Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in α-linolenic acid in rats

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Abstract The activities of hepatic fatty acid oxidation enzymes in rats fed perilla oil rich in α-linolenic acid (α-18:3) were compared with those fed saturated fats or safflower oil (the mixture of safflower oil and olive oil, 94:8, w/w) containing the same amount of polyunsaturated fatty acids with perilla oil exclusively as linoleic acid (18:2). When the rats were fed the diets containing 15% coconut, safflower, and perilla oils for 1 week, the rate of mitochondrial and peroxisomal oxidation of palmitoyl-CoA (16:0-CoA) in the liver homogenates was the highest in rats fed perilla oil. Among the rats fed the diets containing 15% palm, safflower, and perilla oils for 2 weeks, the rates of mitochondrial and peroxisomal oxidations of 16:0-, 18:2-, and α-18:3-CoAs were the highest in rats fed perilla oil, and the rate of oxidation of α-18:3-CoA by both pathways was higher than those of other acyl-CoAs in all groups. Dietary perilla oil relative to palm and safflower oils significantly increased the activities of carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, and 2,4-dienoyl-CoA reductase. The substrate specificity of carnitine palmitoyltransferase appeared to be responsible for the differential rates of the mitochondrial oxidation of acyl-CoAs. The substrate specificity of acyl-CoA oxidase did not account for the preferential peroxisomal oxidation of α-18:3 relative to 18:2. The preferential mitochondrial and peroxisomal β-oxidation of α-18:3-CoA relative to 16:0- and 18:2-CoAs was also confirmed in rats fed laboratory chow irrespective of the substrate/albumin ratios in the assay mixture. It was suggested that both substrate specificities and alterations in the activities of the enzymes in β-oxidation pathway play a significant role in the regulation of the serum lipid concentrations in rats fed a diet rich in α-18:3.—**Ide, T., M. Murata, and M. Sugano.** Stimulation of the activities of hepatic fatty acid oxidation enzymes by dietary fat rich in α-linolenic acid in rats. *J. Lipid Res.* 1996. **37:** 448–463.

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It has been demonstrated that fish oils containing very long-chain n-3 polyunsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids reduce serum lipid concentrations in experimental animals (1–5) and humans (6, 7). Alteration in the rate of oxidation of fatty acids in the liver appears to be a crucial factor accounting for the hypolipidemic effect of fish oil. Yamazaki, Shen, and Schade (1) found that the diet containing fish oil compared to the diet containing safflower oil or a low fat diet increased the activity of acyl-CoA oxidase (an enzyme involved in peroxisomal oxidation of fatty acid). Dietary EPA increased the rates of mitochondrial (3) and peroxisomal (2, 3) oxidation of fatty acid, and raised the activities of carnitine palmitoyltransferase and acyl-CoA oxidase in rat liver (2). DHA also increased the rate of peroxisomal oxidation of fatty acid in rat liver (4). Consistent with these observations, Wong et al. (8) reported that dietary fish oil, relative to safflower oil, enhanced the rate of ketone body production while it decreased the rate of lipogenesis in perfused rat liver. These metabolic changes concomitantly decreased the rate of secretion of triacylglycerol in perfused rat liver (8, 9). Thus, enhanced oxidation of fatty acids by fish oil may, in turn, decrease the hepatic triacylglycerol synthesis and secretion of the lipid molecule as a component of very low density lipoproteins (10–12), and thus, may represent a mechanism by which fish oil decreases serum lipid concentration.

Available information indicated that dietary fats rich in n-3 octadecatrienoic acid (α-linolenic acid) such as linseed (13, 14) and perilla oils (15–17), compared to those rich in n-6 octadecadienoic acid (linoleic acid), decrease serum lipid concentrations in the rat. It is probable that dietary α-linolenic acid, as in the case of fish oil, modifies fatty acid metabolism in the liver and

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thus, decreases serum lipid concentrations. However, information concerning the effect of dietary fat rich in α-linolenic acid on the activities of hepatic enzymes involved in fatty acid metabolism is lacking. In this context, the effect of dietary perilla oil rich in α-linolenic acid on the activities of enzymes involved in fatty acid oxidation was compared to a fat mixture composed of safflower and olive oils and saturated fats (coconut and palm oils) in the present study.

MATERIALS AND METHODS

Materials

[1-^{14}C]palmitic (16:0), linoleic (18:2), and α-linolenic acids (α-18:3) were purchased from Amersham International, Bucks, UK. Various 1-^{14}C-labeled fatty acyl-CoAs as well as non-radiolabeled fatty acyl-CoAs were prepared according to the method of Kawaguchi, Yoshimura, and Okuda (18). Sorboyl-CoA was prepared by the mixed anhydride method (19). Acetyl-CoA was prepared by acetylating CoA with acetic anhydride. [^{14}C]sodium bicarbonate was purchased from New England Nuclear, Boston, MA. Malonyl-CoA was purchased from Sigma Chemical, St. Louis, MO. Bovine serum albumin fraction V (essentially fatty acid-free) and horseradish peroxidase were the products of Boehringer Mannheim, Gmbh, Germany. Coconut and olive oils were purchased from Nakarai Tesque, Kyoto, Japan. Safflower oil, and palm and perilla oils were gifts from Nisshin Oil Co., Tokyo, Japan and Nippon Oil & Fats Co., Tokyo, Japan, respectively.

Animals and diets

Male Sprague-Dawley rats were obtained from Charles River Japan, Kanagawa, Japan at 4 weeks of age. The animals were housed individually in a room with controlled temperature (20–22°C), humidity (55–65%), and lighting (lights on from 0700 to 1900 h), and fed a commercial nonpurified diet (Type NMF; Oriental Yeast, Tokyo, Japan). We followed the guide of our institute for the care and use of laboratory animals. After 5 days of acclimation to the housing conditions, rats were randomly divided into three groups and fed purified experimental diets containing 15% of various dietary fats differing in the degree of unsaturation. The basal composition of experimental diet was (in weight %): fat, 15; casein, 20; corn starch, 15; sucrose, 43; cellulose, 2; mineral mixture (20), 3.5; vitamin mixture (20), 1.0; DL-methionine, 0.3; and choline bitartrate, 0.2. In a first experiment (Expt. 1), animals were fed the diets containing coconut oil, the fat mixture composed of safflower and olive oils (94:8, w/w) or perilla oil for 7 days. In the second experiment (Expt. 2), rats were fed the diets containing palm, safflower-olive and perilla oils for 14 days. As shown in **Table 1,** the composition of polyunsaturated fatty acids was the sole variable and total polyunsaturated fatty acid contents were the same in safflower-olive and perilla oils. Average body weights of rats at the initiation of experiments were 147 ± 2 and 149 ± 2 g for Expts. 1 and 2, respectively.

Enzyme assays

At the termination of the experimental period, rats were lightly anesthetized by diethylether and bled from the abdominal aorta and livers were quickly excised. About 3 g each of 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. The rates of mitochondrial and peroxisomal oxidation of fatty acids were measured using the 500 *g* supernatant fraction of liver homogenates according to the method described by Mannaerts et al. (21). The assay mixture for mitochondrial activity contained 4 mM ATP, 0.5 mM L-carnitine, 0.05 mM CoA, 2 mM dithiothreitol, 7.2 mg/ml bovine serum albumin (fatty acid-free), 0.2 mM 1-^{14}C-labeled fatty acyl-CoA (0.5 μCi/μmol) (acyl-CoA/albumin molar ratio was 1.67) and 0.1 ml of the 500 *g* supernatant (1.3–1.6 mg protein) in modified Krebs-Henseleit bicarbonate buffer (pH 7.4) (21). The assays carried out in the presence of 2 mM KCN served as blanks. The mixture for the assay of peroxisomal activity contained 4 mM ATP, 0.5 mM CoA, 2 mM NAD, 2 mM dithiothreitol, 2 mM KCN, 0.2 mM [1-^{14}C] fatty acyl-CoA (0.5 μCi/μmol), and 0.05 ml of the 500 *g* supernatant (0.65–0.8 mg protein) in the modified

TABLE 1. Fatty acid compositions of dietary fats

Fatty Acid	Dietary Fat			
	Coconut Oil	Palm Oil	Safflower Oil[a]	Perilla Oil
	weight %			
8:0	2.6	–	–	–
10:0	6.8	–	–	–
12:0	23.2	–	–	–
14:0	24.5	1.1	0.2	0.1
16:0	18.6	45.3	7.7	6.3
16:1 (n-7)	–	0.1	0.1	0.2
18:0	6.2	4.4	2.6	2.0
18:1 (n-9)	14.2	39.1	20.4	20.9
18:2 (n-6)	3.4	9.4	67.8	13.3
18:3 (n-3)	0.1	0.3	1.3	56.7
PUFA[b] (18:2 + α-18:3)	3.5	9.7	69.1	69.9

[a]A mixture of safflower and olive oils (94:8, w/w).
[b]PUFA, polyunsaturated fatty acids.

Krebs-Henseleit bicarbonate buffer (pH 7.4). As serum albumin severely inhibits the peroxisomal oxidation of palmitoyl-CoA (21), we did not include albumin for the assay of peroxisomal activity. The assays carried out in the absence of the 500 *g* supernatant served as blanks for peroxisomal activity. The final volumes of the mixture were 2.5 ml for both mitochondrial and peroxisomal assays. The rates of mitochondrial and peroxisomal β-oxidation were assayed under the atmosphere of 5% CO_2–95% O_2 for 10 min in Erlenmeyer flasks with gentle shaking and terminated by pouring the mixture into the test tubes containing 0.625 ml of 30% perchloric acid. After standing the mixture in an ice bath at least for 15 min, the mixture was centrifuged at 2,500 *g* for 10 min. The supernatant was extracted twice with hexane, and an aliquot was taken for the radioactivity measurement by liquid scintillation counting. Preliminary experiments have revealed that the radioactivity found in CO_2 was less than 2% of the acid-soluble radioactivities irrespective of acyl-CoA substrates differing in the degree of unsaturation as described by Mannaerts et al. (21). Therefore, the acid-soluble radioactivity was taken as a measure of total oxidation in the present study. The activity of carnitine palmitoyltransferase (EC 2.3.1.21), which locates in the outer mitochondrial membrane (carnitine palmitoyltransferase I), was measured using freshly isolated mitochondria as an enzyme source according to the method of Bremer et al. (22) as described previously (23). The assay mixture (1.0 ml, pH 7.0) contained 82 mM sucrose, 70 mM KCl, 35 mM imidazole, 35 mM HEPES, 1 mM reduced glutathione, 2 mM KCN, 0.5 mM L-[methyl-^{3}H]carnitine (2,220 dpm/nmol), 4 mg/ml bovine serum albumin (fatty acid-free), 50 μM various acyl-CoA substrates (acyl-CoA/albumin molar ratio was 0.75), and mitochondria (150–250 μg protein). Carnitine palmitoyltransferase activity was also measured spectrophotometrically in the freeze-thawed preparation of isolated mitochondria solubilized with Triton X-100 according to the method of Markwell et al. (24) as described elsewhere (25). This assay may detect not only the activity of the transferase I but also that of the enzyme located in the inner mitochondrial membrane (carnitine palmitoyltransferase II). Acyl-CoA dehydrogenase (EC 1.3.99.3) activity was measured in isolated mitochondria according to the method described by Dommes and Kanau (26) and Dommes, Baumgart, and Kanau (27) except that phenazine methosulfate was used as a primary electron acceptor. Acyl-CoA oxidase (EC 1.3.3.6) activity was measured in the 500 *g* supernatant fraction of liver homogenates as described elsewhere (25, 28). The assay mixture (1 ml) contained 0.82 mM 4-aminoantipyrine, 10 mM phenol, 10 μM flavineadenine dinucleotide, 4 units horseradish peroxidase, 0.2 mg bovine serum albumin (fatty acid-free), 50 μM fatty acyl-CoA (acyl-CoA/albumin molar ratio was 15) in 50 mM potassium phosphate buffer (pH 7.4). 2,4-Dienoyl-CoA reductase (EC 1.3.1.34) activity was measured using sorboyl-CoA as the substrate (29) in both isolated mitochondria and the 500 *g* supernatant. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) (30), fatty acid synthetase (31), and acetyl-CoA carboxylase (EC 6.4.1.2) (32) activities were measured in the 9,000 *g* supernatant fraction of liver homogenates as described previously (33). Activities of marker enzymes for cell organelles including succinate dehydrogenase (EC 1.3.99.1) (mitochondria) (34), catalase (EC 1.11.1.6) (peroxisomes) (35) and lactate dehydrogenase (EC 1.1.1.27) (cytosols) (36) were measured in the 500 *g* supernatant fraction. Succinate dehydrogenase activity was also measured in the isolated mitochondrial fraction to calculate the recovery of mitochondria from the 500 *g* supernatant. The rates of mitochondrial and peroxisomal oxidation of fatty acids and carnitine palmitoyltransferase I activity were measured on the day of the killing using fresh enzyme preparations. The other enzymes were assayed in the enzyme preparations being stored at -40°C up to 7 days. Activities of various enzymes were assayed at 30°C except for the mitochondrial and peroxisomal β-oxidation at 37°C.

Lipid analyses

Liver and serum lipids were extracted and purified (37). Triacylglycerol and phospholipid contents in the extracts were measured as described previously (38). Cholesterol in the liver lipid extract was determined enzymatically (12). Serum cholesterol concentration was assayed using a commercial enzyme kit (Cholesterol C-test Wako, Wako Pure Chemical, Osaka, Japan).

Statistical analysis

Data were analyzed by one-way analysis of variance and the significant differences of the means were inspected at the level of $P < 0.05$ according to the method of Duncan (39). When the activities of fatty acid oxidation enzymes were measured with acyl-CoA substrates differing in the degree of unsaturation (Expt. 2), data were analyzed by two-way analysis of variance to establish the significance of the interactions of types of dietary fats and acyl-CoA followed by statistical inspection as described above.

RESULTS

There were no significant differences in the average food intake among the groups of rats fed various dietary fats both in Expts. 1 (19.0–20.3 g/d) and 2 (20.6–21.3 g/d). The growth of animals was also comparable

among the groups of rats (49–55 g/7 d and 113–115 g/14 d for Expts. 1 and 2, respectively). Liver weight of animals fed safflower oil was slightly but significantly lower than that of the other groups in Expt. 1 (5.76 ± 0.19, 5.36 ± 0.11, and 5.80 ± 0.15 g/100 g body weight for rats fed coconut, safflower, and perilla oils, respectively). Although no significant difference was observed in Expt. 2, the liver weight in rats fed safflower oil (5.11 ± 0.12 g/100 g body weight) tended to be lower than that in the rats fed palm and perilla oils (5.39 ± 0.13 and 5.40 ± 0.15 g/100 g body weight, respectively).

Effects of dietary fats on the oxidation of acyl-CoA in liver homogenates

Table 2 shows the rates of mitochondrial and peroxisomal oxidation of 1-^{14}C-labeled fatty acyl-CoAs by the 500 *g* supernatant fraction of liver homogenates. In Expt. 1, the rates of mitochondrial and peroxisomal oxidation of [1-^{14}C]16:0-CoA were significantly higher in rats fed perilla oil than in those fed other fats both in terms of the specific (nmol/min per mg protein) or total activities (μmol/per liver per 100 g body weight). In Expt. 2, the activities were measured using 18:2- and

TABLE 2. Oxidation of acyl-CoA in liver homogenates of rats fed diets containing various fats differing in the degree of unsaturation

	Acyl-CoA Substrate		
Parameter	16:0	18:2	α-18:3
Expt. 1			
Mitochondrial β-oxidation			
Specific activity (nmol/min per mg protein)			
Coconut oil	0.308 ± 0.048[a]	ND	ND
Safflower oil	0.365 ± 0.087[a]	ND	ND
Perilla oil	0.656 ± 0.053	ND	ND
Total activity (μmol/min per liver per 100 g bw)			
Coconut oil	0.265 ± 0.035[a]	ND	ND
Safflower oil	0.324 ± 0.057[a]	ND	ND
Perilla oil	0.541 ± 0.044	ND	ND
Peroxisomal β-oxidation			
Specific activity (nmol/min per mg protein)			
Coconut oil	3.69 ± 0.27[a]	ND	ND
Safflower oil	3.62 ± 0.16[a]	ND	ND
Perilla oil	5.32 ± 0.09	ND	ND
Total activity (μmol/min per liver per 100 g bw)			
Coconut oil	2.94 ± 0.15[a]	ND	ND
Safflower oil	2.82 ± 0.16[a]	ND	ND
Perilla oil	4.38 ± 0.14	ND	ND
Expt. 2			
Mitochondrial β-oxidation			
Specific activity (nmol/min per mg protein)			
Palm oil	1.67 ± 0.17[a,c]	1.97 ± 0.17[a]	2.42 ± 0.25[a]
Safflower oil	2.15 ± 0.27[a,c]	2.62 ± 0.26[a,c]	3.36 ± 0.28[a]
Perilla oil	2.83 ± 0.27[c]	3.55 ± 0.27[c,d]	5.14 ± 0.53
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	1.03 ± 0.11[a,c]	1.22 ± 0.12[a]	1.54 ± 0.19[a]
Safflower oil	1.25 ± 0.17[a,c]	1.52 ± 0.17[a]	1.95 ± 0.19[a]
Perilla oil	1.83 ± 0.21[c]	2.31 ± 0.23[c]	3.34 ± 0.42
Peroxisomal β-oxidation			
Specific activity (nmol/min per mg protein)			
Palm oil	3.62 ± 0.11[a,c]	3.88 ± 0.10[a,c]	5.84 ± 0.16[a]
Safflower oil	4.44 ± 0.10[a,b,c]	5.38 ± 0.23[a,b,c,d]	7.67 ± 0.29[a,b]
Perilla oil	6.92 ± 0.25[c]	9.02 ± 0.43[c,d]	12.6 ± 0.4
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	2.24 ± 0.11[a,c]	2.41 ± 0.09[a,c]	3.62 ± 0.12[a]
Safflower oil	2.56 ± 0.08[a,c]	3.10 ± 0.16[a,b,c]	4.43 ± 0.20[a,b]
Perilla oil	4.49 ± 0.23[c]	5.84 ± 0.31[c,d]	8.17 ± 0.39

Rats were fed diets containing 15% of a saturated fat (palm and coconut oils for Expts. 1 and 2, respectively), a fat mixture composed of safflower and olive oils (94:8, w/w), or perilla oil. Feeding periods were 1 and 2 weeks for Expts. 1 and 2, respectively. Rates of oxidation of various acyl-CoA substrates were measured using the 500 *g* supernatant fraction of liver homogenates. Values are means ± SE of 8 and 7 rats for Expts. 1 and 2, respectively.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.
[b]Significantly different from the corresponding value in rats fed palm oil at $P < 0.05$.
[c]Significantly different from the corresponding value measured with α-18:3-CoA substrate at $P < 0.05$.
[d]Significantly different from the corresponding value measured with 16:0-CoA substrate at $P < 0.05$. ND, not determined.

α-18:3-CoAs in addition to 16:0-CoA as the substrates. Again, the rates of the mitochondrial and peroxisomal oxidation of 16:0-CoA were significantly higher in rats fed perilla oil than in those fed palm and safflower oils. The same tendencies were observed when 18:2- and α-18:3-CoAs were used as the substrates. In addition, safflower oil, compared to palm oil, slightly but significantly increased the rates of peroxisomal but not mitochondrial oxidation of various acyl-CoA except for one occasion (total activity measured with 16:0-CoA substrate). Although the differences were not always statistically significant, the rates of mitochondrial and peroxisomal oxidation of acyl-CoA were in the order of α-18:3 > 18:2 > 16:0 in the three groups of rats. The mitochondrial activity appeared to be considerably higher in Expt. 2 than in Expt. 1. As a result, the ratio of mitochondrial to peroxisomal activities in Expt. 2 using various acyl-CoA substrates (0.39 to 0.52) was considerably higher than that observed in Expt. 1 using 16:0-CoA substrate (0.09 to 0.12), while the ratio was not essentially modified by types of dietary fats except for one occasion using the 18:2-CoA substrate in Expt. 2. In this occasion, the ratio in rats fed palm oil (0.52 ± 0.05) was significantly higher than that in the animals fed perilla oil (0.39 ± 0.02), but was indistinguishable from that in the animals fed safflower oil (0.47 ± 0.04).

Effects of dietary fats on the activities of enzymes in fatty acid metabolism

Table 3 shows the activities of various enzymes involved in the β-oxidation pathway in rats fed palm, safflower, and perilla oils (Expt. 2). The activities of the enzymes other than acyl-CoA oxidase were measured in isolated mitochondria. To express the activities of enzymes measured in isolated mitochondria in terms of total activity, the activities of a marker enzyme for mitochondria (succinate dehydrogenase) were measured both in the 500 *g* supernatant and mitochondrial fractions and the activities of succinate dehydrogenase found in the isolated mitochondria fraction were used to correct for the recovery of the cell organelles from the 500 *g* supernatant fraction. Recoveries of mitochondria calculated in this way were 73.0 ± 2.8, 70.5 ± 5.5, and 77.9 ± 5.3% for rats fed palm, safflower, and perilla oils, respectively (the differences were statistically insignificant). The amount of mitochondrial protein recovered from the 500 *g* supernatant fraction in rats fed perilla oil (121 ± 8 mg/liver per 100 g body weight) was significantly higher than that in the other groups (89.1 ± 7.1 and 91.9 ± 5.0 mg/liver per 100 g body weight for rats fed palm and safflower oils, respectively). When these values were corrected for the recoveries of mitochondria, the value in rats fed perilla oil (158 ± 10 mg/liver 100 g body weight) was still significantly higher than that in those fed palm oil (123 ± 11 mg/liver 100 g body weight). Although no significant difference was detected for the corrected values between rats fed safflower (135 ± 13 mg/liver 100 g body weight) and perilla oil diets, it was still tended to be higher in the latter.

When 16:0- and 18:2-CoAs were the substrates, the activities of carnitine palmitoyltransferase I were significantly higher in the perilla oil group than in the other groups, both in terms of specific and total activity. However, no significant differences were detected in the specific activity among the groups when α-18:3-CoA was the substrate. As there was a considerable increase in the amount of mitochondrial protein in rats fed perilla oil relative to those fed palm oil and safflower oil, the total activity measured using α-18:3-CoA became significantly higher in the perilla oil group compared to the other groups. The significant difference in the enzyme activity was detected between rats fed palm oil and safflower oil only in case of specific activity measured with 18:2-CoA substrate. The activity of carnitine palmitoyltransferase measured spectrophotometrically using Triton-solubilized mitochondrial preparations was significantly higher in rats fed perilla oil than in those fed other oils irrespective of the units and the types of acyl-CoA substrates employed. Specific activity was also significantly higher in rats fed safflower oil than in those fed palm oil except for one occasion (the activity measured with 18:2-CoA). However, the total activity of this enzyme was not different in these groups. The activities of both carnitine palmitoyltransferase I and carnitine palmitoyltransferase, measured spectrophotometrically, were the highest with α-18:3-CoA, the lowest with 16:0-CoA, and intermediate with 18:2-CoA as the substrates in all groups.

Sensitivities to malonyl-CoA of carnitine palmitoyltransferase I is known to be modified by nutritional and physiological situations (8, 40–45). Thus, the activities of mitochondrial carnitine palmitoyltransferase I were measured with varying concentrations of malonyl-CoA (0.5, 1.0, 3.0, 5.0, and 8.0 μM) using palmitoyl-CoA as an acyl-donor in Expt. 2. The reactions were started by adding mitochondrial to the mixture containing varying concentrations of malonyl-CoA. As expected, malonyl-CoA progressively decreased the activity of this enzyme dose-dependently. The extents of the reductions in the presence of malonyl-CoA at the concentrations higher than 3 μM were significantly lower in rats fed palm oil than in those fed safflower and perilla oils, the values in the latter two groups being indistinguishable (data not shown). The enzyme activity measured in the presence of 8 μM malonyl-CoA was 14.6 ± 0.2% of that observed in the absence of the inhibitor in rats fed the saturated fat. The corresponding values for safflower and perilla oil groups were 21.8 ± 2.3 and 22.6 ± 0.7%, respectively.

The concentration of malonyl-CoA that caused 50% inhibition of the enzyme activity in rats fed palm oil (1.59 ± 0.05 μM) was also significantly lower than in those fed safflower oil (2.19 ± 0.15 μM) and perilla oil (2.09 ± 0.11 μM).

Both specific and total activities of mitochondrial acyl-CoA dehydrogenase in rats fed perilla oil were again significantly higher than in those fed other oils. When the enzyme activity was compared in terms of nmol/min per mg protein, it was significantly lower with the 18:2-CoA substrate than with 16:0- and α-18:3-CoAs in all groups. However, no such substrate-dependent differences were detected when the activities were expressed as total activity except for one occasion (between the activities measured with 16:0-CoA and 18:2-CoA in perilla oil group).

Specific and total activities of acyl-CoA oxidase measured in the 500 *g* supernatant fraction of liver homogenates using various acyl-CoA substrates were significantly higher in the perilla oil group than in the other groups. The specific but not total activities using different acyl-CoA substrates were significantly higher in rats fed safflower oil than in those fed palm oil.

Table 4 summarizes the activities of 2,4-dienoyl-CoA reductase measured in both 500 *g* and mitochondrial fractions of liver homogenates. Polyunsaturated fats relative to palm oil significantly increased both specific and total activities of the enzyme measured in both

TABLE 3. Activities of mitochondrial and peroxisomal enzymes in fatty acid oxidation in the liver of rats fed diets containing various fats differing in the degree of unsaturation (Expt. 2)

	Acyl-CoA Substrate		
Dietary Fat and Enzyme	16:0	18:2	α-18:3
Carnitine palmitoyltransferase I[1]			
Specific activity (nmol/min per mg protein)			
Palm oil	6.15 ± 0.28[a,c]	9.41 ± 0.49[a,c,d]	12.7 ± 0.5
Safflower oil	6.56 ± 0.25[a,c]	10.6 ± 0.5[a,b,c,d]	13.3 ± 0.5
Perilla oil	7.49 ± 0.15[c]	11.7 ± 0.2[c,d]	13.7 ± 0.4
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	0.71 ± 0.08[a,c]	1.13 ± 0.11[a,c,d]	1.55 ± 0.14[a]
Safflower oil	0.88 ± 0.08[a,c]	1.45 ± 0.10[a,c,d]	1.83 ± 0.15[a]
Perilla oil	1.23 ± 0.08[c]	1.90 ± 0.12[d]	2.17 ± 0.16
Carnitine palmitoyltransferase[2]			
Specific activity (nmol/min per mg protein)			
Palm oil	15.6 ± 0.7[a,c]	22.0 ± 0.9[a,c,d]	29.8 ± 1.7[a]
Safflower oil	19.3 ± 0.7[a,b,c]	27.3 ± 1.3[a,c,d]	37.0 ± 0.5[a,b]
Perilla oil	25.5 ± 1.5[c]	40.8 ± 3.1[c,d]	48.4 ± 2.2
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	2.01 ± 0.22[a,c]	2.62 ± 0.32[a,c]	4.03 ± 0.41[a]
Safflower oil	2.58 ± 0.23[a,c]	3.67 ± 0.39[a,c]	4.99 ± 0.47[a]
Perilla oil	3.84 ± 0.31[c]	6.40 ± 0.55[c,d]	7.84 ± 0.63
Acyl-CoA dehydrogenase			
Specific activity (nmol/min per mg protein)			
Palm oil	53.2 ± 1.7[a]	41.8 ± 2.2[a,c,d]	51.6 ± 1.6[a]
Safflower oil	50.2 ± 2.2[a]	40.4 ± 2.6[a,c,d]	49.4 ± 1.5[a]
Perilla oil	64.1 ± 2.5	51.1 ± 2.8[c,d]	61.4 ± 2.7
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	6.65 ± 0.51[a]	5.45 ± 0.66[a]	6.32 ± 0.53[a]
Safflower oil	6.98 ± 0.71[a]	5.80 ± 0.77[a]	6.74 ± 0.77[a]
Perilla oil	9.94 ± 0.61	7.77 ± 0.47[d]	9.46 ± 0.59
Acyl-CoA oxidase			
Specific activity (nmol/min per mg protein)			
Palm oil	2.49 ± 0.10[a,c]	3.64 ± 0.12[a,d]	3.50 ± 0.12[a]
Safflower oil	3.04 ± 0.15[a,b,c]	4.99 ± 0.19[a,b,d]	4.55 ± 0.37[a,b]
Perilla oil	3.72 ± 0.20[c]	7.48 ± 0.57[d]	6.57 ± 0.44
Total activity (μmol/min per liver per 100 g bw)			
Palm oil	1.59 ± 0.08[a,c]	2.31 ± 0.11[a,d]	2.30 ± 0.14[a]
Safflower oil	1.75 ± 0.10[a,c]	2.79 ± 0.15[a,d]	2.62 ± 0.22[a]
Perilla oil	2.35 ± 0.14[c]	4.65 ± 0.31[d]	4.10 ± 0.32

Rats were fed diets containing 15% of palm oil, a fat mixture composed of safflower and olive oils (94:8, w/w), or perilla oil for 2 weeks. Values are means ± SE of 7 rats. Activities of various enzymes except for acyl-CoA oxidase were assayed using isolated mitochondria as the enzyme source. Acyl-CoA oxidase activity was measured using the 500 *g* supernatant fraction of liver homogenates.

[1]Carnitine palmitoyltransferase activity measured radiochemically using freshly isolated mitochondria.

[2]Carnitine palmitoyltransferase activity measured spectrophotometrically using Triton-solubilized mitochondria.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed palm oil at $P < 0.05$.

[c]Significantly different from the corresponding value measured with α-18:3-CoA substrate at $P < 0.05$.

[d]Significantly different from the corresponding value measured with 16:0-CoA substrate at $P < 0.05$.

TABLE 4. Activities of 2,4-dienoyl-CoA reductase measured in the 500 *g* supernatant and mitochondrial fractions of liver homogenates in rats fed diets containing various fats differing in the degree of unsaturation (Expt. 2)

Fraction	Dietary Fat		
	Palm Oil	Safflower Oil	Perilla Oil
500 g Supernatant			
Specific activity (nmol/min per mg protein)	8.73 ± 0.77[a]	17.7 ± 1.4[a,b]	26.4 ± 3.1
Total activity (μmol/min per liver per 100 g bw)	4.99 ± 0.18[a]	10.2 ± 0.8[a,b]	16.9 ± 1.8
Mitochondria			
Specific activity (nmol/min per mg protein)	32.7 ± 2.2[a]	44.6 ± 3.0[a,b]	53.4 ± 3.1
Total activity (μmol/min per liver per 100 g bw)	3.71 ± 0.41[a]	5.41 ± 0.50[a,b]	8.36 ± 0.57

Experimental condition was the same as described in the footnote of Table 3. Values are means ± SE of 7 rats.

[a]Significantly different from the corresponding values in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed palm oil at $P < 0.05$.

fractions as the enzyme sources. The extent of the increase was more greater with perilla oil than with safflower oil. The mitochondrial activity accounted for 75.7 ± 7.4% of the activity found in the 500 *g* supernatant fraction in rats fed palm oil, while it was significantly lower with polyunsaturated fats (59.2 ± 5.1 and 51.4 ± 3.1% for rats fed safflower and perilla oils, respectively).

As shown in **Table 5,** polyunsaturated fats relative to saturated fats significantly decreased the activities of enzymes in fatty acid synthesis both in Expts. 1 and 2. However, no significant differences in the activities of various enzymes were observed between rats fed safflower and perilla oils in both trials.

Effects of dietary fats on the activities of marker enzymes for cell organelles

Table 6 summarizes the activities of marker enzymes for cell organelles measured in the 500 *g* supernatant fraction of liver homogenates. Although the differences were statistically insignificant, both specific and total activities of succinate dehydrogenase (a marker enzyme for mitochondria) increased as the degree of unsaturation of dietary fat increased. The specific activities of succinate dehydrogenase measured in isolated mitochondria (329 ± 12, 307 ± 13, and 329 ± 19 nmol/min per mg protein for rats fed palm, safflower, and perilla oils, respectively) were 4 to 5 times higher than those measured in the 500 *g* supernatant fraction, and no significant dietary fat-dependent differences were detected. Two types of polyunsaturated fats compared to palm oil significantly increased the specific and total activities of catalase (a marker enzyme of peroxisomes). Perilla oil compared to safflower oil was more effective in this respect, and the difference between these groups was also statistically significant. Dietary fat did not influence the specific and total activities of lactate dehydrogenase (a marker enzyme of cytosols).

Effects of dietary fats on tissue lipid concentrations

Table 7 summarizes the results of liver and serum lipid analyses in rats fed various fats. Polyunsaturated fats compared to saturated fats significantly decreased the concentration and content of triacylglycerol in the liver in both experiments. In Expt. 1, the hepatic choles-

TABLE 5. Activities of enzymes in fatty acid synthesis in the liver of rats fed diets containing various fats differing in the degree of unsaturation

Dietary Fat	Glucose 6-Phosphate Dehydrogenase		Fatty Acid Synthetase		Acetyl-CoA Carboxylase	
	Specific Activity (nmol/min per mg protein)	Total Activity (μmol/min per liver per 100 g bw)	Specific Activity (nmol/min per mg protein)	Total Activity (μmol/min per liver per 100 g bw)	Specific Activity (nmol/min per mg protein)	Total Activity (μmol/min per liver per 100 g bw)
Expt. 1						
Coconut oil	76.4 ± 10.0[a]	43.4 ± 3.8[a]	19.2 ± 2.8[a]	10.9 ± 1.0[a]	2.02 ± 0.20[a]	1.12 ± 0.08[a]
Safflower oil	54.0 ± 4.12[b]	24.7 ± 2.2[b]	8.67 ± 0.83[b]	4.30 ± 0.61[b]	1.49 ± 0.13[b]	0.78 ± 0.07[b]
Perula oil	58.0 ± 5.2	27.7 ± 1.9	8.86 ± 0.99	4.48 ± 0.40	1.49 ± 0.12	0.77 ± 0.04
Expt. 2						
Palm oil	57.4 ± 9.6[a]	33.6 ± 5.9[a]	13.1 ± 1.3[a]	7.02 ± 0.80[a]	ND	ND
Safflower oil	27.0 ± 2.7[b]	13.7 ± 1.5[b]	6.62 ± 0.76[b]	3.72 ± 0.40[b]	ND	ND
Perilla oil	29.4 ± 3.1	15.0 ± 2.0	8.76 ± 0.58	4.55 ± 0.28	ND	ND

Experimental conditions was the same as described in the footnote of Table 2. Values are means ± SE of 8 and 7 rats for Expts. 1 and 2, respectively.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed saturated fats (coconut and palm oils for Expts. 1 and 2, respectively) at $P < 0.05$.

TABLE 6. Activities of marker enzymes for liver cell organelles in rats fed diets containing various fats differing in the degree of unsaturation (Expt. 2)

Enzyme	Dietary Fat		
	Palm Oil	Safflower Oil	Perilla Oil
Succinate dehydrogenase			
Specific activity (nmol/min per mg protein)	65.3 ± 5.7	71.2 ± 5.9	79.7 ± 6.6
Total activity (μmol/min per liver per 100 g bw)	40.9 ± 4.3	41.3 ± 4.0	51.8 ± 5.1
Catalase			
Specific activity (κ/mg protein)	0.13 ± 0.01[a]	0.18 ± 0.01[a,b]	0.21 ± 0.01
Total activity (κ/per liver per 100 g bw)	79.1 ± 7.4[a]	102 ± 7[a,b]	138 ± 6
Lactate dehydrogenase			
Specific activity (μmol/min per mg protein)	3.34 ± 0.10	3.45 ± 0.11	3.33 ± 0.14
Total activity (μmol/min per liver per 100 g bw)	2082 ± 111	1985 ± 54	2157 ± 109

Experimental condition was the same as that described in the footnote of Table 3. Values are means ± SE of 7 rats.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed palm oil at $P < 0.05$.

terol concentration but not the content was significantly higher in rats fed the perilla oil than in those fed other oils, while both concentration and content became comparable among the groups when rats were fed various fats for 2 weeks (Expt. 2). The phospholipid concentration and content became significantly higher in rats fed perilla oil than in the other groups in both experiments. Polyunsaturated fats relative to either coconut (Expt. 1) or palm oil (Expt. 2) significantly decreased the concentrations of triacylglycerol, cholesterol, and phospholipid in the serum. Perilla oil was more effective than safflower oil in decreasing these lipids in both experiments. Free fatty acid concentrations in rats fed perilla oil were significantly lower than in those fed other oils irrespective of the feeding period.

Table 8 shows the fatty acid compositions of liver triacylglycerol. Dietary polyunsaturated fats relative to saturated fats greatly increased the proportions of polyunsaturated fatty acids in both experiments. However, the extent of the increase was attenuated in rats fed perilla oil compared to safflower oil.

Effects of serum albumin on the rate of oxidation of acyl-CoA substrates and the activity of carnitine palmitoyltransferase I

We used the method of Mannaerts et al. (21) to measure the mitochondrial and peroxisomal β-oxidation in the 500 *g* supernatant fraction of liver homogenates. They reported that mitochondrial activity is stimulated by albumin, while the peroxisomal activity is maximal in the absence of albumin. Thus, in the present study we added 7.2 mg/ml albumin, which corresponds to the substrate/albumin ratio of 1.67, to measure the mitochondrial oxidation of various fatty acyl-CoAs. On

TABLE 7. Concentrations of liver and serum lipids in rats fed diets containing various fats differing in the degree of unsaturation

Lipid;	Dietary Fat					
	Expt. 1			Expt. 2		
	Coconut Oil	Safflower Oil	Perilla Oil	Palm Oil	Safflower Oil	Perilla Oil
Liver lipids						
Concentration (μmol/g)						
Triacylglycerol	51.9 ± 4.2[a]	30.2 ± 1.5[b]	28.9 ± 2.4	56.8 ± 6.2[a]	32.3 ± 2.0[b]	24.4 ± 1.0
Cholesterol	7.30 ± 0.58[a]	7.04 ± 0.30[a]	7.70 ± 0.32	6.60 ± 0.33	6.50 ± 0.20	6.30 ± 0.44
Phospholipid	37.4 ± 1.0[a]	37.2 ± 0.4[a]	40.3 ± 0.7	38.9 ± 0.2[a]	39.4 ± 0.6[a]	43.7 ± 0.9
Content (μmol/g per 100 g bw)						
Triacylglycerol	304 ± 4.2[a]	157 ± 9[b]	169 ± 14	313 ± 39[a]	168 ± 10[b]	132 ± 8
Cholesterol	42.3 ± 3.4	38.9 ± 1.2	43.5 ± 2.0	37.5 ± 1.6[a]	33.3 ± 1.5	32.0 ± 1.6
Phospholipid	215 ± 2[a]	200 ± 5[a,b]	230 ± 3	211 ± 5[a]	204 ± 5[a]	233 ± 7
Serum lipids						
Concentration (μmol/dl)						
Triacylglycerol	456 ± 32[a]	212 ± 37[a,b]	126 ± 20	574 ± 44[a]	223 ± 21[a,b]	129 ± 19
Cholesterol	346 ± 10[a]	288 ± 10[a,b]	238 ± 14	336 ± 16[a]	275 ± 8[a,b]	225 ± 7
Phospholipid	356 ± 15[a]	259 ± 7[a,b]	223 ± 9	270 ± 4[a]	193 ± 5[a,b]	154 ± 4
Free fatty acid	137 ± 9[a]	134 ± 27[a]	82.3 ± 11	193 ± 15[a]	133 ± 11[a,b]	91.8 ± 11.2

Experimental condition was the same as those described in the footnote of Table 2. Values are means ± SE of 8 and 7 rats for Expts. 1 and 2, respectively.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed saturated fats (coconut and palm oils for Expts. 1 and 2, respectively) at $P < 0.05$.

TABLE 8. Fatty acid compositions of triacylglycerol in the liver of rats fed diets containing various fats differing in the degree of unsaturation

	Dietary Fat					
	Expt. 1			Expt. 2		
Fatty Acid	Coconut Oil	Safflower Oil	Perilla Oil	Palm Oil	Safflower Oil	Perilla Oil
	weight %					
12:0	1.37 ± 0.20[a]	0.02 ± 0.00[b]	0.01 ± 0.00	0.03 ± 0.01[a]	0.01 ± 0.00[b]	0.01 ± 0.00
14:0	5.23 ± 0.27[a]	0.52 ± 0.03[b]	0.48 ± 0.02	1.42 ± 0.06[a]	0.69 ± 0.03[a,b]	0.54 ± 0.03
16:0	39.4 ± 0.6[a]	25.3 ± 1.0[b]	26.9 ± 1.0	36.6 ± 0.6[a]	24.4 ± 1.0[b]	25.3 ± 1.2
16:1 (n-7)	10.4 ± 0.4[a]	2.46 ± 0.25[a,b]	4.22 ± 0.36	8.29 ± 0.35[a]	2.40 ± 0.23[a,b]	3.87 ± 0.31
18:0	2.42 ± 0.08	2.08 ± 0.11[a,b]	2.73 ± 0.14	1.75 ± 0.06[a]	2.00 ± 0.08[a]	3.00 ± 0.15
18:1 (n-9)	39.5 ± 0.5	25.0 ± 0.7[a,b]	38.9 ± 1.1	48.3 ± 0.8[a]	23.2 ± 0.9[a,b]	36.1 ± 1.5
18:2 (n-6)	1.43 ± 0.12[a]	40.7 ± 1.6[a,b]	10.6 ± 0.6	3.19 ± 0.17[a]	41.5 ± 1.6[a,b]	10.9 ± 0.5
18:3 (n-6)	0.01 ± 0.00	0.87 ± 0.04[a,b]	0.06 ± 0.02	0.06 ± 0.00	0.69 ± 0.05[a,b]	0.04 ± 0.01
18:3 (n-3)	0.19 ± 0.02[a]	0.34 ± 0.06[a]	14.6 ± 1.2	0.21 ± 0.02[a]	0.49 ± 0.05[b]	17.8 ± 1.7
20:3 (n-6)	0.01 ± 0.00	0.30 ± 0.05[a,b]	0.08 ± 0.00	0.01 ± 0.01	0.47 ± 0.08[a,b]	0.09 ± 0.02
20:4 (n-6)	0.05 ± 0.01	1.38 ± 0.20[a,b]	0.24 ± 0.04	0.12 ± 0.01	2.37 ± 0.33[a,b]	0.36 ± 0.06
20:5 (n-3)	0.01 ± 0.01[a]	0.03 ± 0.01[a]	0.48 ± 0.16	0.06 ± 0.01[a]	0.10 ± 0.02[a]	0.86 ± 0.24
22:4 (n-6)	0.01 ± 0.00	0.54 ± 0.08[a,b]	0.01 ± 0.00	0.01 ± 0.01	1.02 ± 0.18[a,b]	0.03 ± 0.01
22:5 (n-6)	0.01 ± 0.00	0.28 ± 0.05[a,b]	0.01 ± 0.00	0.00 ± 0.00	0.45 ± 0.09[a,b]	0.00 ± 0.00
22:5 (n-3)	0.01 ± 0.00[a]	0.03 ± 0.01[a]	0.44 ± 0.15	0.00 ± 0.00[a]	0.04 ± 0.01[a]	0.59 ± 0.27
22:6 (n-3)	0.02 ± 0.01[a]	0.16 ± 0.02[b]	0.25 ± 0.07	0.00 ± 0.00[a]	0.15 ± 0.03[a]	0.49 ± 0.16
Total PUFA	1.75 ± 0.14[a]	44.7 ± 1.9[a,b]	26.8 ± 2.1	3.66 ± 0.20[a]	47.3 ± 2.1[a,b]	31.2 ± 2.8

Experimental condition was the same as described in the footnote of Table 2. Values are means ± SE of 8 and 7 rats for Expts. 1 and 2, respectively.

[a]Significantly different from the corresponding value in rats fed perilla oil at $P < 0.05$.

[b]Significantly different from the corresponding value in rats fed saturated fats (coconut and palm oils for the Expts. 1 and 2, respectively) at $P < 0.05$.

the other hand, peroxisomal activity was assayed in the absence of albumin to measure its maximal activity. The results obtained indicated that α-18:3-CoA is oxidized faster than 16:0-CoA and 18:2-CoA in both mitochondrial and peroxisomal pathways (see Table 2). To confirm this observation, the rates of oxidation of acyl-CoAs in liver homogenates were measured in the presence of 0.2 mM substrates and varying concentrations of serum albumin. The rats at 7–8 weeks of age given a commercial nonpurified diet were used in this experiment. As shown in **Fig. 1,** maximal mitochondrial activities measured with various acyl-CoAs were obtained in the presence of 7.2 mg/ml of albumin (fatty acyl-CoA/albumin molar ratio of 1.67), and the activities decreased as the albumin concentrations decreased. No detectable activities were noted in the absence of albumin in all acyl-CoA substrates examined. On the contrary, the peroxisomal activity progressively increased as the concentrations of albumin decreased and the maximal value was obtained in the absence of albumin. Mitochondrial and peroxisomal activities obtained with α-18:3-CoA were significantly higher ($P < 0.05$) than those observed with 16:0-CoA and 18:2-CoA at various substrate/albumin ratios except for the mitochondrial activity measured in the absence of albumin. The rates obtained with 18:2-CoA at the various substrate/albumin ratios were also higher than those measured with 16:0-CoA, and significant differences were obtained at the substrate/albumin molar ratios of 1.67, 3.33, and 6.67 for mitochondrial and of 0.833, 1.67, 3.33 and in the absence of albumin for peroxisomal activities, respectively. The mitochondrial activities obtained with various acyl-CoA substrates at the substrate/albumin ratio of 1.67 were more than 5 times higher than the corresponding values for peroxisomal activities measured at the same substrate/albumin ratio. However, the peroxisomal activities measured in the absence of albumin were even higher than mitochondrial activities measured at the substrate/albumin ratio of 1.67 giving the maximal activity in all acyl-CoA substrates.

Acyl-CoA oxidase activities were not necessarily high enough to account for the peroxisomal oxidation of various acyl-CoAs measured in the absence of albumin in all groups of rats (compare Tables 2 and 3). However, we measured peroxisomal β-oxidation and the activity of acyl-CoA oxidase at different temperatures (37° and 30°C for the former and the latter, respectively) in this study. The acyl-CoA oxidase activities measured at 37°C using the 500 *g* supernatant fraction of liver homogenates from rats fed laboratory chow (n = 4) were approximately two times higher than those measured at 30°C (5.0 ± 0.4, 6.6 ± 0.2, and 6.9 ± 0.6 vs. 2.5 ± 0.1, 3.6 ± 0.2, and 3.6 ± 0.2 nmol/min per mg protein using 16:0-, 18:2-, and α-18:3-CoAs as the substrates, respectively). The former values were higher than the rates of the oxidation of various acyl-CoA substrates via the peroxisomal pathway measured at 37°C in the absence of albumin (2.7 ± 0.2, 3.3 ± 0.1, and 5.3 ± 0.1 nmol/min per mg protein for 16:0-, 18:2-, and α-18:3-CoAs, respectively). Serum albumin added at the concentration of 0.2

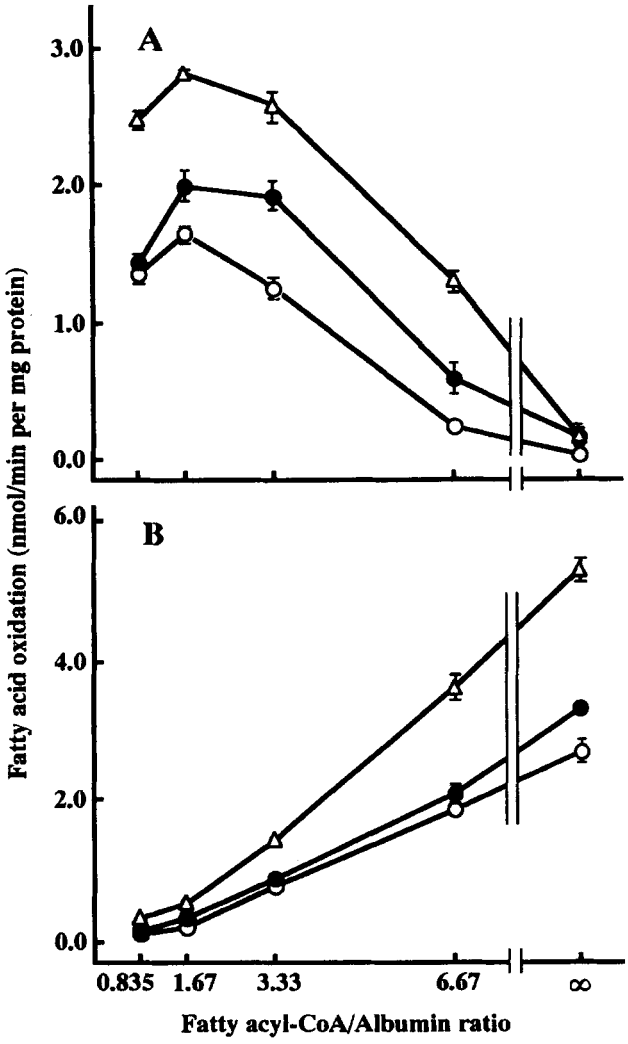

Fig. 1. Rates of mitochondrial and peroxisomal oxidation of fatty acyl-CoA substrates differing in the degree of unsaturation in the 500 *g* supernatant fraction of liver homogenates as a function of the substrate/albumin molar ratio. The oxidation rates of fatty acyl-CoAs added to the assay media at the concentration of 0.2 mM were measured in the presence of varying concentrations of serum albumin (0–14.4 mg/ml, the substrate/albumin molar ratios ranging from 0.835 to ;in). Panels A and B showed mitochondrial and peroxisomal oxidation of fatty acyl-CoA substrates, respectively; ○, 16:0-CoA; ●, 18:2-CoA; △, α-18:3-CoA. Values represent means ± SE of 5 and 4 rats for mitochondrial and peroxisomal activities, respectively.

mg/ml in the assay mixture for the analysis of acyl-CoA oxidase (see Materials and Methods) did not affect the activities irrespective of the assay temperature in all substrates (data not shown).

We measured the carnitine palmitoyltransferase I activities in the presence of 50 μM of various acyl-CoA substrates and 4 mg/ml of serum albumin (acyl-CoA/albumin ratio of 0.75), and found that the activities were dependent on the types of the acyl-CoA substrates. It has also been reported that the concentration of albumin is a factor influencing the activity of carnitine palmitoyltransferase I (46). Thus, we measured the enzyme activity in rats fed a commercial diet in the presence of varying concentrations of serum albumin. As shown in **Fig. 2,** the enzyme activities measured with various acyl-CoAs increased as the substrate/albumin ratio increased. The activities obtained with α-18:3-CoA were significantly higher ($P < 0.05$) than those observed with 16:0- and 18:2-CoAs under various substrate/albumin ratios. Although the differences were not statistically insignificant, the values obtained with 18:2-CoA also tended to be higher than those measured with 16:0-CoA at the various substrate/albumin ratios.

DISCUSSION

Effect of dietary α-linolenic acid on fatty acid oxidation enzymes in rat liver

The present study for the first time presented the evidence that dietary perilla oil rich in α-linolenic acid increased the activities of peroxisomal and mitochondrial enzymes involved in fatty acid oxidation pathway

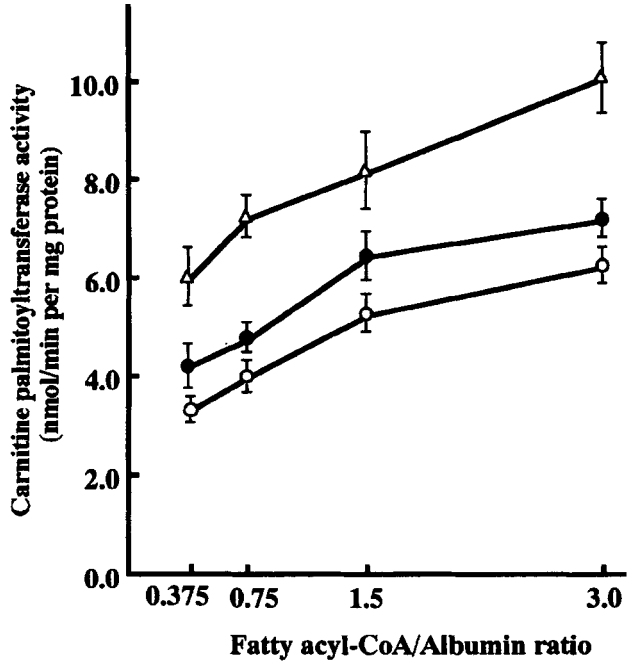

Fig. 2. Activities of carnitine palmitoyltransferase I in isolated mitochondria measured with fatty acyl-CoA substrates differing in the degree of unsaturation as a function of the substrate/albumin molar ratio. The activity of carnitine palmitoyltransferase I in isolated mitochondria in the presence 50 μM of fatty acyl-CoA was measured in the presence of varying concentrations of serum albumin (1–8 mg/ml, the substrate/albumin molar ratios ranging from 0.375 to 3); ○, 16:0-CoA; ●, 18:2-CoA; △, α-18:3-CoA. Values represent means ± SE

in the liver. Thus, it is apparent that not only 20:5 (n–3) and 22:6 (n–3) acids (1–5, 8, 9) but also 18:3 (n–3) can induce hepatic fatty acid oxidation pathway.

We measured the rates of mitochondrial and peroxisomal β-oxidation of fatty acids using the 500 *g* supernatant fraction of liver homogenates in rats fed purified diets containing various fats according to the method of Mannaerts et al. (21). They reported that serum albumin differently affected the mitochondrial and peroxisomal oxidation of palmitoyl-CoA and oleic acid in liver homogenates. Thus, mitochondrial β-oxidation was increased by added albumin, but the peroxisomal activity was maximum in the absence of albumin. When both mitochondrial and peroxisomal activities were assayed in the presence of 0.2 mM palmitoyl-CoA and 7.2 mg/ml albumin (substrate/albumin molar ratio of 1.67), the rate of oxidation of palmitoyl-CoA via the peroxisomal pathway in the liver homogenates was approximately 30% of the mitochondrial activities in rats fed a laboratory chow. However, the peroxisomal activity measured in the absence of albumin was 3 times higher than the activity of the mitochondrial pathway measured in the presence of 7.2 mg/ml albumin. This finding was essentially confirmed in the present study, in which the peroxisomal activity measured in the absence of albumin was much higher than the mitochondrial activity measured with albumin at the concentration corresponding to the substrate/albumin molar ratio of 1.67. The peroxisomal activity measured in the absence of albumin may represent the maximal capacity of this organell to oxidize fatty acids but may not represent the actual in vivo rate in the liver. It has been considered that the peroxisomal β-oxidation pathway is mainly concerned in the metabolism of very long-chain fatty acids, dicarboxylic acid, prostaglandins, xenobiotics, and bile acids rather than that of regular fatty acids (40). In fact, the early studies using isolated hepatocytes or perfused liver indicated the minor importance of the peroxisomal pathway for β-oxidation of fatty acids (21, 47).

As the first step of peroxisomal but not mitochondrial β-oxidation involves the reduction of O_2 to H_2O_2, Mannaerts et al. (21) measured the rate of the H_2O_2 formation in the presence of fatty acid substrates to estimate the contribution of the peroxisomal pathways in isolated rat hepatocytes. This study suggested that the contribution of peroxisomes to the oxidation of palmitate or oleate was less than 10% in hepatocytes isolated from control and clofibrate-fed rats. Foerster et al. also (47) determined the H_2O_2 formation after the addition of various fatty acids differing in chain-length in perfused rat liver. They showed that H_2O_2 production accounted for less than one acetyl unit formation by peroxisomes per mole of added fatty acids even when rats were treated with a peroxisomal proliferator, benzafibrate.

However, more recent studies (48–50) indicated that peroxisomes make a significant contribution to fatty acid oxidation in the liver. Handler and Thurman (48) compared the rates of ketone body and H_2O_2 productions in the presence of varying concentrations of oleate as the markers for mitochondrial and peroxisomal oxidation of fatty acids in perfused rat liver, and found that the rates of ketone body production from oleate were half-maximal at around 0.13 mM, while the rates of H_2O_2 production required higher concentrations of oleate with a half-maximal at around 0.65 mM. They suggested that fatty acids are preferentially transported to mitochondria for oxidation when available at low concentrations but may also be handled by peroxisomes when available at high concentrations. Based on these observations, Handler and Thurman (48) estimated that hepatic fatty acid oxidation was mediated about 25% via the peroxisomal pathway in the presence of 1–2 mM oleate. They also suggested that a low yield of H_2O_2 after the addition of fatty acids in the study of Foerster et al. (47) was due to the nonphysiologically low concentrations of fatty acids used.

Kondrup and Lazarow (49), using a novel approach to compare the metabolic fates of 1-^{14}C-labeled acetate, butyrate, and palmitate, estimated that approximately 32% of palmitate oxidation took place in peroxisomes in hepatocytes isolated from rats fed laboratory chow. Rognstad (50) monitored the relative importance of peroxisomal and mitochondrial pathways using different techniques and estimated that 18–23% of palmitate oxidation was started by peroxisomes in hepatocytes isolated from rats fed laboratory chow. In hepatocytes from clofibrate-fed rats, the amount of palmitate or oleate handled by peroxisomal pathway was comparable with that handled by mitochondria (50). Thus, although the relative contribution of peroxisomes to fatty acid oxidation is still controversial, the recent studies at least indicate a significant contribution by the peroxisome pathway to fatty acid oxidation in rat liver.

The present and other observations (1–5) that the types of the dietary fats affect the activities of fatty acid oxidation enzymes both in mitochondrial and peroxisomal pathways imply a significant role of peroxisomes in metabolizing dietary fatty acids. However, in contrast to the situation in rats fed clofibrate (21), the ratio of the mitochondrial to peroxisomal activity was not essentially modified by the dietary fat in our study. It is therefore likely that dietary α-linolenic acid coordinately induces both mitochondrial and peroxisomal enzymes. At any rate, experiments using intact organ or isolated hepatocytes are required to assess the peroxisomal contribution in rats fed various dietary fats.

Degradation of unsaturated fatty acids via β-oxidation pathway to yield acetyl-CoA requires several auxiliary

enzymes in addition to those required for β-oxidation of saturated fatty acids (40, 51, 52). 2,4-Dienoyl-CoA reductase is a enzyme that locates both in mitochondria and peroxisomes (27, 40, 51–55) and is required for the degradation by β-oxidation pathway of unsaturated fatty acids having double bonds at even-numbered positions (40, 51, 52). Also, a recent study indicates the participation of 2,4-dienoyl-CoA reductase in the β-oxidation of unsaturated fatty acids with double bonds at odd-numbered positions in isolated rat liver mitochondria (56). Available evidence indicates that the reaction catalyzed by this enzyme is a rate-limiting step in the β-oxidation of polyunsaturated fatty acids both in isolated mitochondria (55, 57) and in peroxisomes (58).

It is thus expected that 2,4-dienoyl-CoA reductase activity could be modified by dietary fats. Consistent with this assumption, Borrebaek et al. (53) reported that the experimental diets containing 15% of partially hydrogenated marine oil and soybean oil relative to a low fat diet (laboratory chow) increased the reductase activity in rat liver mitochondria. The present study showed that the activity of this enzyme measured both in 500 *g* supernatant and mitochondrial fractions of the liver increased as the degree of unsaturation of dietary fats increased. This observation indicates a crucial role of the reductase in degradating dietary polyunsaturated fatty acids. Contribution of the mitochondrial activity to the activity of 500 *g* supernatants became significantly lower in rats fed polyunsaturated fats compared to those fed saturated fat in the present study, suggesting that the peroxisomal β-oxidation pathway plays a significant role in the degradation of polyunsaturated fatty acids. The mitochondrial activity represented merely 50% of the enzyme activity that was found in the 500 *g* supernatant of liver homogenates when rats were fed polyunsaturated fats. This is not surprising because of the observation (59) that the total activities of peroxisomal 2,4-dienoyl-CoA reductase exceeded that of the mitochondrial activity even in rats fed laboratory chow.

Malonyl-CoA-mediated inhibition of carnitine palmitoyltransferase I represents a mechanism by which mitochondrial oxidation of long-chain fatty acids is regulated (40). The sensitivity of the enzyme to this inhibitor is influenced by the nutritional and physiological states of the animals (8, 40–45). The present study demonstrated that polyunsaturated fats compared to a saturated fat (palm oil) lowered the sensitivity of the enzyme to malonyl-CoA. However, the magnitude of the inhibition by varying concentrations of malonyl-CoA was comparable between safflower and perilla oils. Thus, malonyl-CoA-mediated mechanism regulating hepatic fatty acid oxidation cannot account for the serum lipid-lowering effect of α-18:3 relative to 18:2.

Substrate specificities of fatty acid oxidation enzymes

This study confirmed previous observations (23, 58, 60, 61) that fatty acid oxidation enzymes in mitochondria and peroxisomes can discriminate the unsaturation of fatty acid substrates. On the substrate specificity of the mitochondrial pathway, Clouet, Niot, and Bézard (60) reported that the rate of the oxidation of unsaturated fatty acids in rat liver mitochondria was in the order of α-18:3 > 18:2 > 18:1. Gavino and Gavino (61) demonstrated that activity of carnitine palmitoyltransferase I measured with α-18:3 acid and its CoA ester was higher than that observed with 16:0, 18:1, 18:2, 20:4, 20:5, and 22:6 acids and their CoA esters. We also observed that the activity of the transferase measured with α-18:3-CoA was higher than with 16:0-, 18:1-, 18:2-, and γ-18:3-CoAs (23). The present study confirmed these observations and the mitochondrial carnitine palmitoyltransferase (both measured in the intact and freeze-thawed mitochondrial preparations solubilized with Triton) was the highest with α-18:3-CoA and the lowest with 16:0-CoA, being intermediate with 18:2-CoA. Thus, the substrate specificity of this enzyme may account for the differences in the oxidation rates of the different fatty acyl-CoA substrates in mitochondria. Hiltunen et al. (58) compared the rates of the oxidation of various polyunsaturated fatty acids in isolated rat liver peroxisomes and showed that γ-18:3 acid was oxidized at the rate higher than 18:1, 18:2, and 20:4 acids. However, no information has hitherto been available regarding the rate of oxidation of α-18:3 in the peroxisomal pathway.

The activity of acyl-CoA oxidase, the enzyme that catalyzes the first step of the peroxisomal fatty acid oxidation pathway, was higher with 18:2- and α-18:3-CoAs than with 16:0-CoA. However, the activity observed with α-18:3-CoA was indistinguishable from that observed with 18:2-CoA. Thus the substrate specificity of this enzyme cannot necessarily account for the differences in the rates of the peroxisomal oxidation of acyl-CoA substrates differing in the degree of unsaturation.

In the present study, serum albumin was included in the incubation mixture to measure the activities of some enzymes involved in the fatty acid oxidation pathway. Serum albumin tightly binds fatty acyl-CoA, and thus only a small proportion of fatty acyl-CoAs added exists as a free form in solution. Therefore, a limited amount of the substrate is available for the enzyme reaction when the albumin is added to the assay mixture (21). The relative affinity to serum albumin of various fatty acids appears to be different and the kinetic study (62) showed that the affinity of the strongest fatty acid binding site of serum albumin to various fatty acids was in

the order of 16:0 > 16:1 > 18:1 > 18:2 > 14:0. Although this order was not necessarily consistent with the affinity of weaker binding sites, 16-carbon fatty acids (16:0 and 16:1) bind most firmly at the various fatty acid binding sites of albumin (sites 1 to 6). Although the information regarding the relative affinity to serum albumin of various acyl-CoAs is lacking, these observations raise the possibility that the dependence of the enzymatic activity on the source of acyl-CoA represents the differences in the relative affinity to serum albumin of the acyl-CoA substrate rather than those in the specificities of fatty acid oxidation enzymes. Because of the consideration that free fatty acids and their CoA esters presumably tightly bind to protein in vivo in the liver (21, 63, 64), it is necessary to examine whether the observed differences in the activities of enzymes using various acyl-CoA substrates can be reproduced in the presence of varying amounts of serum albumin in the assay media.

Clouet et al. (60) reported that the rate of β-oxidation of α-18:3 acid by isolated mitochondria was higher than that of 18:1 and 18:2 at various fatty acid/albumin molar ratios. This observation was essentially confirmed in the present study in which mitochondrial β-oxidation was measured with CoA esters of 16:0, 18:2, and α-18:3 using the 500 *g* supernatant fraction of liver homogenates as an enzyme source. Gavino and Gavino (61) compared the dissociation rate of various fatty acids from albumin and the activities of carnitine palmitoyltransferase at various fatty acid/albumin molar ratios. They found that even in the situations where the dissociation rate from albumin of 16:0, 18:1, 20:4, 20:5, and 22:6 acids was higher than that of α-18:3 acid, the activity of the transferase toward α-18:3 was higher than other fatty acids. This result indicated that the fatty acid-dependent differences in the activities of this enzyme represent its specificity but not the differences in the relative affinity of various fatty acids to serum albumin. The current observation that the transferase activity measured with α-18:3-CoA was distinctly higher than with 16:0- and 18:2-CoAs at various substrate/albumin ratios supports this concept.

Less is known concerning the effect of albumin on the oxidation rate of various fatty acids by peroxisomes. According to Mannaerts et al. (21), 16:0-CoA and 18:1 acid were oxidized at a comparable rate by peroxisomes at various substrate/albumin ratios in spite of the fact that 16:0-CoA compared to 18:1 binds more tightly to serum albumin. The present study showed that α-18:3-CoA was oxidized at the rate higher than 16:0- and 18:2-CoAs at various substrate/albumin ratios. Therefore, the difference in the affinity of fatty acid substrates to albumin may also not be the crucial factor that affects the rate of peroxisomal oxidation of fatty acids or their CoA esters differing in the degree of unsaturation.

Role of the fatty acid oxidation pathway in regulating serum lipid concentration in rats fed α-linolenic acid

Using linseed oil (13, 14) or perilla oil (15–17) as the sources of α-18:3, it has been reported that dietary α-18:3 in relation to both saturated fatty acids and 18:2 reduces serum lipid concentrations in rats as currently confirmed. However, the mechanism(s) by which dietary α-18:3 reduces serum lipid concentrations is not clear. The results of the present study indicated that both substrate specificities and changes in the activities of the β-oxidation enzymes play crucial roles in reducing serum lipid concentrations in rats fed α-18:3. The experiment with isolated liver perfusion unequivocally demonstrated that the rate of fatty acid oxidation is a decisive regulatory factor for triacylglycerol synthesis and secretion of very low density lipoproteins in the liver (10–12). As α-18:3 compared to 16:0 and 18:2 is the preferred substrate for the β-oxidation enzymes, dietary α-18:3 is considered to be preferentially oxidized rather than esterified to form triacylglycerol in the liver.

The present study showed that the proportion of polyunsaturated fatty acids in liver triacylglycerol of rats fed perilla oil was significantly lower than that in the animals fed safflower oil although the same amount of polyunsaturated fatty acids was supplied. The ratio of 18:2 to α-18:3 in dietary perilla oil was 0.37 (Table 1) while it was definitely higher in liver triacylglycerol in rats fed perilla oil (0.73 and 0.61 in the Expts. 1 and 2, respectively) (Table 8). Thus, α-18:3 compared to 18:2 appears to be preferentially degraded via the β-oxidation pathway. Moreover, the stimulation by dietary α-18:3 of the β-oxidation pathway in turn reduces the availability of fatty acid as the substrate for triacylglycerol synthesis in the liver. The significant reduction by perilla oil of serum free fatty acid concentration may also support the view that dietary α-18:3 induces fatty acid oxidation in the liver.

Thus, dietary α-18:3 is considered to reduce triacylglycerol synthesis, thereby decreasing assembly and secretion of triacylglycerol-rich lipoproteins in the liver. Consequently, serum lipid concentrations decrease as observed in the present study. The alteration in the rate of fatty acid synthesis also appears to be a factor in regulating hepatic synthesis and secretion of triacylglycerol (65, 66). Although both safflower and perilla oil diets compared to saturated fat diets decreased the activities of enzymes in fatty acid synthesis in the present study, there were no differences in the activities between the two polyunsaturated fatty acid diets. Thus, the alteration in the fatty acid synthesis rate may not be a decisive factor responsible for the serum lipid-lowering effect of dietary α-18:3 relative to 18:2.

The mechanism by which dietary fat rich in α-18:3 induced the activities of enzymes in fatty acid oxidation is not clear at present. As α-18:3 is metabolized to 20:5 (n–3) and 22:6 (n–3), it is plausible that these metabolites rather than α-18:3 per se induce hepatic fatty acid oxidation. Further studies are needed to clarify this point.

In conclusion, dietary fat rich in α-18:3 compared to saturated fats and a fat blend that contained the same amount of polyunsaturated fatty acids almost entirely as 18:2 significantly increased the activities of fatty acid oxidation enzymes in rat liver. Also, α-18:3 was degraded at a rate faster than 16:0 and 18:2 in both mitochondrial and peroxisomal pathways. Thus, both the substrate specificities and alterations in the activities of the β-oxidation enzymes may account for the serum lipid-lowering effect of dietary α-18:3 in this animal model.

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