybean oil added to coconut and safflower oil diets except for one occasion. Soybean phospholipid compared with soybean oil added to coconut oil diet significantly decreased the concentrations of triacylglycerol, choles-terol and phospholipid in the serum and of triacylglycerol and cholesterol in the liver. However, the dietary phospholipid added to safflower oil diet failed to alter these values. These results suggested that the alteration in the rate of fatty acid synthesis, but not oxidation, in the liver is responsible for the lipid-lowering effect of dietary soybean phospholipid added to a saturated fat diet.

Key Words soybean phospholipid, fatty acid synthesis, fatty acid oxidation, rat liver

Dietary phospholipids from various origins exert a potent lipid-lowering effect not only in experimental animals (1-3) but also in humans (4, 5). Regarding the mechanism by which phospholipids reduce serum lipid concentrations, we have previously demonstrated that the diets containing soybean phospholipid and egg

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yolk phospholipid compared to those containing soybean oil or the fat blend simulating fatty acid composition of soybean phospholipid profoundly reduced various parameters for triacylglycerol synthesis in the rat liver (6–9). Analyses of the concentrations of intermediates in triacylglycerol synthesis and the activities of enzymes in triacylglycerol and fatty acid synthetic pathways indicated that the reduction in the availability of fatty acids due to decreased fatty acid synthesis is the primary factor responsible for the soybean phospholipid-dependent reduction in triacylglycerol synthesis in the liver (6, 7, 9). The results obtained using hepatocytes isolated from rats fed soybean phospholipid also support this consideration (8). Thus, it is probable that impairment of the assembly and secretion of triacylglycerol-rich lipoprotein due to the reduction of triacylglycerol synthesis through the inhibition of fatty acid synthesis is the primary factor responsible for the hypolipidemic effect of dietary soybean phospholipid. An alternate plausible factor to modify the hepatic triacylglycerol synthesis and the secretion of triacylglycerol rich lipoprotein is the alteration in the rate of fatty acid oxidation. It has been well demonstrated that the alteration in the rate of fatty acid oxidation either in mitochondrial (10) or peroxisomal pathway (11) in the liver modify the availability of fatty acids for triacylglycerol synthesis and alter the very low density lipoprotein production by the liver. Moreover, there are evidences to support the concept that the rates of fatty acid oxidation and synthesis in the liver are inversely proportional to each other under various nutritional and pathological conditions (12–18). Thus, there is a possibility that the ingestion of phospholipid alters not only the rate of the fatty acid synthesis but also the rate of the fatty acid oxidation and these changes contribute to the hypolipidemic effect of soybean phospholipid. However, the activities of enzymes in fatty acid oxidation in rats fed soybean phospholipid remain to be studied. If the modification in the fatty acid synthetic pathway, and not the fatty acid oxidation pathway, is the primary factor responsible for the physiological activity of dietary phospholipid, its hypolipidemic effect may be altered under the condition in which hepatic fatty acid synthesis is modified. It has been well established that alterations in the fatty acid composition of diets causes alterations in the rate of fatty acid synthesis in the rat liver (12, 19). In these contexts, in the present study, we compared the effect of dietary soybean phospholipid and soybean oil (3% fatty acid levels in the diets) to the diets containing 12% fatty acid levels of saturated (coconut oil) and polyunsaturated fats (safflower oil) on the activities of enzymes in fatty acid synthesis and oxidation pathways in the rat.

MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats obtained from a commercial breeder (Charles River Japan, Kanagawa) at 4 weeks of age were used in the present study. After 6 days of acclimatization to our housing conditions, rats were given purified experimental diets. All the experimental diets contained dietary...
lipids at levels corresponding to 15% fatty acids. The rats were randomly divided into 4 groups of 7 animals each. Two groups of rats were fed the diets containing 12% fatty acids as coconut oil together with 3% fatty acids as either soybean oil or soybean phospholipid, whereas the other two groups were fed the diets containing 12% fatty acids as safflower oil together with 3% fatty acids as either soybean oil or soybean phospholipid. The experimental period was 15 days. Body weights of animals at the initiation of the experiments was 120–135 g. The basal composition of the experimental diet was (in weight %): casein, 20; corn starch, 15; cellulose, 2; mineral mixture (20), 3.5; vitamin mixture (20), 1.0; DL-methionine, 0.3; choline bitartrate, 0.2; and dietary lipids corresponding to 15% fatty acids and sucrose to make 100%. The fatty acid compositions of the various dietary lipids employed in the present study are summarized in Table 1. The fatty acid content of soybean, coconut and safflower oils ranged from 940 to 955 mg/g, while the value for soybean phospholipids was 607 mg/g. The phosphorous content of soybean phospholipid was 1.03 µmol/g, mainly consisting (mol%) of phosphatidylcholine (35.4%), phosphatidylethanolamine (33.2%), phosphatidylinositol (16.1%) and phosphatidic acid (13.7%).

**Enzyme assays.** At the end of the experimental period, rats were anesthetized with diethylether and killed by bleeding from the abdominal aorta, and livers were quickly excised. About 3 g of each liver was homogenized with 7 volumes of 0.25 M sucrose and centrifuged at 500 × g for 10 min. The supernatant was recentrifuged at 9,000 × g for 10 min to isolate mitochondria. Mitochondrial fraction was washed twice with 0.25 M sucrose containing 1 mM EDTA and 3 mM Tris-HCl (pH 7.0) and finally suspended in the same medium to give a protein concentration of 20–25 mg/ml. Carnitine palmitoyltransferase (EC 2.3.1.21) activity was measured spectrophotometrically in isolated mitochondria according to the method of Markwell et al. (21) as described previously (22). Acyl-CoA dehydrogenase (EC 1.3.99.3) activity was measured in isolated mitochondria according to the method of Dommes and Kunau (23) except that phenazine methosulfate was used as a

| Table 1. Fatty acid composition of dietary lipids. |
|-----------------------------------------------|
| Soybean phospholipid | Soybean oil | Coconut oil | Safflower oil |
|----------------------|------------|-------------|--------------|
| 8:0                  | —          | —           | 2.6          | —            |
| 10:0                 | —          | —           | 6.8          | —            |
| 12:0                 | —          | —           | 23.2         | —            |
| 14:0                 | 0.3        | 0.2         | 24.5         | 0.2          |
| 16:0                 | 15.9       | 10.0        | 18.6         | 8.0          |
| 16:1                 | 0.1        | —           | —            | —            |
| 18:0                 | 3.3        | 3.5         | 6.2          | 2.7          |
| 18:1                 | 12.8       | 24.4        | 14.2         | 16.3         |
| 18:2                 | 59.3       | 53.5        | 3.4          | 71.6         |
| 18:3                 | 7.8        | 8.2         | 0.1          | 1.0          |

(weight %)
Acyl-CoA oxidase (EC 1.3.99.3) activity was measured in 500×g supernatant fraction of liver homogenate as described elsewhere (22,24). Fatty acid synthetase, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and malic enzyme (EC 1.1.1.40) are the cytosolic enzymes but the activities of these enzymes were conveniently measured in the 9,000×g supernatant of liver homogenate as described previously (7,9). Preliminary experiments have revealed that the activities of these enzymes in fatty acid synthesis assayed in this subcellular fraction were indistinguishable from those measured in cytosolic fraction (105,000×g supernatant fraction of liver homogenate) when they were expressed per gram of tissue. Epididymal adipose tissues were homogenized with 3 volumes of 0.25 M sucrose containing the inhibitors for proteolytic enzymes (25) and centrifuged at 9,000×g for 10 min. The infranatant between the floating fat layer and sedimenting cell particles was taken for measurement of the activities of glucose-6-phosphate dehydrogenase and malic enzyme.

Lipid analyses. Liver and serum lipids were extracted and purified (26). Triacylglycerol and phospholipid contents in the extracts were determined as described previously (27). Cholesterol content in the liver lipid extracts was determined enzymatically as described elsewhere (11). The serum cholesterol concentration was assayed using a commercially available kit (Cholesterol C-test Wako, Wako Pure Chemical, Kyoto).

Statistical analyses. Data were analyzed by two-way analysis of variance and significant differences of means were evaluated using Duncan’s multiple range test (28) at the level of p<0.05.

RESULTS

There were no significant differences in average food intake due to the types of dietary lipids (20.4–22.5 g/day). Also, no significant differences in average body weights (285–299 g) and liver weights (4.73–5.24 g/100 g body weight) of animals at the time of sacrifice were not detected among the groups.

Table 2 shows the activities of enzymes in fatty acid oxidation and fatty acid synthesis in the liver. Also, the activities of enzymes involved in fatty acid synthetic pathway in epididymal adipose tissue are presented in this table. The protein contents of subcellular fractions of the liver employed as the enzyme sources were 147–156, 134–137 and 21–26 mg/g liver for 500×g supernatant, 9,000×g supernatant and mitochondrial fractions, respectively. No differences were found for these values among the groups. The activities of mitochondrial carnitine palmitoyltransferase and acyl-CoA dehydrogenase in two groups of rats fed coconut oil diets were significantly lower than the corresponding values in the animals fed safflower oil diets. However, soybean phospholipid compared to soybean oil failed to modify these enzyme activities whether it was added to the coconut oil or safflower oil diet. Safflower oil, compared to coconut oil, slightly but significantly increased acyl-CoA oxidase activity in the rats fed the diets containing 3% fatty acids as soybean oil.
Table 2. Activities of the enzymes in fatty acid oxidation in the liver and of those in fatty acid synthesis in the liver and epididymal adipose tissue of rats fed coconut and safflower oil diets containing soybean oil and soybean phospholipid.

| Enzymes in fatty acid oxidation | Dietary lipids | Dietary lipids |
|-------------------------------|----------------|----------------|
|                               | Coconut oil diet | Safflower oil diet |
|                               | Soybean oil | Soybean phospholipid | Soybean oil | Soybean phospholipid |
| Carnitine palmitoyltransferase | 11.3±1.1** | 12.2±1.0** | 16.6±1.1 | 15.2±1.0 |
| Acyl-CoA dehydrogenase         | 34.8±2.5** | 35.7±2.8** | 45.4±1.4 | 42.5±1.8 |
| Acyl-CoA oxidase               | 2.90±0.17** | 2.54±0.14 | 3.32±0.16 | 2.41±0.08* |

Values represent M±SE of 7 rats. *Values are significantly different from those in the soybean oil group at p<0.05. **Values are significantly different from corresponding values in the rats fed safflower oil diets at p<0.05.

However, no such dietary fat-dependent difference was detected between the groups of rats fed the diet containing soybean phospholipid. Soybean phospholipid, compared to soybean oil, slightly decreased the acyl-CoA oxidase activity both in the rats fed coconut and safflower oil diets. And the difference was statistically significant in the animals fed the polyunsaturated fat diet.

Soybean phospholipid compared to soybean oil significantly reduced the activities of various enzymes in fatty acid synthesis in the liver whether the phospholipid was added to the coconut oil diet or the safflower oil diet. Between the two groups of rats fed the diets containing 3% fatty acids as soybean oil, the activity of fatty acid synthetase but not of glucose-6-phosphate dehydrogenase and malic enzyme was higher in the rats fed coconut oil diet than in the rats fed safflower oil diet. When comparisons were made between the two groups of rats fed soybean phospholipid, the activity of glucose-6-phosphate dehydrogenase was significantly higher in the animals given coconut oil diet compared to those given safflower oil diet. However, the activities of fatty acid synthetase and malic enzyme were only slightly and insignificantly higher in the former compared to the latter. In contrast to the situation in the liver, glucose-6-phosphate dehydrogenase and malic enzyme...
Table 3. The concentrations of liver and serum lipids in rats fed coconut and safflower oil diets containing soybean oil and soybean phospholipids.

| Dietary lipids          | Coconut oil diet | Safflower oil diet |
|-------------------------|-----------------|-------------------|
|                         | Soybean oil     | Soybean phospholipids | Soybean oil  | Soybean phospholipids  |
| Liver lipids (μmol/g)   |                 |                   |               |                   |
| Triacylglycerol         | 47.9±4.5**      | 28.5±2.4*         | 32.9±2.0      | 33.8±4.1           |
| Cholesterol            | 5.85±0.28       | 4.41±0.08*        | 6.18±1.4      | 5.95±0.17          |
| Phospholipids          | 39.5±1.0        | 39.4±1.7          | 39.0±0.5      | 41.9±0.4           |
| Serum lipids (μmol/100 ml) |             |                   |               |                   |
| Triacylglycerol         | 373±48**        | 216±30*           | 153±15        | 176±19             |
| Cholesterol            | 294±14**        | 212±21*           | 226±16        | 188±13             |
| Phospholipids          | 312±12**        | 257±33*           | 253±11        | 201±15             |

Values represent M±SE of 7 rats. *Values are significantly different from those in the soybean oil group at p<0.05. **Values are significantly different from corresponding values in the rats fed safflower oil diets at p<0.05.

activities expressed on the bases of the enzyme protein in adipose tissue were comparable among the groups of rats. No significant differences in the activities of these enzymes among the groups were detected even if they were expressed on the bases of cellular DNA (data not shown). There were no significant differences in the contents of protein in the 9,000×g supernatant fraction (12.6–13.7 mg/g) and of DNA in epidydimal adipose tissue (86.8–117 μg/g) among the groups.

Soybean phospholipid compared to soybean oil significantly reduced hepatic concentrations of triacylglycerol and cholesterol when it was added to the coconut oil diet (Table 3). However, soybean phospholipid failed to alter these parameters when it was added to the diet containing 12% fatty acids as safflower oil. Dietary soybean phospholipid compared to soybean oil added to the coconut oil diets significantly reduced the concentrations of triacylglycerol, cholesterol and phospholipid in the serum. However, the phospholipid failed to modify these parameters when it was added to the diet containing 12% fatty acids as safflower oil.

DISCUSSION

We have previously demonstrated (6–9) that diets containing soybean phospholipid as the sole dietary lipid source at the amounts corresponding to 2–20% fatty acids compared to the diets containing the same amounts of fatty acids either as soybean oil or the fat blend simulating fatty acid composition of soybean phospholipid profoundly reduced various parameters of triacylglycerol synthesis in the liver. As dietary soybean phospholipid profoundly reduced the activities of enzymes in fatty acid synthesis in liver homogenate (7, 9) and the rate of incorpor-
ration of [1-\textsuperscript{14}C]acetate into fatty acid in isolated hepatocytes (8), the reduction in the availability of fatty acid through the depression of fatty acid synthesis appeared to be primary factors responsible for triacylglycerol synthesis in the liver. The reductions of the activities of hepatic enzymes in fatty acid synthesis in rats fed soybean phospholipid were confirmed in the present study in which the phospholipid corresponding to the dietary level of 3% fatty acids was served together with coconut and safflower oils corresponding to dietary levels of 12% fatty acids. However, the activities of enzymes in fatty acid synthesis in epididymal adipose tissue were in no way modified by the dietary soybean phospholipid in the present study. Thus, the enzymes in fatty acid synthesis in the liver and adipose tissue are regarded to be under different control mechanisms. In this context, Clarke et al. (19) reported that dietary polyunsaturated fatty acids decreased activities of enzyme in fatty acids synthesis in the liver but not those in the epididymal adipose tissue.

An alternate plausible factor to modify the availability of fatty acids for triacylglycerol synthesis is the alteration in the rate of fatty acid oxidation (10,11). In addition, there is evidence to support the concept that nutritional and pathological conditions which cause a decrease in fatty acid synthesis are generally accompanied by the enhancement of fatty acid oxidation in the liver (12–18). Therefore, it is plausible that the inhibition of fatty acid synthesis by dietary phospholipid consequently cause an induction of the enzymes in the fatty acid oxidation pathway. However, this hypothesis was not supported by the present findings. Safflower oil diets which contain either soybean oil or soybean phospholipid compared to the corresponding coconut oil diets significantly increased the activities of both carnitine palmitoyltransferase and acyl-CoA dehydrogenase in the isolated mitochondria. Therefore, there is the possibility that enhanced oxidation of fatty acid in mitochondria may be, in part, responsible for the hypolipidemic effect of polyunsaturated fats. However, Halinski et al. (16) reported that the rates of mitochondrial and peroxisomal oxidation of fatty acids in rats fed the diets containing 10% safflower and palm oils were indistinguishable from each other. Thus, detailed studies regarding the effect of various dietary fats on the activities of enzymes in fatty acid oxidation are still required to draw a definite conclusion.

Although we could not observe the alterations of the activities of the enzymes in fatty acid oxidation in the liver of rats fed soybean phospholipid, the observation does not necessarily rule out the possibility that enhanced oxidation of fatty acids in the liver is responsible for the lipid-lowering effect of dietary soybean phospholipid. Malonyl-CoA-mediated inhibition of carnitine palmitoyltransferase represents a mechanism by which mitochondrial oxidation of long-chain fatty acids is regulated (29). As malonyl-CoA is the enzyme product of acetyl-CoA carboxylase, its concentration in the liver proportionally decreases as the rate of fatty acid synthesis decreases (30). Thus, depression by soybean phospholipid of hepatic fatty acid synthesis should decrease the malonyl-CoA concentration in the liver and in turn enhance the rate of fatty acid oxidation even when the activities of the
mitochondrial fatty acid oxidation enzymes remain unaltered. However, our previous study (8) using isolated rat hepatocytes does not support this hypothesis. Although the rates of the incorporation of [1-14C]acetate into fatty acids and of [2-3H]glycerol into triacylglycerol were lower in hepatocytes isolated from rats fed soybean phospholipid than those in the cells isolated from the animals fed soybean oil, the rate of ketone body production from oleate substrate added to the incubation mixture was higher in the former compared to the latter. Thus, the malonyl-CoA-mediated mechanism in regulating fatty acid oxidation does not seem to operate at least in rats fed soybean phospholipid.

If the depression of fatty acid synthesis is a factor primarily responsible for the lipid-lowering effect of soybean phospholipid (1-9), the lipid-lowering effect of the phospholipid should be diminished under the condition in which hepatic fatty acid synthesis is hampered. Dietary polyunsaturated fatty acids compared to saturated fatty acids has been reported to be more competent in reducing the rate of fatty acid synthesis in the liver (12,19). Although the activities of glucose-6-phosphatase dehydrogenase and malic enzyme were similar between rats fed the coconut and safflower oil diets containing 3% dietary fatty acid level of soybean oil in the present study, fatty acid synthetase activity in rats fed the safflower oil diet containing soybean oil was much lower than the value in the animals fed the corresponding coconut oil diet. The results indicate that the rate of fatty acid synthesis in the liver is much lower in rats fed safflower oil diet containing soybean oil than in the animals fed the corresponding coconut oil diet. The replacement of soybean oil by soybean phospholipid significantly reduced the activities of various enzymes in fatty acid synthesis both in rats fed coconut and safflower oil diets. Under this situation, dietary soybean phospholipid added to the coconut oil diet significantly reduced the liver concentrations of triacylglycerol and cholesterol and serum lipid concentrations. However, no such alterations can be detected in rats fed safflower oil diets. This observation supports the consideration that the reduction in fatty acid synthesis in the liver is a crucial factor responsible for the lipid-lowering effect of soybean phospholipid in rats fed the coconut oil diet, as observed in the present study.

It has been well demonstrated that dietary phospholipid decreases serum cholesterol concentration in the rat (1-3). The available evidence indicates that phosphatidylethanolamine is the phospholipid class which is responsible for the cholesterol lowering effect of dietary soybean phospholipid (1,31). Our previous study (9) presented evidence that phosphatidylethanolamine is the component which is responsible for the reduction by dietary soybean and egg yolk phospholipids of the activities of enzymes in fatty acid synthesis, as well. It has been demonstrated that prominent proportions of the ethanolamine moiety of the dietary phosphatidylethanolamine are hydrolyzed in the small intestine and are absorbed through the portal vein (32). In fact, Imaizumi et al. (31) demonstrated that dietary phosphatidylethanolamine increased the circulating level of ethanolamine. In addition, they showed that ethanolamine added to the culture medium suppress-
ed fatty acid synthesis from \[^{[3]}H\]serine in rat hepatocytes (31). Therefore, it is plausible that ethanolamine contained in the dietary soybean phospholipid as a base moiety is responsible for the reductions in the activities of enzymes in fatty acid synthesis as observed in the present study. Further studies are required to clarify this point.

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