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COMPARATIVE EFFECTS OF OXYGEN AND SULFUR-SUBSTITUTED FATTY ACIDS ON SERUM LIPIDS AND MITOCHONDRIAL AND PEROXISOMAL FATTY ACID OXIDATION IN RAT

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Abstract—Feeding tetradecyloxyacetic acid (a 3-oxa fatty acid) to rats led to decreased serum cholesterol and decreased serum triacylglycerol, resembling the effects of the corresponding 3-thia fatty acid (tetradecylthioacetic acid). The 3-oxa fatty acid inhibited strongly the mitochondrial fatty acid oxidation and led to the development of fatty liver, while the 3-thia fatty acid stimulated the mitochondrial fatty acid oxidation. Feeding tetradecyloxypropionic acid (a 4-oxa fatty acid) had less effect on the serum lipids. It stimulated fatty acid oxidation in the mitochondria and lowered the hepatic level of triacylglycerol. The corresponding 4-thia fatty acid (tetradecylthiopropionic acid) inhibited mitochondrial fatty acid oxidation and induced development of fatty liver. All these compounds, both the oxa and the thia fatty acids, induced some increase in the activity of the peroxisomal acyl-CoA oxidase. Repeated administration of 3-oxadicarboxylic acid to rats resulted in no lipid lowering effects, and marginal changes of fatty acyl-CoA oxidase activity. Oxidation of the S-atom of the 3-thia fatty acid to the corresponding sulfoxide or sulfone eliminated the metabolic effects of the thia fatty acid. The study has shown that the effects of 3- and 4-oxa fatty acids are in some ways opposite to those of the 3- and 4-thia fatty acids. The possibility that the lipophilicity of the fatty acid analogues may be an important factor behind the differences observed are discussed. It is suggested that these oxa- and thia-analogues of fatty acids may be useful in studies on the regulation of fatty acid metabolism.

Administration of 3-thia fatty acid (tetradecylthioacetic acid) to rats decreases their serum concentrations of triacylglycerol and cholesterol, induces proliferation of peroxisomes and increases peroxisomal oxidation of fatty acids [1–3]. The mitochondrial fatty acid oxidation is also enhanced, as are the activities of the carnitine acyltransferases [4, 5]. Thus, the lowering of serum triacylglycerol is at least partially due to increased fatty acid oxidation in the liver [6].

In contrast, a 4-thia fatty acid (tetradecylthiopropionic acid) causes fatty liver, and liver mitochondria isolated from rats fed with this fatty acid for a week show decreased capacity to oxidize fatty acids [2, 4–6]. The fatty liver which develops is probably caused by the inhibition of fatty acid oxidation and an accelerated triacylglycerol biosynthesis due to stimulation of the enzyme phosphatidate phosphohydrolase [6].

The dicarboxylic acid, 3-thiadicarboxylic acid, is a more potent peroxisome proliferator, but has the same hypolipidemic effect as tetradecylthioacetic acid. It is conceivable that both the hydrophobicity, and the availability for β- and ω-oxidation may determine the metabolic effects of these fatty acid analogues.

Because of the distinct metabolic differences between these chemically closely related fatty acid analogues we have now extended the feeding studies to 3- and 4-oxa fatty acids and 3-oxadicarboxylic acid (Table 1). Since the 3-thia fatty acids (besides being ω-oxidized) are oxidized biologically to their sulfoxides [7], we have also included the sulfoxide and the sulfone of a thia fatty acid (tetradecylthiacetic acid) in the study.

MATERIALS AND METHODS

Chemicals. [1-^{14}C]palmitoyl-CoA, [1-^{14}C]palmitoyl-L-carnitine and [1-^{14}C]carnitine were purchased from New England Nuclear (Boston, MA, U.S.A.). Palmitoyl-CoA, palmitoyl-L-carnitine, L-carnitine, CoASH and Hepes‖ were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of analytical grade or of the highest purity available.

Synthesis of oxy- and thio-substituted fatty acid analogues. Tetradecylthioacetic acid (alkylthioacetic acid), and tetradecylthiopropionic acid (alkylthiopropionic acid), tetradecylsulfinylacetic acid and tetradecylsulfonylacetic acid were prepared as described earlier [8, 9].

Tetradecyloxyacetic acid was made by alkaline hydrolysis of the corresponding ethyl ester which

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‖ Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; CPT, carnitine palmitoyltransferase; POCA, 2(5(4-chlorophenyl)pentyl)oxirane-2-carbonyl-CoA.

Table 1.

Structure of compounds	Systematic names	Trivial names
$CH_3—CH_2—(CH_2)_{12}—O—CH_2—COOH$	Tetradecyloxyacetic acid	Alkyloxyacetic acid
$CH_3—CH_2—(CH_2)_{12}—O—CH_2—CH_2—COOH$	Tetradecyloxypropionic acid	Alkyloxypropionic acid
$HOOC—CH_2—O—(CH_2)_{10}—O—CH_2—COOH$	3,14-Dioxahexadecanedioic acid	3-Oxadicarboxylic acid
$CH_3—CH_2—(CH_2)_{12}—S—CH_2—COOH$	Tetradecylthioacetic acid	Alkylthioacetic acid
$CH_3—CH_2—(CH_2)_{12}—S—CH_2—CH_2—COOH$	Tetradecylthiopropionic acid	Alkylthiopropionic acid
$HOOC—CH_2—S—(CH_2)_{10}—S—CH_2—COOH$	3,14-Dithiahexadecanedioic acid	3-Thiadicarboxylic acid
$CH_3—CH_2—(CH_2)_{12}—\overset{\overset{O}{\|}}{S}—CH_2—COOH$	Tetradecylsulfinylacetic acid	Alkylsulfinylacetic acid
$CH_3—CH_2—(CH_2)_{12}—\overset{\overset{O}{\|}}{S}—CH_2—COOH$	Tetradecylsulfonylacetic acid	Alkylsulfonylacetic acid

was synthesized by Lewis acid-catalysed carboxymethylation of tetradecanol. Tetradecyloxypropionic acid was made by hydrolysis of the corresponding nitrile which in turn was made by base-catalysed addition of tetradecanol and acrylonitrile.

The NMR spectra were recorded on a Varian XL 300 instrument using TMS as internal standard. The i.r. spectra were recorded on a Perkin–Elmer 1310 Infrared Spectrophotometer. The mass spectra were obtained with a VG Micromass 7070 F. The melting points were obtained with a Reichert Thermopan melting point microscope and are uncorrected.

Tetradecyloxyacetic acid ethyl ester. Tetradecanol (23.6 g, 0.110 mol) and diazoacetic acid ethyl ester (16 g, 0.14 mol) was dissolved in dichloromethane (140 mL) and cooled to 0° in an ice/water bath. A solution of boron trifluoride etherate (3.0 mL) in dichloromethane (10 mL) was added dropwise for 10 min. After the addition of boron trifluoride the reaction mixture was stirred at room temperature for 45 min. The dichloromethane solution was washed with water and dried ($MgSO_4$). After filtration and evaporation the product was purified by distillation. Yield 20.5 g (62%), b.p. 122–125°/0.01 mmHg. i.r. (film): 1750 cm^{-1} (CO). NMR and mass spectroscopic results were in accordance with the structure.

Tetradecyloxyacetic acid. Tetradecyloxyacetic acid ethyl ester (18 g, 0.060 mol) was dissolved in methanol (100 mL). A solution of sodium hydroxide (8.0 g, 0.2 mol) in water (50 mL) was added and the mixture was refluxed for 24 hr. The reaction mixture was cooled and acidified with hydrochloric acid. Precipitated material was extracted into ether and the ether solution was washed several times with water. The ether solution was dried ($MgSO_4$), filtered and evaporated. The product was purified by recrystallization from ether/hexane. Yield 12.5 g (76%) m.p. 58–59°. i.r. (KBr): 3500–2500 cm^{-1} (OH), 1700 cm^{-1} (CO). ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (3H, t, J 6.7 Hz [CH_3]), 1.2–1.4 (22H, [CH_2]), 1.60 (2H, p, [CH_2—C—O]), 3.53 (2H, t, J 6.7 Hz, [R-CH_2—O]), 4.10 (2H, s, [O—CH2—COO]), 11.2 (1H, broad s, [COOH]). ^{13}C NMR (75 MHz, $CDCl_3$):δ 14.0 (CH_3), 22.6–31.8 (CH_2), 67.6 (CH_2—O), 72.0 (CH_2—O), 175.5 (COOH). MS (IP 70 eV, $m/z > 100$, rel.int > 5%):272 (8%,M), 111 (17%). Table 1 gives the structure of the different compounds.

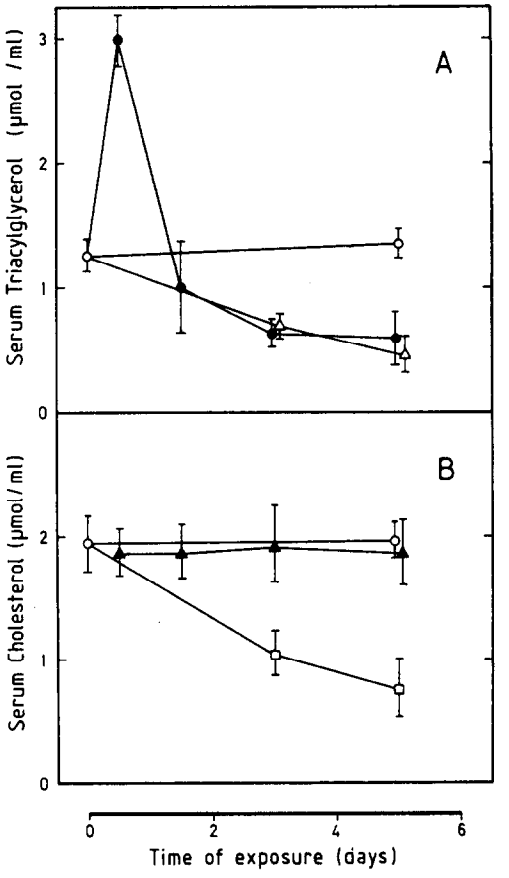

Fig. 1. Effect of tetradecyloxyacetic acid exposure on serum triacylglycerol (A) and cholesterol (B) at doses of 150 (●, ▲) and 250 (△, □) mg/day/kg body weight. Changes in serum lipids in control animals during the 5-day period (○).

Tetradecyloxypropionitrile. 1-Tetradecanol (21.4 g, 0.100 mol) was dissolved in *t*-butanol (15 mL)

at 50°. A solution of tetrabutyl ammonium hydroxide in methanol (25%, 0.3 mL) was added and the mixture was cooled to 35°. Acrylonitrile (6.38 g, 0.120 mol) was added dropwise for 10 min and thereafter the reaction mixture was stirred at 35° for 30 min. During the addition of acrylonitrile and while stirring a further 1.0 mL of tetrabutyl ammonium hydroxide in methanol was added continuously. The reaction mixture was poured into water and the product was taken up in ether. The ether solution was washed with water and dried ($MgSO_4$). After filtration ether was removed and the product was purified by distillation. Yield 14.1 g (53%), b.p. 128–130°/0.01 mmHg. MS (IP 70 eV, $m/z > 200$, rel.int ≥3%): 267 (3%,M), 238 (8%), 224 (7%), 210 (8%).

Tetradecyloxypropionic acid. A mixture of tetradecyloxypropionitrile (13 g, 0.049 mol), glacial acetic acid (40 mL) and concentrated hydrochloric acid (40 mL) was refluxed for 4 hr. The mixture was cooled and extracted with ether. The ether solution was washed with water until the washings became neutral, dried ($MgSO_4$) and evaporated. The residue was crystallized from ethanol/water. Yield 11.4 g (81%), m.p. 65.0–65.5°. i.r. (KBr): 3200–2500 cm^{-1} (OH), 1700 cm^{-1} (CO). ^1H NMR (300 MHZ, $CDCl_3$): δ 0.88 (3H, t, [CH_3]), 1.2–1.4 (22H, [CH_2]), 1.56 (2H, p, [R-CH_2—C—O]), 2.63 (2H, t, J 6.4 Hz, [CH_2—COO]), 3,45 (2H, t, J 6.7 Hz, [R-CH_2—O]), 3.70 (2H, t, J 6.4 Hz, [O—CH_2—C—COO]), 11.3 (1H, broad s, [COOH]). ^{13}C NMR (75 MHz, $CDCl_3$):δ 14.0 (CH_3), 22.6–31.8 (CH_2), 65.6 (CH_2—O), 71.2 (CH_2—O), 177.4 (COOH). MS (CI NH_3): 304 (100%, M·NH_4), 287 (7%, M·H, 286 (4%, M).

3-Oxadicarboxylic acid. The preparation was based upon the reaction between the disodium salt of the 1.10-diol and the potassium salt of chloroacetic acid, 2 mol, in diglyme (diethyleneglycol dimethyl ether) in an atmosphere of dry argon to prevent atmospheric moisture.

Diol (17.4 g, 0.1 mol) was dissolved in 50 mL diglyme at 40–50°. Two equivalents of metallic sodium, 4.6 g, was added and dissolved for 48 hr at 70–90°. To the clear solution was then added in small portions 26.5 g $ClCH_2COOK$. After the addition was completed the reaction mixture was kept at around 70° under constant stirring for 3–4 hr. After cooling to room temperature 100 mL water was added and the pH adjusted to 1–2 with hydrochloride acid. After two nights at 0° the reaction mixture was filtered. The solid was washed with water to pH 5, dried in air and crystallized from methanol and then from ethanol. This sample of the desired bis-acetic acid gave a clear solution when dissolved in acetone. The compound was finally converted into its dipotassium salt with an aqueous solution of KOH, the solution carefully washed with diethylether and liberated with hydrochloride acid as indicated above. After drying in air, crystallization from methanol and ethanol and drying at 1 mmHg to constant weight, the correct molecular weight was obtained by a titration with sodium hydroxide in ethanol. The total yield of pure product ranged from 40 to 50%.

Animals and diets. Male Wistar rats from Möllegaard Breeding Laboratory, Ejby, Denmark, weighing 150–170 g, were housed in metal wire cages in a room maintained under a 12 hr light–dark cycle and a constant temperature of 20 ± 3°. The animals were acclimatized for at least 5 days under these conditions before the start of the experiments. The fatty acid analogues were suspended in 0.5% sodium carboxymethyl cellulose. The individual analogues were administered by gastric intubation in a volume of 0.7–1.0 mL once a day (see legends to figures and tables).

The control animal groups received only sodium

Table 2. Serum lipid composition in male rats treated with oxygen- and sulphur-substituted fatty acid analogues

Treatment	Dose (mg/day/kg body weight)	Serum lipids	
		Triacylglycerols	Cholesterol
Exp. I			
Control	0	1.25 ± 0.30	1.98 ± 0.25
Alkyloxyacetic acid	150	0.74 ± 0.20*	1.75 ± 0.20
	250	0.36 ± 0.15*	0.83 ± 0.15*
Exp. II			
Control	0	0.74 ± 0.07	1.46 ± 0.25
Alkyloxyacetic acid	400	0.21 ± 0.09*	0.53 ± 0.22*
Alkyloxypropionic acid	250	0.91 ± 0.20	1.63 ± 0.42
	400	0.44 ± 0.14*	1.55 ± 0.40
Exp. III			
Control	0	1.32 ± 0.19	2.01 ± 0.36
3-Oxadicarboxylic acid	150	0.97 ± 0.20	1.89 ± 0.10
	250	1.30 ± 0.30	1.94 ± 0.40
	400	0.99 ± 0.05	1.62 ± 0.15
Exp. IV			
Control	0	1.18 ± 0.25	2.05 ± 0.20
Alkylsulfinylacetic acid	250	1.05 ± 0.30	1.88 ± 0.15
Alkylsulfonylacetic acid	250	1.15 ± 0.20	1.98 ± 0.30

The analogues were administered for 5 days at the dose levels shown.

Values are reported as the means ± SD for three to six rats and are expressed as μmol/mL serum. *$P < 0.05$.

Table 3. Liver mass and hepatic protein content in male rats following oxygen- and sulphur-substituted fatty acid analogues treatment for 5 days

Treatment	Dose (mg/day/kg body weight)	Liver mass		Protein (mg/g liver)
		g wet weight	Relative %, body weight	
Exp. I				
Control	0	5.75 ± 0.45	3.41 ± 0.20	165.0 ± 8.2
Alkyloxyacetic acid	150	6.43 ± 0.57	4.06 ± 0.23*	152.6 ± 4.1
	250	6.85 ± 0.36	3.97 ± 0.11*	135.3 ± 2.1*
Exp. II				
Control	0	6.08 ± 0.28	3.36 ± 0.30	155.2 ± 6.6
Alkyloxyacetic acid	400	5.75 ± 0.25	4.02 ± 0.21*	138.6 ± 3.1*
Alkyloxypropionic acid	250	6.07 ± 0.30	3.43 ± 0.35	169.4 ± 6.4
	400	6.30 ± 0.70	3.60 ± 0.40	167.3 ± 8.5
Exp. III				
Control	0	6.82 ± 0.91	3.08 ± 0.30	176.2 ± 8.9
3-oxadicarboxylic acid	150	6.63 ± 0.30	3.00 ± 0.17	163.4 ± 5.2
	250	6.98 ± 0.54	3.09 ± 0.12*	160.1 ± 3.2*
	400	7.34 ± 0.73	3.0 ± 0.11	159.8 ± 4.1
Exp. IV				
Control	0	6.05 ± 0.45	3.25 ± 0.20	167.2 ± 6.9
Alkylsulfinylacetic acid	250	5.98 ± 0.62	3.40 ± 0.10	165.6 ± 7.1
Alkylsulfonylacetic acid	250	6.16 ± 0.37	3.30 ± 0.20	168.1 ± 5.3

Values are expressed as the means ± SD for three experimental rats and six control animals. *$P < 0.05$.

carboxymethyl cellulose. All animals had free access to water and food. The body weights were measured daily. At the end of the experiments the fasted rats (12 hr) were lightly anesthesized and cardiac puncture was performed. The livers were removed and immediately chilled on ice and weighed.

All animals treated with the fatty acid analogues at various doses and as a function of time gained in body weight at the same rate as controls. Rats in experimental groups consumed similar amounts of food (20–24 g/day) irrespective of the dietary regime, indicating that the appetite was not affected and the drugs were well tolerated. Drug-treated rats appeared healthy and looked and behaved like normal animals.

Preparation of total homogenate and cellular fractions. The livers from individual rats were homogenized in ice-cold sucrose-medium (0.25 M, sucrose in 10 mM Hepes buffer, pH 7.4, and 2 mM EDTA). The postnuclear fraction was used for further analysis by differential centrifugation. Samples from three animals were pooled and mitochondrial-enriched, peroxisome-enriched, microsomal and cytosolic fractions were isolated [1].

The variation of the response from animal to animal was estimated separately for selected enzymes in the group of control animals.

Other analytical methods. The enzymatic activity of palmitoyl-CoA hydrolase and acyl-CoA oxidase activity were determined in accordance with earlier descriptions (1–4). Palmitoyl-CoA- and palmitoyl-L-carnitine oxidation was evaluated by measuring the appearance of acid-soluble products [4]. Total CPT activity was measured as in Ref. 10. Protein was assayed by Bio-Rad protein assay kit (Bio-Rad, Richmond, CA). Lipid analyses were performed as recommended (the monotest cholesterol enzymatic kit, Boehringer Mannheim, Germany and Biopak triglyceride enzymatic kit, Biotrol, Paris, France). Results are expressed as mean ± SD. Statistical analysis was by Student's *t*-test and $P > 0.05$ was taken to be statistically insignificant.

RESULTS

Serum lipids

The chemical structures of the oxygen-substituted fatty acid analogues used in this study suggest that the tetradecyloxyacetic acid cannot be β-oxidized, whereas the tetradecyloxypropionic acid can undergo one cycle of β-oxidation. The 3-oxadicarboxylic acid is blocked for both ω- and β-oxidation (Table 1).

Administration of tetradecyloxyacetic acid to rats caused a significant reduction in the triacylglycerol level with a dose of 150 mg/day/kg body weight. A transient increase in serum triacylglycerol level was, however, seen after 12 hr of feeding (Fig. 1). A significant hypocholesterolemic effect was seen with a dose of 250 mg/day/kg body weight or above (Table 2). The lipid-lowering effect was in both cases established during the third day of treatment (Fig. 1).

Tetradecyloxypropionic acid and 3-oxadicarboxylic acid had smaller effects on the serum lipids. Only with the highest dose used, 400 mg/day/kg body weight, did tetradecyloxypropionic acid have some lowering effect on serum triacylglycerol while the effect on cholesterol was not significant (Table 2).

The alkylsulfinyl- and alkylsulfonylacetic acids had no significant effects on the blood lipids (Table 2).

Liver weight and protein content

Administration of alkyloxyacetic acid significantly increased the relative liver weight whereas the protein content was decreased with doses above 250 mg/day/kg body weight. With alkyloxypropionic

acid and 3-oxadicarboxylic acid feeding the protein content remained unchanged and no hepatomegaly was observed. No hepatomegaly was observed in alkylsulfinyl- and alkylsulfonylacetic acid-fed animals (Table 3).

Liver lipids

Table 4 shows that alkyloxyacetic acid gave a 5–6-fold elevation of liver triacylglycerol even at the lowest dose used. Liver cholesterol was also increased, more than 2-fold, with the higher doses used. Figure 2 shows that the lipidosis was already under development after 12 hr with a dose of 150 mg/kg body weight.

Alkyloxypropionic acid caused no lipidosis. The highest dose even seemed to lower the liver content of triacylglycerol (Table 4).

3-Oxadicarboxylic acid only marginally affected the hepatic levels of triacylglycerol and cholesterol, and the alkylsulfinyl- and alkylsulfonylacetic acids had no significant effects on liver lipids (Table 4).

Fatty acid oxidation

Feeding rats alkyloxyacetic acid increased the activity of the peroxisomal acyl-CoA oxidase. With a dose of 150 mg/day/kg body weight the increased activity of the peroxisomal enzyme was already established during the first day of treatment (Fig. 3A).

This fatty acid analogue had a qualitatively different effect on the mitochondrial fatty acid oxidation. The animals fed alkyloxyacetic acid showed more than 90% inhibition of the oxidation of palmitoylcarnitine in isolated liver mitochondria (Fig. 4). Experiments with palmitoyl-CoA as substrate showed a similar degree of inhibition (data not shown). After treatment of rats with 150 mg/

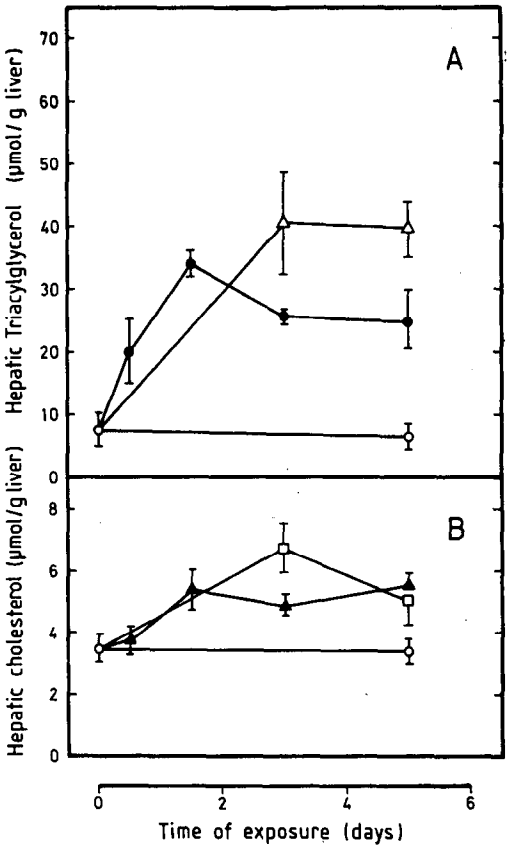

Fig. 2. Effect of tetradecyloxyacetic acid exposure on hepatic triacylglycerol (A) and cholesterol (B) at doses of 150 (●, ▲) and 250 (△, □) mg/day/kg body weight. Changes in hepatic lipids in control rats (○).

Table 4. Hepatic lipids in male rats treated with oxygen- and sulphur-substituted fatty acid analogues

Treatment	Dose (mg/day/kg body weight)	Liver lipids	
		Triacylglycerols	Cholesterol
Exp. I			
Control	0	6.3 ± 1.0	3.3 ± 0.5
Alkyloxyacetic acid	150	29.6 ± 8.7*	5.3 ± 0.5*
	250	39.4 ± 4.0*	5.0 ± 0.8*
Exp. II			
Control	0	8.8 ± 2.2	1.9 ± 0.5
Alkyloxyacetic acid	400	37.5 ± 8.7*	7.7 ± 2.9*
Alkyloxypropionic acid	250	7.7 ± 1.1	2.9 ± 0.1*
	400	5.0 ± 0.9*	1.8 ± 0.2
Exp. III			
Control	0	6.8 ± 1.3	3.1 ± 0.4
3-Oxadicarboxylic acid	150	6.5 ± 1.6	2.5 ± 0.3
	250	6.9 ± 0.9	2.6 ± 0.4
	400	6.9 ± 1.4	3.8 ± 0.4
Exp. IV			
Control	0	6.9 ± 1.2	3.5 ± 0.3
Alkylsulfinylacetic acid	250	6.5 ± 0.5	3.6 ± 0.3
Alkylsulfonylacetic acid	250	6.7 ± 1.5	3.4 ± 0.3

The analogues were administered for 5 days at the dose levels shown.

Values are reported as the means ± SD for three to six rats and are expressed as μmol/g liver. *$P < 0.05$.

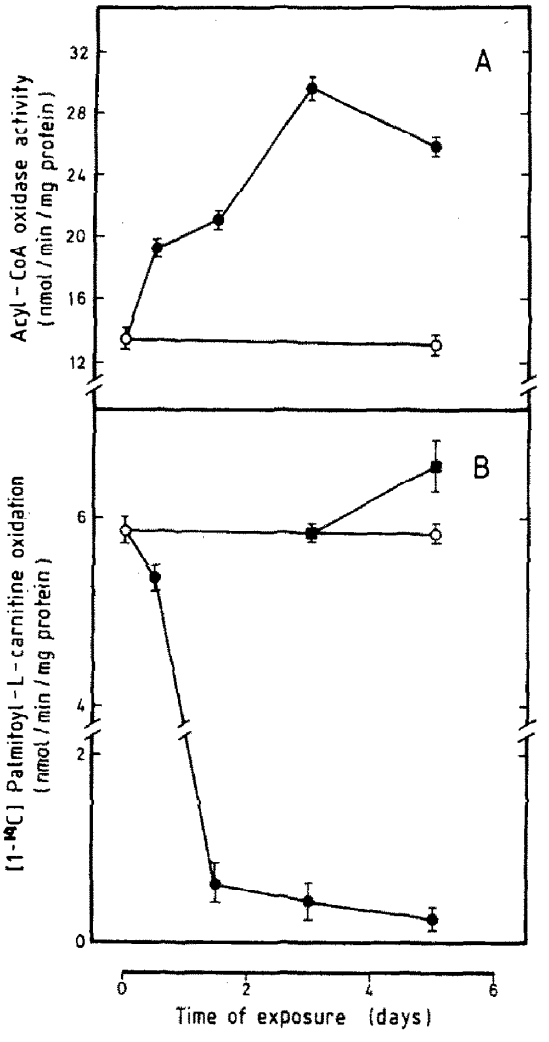

Fig. 3. Effect of tetradecyloxyacetic acid (150 mg/day/kg body weight) exposure on the activity of acyl-CoA oxidase in the peroxisomal fraction (●) (A). Effect on [1-^{14}C]-palmitoyl-L-carnitine oxidation in the mitochondrial fraction after exposure to tetradecyloxyacetic acid (150 mg/day/kg body weight) (●) and after exposure to 3-oxadicarboxylic acid (150 mg/day/kg body weight) (■) (B). Changes in activity in control rats (○).

day/kg body weight alkyloxyacetic acid, the inhibited fatty acid oxidation was established after 36 hr of treatment (Fig. 3B). There was, however, no effect on the mitochondrial β-oxidation after 12 hr treatment, when substantial effects on serum and hepatic triacylglycerol levels were seen.

Mitochondria from animals fed alkyloxypropionic acid showed increased rates of palmitoylcarnitine oxidation (Fig. 4). The effects of the corresponding alkylthio acids were reversed. Liver mitochondria from rats fed alkylthiopropionic acid showed decreased rates of palmitoylcarnitine oxidation while mitochondria from rats fed alkylthioacetic acid had an increased rate of palmitoylcarnitine oxidation.

The activity of CPT always changed in the same direction as the peroxisomal acyl-CoA oxidase. 3-Oxadicarboxylic acid and the alkylsulfinyl- and alkylsulfonylacetic acids had only minor effects on fatty acid oxidation (Figs 3 and 4).

DISCUSSION

The presented results show that oxa fatty acids, like thia fatty acids, may have hypocholesterolemic and hypotriglycerolemic effects in normal rats. The alkyloxyacetic acid had, in contrast to alkyloxypropionic acid and 3-oxadicarboxylic acid, a strong lowering effect on plasma lipids, depending on the dose administered to the rats. Furthermore, alkyloxyacetic acid strongly inhibited mitochondrial fatty acid oxidation which resulted in the formation of fatty liver. Some stimulation of the peroxisomal acyl-CoA oxidase evidently was not sufficient to prevent this accumulation of hepatic triglycerides.

Another fatty acid analogue, POCA, has also been shown to be hypolipidemic in rats and to induce a limited proliferation of peroxisomes [11, 12]. The CoA ester of POCA inhibits mitochondrial β-oxidation at the level of CPT I, and probably also directly inhibits fatty acid synthase [13]. None of the sulphur- and oxygen-substituted fatty acids used in this study has a similar effect on CPT. The activity of this enzyme is increased somewhat after feeding with these analogues as shown in this and earlier studies [1, 5].

It is reasonable to assume that increased fatty acid oxidation in the liver will lower the formation and export of very-low-density lipoprotein and therefore lower the plasma triacylglycerol level. The hypolipidemic effect of 3-thiadicarboxylic acid and alkylthioacetic acid has been ascribed partly to increased fatty acid oxidation [6]. The different observations mentioned above regarding alkyloxyacetic acid and POCA show however, that fatty acid analogues may have a lowering effect on plasma lipids independent of their effects on fatty acid oxidation, pointing to rather diverse mechanisms behind the hypolipidemic effect.

Furthermore, both alkyloxyacetic acid and alkylthiopropionic acid reduce the plasma level of triglycerides at a feeding dose where the cholesterol level is unchanged, an effect which has not been seen with the other hypolipidemic analogues. This indicates that the plasma levels of triglycerides and cholesterol can be regulated independently, and that fatty acid analogues interfere with this regulation at different sites.

The mechanism behind the inhibition of mitochondrial β-oxidation by alkyloxyacetic acid and alkylthiopropionic acid is not fully elucidated. It has been shown recently that 4-thia-acyl-carnitine derivatives inhibits fatty acid oxidation both *in vitro* and *in vivo* [5]. It was suggested that apparently irreversible inhibitors were generated during β-oxidation of these fatty acid derivatives.

Lau *et al.* [14, 15] have shown that 4-thia-acyl-CoA is a better substrate for the mitochondrial medium-chain acyl-CoA dehydrogenase than 3-thia-acyl-CoA. The 4-thia-2-*trans*-enoyl-CoA formed binds strongly to the dehydrogenase, which might explain the inhibitory action of the alkylthiopropionic acid. The 3-oxa-acyl-CoA derivative is, however, a much poorer substrate for the medium-chain acyl-

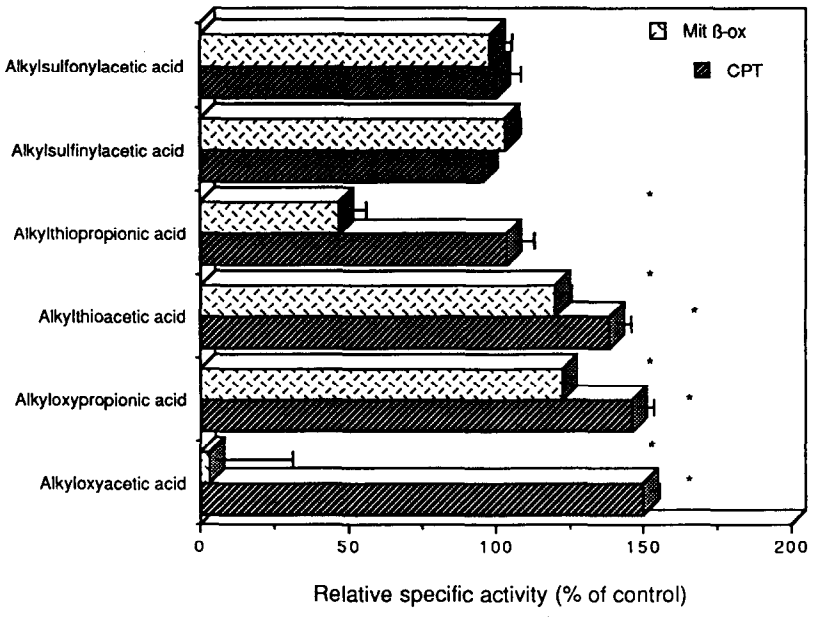

Fig. 4. Relative specific activities of CPT and [1-^{14}C]palmitoyl-L-carnitine oxidation (mit. β-ox.) in liver mitochondria of male rats. Rats were fed fatty acid analogues for 5 days (250 mg/day/kg body weight). Control activities (100%) were 21.1 ± 2.5 and 5.95 ± 0.5 nmol/min/mg protein for CPT and mit.β-ox., respectively. *$P < 0.05$ compared to control.

CoA dehydrogenase than 4-thia-acyl-CoA [14]. This may indicate that the mechanisms behind the inhibition of β-oxidation by the oxa- and thia-analogues are different.

When comparing the analogues used in this study, it can be seen that changing the hetero-atom substituent or moving it one position in the carbon chain, profoundly influences the properties of the fatty acids. It is striking that 3-oxadicarboxylic acid has no effect on plasma and hepatic lipids and fatty acid oxidation, parameters that are strongly influenced by the corresponding sulfur derivative, 3-thiadicarboxylic acid [1, 2]. Alkylthioacetic acid increases fatty acid oxidation while the corresponding oxa fatty acid inhibits fatty acid oxidation and induces fatty liver, in this respect resembling the 4-thia derivative, alkylthiopropionic acid. It is at present hard to envisage which physico-chemical properties of the fatty acid analogues may contribute to these contrasting effects. Some ideas as to which properties may be important can however be put forward.

In a recent study [16] we have shown that there is no correlation between the rate of CoA esterification of the different fatty acid analogues, and their hypolipidemic or peroxisome proliferating capacity. The alkylthioacids are more efficient inducers of peroxisomal activity than the alkyloxyacids. The alkylsulfinyl- and alkylsulfonylacetic acids have no effect. Presumably both polarity and the acid strength of these compounds increase in the same order. These physical properties may influence their ability to activate the genes of peroxisomal enzymes. It is tempting to suggest that when the alkylthioacetic acid has entered into hepatocytes it is bound to the endoplasmic reticulum due to its lipophilicity, and thereby undergoes sulfur oxidation and ω-oxidation as sulfinyl metabolites. The proposed metabolic pathway is an initial ω-hydroxylation followed by oxidation to a dicarboxylic acid and β-oxidation from the ω-end [7]. It is apparent that when the drugs have become sufficient polar, like tetradecylsulfinylacetic acid and tetradecylsulfonylacetic acid, they are not captured by the endoplasmic reticulum but becomes substrates for soluble enzymes. The hydrophobic character of the peroxisome-proliferating fatty acids may thus play an important role in determining the choice of enzyme systems used and consequently the type of metabolism.

The different effects observed make some of these fatty acid analogues interesting tools in studies on the regulation of fatty acid metabolism.

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