Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation

Lise Madsen,[1] Livar Frøyland, Endre Dyrøy, Kari Helland, and Rolf K. Berge

Department of Clinical Biochemistry, University of Bergen, Haukeland Hospital, N-5021 Bergen, Norway

Abstract The 3-thia fatty acids, tetradecylthioacetic acid and 3,10-dithiadicarboxylic acid are mitochondrion and peroxisome proliferators. Administration of these promotes an increased transport of endogenous fatty acids to these organelles and a higher capacity of β-oxidation. Administration of 3-thia fatty acids to rats resulted in a significant decrease of the hepatic level of docosahexaenoic acid (DHA) (17–24%) and especially eicosapentaenoic acid (EPA) (40–80%) accompanied by increased gene expression of mitochondrial 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase. The mitochondrial oxidation of EPA was increased more than 4-fold after administration of 3-thia fatty acids. EPA-CoA was a good substrate for mitochondrial carnitine acyltransferase-I and treatment with 3-thia fatty acids increased the activity 1.7-fold. DHA was a poor substrate for both mitochondrial and peroxisomal β-oxidation. DHA-CoA was a very poor substrate for mitochondrial carnitine acyltransferase-I and -II, and the activity did not increase after treatment. However, the peroxisomal DHA-CoA oxidase increased 10-fold after 3-thia fatty acid treatment, whereas the peroxisomal EPA-CoA oxidase increased only 5-fold. In isolated hepatocytes, 16% of total metabolized EPA was oxidized and 76% was incorporated into glycerolipids, whereas DHA was oxidized very little. We conclude that under conditions of increased mitochondrial and peroxisomal proliferation by 3-thia fatty acids, a relatively higher oxidation rate of polyunsaturated n–3 fatty acids might result in a decreased hepatic level of EPA and DHA. Under these conditions DHA seems to be oxidized by the peroxisomes, whereas EPA, which can be oxidized in both organelles, is mainly oxidized by mitochondria.—**Madsen, L., L. Frøyland, E. Dyrøy, K. Helland, and R. K. Berge.** Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. *J. Lipid Res.* **39:** 583–593.

Supplementary key words 3-thia fatty acids • fatty acyl-CoA oxidase • carnitine acyltransferase • liver • EPA • DHA

Membrane fatty acids have key roles in cellular processes, as receptor-mediated action and the general mobility and the activity of a number of membrane proteins, including protein receptors, might be affected by the membrane fluidity. Changing the fatty acid composition might, therefore, have serious consequences. It is well known that the fatty acid composition is changed by dietary fat manipulation (1) and administration of peroxisome proliferators such as clofibrate (2) and 3-thia fatty acids (3). The mechanism is, however, not elucidated.

Fatty acid oxidation and triacylglycerol biosynthesis are the major competitors for the utilization of fatty acids as substrates. Administration of hypolipidemic mitochondrion and peroxisome proliferating 3-thia fatty acids influences this balance, by increasing the fatty acid oxidation rate (4, 5). At the same time, the 3-thia fatty acids that are blocked for normal β-oxidation, due to a sulfur substitution in 3-position, are incorporated into hepatic glycerolipids of rats after administration and therefore change the hepatic fatty acid composition (6). Also, a Δ^9-desaturated metabolite of C14-S-acetic acid accumulates in the liver (7).

Administration of C14-S-acetic acid to rats causes a decrease in the hepatic level of n–3 fatty acids and we have suggested that oleic acid and the Δ^9-desaturated metabolite of C14-S-acetic acid might displace the n–3 fatty acids from the *sn*-2 position in glycerolipids which renders them available for further metabolism by mitochondrial fatty acid oxidation (8).

The n–3 fatty acids affect plasma lipids and lipid metabolism profoundly and the mechanisms behind these effects are not fully elucidated although intensively investigated. Furthermore, n–3 fatty acids are incorporated into cell membranes in a highly selective way where they act as structural components influencing

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CAT, carnitine acyltransferase; TD, dithiadicarboxylic acid (1,10 bis (carboxymethylthio) decane.

[1]To whom correspondence should be addressed.

fluidity of the membrane (9). The n–3 fatty acids also offer themselves for enzymatic transformations into eicosanoids that act as intracellular signals and, finally, they become involved in the activity of membrane enzymes and ion channels (10).

The main n–3 fatty acids, EPA and DHA, have earlier been referred to as fish oils without any further distinction. However, studies performed in recent years demonstrate that EPA and DHA both possess different hypolipidemic and metabolic properties (9, 11–13).

This study was, therefore, undertaken first, to further elucidate the mechanism by which two different mitochondrion and peroxisome proliferators, tetradecylthioacetic acid (C14-S-acetic acid) and 1,10 bis(carboxymethylthio)decane (TD), change the levels of EPA and DHA in rat liver after administration and especially focusing on the oxidation rates of EPA and DHA compared to the oxidation rates of palmitic and oleic acids; and second, to establish differences in the metabolism of EPA, DHA, palmitic and oleic acids during mitochondrion and peroxisome proliferation.

MATERIALS AND METHODS

Chemicals and drugs

[^{32}Pα]dCTP (3000 mCi/mmol) l-[methyl-^{14}C]carnitine hydrochloride (54 mCi/mmol), [1-^{14}C]palmitoyl-l-carnitine (54 mCi/mmol), and [1-^{14}C]palmitoyl-CoA (54 mCi/mmol), were purchased from the Radiochemical Centre, Amersham, England. [1-^{14}C]palmitic acid (50 mCi/mmol), [1-^{14}C]oleic acid (50 mCi/mmol), [1-^{14}C]eicosapentaenoic acid (50 mCi/mmol), and [1-^{14}C] docosahexaenoic acid (50 mCi/mmol) were obtained from New England Nuclear, Boston, MA. Nylon membranes and slot-blot equipment were obtained from Schleicher & Schuell, Dassel, Germany, and leucodichlorofluorescein was obtained from Eastman Kodak Company, Rochester, NY. The sulfur-substituted fatty acids were prepared at Department of Chemistry, University of Bergen, as earlier described (14).

[1-^{14}C]tetradecylthioacetic acid was prepared at the Department of Chemistry, University of Bergen. 1-Tetradecanethiol was distilled at 84–86°C/0.01 mm Hg and [1-^{14}C]bromoacetic acid [1 mCi bromoacetic acid (55 mCi/mmol)] was distilled at 117–118°C/15 mm Hg. All other chemicals were flushed with argon. Tetradecanethiol (0.042 g) was placed in a round-bottom flask; 2 ml methanol was added and the mixture was stirred under argon to prevent oxidation of the sulfides. After 15 min, 1.7 ml of 0.75 m KOH in methanol was carefully added. The reaction mixture was then stirred for 20 min. [1-^{14}C]bromoacetic acid (0.019 g) was dissolved in 10 ml methanol. The solution was allowed to evaporate to 5 ml under a flush of nitrogen after which 1.68 ml of 0.757 m KOH was added. The reaction mixture was bubbled through with argon and boiled for 24 h; 0.048 g of 37% HCl in 10 ml water was then added. This led to precipitation of the product. Before the mixture was set at 4°C to continue precipitation, pH was measured and seen to have a value of 4–5. [1-^{14}C]tetradecylthioacetic acid was transferred to a G-S filter, washed with distilled water, and recrystalized from dichloromethane in 10% pentane. It had a melting point of 63°C and the yield was 78%.

Sorbityl-CoA, eicosapentaenoic acyl-CoA, docosahexaenoic acyl-CoA, and tetradecylthioacetyl-CoA were synthesized as described by Kawaguchi et al. (15). All other chemicals and solvents were of reagent grade from common commercial sources

Treatment of animals

Male Wistar rats, weighing 260–300 g, were obtained from Møllegard Breeding Laboratory, Ejby, Denmark. They were housed in pairs in metal wire cages, and maintained on a 12-h cycle of light and dark at 20 ± 3°C. The rats had free access to standard rat pellet food and water during the experiment. They were acclimatized under these conditions for at least 1 week before the experiments. Each test and control group consisted of four animals. Palmitic acid and the thia fatty acids were suspended in 0.5% sodium carboxymethyl cellulose (CMC). The control animals received only CMC. Different doses (150 and 300 mg/kg body weight) were administered by gastric intubation once a day in a volume of 0.7–1.0 ml for 1 week. All animals had free access to water and standard rat pellet food during the experiment. After 12 h fasting, the rats were anesthetized with 0.2 ml Hypnorm-Dormicum® (fluanisone-fentylmidazolam)/100 g body weight. Cardiac puncture was performed and blood was collected in Vacutainers containing EDTA. The livers and hearts were removed, weighed, and parts of them were immediately chilled on ice, while the remainder was freeze-clamped and stored at −80°C. The use of the animals was approved by the Norwegian State Board of Biological Experiments with Living Animals.

Preparation of subcellular fractions and protein measurements

The livers from individual rats were homogenized in ice-cold sucrose medium [0.25 m sucrose, 10 mm HEPES (pH 7.4) and 2 mm EDTA]. Subcellular fractions were prepared according to DeDuve et al. (16) using preparative differential centrifugation. Modifications, purity, and yield are described earlier (17). Bio-Rad protein

kit (Bio-Rad, Richmond, CA) was used for protein measurement. BSA dissolved in distilled water was used as a standard. The absorbance was read on a LKB Bromma Ultrolab System 2074 Calculating Absorptiometer.

Measurement of enzyme activities

Acid-soluble products were measured using palmitoyl-CoA and palmitoyl-l-carnitine as substrates, as described elsewhere (11). Acid-soluble products were also measured using fatty acids as substrate. The assay medium (0.25 ml) contained 13.3 mm HEPES, pH 7.3, 83.3 mm KCl, 16.7 mm $MgCl_2$, 13.3 mm dithiothreitol, 0.7 mm EDTA, 1.25 mm l-carnitine, 0.2 mm NAD^+, 2.0 mm ATP, 0.4 mm CoASH, 150 μm [1-^{14}C]fatty acid, and 0.25–0.5 mg protein. All samples were preincubated for 3 min at 30°C. After incubation for 4 min (mitochondrial fraction) or 10 min (peroxisomal fraction), the reaction was terminated by addition of 150 μl 1.5 m KOH. Twenty-five μl fatty acid-free BSA (100 mg/ml) was added to the suspension in order to bind non-oxidized substrates. 500 μl 4 m $HClO_4$ was added to precipitate protein and non-oxidized substrates bound to BSA. The total solution was centrifuged at 2010 *g* for 10 min. Aliquots of 500 μl were assayed for radioactivity. Carnitine acyltransferase (CAT)-I activity was measured essentially as described by Bremer (18). The assay for CAT-I contained 20 mm HEPES, pH 7.5, 70 mm KCl, 5 mm KCN, 100 μm acyl-CoA, 10 mg BSA/ml, and 0.6 mg tissue protein/ml. The reaction was started with 200 μm [methyl-^{14}C]l-carnitine (200 cpm/nmol). When included, malonyl-CoA was added prior to the start of reaction. Assay conditions for CAT-II were identical except that BSA was omitted and 0.01% Triton X-100 was included. Tissue protein concentration was 0.1 mg/ml.

The enzymatic activity of peroxisomal fatty acyl-CoA oxidase was measured in the peroxisomal fraction by the coupled assay described by Small, Burdett, and Connock (19). The production of H_2O_2 was measured by monitoring the increase in dichlorofluorescein absorbance in the presence of palmitoyl-CoA using a Varian 2300 spectrophotometer.

2,4-Dienoyl-CoA reductase activity was determined in the mitochondrial and peroxisomal fraction according to Kunau and Dommes (20), with some minor modifications described earlier (8).

Acyl-CoA synthase activity was measured according to Bar-Tana, Rose, and Shapiro (21) with some modifications. The reaction mixture at a total volume of 250 μl contained 120 mm Tris-HCl buffer, pH 7.4, 0.05% Triton X-100, 2 mm EDTA, 5 mm $MgCl_2$, 2 mm ATP, 0.5 mm CoA, 8 mm DDT, and 10–20 μg protein. The reaction was started with 25 μl 1.5 mm [1-^{14}C] fatty acid. Incubations were carried out for 3 min at 37°C and terminated by 3.25 ml methanol–chloroform–heptane 141:125:100. The contents were mixed for 5 min and centrifuged; 0.5 ml of the water phase was assayed for radioactivity.

Purification of RNA and hybridization analysis

Total RNA was isolated using the guanidinium thiocyanate–phenol method (22). A Schleicher & Schuell aperture was used to transfer RNA to a nylon-filter for hybridization as earlier described (23). Three different RNA concentrations were applied. Hybridization reactions were performed as described by Sambrook, Fritsch, and Maniatis (24). Kodak XAR-5 X-ray films were exposed to the membranes at −80°C in the presence of intensifying screens, for an adequate exposure (3 days to 2 weeks). Autoradiograms were obtained using an LKB Ultrogel laser densitometer. The relative level of mRNA expression was estimated as the amount of radioactive probe hybridized to each sample of RNA relative to the level of 28S rRNA in each sample.

Preparation of hybridization probes

The appropriate DNA fragments were excised from plasmids by restriction enzymes. Purified fragments

TABLE 1. Effect of palmitic, C14-S-acetic acid, and TD on the hepatic fatty acid composition

Treatment		Fatty Acid				
Fatty Acid	Dose	Palmitic Acid	Oleic Acid	EPA	DHA	C14-S-Acetic Acid
	mg/day/kg body weight			*mg fatty acid/g liver*		
Control		7.3 ± 0.7	3.2 ± 0.3	0.5 ± 0.1	2.9 ± 0.3	ND
Palmitic acid	150	7.5 ± 0.4	3.0 ± 0.5	0.5 ± 0.1	2.8 ± 0.3	ND
C14-S-acetic acid	150	8.4 ± 1.2	4.2 ± 1.2[a]	0.3 ± 0.1[a]	2.4 ± 0.3[a]	0.6 ± 0.1[a]
	300	7.5 ± 1.6	4.9 ± 1.5[a]	0.1 ± 0.1[a]	1.7 ± 0.2[a]	1.1 ± 0.5[a]
TD	150	9.0 ± 0.6	3.5 ± 0.4	0.1 ± 0.0[a]	2.2 ± 0.2[a]	ND
	300	9.0 ± 0.6	3.6 ± 0.3	0.1 ± 0.0[a]	2.0 ± 0.3[a]	ND

The values represent means ± SD from four animals in each group; TD, dithiadicarboxylic acid; ND, not determined.
[a]Significantly different from control: $P < 0.05$.

were then ^{32}P-labeled using the oligolabeling technique (25), resulting in specific activities ranging from 0.8 to 5 $\times$ 10^9 cpm/mg. The 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase probes were a kind gift from Dr. J. K. Hiltunen (University of Oulu, Finland).

Determination of fatty acid composition

Total lipids were extracted from liver as described by Lie, Lied, and Lambertsen (26). The lipids were evaporated, saponified, and esterified prior to separation using a Carlo Erba 2900 gas-chromatograph as earlier described (8).

Hepatocyte isolation and culture conditions

Rat liver parenchymal cells were isolated as described by Berry and Friend (27) with modifications according to Seglen (28). The hepatocytes were plated at a density of 2.0 $\times$ 10^6/dish (1–1.5 mg cell protein) in 2 ml of DMEM containing 20 mm HEPES, Ultroser G (2%), 0.5 mm l-carnitine, and gentamicin (50 μg/ml). Cultures were maintained in a humidified incubator at 37°C in an atmosphere containing 5% CO_2. After overnight incubation, the medium was replaced with DMDM (2 ml/dish) containing ^{14}C-labeled fatty acids (0.25 μCi/ml) bound to BSA. The molar ratio between fatty acids and BSA was 2.5:1. Harvesting of the cells, determination of fatty acid oxidation by acid soluble radioactivity, and extraction and separation of radiolabeled lipids was performed as earlier described (29).

Statistical analysis and presentation of data

The data are presented as mean ± standard deviation (SD) from 4 animals and were evaluated by a two-sample variance Student's *t*-test (two-tailed distribution) where relevant. The level of statistical significance was set at $P < 0.05$.

RESULTS

Table 1 shows the hepatic amount of EPA, DHA, palmitic, oleic, and C14-S-acetic acids, before and after administration of two different hypolipidemic sulfur-substituted fatty acids. The monocarboxylic C14-S-acetic acid, which is blocked for β-oxidation acid, was incorporated in the liver in a dose-dependent manner, whereas the more effective peroxisome proliferator 3,10-dithiadicarboxylic acid (TD) which is blocked for both β- and ω-oxidation, could not be detected. Administration of both TD and C14-S-acetic acid caused a decrease in the hepatic amount of both DHA and especially EPA. After C14-S-acetic acid and TD treatment at the low dose, the EPA level was decreased 40 and 80%, respectively. The DHA level decreased 17 and 24%. Confirming earlier studies, C14-S-acetic acid treatment increased the amount of oleic acid more effectively than TD treatment. Neither C14-S-acetic acid nor TD administration significantly changed the amount of palmitic acid in the liver.

In untreated control rats, the order of the fatty acid oxidation measured as acid-soluble products, in isolated mitochondria, toward individual fatty acids, when measured under an equal fatty acid concentration, was palmitic acid > oleic acid > EPA >> DHA (**Fig. 1A**). The oxidation of palmitic acid in isolated mitochondria from rats treated with C14-S-acetic acid and TD was increased 2.2- and 1.8-fold, respectively, whereas

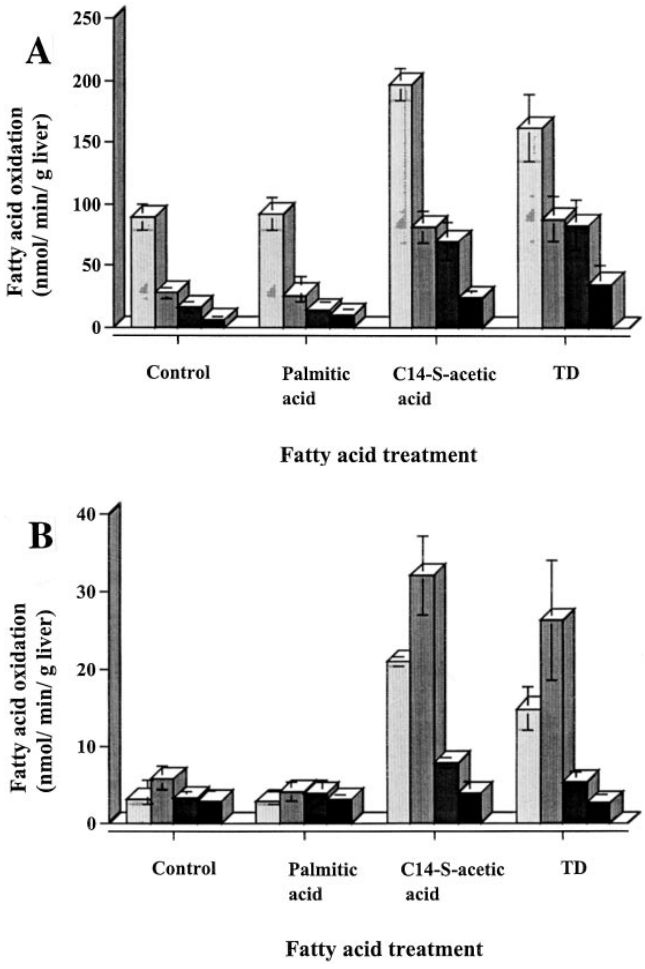

Fig. 1. Oxidation of different fatty acids (□, palmitic acid; ▩, oleic acid; ■, EPA; and ■, DHA) measured as acid-soluble products as described in Materials and Methods in the mitochondrial (A) and peroxisomal (B) fractions prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

TABLE 2. Effect of palmitic, C14-S-acetic acid, and TD on hepatic 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase

Treatment		2,4-Dienoyl-CoA Reductase			Enoyl-CoA Isomerase
		Activity			
Fatty Acid	Dose	Mitochondrial Fraction	Peroxisomal Fraction	mRNA	mRNA
	mg/day/kg body weight	*μmol/min/g liver*		*fold increase*	
Control		7.4 ± 2.5	0.5 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
Palmitic acid	150	8.1 ± 3.1	0.7 ± 0.2	0.8 ± 0.1	1.2 ± 0.2
C14-S-acetic acid	150	ND	ND	2.1 ± 0.7[a]	2.5 ± 0.4[a]
	300	20.2 ± 1.4[a]	1.3 ± 0.4[a]	2.7 ± 0.7[a]	2.8 ± 0.3[a]
TD	150	ND	ND	2.2 ± 0.3[a]	2.9 ± 0.4[a]
	300	18.4 ± 3.4[a]	1.5 ± 0.1[a]	2.9 ± 0.6[a]	3.3 ± 0.7[a]

The values represent means ± SD from four animals in each group; TD, dithiadicarboxylic acid; ND, not determined.

[a]Significantly different from control: $P < 0.05$.

the increased oxidation rate of oleic acid was 2.9- and 3.1-fold, respectively. It was, however, interesting to note that C14-S-acetic acid and TD administration increased the oxidation capacity of EPA as much as 4.4- and 5.1-fold, respectively. The oxidation of DHA was increased 3- and 4-fold (Fig. 1A).

In the peroxisomal fraction from control animals, in contrast to the mitochondrial fraction, there was no significant difference in the order of fatty acid oxidation toward individual fatty acids when measured under equal concentrations (Fig. 1B). C14-S-acetic acid and TD treatment increased the oxidation capacity of oleic acid 5.9- and 4.2-fold, respectively. The increased oxidation of palmitic acid and EPA was 6.9- and 6-fold and 2.3- and 1.5-fold, respectively. The oxidation of DHA tended to increase but this was not statistically significant.

Table 2 shows that the two enzymes that are necessary to oxidize unsaturated fatty acids, 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase, were up-regulated at the mRNA level after both C14-S-acetic acid and TD treatment. In addition, 2,4-dienoyl-CoA reductase activity increased significantly in both mitochondrial and peroxisomal fractions after 3-thia fatty acid treatment.

Even though C14-S-acetic acid was not oxidized, C14-S-acetic acid was a good substrate for mitochondrial and peroxisomal fatty acyl-CoA synthase (**Fig. 2A** and **B**). In contrast, DHA was a very poor substrate. Palmitic acid was, however, the best substrate in both treated and untreated animals. In general, the fatty acyl-CoA synthases seemed to follow the same pattern as the oxidation rate of the respective fatty acids with regard to substrate specificity, except for the non-oxidable 3-thia fatty acids. Thus, the order of the mitochondrial and peroxisomal fatty acyl-CoA synthase activities toward the various fatty acids was 16:0 > 18:1 (n–9) = C14-S-acetic acid > 20:5 (n–3) >> 22:6 (n–3). In C14-S-acetic acid-treated rats, the peroxisomal C14-S-acyl-CoA synthase was increased more than the mitochondrial enzyme. In contrast, DHA was a very poor sub-

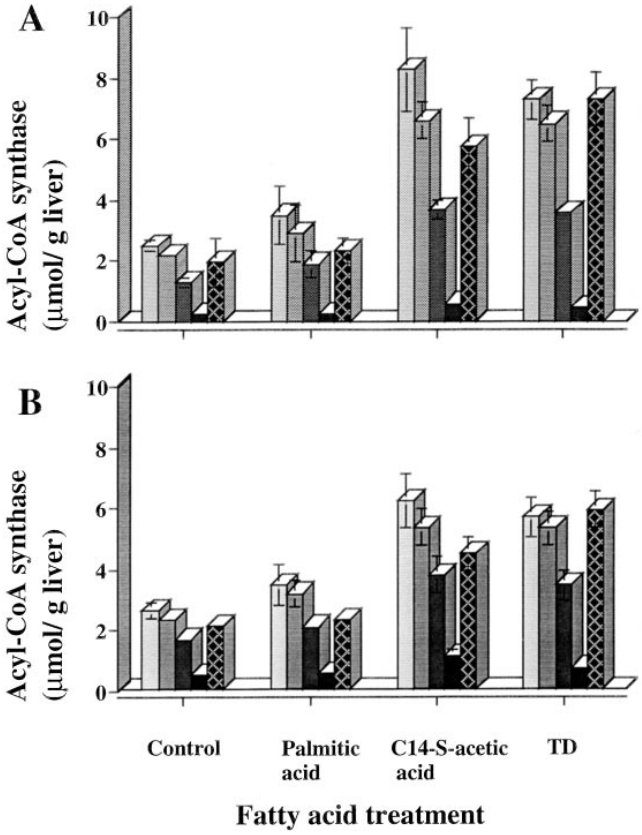

Fig. 2. Acyl-CoA synthase activities measured with different substrates (▩, palmitic acid; ▩, oleic acid; ■, EPA; ■, DHA; and ▩, C14-S-acetic acid) as described in Material and Methods in the mitochondrial (A) and peroxisomal (B) fractions prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

TABLE 3. Effects of palmitic and C14-S-acetic acid on mitochondrial palmitoyl-CoA oxidation and carnitine palmitoyltransferase-I and -II activities

	Palmitoyl-CoA Oxidation		Carnitine Palmitoyltransferase -I		Carnitine Palmitoyltransferase -II	
Fatty Acid Treatment	−Malonyl-CoA	+Malonyl-CoA	−Malonyl-CoA	+Malonyl-CoA	−Aminocarnitine	+Aminocarnitine
	nmol/min/mg mitochondrial protein					
Control	0.92 ± 0.07	0.36 ± 0.08	1.32 ± 0.15	0.82 ± 0.02	26 ± 3	10 ± 3
Palmitic acid	1.28 ± 0.10	0.51 ± 0.05	1.23 ± 0.18	0.85 ± 0.09	33 ± 3	12 ± 3
C14-S-acetic acid	2.20 ± 0.47	0.92 ± 0.15	1.20 ± 0.10	0.90 ± 0.08	69 ± 16	23 ± 7

The values represent means ± SD for four animals in each group. Palmitoyl-CoA oxidation was measured in the presence and absence of 12 μm malonyl-CoA and carnitine palmitoyltransferase-I was measured in the presence and absence of 6 μm malonyl-CoA. Carnitine palmitoyltransferase-II was measured in the presence and absence of 6 μm aminocarnitine.

strate for peroxisomal and especially mitochondrial acyl-CoA synthase in both treated and untreated animals. In the treated animals, the DHA-CoA synthase activity, however, was 3-fold higher in the peroxisomal fraction compared to the mitochondrial. In contrast, the activity of EPA-CoA synthase was stimulated to the same extent in mitochondrial and peroxisomal fractions in treated animals.

The mitochondrial oxidation of palmitoyl-CoA and palmitoyl-l-carnitine was stimulated after 3-thia fatty acid treatment (**Table 3**). Palmitoyl-CoA oxidation and carnitine palmitoyltransferase-I were malonyl-CoA-sensitive. To enter the mitochondria for fatty acid oxidation, the activated fatty acids must be channeled through the carnitine acyltransferase (CAT) system which is also considered to be the rate-limiting step. There was no significant difference with regard to the substrate specificity of palmitoyl-CoA, oleoyl-CoA, and EPA-CoA, at equal concentrations, for CAT-I activity in isolated mitochondria from control animals (**Fig. 3A**). DHA-CoA was, however, a very poor substrate. It is noteworthy that the CAT-I activities were not induced with palmitoyl-CoA and oleoyl-CoA after 3-thia fatty acid treatment, whereas the activity measured with EPA-CoA was induced 1.7-fold. In contrast, the CAT-I activity was significantly decreased in 3-thia fatty acid-treated animals, compared to controls when C14-S-acetyl-CoA was used as a substrate.

Malonyl-CoA-sensitive CAT-I activity has also been detected in peroxisomes and we therefore investigated the CAT-I activity with the various individual fatty acyl-CoAs as substrates in the peroxisomal fraction (Fig. 3B). There was no difference with regard to substrate specificity with palmitoyl-CoA and oleoyl-CoA for CAT-I activity in the peroxisomal fraction from control animals, whereas EPA-CoA and C14-S-acetyl-CoA were poorer substrates. In contrast to the mitochondrial CAT-I activity, the peroxisomal activities were induced after 3-thia fatty acid treatment when palmitoyl-CoA, oleoyl-CoA, and C14-S-acetyl-CoA were used as substrates, whereas the activity was unchanged when EPA-CoA was used as a substrate. DHA-CoA was a very poor substrate for peroxisomal CAT-I activity, but was induced 2- and 3-fold after C14-S-acetic acid and TD treatment.

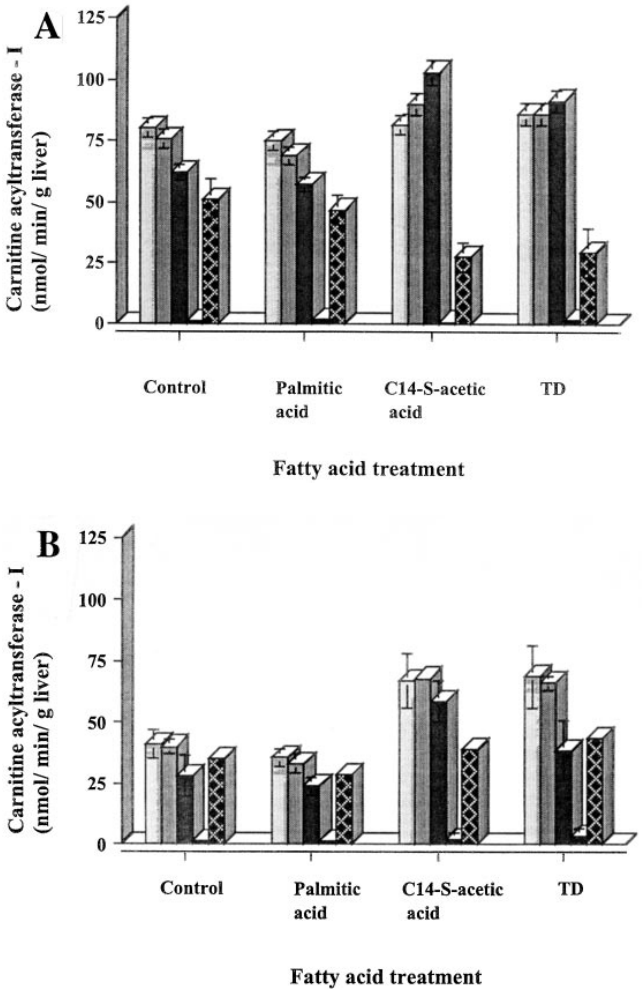

Fig. 3. Carnitine palmitoyltransferase-I activities measured with different substrates (▩, palmitoyl-CoA; ▩, oleoyl-CoA; ■, EPA-CoA; ■, DHA-CoA; and ▩, C14-S-acetyl-CoA) as described in Material and Methods in the mitochondrial (A) and peroxisomal (B) fractions prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

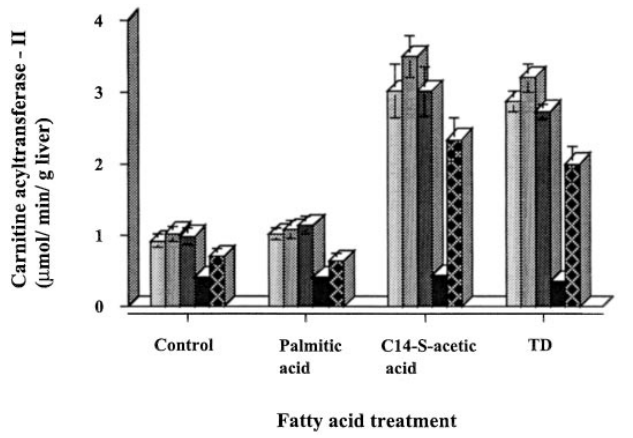

Fig. 4. Carnitine palmitoyltransferase-II activities measured with different substrates (□, palmitoyl-CoA; ▩, oleoyl-CoA; ■, EPA-CoA; ■, DHA-CoA; and ▩, C14-S-acetyl-CoA) as described in Material and Methods in the mitochondrial fraction prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

Figure 4 shows that mitochondrial CAT-II was increased 2.5-fold, when C14-S-acetyl-CoA was used as a substrate, in isolated mitochondria after 3-thia fatty acid treatment, compared to controls. When palmitoyl-CoA, oleoyl-CoA, and EPA-CoA were used as substrates, the CAT-II activities were increased to the same extent. Regarding the substrate specificity, no significant differences were observed in control animals except that DHA-CoA was a very poor substrate.

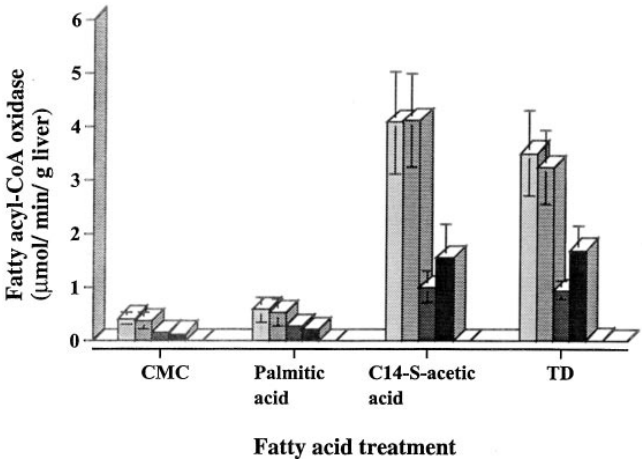

Fig. 5. Fatty acyl-CoA oxidase activities measured with different substrates (□, palmitoyl-CoA; ▩, oleoyl-CoA; ■, EPA-CoA; ■, DHA-CoA; and ▩, C14-S-acetyl-CoA) as described in Material and Methods in the peroxisomal fraction prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

Compared to the other substrates used, DHA-CoA was also a poor substrate for the peroxisomal fatty acyl-CoA oxidase in control animals (**Fig. 5**). However, after C14-S-acetic acid and TD treatment, the activity increased 10- and 11-fold, respectively. At an equal concentration, EPA-CoA was also a relatively poor substrate for fatty acyl-CoA oxidase and, in contrast to when DHA-CoA was used as a substrate, the activity increased only 5.7- and 5.3-fold after C14-S-acetic acid and TD treatment. Palmitoyl-CoA and oleoyl-CoA were better substrates at this concentration.

As palmitic acid, oleic acid, EPA, DHA, and the 3-thia fatty acids are probably differently metabolized in vivo, we examined the metabolism of these acids in isolated rat hepatocytes. When 100 μm of labeled palmitic acid was added to isolated rat hepatocytes, 22% of the total metabolized acid was oxidized, whereas 73% was incorporated into glycerolipids. EPA was metabolized in a similar manner (16% oxidized and 76% incorporated) (**Table 4**). DHA and C14-S-acetic acid seemed to be almost exclusively shuttled into glycerolipid synthesis.

Activation of fatty acids to their respective CoA esters is a necessary step prior to both oxidation and incorporation into glycerolipids. **Figure 6** shows the acyl-CoA synthase activity in the microsomal fraction. Palmitic acid and oleic acid were better substrates than EPA and C14-S-acetic acid for the microsomal acyl-CoA synthase. Again, DHA was a poor substrate. C14-S-acetic acid and TD administration increased the activities of the acyl-CoA synthases in all fractions, but the induction was greater in the mitochondrial- and peroxisomal-enriched fractions than the microsomal fraction, which indicates that the balance between fatty acid incorporation into glycerolipids and fatty acid oxidation is shifted towards oxidation. **Figure 7** shows that addition of C14-S-acetic acid to the medium for cultured hepatocytes influences this balance. Whereas only 22% of total metabolized palmitic acid was oxidized in untreated cells, 48–52% was oxidized when C14-S-acetic acid was added to the medium. The % of total palmitic acid incorporated into glycerolipids (both triacylglycerol and phospholipids) was decreased.

TABLE 4. Metabolism of different labeled fatty acids in cultured hepatocytes

	Fatty Acid				
	Palmitic Acid	Oleic Acid	EPA	DHA	C14-S-Acetic Acid
	% of total metabolized				
Oxidized	22	9	16	1	5
Triacylglycerol	40	50	49	35	35
Phospholipids	33	34	27	59	47

Data are expressed as mean of two separate experiments. Both experiments were run in triplicate.

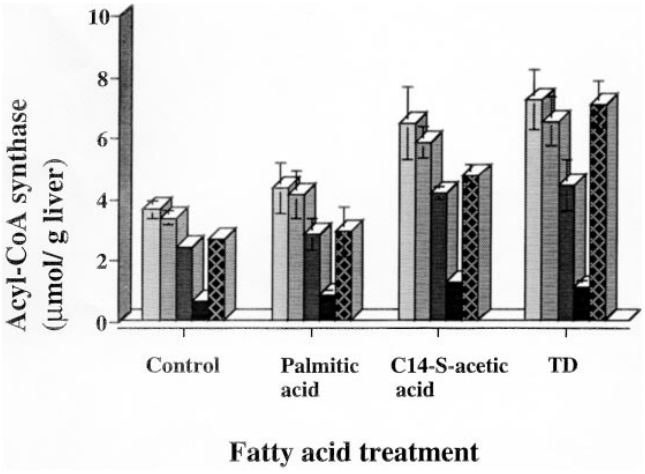

Fig. 6. Acyl-CoA synthase activities measured with different substrates (▩, palmitic acid; ▩, oleic acid; ■, EPA; ■, DHA; and ▩, C14-S-acetic acid) as described in Material and Methods in the microsomal fraction prepared from the liver of rats treated with palmitic or 3-thia fatty acids, at a dose of 300 mg/day per kg body weight for 1 week. The values represent mean ± SD from four animals in each group.

DISCUSSION

The hepatic content of polyunsaturated n–3 fatty acids, especially EPA, decreased after administration of the hypolipidemic 3-thia fatty acids (Table 1), confirming earlier experiments (8). Conversion of EPA to docosahexaenoic acid (DHA) cannot explain the decreased content of EPA, as the hepatic content of DHA also decreased (Table 1). It has been reported that C14-S-acetic acid up-regulates the hepatic Δ^9-desaturase activity as well as the mRNA levels, which results in an increased level of oleic acid in the liver (7). Administration of the more effective peroxisome proliferator, TD, neither stimulated Δ^9-desaturase activity nor decreased the hepatic oleic acid content (7). This study, however, shows that TD administration decreased the hepatic content of EPA and DHA as effectively as C14-S-acetic acid administration (Table 1). Thus, replacement of oleic acid in the *sn*-2 position of glycerolipids (8) cannot be the sole explanation for the decreased EPA and DHA content.

In treated animals, the activities of acyl-CoA synthases were increased in all subcellular fractions, but the increase was greater in mitochondrial and peroxisomal fractions, than in the microsomal fraction (Fig. 2 and Fig. 6). Consequently, the 3-thia fatty acids may influence the balance between fatty acid oxidation and esterification of fatty acids into glycerolipids towards fatty acid oxidation. Also in vitro experiments (Fig. 7) with cultured hepatocytes show that C14-S-acetic acid shifted this balance towards oxidation. Figure 1 shows that the oxidation of oleic and palmitic acids increased both in the mitochondrial and the peroxisomal fractions. As oleic acid was a good substrate for CAT-I and CAT-II (Figs. 3A and 4) and peroxisomal fatty acyl-CoA oxidase (Fig. 5), the overall kinetics suggest that oleic acid as well as palmitic acid can be oxidized in both peroxisomes and mitochondria.

The decreased hepatic levels of n–3 fatty acids may result from a selective increased β-oxidation (8). Table 2 shows that the 2,4-dienoyl-CoA reductase activity, which is necessary to oxidize polyunsaturated fatty acids (30), was increased in both the mitochondrial and the peroxisomal fractions, after both C14-S-acetic acid and TD treatments (Table 2). Increased mRNA levels of mitochondrial 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase and a parallel increase in 2,4-dienoyl-CoA reductase activity (Table 2) indicate that the 3-thia

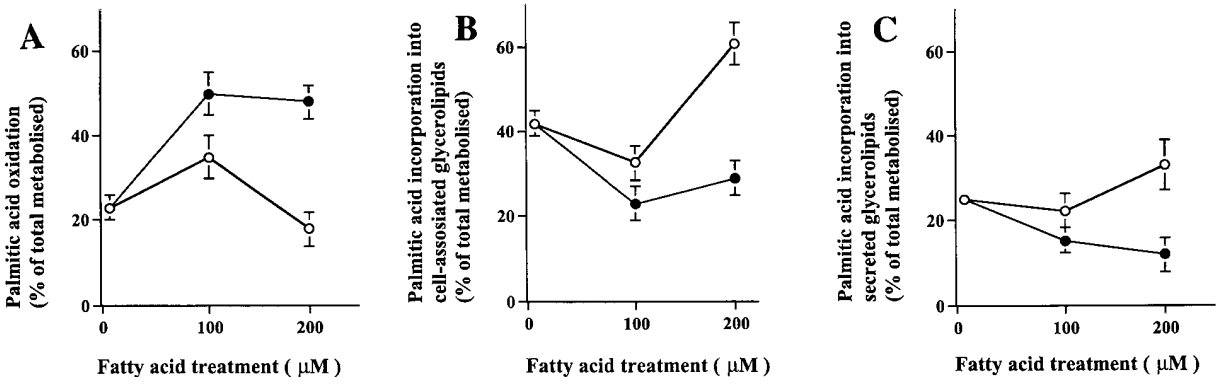

Fig. 7. Effect of palmitic acid —○— and C14-S-acetic acid ···●··· on (A) palmitic acid oxidation; (B) incorporation of palmitic acid into cell-associated glycerolipids; and (C) incorporation of palmitic acid into secreted glycerolipids. Hepatocytes were cultured and treated as described in Materials and Methods. The values represent mean of three different experiments. Each experiment was run in triplicate.

fatty acids influence the mitochondrial oxidation of polyunsaturated fatty acids at the transcriptional level.

DHA was converted to its CoA ester in all subcellular fractions (Fig. 2 and Fig. 6), but was a poor substrate for oxidation in both the mitochondrial and the peroxisomal fractions (Fig. 1A and B). The oxidation was increased 3.1-fold in the mitochondrial fraction after C14-S-acetic acid treatment (Fig. 1A), suggesting oxidation by the mitochondria. However, the finding of catalase (17%) and urate oxidase (10%) in the mitochondrial fraction indicate contamination of peroxisomes (17). Compared to EPA-CoA, DHA-CoA was more effectively synthesized in the peroxisomal than the mitochondrial fraction (Fig. 2), especially in treated animals. It has been reported that very long chain fatty acyl-CoA synthases, such as lignoceryl-CoA synthase, are absent in mitochondria (31), and DHA-CoA synthase activity measured in the mitochondrial fraction might also be due to contamination by peroxisomes.

The oxidation of DHA tended to increase in the peroxisomal fraction; however, this was not statistically significant (Fig. 1B). On the other hand, DHA-CoA was a substrate for peroxisomal fatty acyl-CoA oxidase and the activity of DHA-CoA oxidase increased 10-fold after C14-S-acetic acid treatment (Fig. 5). Noteworthy, in contrast to EPA-CoA, DHA-CoA was a very poor substrate, if at all, for mitochondrial CAT-I and CAT-II (Fig. 3A and Fig. 4). In addition, neither mitochondrial CAT-I nor CAT-II activity increased after 3-thia fatty acid treatment (Fig. 3A and Fig. 4) when DHA-CoA was used as a substrate. This is consistent with our earlier finding that the oxidation of DHA, in contrast to the oxidation of EPA and oleic acid, was not significantly inhibited by the CAT-II inhibitor aminocarnitine (8). It might therefore be considered that DHA cannot be oxidized by the mitochondria.

The peroxisomal CAT-I activity was approximately 50% of the mitochondrial activity in control animals. The role of CAT-I in peroxisomes is not elucidated, but unlike in mitochondria, CoA esters do not need carnitine for their penetration into the peroxisomes (32). One might speculate that acylcarnitines formed in peroxisomes are delivered to the mitochondria for further oxidation. This, however, needs to be further investigated. Interestingly, when DHA-CoA was used as a substrate, the peroxisomal CAT-I activity was induced 2-fold in 3-thia fatty acid-treated animals. No induction was found when EPA-CoA was used as a substrate (Fig. 3B). Taking all these results together, the overall kinetics favor peroxisomal oxidation of DHA.

C14-S-acetic acid treatment increased the mitochondrial oxidation of palmitic acid 2.2-fold and the oxidation of oleic acid 2.8-fold in the mitochondrial fraction, whereas the oxidation of EPA increased more than 4-fold (Fig. 1A). There was no significant increase of mitochondrial CAT-I activity when palmitoyl-, oleoyl- as well as DHA-CoA were used as substrates. Noteworthy, about 2-fold increase was observed when EPA-CoA was used as a substrate. Moreover, whereas oleoyl- and palmitoyl-CoA oxidase was increased approximately 5-fold, the peroxisomal EPA-CoA oxidase was increased only 2-fold. This indicates that EPA becomes a relatively better substrate for mitochondrial fatty acid oxidation after 3-thia fatty acid treatment. The different metabolism of EPA and DHA is consistent with our earlier findings that the oxidation of EPA and not DHA in cultured hepatocytes is strongly inhibited by the CAT-II inhibitor aminocarnitine (8). The overall kinetics suggest that EPA can be oxidized in both peroxisomes and mitochondria, but is preferably oxidized by mitochondria. The different metabolism of EPA and DHA was confirmed using cultured hepatocytes (Table 4). DHA was almost exclusively incorporated into glycerolipids, especially the phospholipid species, whereas EPA was more easily oxidized.

3-Thia fatty acids increased oxidation of the different fatty acids, but the oxidation of palmitic and oleic acids and EPA in the peroxisomal fraction was only 5–20% of the mitochondrial fatty acid oxidation. Thus, the capacity of the mitochondria to oxidize long-chain fatty acids is of quantitative major importance. Seen as a whole, our results suggest that EPA is preferentially oxidized by mitochondria, while DHA is preferentially oxidized by peroxisomes. This might explain why the EPA level was decreased 40–80% after 3-thia fatty acid treatment whereas the DHA level was decreased only 17–24%.

When EPA or DHA is fed to rats, the acids accumulate in different organs (9). However, DHA feeding also leads to an accumulation of EPA (9). As DHA seems difficult to metabolize, one might speculate that DHA needs to be converted to EPA for further metabolism.

In contrast to prolonged DHA feeding, EPA treatment leads to proliferation of mitochondria. On the other hand, DHA acts as a peroxisome proliferator (33). Thus, different effects of these polyunsaturated n–3 fatty acids also imply different metabolic properties, i.e., they affect organelle proliferation in relation to the substrate preference.

It has been suggested that 3-thia fatty acids (34, 35) and EPA, which is the hypotriacylglycerolipidemic component of fish oil (9, 11, 12), may mediate their hypotriacylglycerolipidemic effect by increasing the mitochondrial β-oxidation. In addition, we have obtained evidence that the mitochondria are the principal target for nutritional and pharmacological control for the hypolipidemic effect of EPA, fibrates, and C14-S-acetic acid in rats and rabbits (33).

Both EPA and C14-S-acetic acid are converted to

their respective CoA esters in mitochondria (Fig. 2 and Fig. 6). Furthermore, in contrast to DHA-CoA, EPA-CoA and C14-S-acetyl-CoA are easily transferred into the mitochondria by the CAT system (Fig. 3A). EPA is more difficult to oxidize than saturated and monounsaturated fatty acids, due to the double bonds, and C14-S-acetic acid is non-oxidizable by β-oxidation, due to the sulfur atom in 3-position. Thus, accumulation of their respective CoA esters in the mitochondria might give a "fatty acid overload" signal leading to increased mitochondrial fatty acid oxidation. C14-S-acetic acid mimics the effects of peroxisome proliferators such as the fibrates and it was recently shown that it may be a ligand for the peroxisome proliferating activated receptor (PPAR)α (36). As administration of the 3-thia fatty acids seems to force EPA to the mitochondria, an additional "fish oil effect" might be seen.

It is striking that C14-S-acetic acid only marginally affects the mitochondrial CAT-I activity except when EPA-CoA was used as a substrate (Fig. 3). This indicates that the CAT-I capacity is sufficient to overcome the increased fatty acid oxidation. However, both oxidation of palmitic acid and palmitoyl-CoA increased after 3-thia fatty acid treatment (Fig. 2 and Table 3). On the other hand, mitochondrial CAT-II activity increased 3-fold (Fig. 4). In fact, the oxidation rate seems to follow the CAT-II activity, not CAT-I. It is tempting to suggest that the role of CAT-II might be underestimated under mitochondrion and peroxisome proliferation.

In conclusion, we have demonstrated that the polyunsaturated n–3 fatty acids, EPA and DHA, are differently metabolized in rat liver. As palmitic and oleic acids, EPA is oxidized by both peroxisomes and mitochondria; however, during mitochondria and peroxisome proliferation, EPA seems to be preferentially oxidized by the mitochondria whereas DHA is most likely oxidized by the peroxisomes.

This work was supported by the Norwegian Cancer Society, Research Council of Norway, and Astri og Edvard Riisøens legat. The authors are obliged to Ms. Kari Williams, Ms. Randi Sandvik, and Mr. Svein Kruger for excellent technical assistance. The assistance of Tore-Jacob Raa at Gade Institute is also appreciated. We are also grateful to Dr. J. K. Hiltunen (University of Oulu, Finland) for generously providing the cDNAs for 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase.

Manuscript received 16 July 1997 and in revised form 3 November 1997.

REFERENCES

1. Irtani, N., and R. Narita. 1984. Changes of arachidonic acid and n–3 polyunsaturated fatty acids of phospholipid classes in liver, plasma and platelets during dietary fat manipulation. *Biochim. Biophys. Acta.* **441:** 441–447.
2. Kawashima, Y., A. Hirose, and H. Kozuka. 1984. Modification by clofibric acid of acyl composition of glycerolipids in rat liver. *Biochim. Biophys. Acta.* **795:** 543–551.
3. Asiedu, D. K., L. Frøyland, H. Vaagenes, Ø. Lie, A. Demoz, and R. K. Berge. 1996. Long term effect of tetradecylthioacetic acid: a study on lipid profile and fatty acid composition and oxidation in different rat organs. *Biochim. Biophys. Acta.* **1300:** 86–96.
4. Asiedu, D., A. Aarsland, J. Skorve, A. Svardal, and R. K. Berge. 1990. Fatty acid metabolism in liver of rats treated with hypolipidemic sulfur-substituted fatty acid analogues. *Biochim. Biophys. Acta.* **1044:** 211–221.
5. Asiedu, D., J. Skorve, A. Demoz, N. Willumsen, and R. K. Berge. 1992. Relationship between translocation of long-chain acyl-CoA hydrolase, phosphatidate phosphohydrolase and CTP: phosphocholine cytidyltransferase and the synthesis of triglycerides and phosphatidylcholine in rat liver. *Lipids.* **27:** 241–247.
6. Grav, H., D. Asiedu, and R. K. Berge. 1994. Gas chromatographic measurement of 3- and 4-thia fatty acids incorporated into various classes of rat liver lipids during feeding experiments. *J. Chromatogr. B.* **658:** 1–10.
7. Madsen, L., L. Frøyland, H. Grav, and R. K. Berge. 1997. Up-regulated Δ^9-desaturase gene expression by hypolipidemic peroxisome proliferating fatty acids resulted in increased oleic acid content in liver and VLDL: accumulation of a Δ^9-desaturated metabolite of tetradecylthioacetic acid. *J. Lipid Res.* **38:** 554–563.
8. Frøyland, L., L. Madsen, W. Sjursen, A. Garras, Ø. Lie, J. Songstad, A. C. Rustan, and R. K. Berge. 1997. Effect of 3-thia fatty acids on the lipid composition of rat liver, lipoproteins, and heart. *J. Lipid Res.* **38:** 1522–1534.
9. Frøyland, L., H. Vaagenes, D. Asiedu, A. Garras, Ø. Lie, and R. K. Berge. 1996. Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholesterol and changed the fatty acid composition in rat blood and organs. *Lipids.* **31:** 169–178.
10. Drevon, C. 1992. Marine oils and their effects. *Nutr. Rev.* **50:** 38–45.
11. Willumsen, N., S. Hexeberg, J. Skorve, M. Lundquist, and R. K. Berge. 1993. Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. *J. Lipid Res.* **34:** 13–22.
12. Willumsen, N., J. Skorve, S. Hexeberg, A. C. Rustan, and R. K. Berge. 1993. The hypotriglyceridemic effect of eicosapentaenoic acid in rats is reflected in increased mitochondrial fatty acid oxidation followed by diminished lipogenesis. *Lipids.* **28:** 683–689.
13. Willumsen, N., H. Vaagenes, D. Asiedu, Ø. Lie, A. C. Rustan, and R. K. Berge. 1996. Eicosapentaenoic acid but not docosahexaenoic acid (both as ethyl esters) increases mitochondrial fatty acid oxidation and up-regulates 2,4-dienoyl-CoA reductase gene expression. A potential mechanism for the hypolipidemic action of fish oil in rats. *Lipids.* **31:** 579–592.
14. Spydevold, Ø., and J. Bremer. 1989. Induction of peroxisomal β-oxidation in 7800 C1 Morris hepatoma cells in steady state by fatty acids and fatty acid analogues. *Biochim. Biophys. Acta.* **1003:** 72–79.
15. Kawaguchi, A., S. Tsubotani, Y. Seyama, T. Yamakawa, T. Osumi, T. Hashimoto, M. Ando, and S. Okuda. 1980. Stereochemistry of dehydrogenation catalyzed by acyl-CoA oxidase. *J. Biochem.* **88:** 1481–1486.

16. DeDuve, C., B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Applemans. 1955. Intracellular distribution patterns of enzymes in rat liver tissue. *Biochem. J.* **60:** 604–617.
17. Garras, A., D. K. Asiedu, and R. K. Berge. 1995. Subcellular localisation and induction of NADH-sensitive acetyl-CoA hydrolase and propionyl-CoA hydrolase activities in rat liver under lipogenic conditions after treatment with sulfur-substituted fatty acids. *Biochim. Biophys. Acta.* **1255:** 154–160.
18. Bremer, J. 1981. The effect of fasting on the activity of liver carnitine palmitoyltransferase and its inhibition by malonyl-CoA. *Biochim. Biophys. Acta.* **665:** 628–631.
19. Small, G. M., K. Burdett, and M. J. Connock. 1985. A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase. *Biochem. J.* **227:** 205–210.
20. Kunau, W-H., and P. Dommes. 1978. Degradation of unsaturated fatty acids. Identification of intermediates in the degradation of *cis*-4-decenoyl-CoA by extracts of beef-liver mitochondria. *Eur. J. Biochem.* **91:** 533–544.
21. Bar-Tana, J., G. Rose, and B. Shapiro. 1971. The purification and properties of microsomal palmitoyl-coenzyme A synthetase. *Biochem. J.* **122:** 353–362.
22. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162:** 156–159.
23. Aasland, R., L. Akslen, J. E. Varhaug, and J. R. Lillehaug. 1990. Co-expression of the genes encoding transforming growth factor-α and its receptor in papillary carcinomas of the thyroid. *Int. J. Cancer.* **46:** 382–387.
24. Sambrook, J., E. Fritsch, and T. Maniatis. 1989. Molecular Cloning: a Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York. 86–94.
25. Feinberg, A., and P. Vogelstein. 1983. A technique for radiolabeling DNA restriction fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.
26. Lie, Ø., E. Lied, and G. Lambertsen. 1987. Lipid digestion in cod. *Comp. Biochem. Physiol.* **88B:** 697–700.
27. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* **43:** 506–520.
28. Seglen, P. 1973. Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exp. Cell Res.* **82:** 391–398.
29. Frøyland, L., K. Helland, G. K. Totland, H. Kryvi, and R. K. Berge. 1996. A hypolipidemic peroxisome proliferating fatty acid induces polydispersity of rat liver mitochondria. *Biol. Cell.* **87:** 105–112.
30. Schulz, H., and W. H. Kunau. 1987. Beta-oxidation of unsaturated fatty acids: a revised pathway. *Trends Biochem. Sci.* **12:** 403–406.
31. Lazo, O., M. Contreras, Y. Yoshida, A. K. Singh, W. Stanley, M. Weise, and I. Singh. 1990. Cellular oxidation of lignoceric acid is regulated by the subcellular localization of lignoceroyl-CoA ligases. *J. Lipid Res.* **31:** 583–595.
32. Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. DeShepper. 1979. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J. Biol. Chem.* **254:** 4585–4595.
33. Frøyland, L., L. Madsen, H. Vaagenes, G. K. Totland, J. Auwerx, H. Kryvi, B. Staels, and R. K. Berge. 1997. Mitochondrion is the principal target for nutritional and pharmacological control for triglyceride metabolism. *J. Lipid Res.* **38:** 1851–1858.
34. Asiedu, D. K., A. Al-Shurbaji, A. C. Rustan, I. Björkhem, L. Berglund, and R. K. Berge. 1995. Hepatic fatty acid metabolism as a determinant of plasma and liver triacylglycerol levels. *Eur. J. Biochem.* **227:** 715–722.
35. Asiedu, D. K., A. Demoz, J. Skorve, H. J. Grav, and R. K. Berge. 1995. Acute modulation of rat hepatic lipid metabolism by sulphur-substituted fatty acid analogues. *Biochem. Pharmacol.* **49:** 1013–1022.
36. Foreman, B. M., J. Chen, and R. M. Evans. 1996. The peroxisome proliferator-activated receptors: ligands and activators. *Ann. N.Y. Acad. Sci.* **804:** 266–275.