Up-regulated Δ^9-desaturase gene expression by hypolipidemic peroxisome-proliferating fatty acids results in increased oleic acid content in liver and VLDL: accumulation of a Δ^9-desaturated metabolite of tetradecylthioacetic acid

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Abstract In the liver of rats, monocarboxylic 3-thia fatty acids, tridecylthioacetic acid (C_{13}-S-acetic acid) and tetradecylthioacetic acid (C_{14}-S-acetic acid), increase the mRNA levels of Δ^9-desaturase both in a time- and dose-dependent manner. The increased Δ^9-desaturase mRNA levels were accompanied by increased Δ^9-desaturase activity and increased amounts of oleic acid (18:1 n–9) and Δ^9-desaturated C_{14}-S-acetic acid. Δ^9-Desaturated C_{14}-S-acetic acid was only detected in phospholipid and cholesterolester species after C_{14}-S-acetic acid treatment. In contrast, C_{14}-S-acetic acid was detected in all the different hepatic lipid fractions, but mainly in the phospholipids. Moreover, C_{13}-S-acetic acid and C_{14}-S-acetic acid were detected in both liver and very low density lipoprotein (VLDL). No Δ^9-desaturated 3-thia fatty acid products, however, were found in VLDL. Administration of mono- and dicarboxylic 3-thia fatty acids to rats induced liver expression of the fatty acyl-CoA oxidase gene. After 1 week of C_{14}-S-acetic acid treatment, the levels of fatty acyl-CoA oxidase mRNA increased 5-fold, whereas the Δ^9-desaturase mRNA was increased about 1.8-fold. Both fatty acyl-CoA oxidase and Δ^9-desaturase mRNA increased about 8-fold after 12 weeks of treatment with C_{14}-S-acetic acid. In conclusion, this study demonstrates that C_{14}-S-acetic acid increases rat Δ^9-desaturase gene expression and activity and that changes in hepatic lipids, e.g., 18:1 n–9, are reflected in the VLDL. The peroxisome-proliferating monocarboxylic thia fatty acids are good substrates for desaturases, as Δ^9-desaturated metabolites of monocarboxylated thia acids were formed in the liver. Modification of Δ^9-desaturation, however, appears not to be related to peroxisome proliferation.—**Madsen, L., L. Frøyland, H. J. Grav, and R. K. Berge.** UP-regulated Δ^9-desaturase gene expression by hypolipidemic peroxisome-proliferating fatty acids results in increased oleic acid content in liver and VLDL. *J. Lipid Res.* 1997. **38:** 554–563.

Supplementary key words 3-thia fatty acids • fatty acid composition • Δ^9-desaturase • fatty acyl-CoA oxidase • liver • VLDL

The 3-thia fatty acids, tetradecylthioacetic (C_{14}-S-acetic) acid and 1,10 bis(carboxymethylthio)decane (TD) have hypolipidemic effects in normolipidemic (1) and hypertriglyceridemic rats (2). They probably exert their effects by interfering with fatty acid and very low density lipoprotein (VLDL) metabolic cycles (3). Despite their clear effects on lipoprotein levels, such as reducing plasma VLDL and increasing high density lipoprotein (HDL) concentrations, lipoprotein lipase is not activated (4). Therefore, they do not seem to act mainly by increasing VLDL catabolism. Although the 3-thia fatty acids act mainly by decreasing the VLDL production, the molecular basis of these phenomena has not been defined.

Many eucaryotic cells have the capacity for 2-carbon chain elongation both of endogenously synthesized fatty acids and of exogenous, dietary fatty acids. Monounsaturated fatty acids are formed in mammalian systems by direct oxidative desaturation of a preformed long-chain saturated fatty acid. The Δ^9-desaturase is usually the predominant, if not exclusive, desaturation enzyme of saturated fatty acids in liver, mammary gland, brain, testis, and adipose tissue.

3-Thia fatty acids influence the balance between fatty acid oxidation and esterification into glycerolipids. At the same time, 3-thia monocarboxylic fatty acids are themselves incorporated into phospholipids (5) and it is therefore possible that they change the fatty acid composition of these glycerolipids. As tetradecylthioacetic acid seems to resemble normal saturated fatty acids in glycerolipid biosynthesis, we were interested in studying

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; PPAR, peroxisome proliferator-activated receptor.

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whether these changes of fatty acid composition are due to the concerted activities of elongation and desaturation enzymes. Given that the fatty acid composition of glycerolipids has an important role in controlling the lipoprotein metabolism, we now report that C_{14}-S-acetic acid up-regulates Δ^9-desaturase mRNA levels and activity in rat liver concomitant with the formation of a Δ^9-desaturated metabolite of C_{14}-S-acetic acid, i.e., the saturated 3-thia fatty acid can be converted to a monoenic 3-thia fatty acid in vivo.

MATERIALS AND METHODS

Chemical and drugs

[^{32}Pa]dCTP (3000 mCi/mmol) was purchased from the Radiochemical Center, Amersham, England, and stearoyl-(carbonyl-^{14}C)CoA was from Sigma Chemical Co., St. Louis, MO. Nylon membranes and slot-blot equipment were obtained from Schleicher & Schuell, Dassel, Germany, and leucodichlorofluorescein was obtained from Eastman Kodak Company, Rochester, NY. Thioglycolic acid, 1-bromotetradecane, 1-bromotridecane, and 1,10-dibromodecane were purchased from Fluka Chemika-BioChemika, Buchs, Switzerland. All other chemicals and solvents were of reagent grade from common commercial sources.

Synthesis of 3-thia fatty acids

The sulfur-substituted fatty acids were prepared at the Department of Chemistry, University of Bergen. Tetradecylthioacetic acid was synthesized as follows: 3.84 g thioglycolic acid was dissolved in 50 ml methanol and 6.1 g potassium hydroxide dissolved in 50 ml argon-flushed methanol was added. After 20 min 11.53 g 1-bromotetradecane, dissolved in methanol, was slowly added. The solution was kept at reflux temperature for 25 h whereupon 7.0 g 37% hydrochloric acid in 150 ml water was added to ensure a pH of the solution below 2. The microcrystalline product was washed with distilled water. The product was dried at room temperature for 4 h and then dissolved in a minimum amount of diethylether. The solution was filtrated and put aside at −20°C for several hours to ensure a complete precipitation from diethyl ether in which the compound is very soluble. The compound was finally crystallized twice from approximately 200 ml methanol and dried. Tridecylthioacetic acid was synthesized by the same procedure, using 10.95 g 1-bromotridecane. 1,10-Bis(carboxymethylthio)decane was synthesized as follows: 28 g potassium hydroxide and 15 ml thioglycolic acid were dissolved in 300 ml methanol. 29 g 1,10-dibromodecane was added in portions with stirring. The solution was slowly heated to 50°C and 2 volumes of water were added after 3 h. Upon cooling of the solution to 3–4°C, the potassium salt of 1,10 bis(carboxymethylthio)decane precipitated and was isolated by filtration. The precipitate was recrystallized from hot acetone–water 4:1.

Treatment of animals

Male Wistar rats, weighing 260–300 g, were obtained from Møllegard Breeding Laboratory, Ejby, Denmark. They were housed in metal wire cages, in pairs, 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 to nine animals.

The 3-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 and 12 weeks. After 12 h fasting, the rats were anesthetized with 0.2 ml Hypnorm-Dormicum/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 other part was freeze-clamped and stored at −80°C. The protocol was approved by the Norwegian State Board of Biological Experiments with Living Animals.

Isolation of VLDL

VLDL was isolated using ultracentrifugation as described by Lopes-Virella et al. (6). Plasma was adjusted to a relative density of 1.006 g/ml using NaCl. The solution was centrifuged for 18 h at 18°C at 40,000 rpm using a Centrikon T-2060 ultracentrifuge with TFT 45.6 rotor.

Preparation of post-nuclear fraction and measurement of protein and enzyme activities

The livers from individual rats were homogenized in ice-cold sucrose medium (0.25 M sucrose, 10 mM HEPES, and 1 mM Na_2 EDTA, pH 7.4) as described earlier (7). The homogenate was centrifuged at 3000 rpm at 4°C in a Sorvall RC-5 Superspeed Refrigerated Centrifuge using a SS34 rotor, resulting in nuclear and post-nuclear fractions. A Bio-Rad protein assay was used for protein measurement. BSA solved in distilled water was used as a standard. The enzymatic activity of peroxisomal fatty acyl-CoA oxidase was measured in the post-nuclear fraction of rat livers by the coupled assay described by Small, Burdett, and Connock (8). The production of H_2O_2 was measured by monitoring

the increase in dichlorofluorescein absorbance in the presence of palmitoyl-CoA. The Δ^9-desaturase activity was measured essentially as described in (9) with following modifications. The reaction mixture contained: 50 μM [1-^{14}C]stearoyl-CoA (0.2 μCi/ml), 2 mM NADH, and 100 mM Tris-HCl (pH 7.4) in a total volume of 1.5 ml. The reaction was started with 1.5 mg microsomal protein and the incubation time was 7 min (37°C). The reaction was stopped with 3 ml 10% methanolic KOH and saponification took place for 30 min at 70°C. Acidification was achieved by 6 M HCl and the fatty acids were extracted with 2 × 2 ml hexane and evaporated to dryness under nitrogen. The fatty acids were converted into methyl esters by adding 0.5 ml 1 M HCl–methanol followed by incubation at 56°C for 15 min. Two ml water was added and the methylated products were extracted with 2 × 3 ml hexane and evaporated to dryness under nitrogen and finally dissolved in 100 μl hexane. Stearate was separated from oleate by thin-layer chromatography on $AgNO_3$ silica gel (Merck). The solvent system contained diethyl ether–hexane 1:9. The fractions were scraped into scintillation vials and counted by liquid scintillation.

Extraction of lipids from homogenates and preparation of samples for gas chromatography

Lipids were extracted from liver tissue (1 g) and plasma (400 μl) by the procedure of Folch, Lees, and Sloane Stanley (10). During extraction of the liver, known amounts of heptadecanoic acid, triheptadecanoylglycerol, and L-α-phosphatidylcholine-diheptadecanoyl were added as internal standards; the plasma samples were fortified with heptadecanoic acid. Each liver fraction was than subjected to liquid anion chromatography (11) on Superclean™ LC-NH_2 SPE columns (Supelco SA, Gland, Switzerland) to effect lipid class separation. The fractions containing triacylglycerol, cholesteryl esters, and phospholipids as well as the plasma lipid extracts then underwent hydrolysis in 15% methanolic KOH for 45 min at 65°C, followed by acidification with HCl and extraction of the liberated fatty acids with hexane. The fatty acids of each fraction were converted to picolinyl esters (12).

Gas chromatography–mass spectrometry

The picolinyl esters were separated by capillary gas–liquid chromatography on a 50 m BPI 0.22 on i.d. column (S.G.E. International, Ringwood, Victoria, Australia) on a Carlo Erba Model 4150 gas chromatograph fitted with a flame ionization detector (FID). One-ml samples were injected in the split mode (closed split 40 sec; injector temperature: 290°C). Column temperature was held at 140°C for 5 min, then increased at a rate of 4°C/min to 250°C, which was maintained for 30 min. Fatty acids were quantified using the internal standard method (13) relative to the heptadecanoic acid released from the added internal standard. Results were calibrated with decanoic acid (its response factor was set to 1.0). All response factors were determined in duplicate at different concentrations. The response factor of the Δ^9-desaturated metabolite was assumed to be the same as corresponding thia fatty acid, as standards are not available.

The identities of thia fatty acyl components were verified by subjecting picolinyl esters (5) to GC–MS using a Shimadzu GCMS QP2000 instrument equipped with non-polar 40 m DB1 quartz capillary column (0.18 mm internal diameter, J & W Scientific, Folsom, CA).

Purification of RNA and hybridization analysis

Total RNA was isolated using the guanidinium thiocyanate-phenol method (14) and the RNA concentrations were determined by measuring the absorbance at 260 nm. The degree of RNA degradation was tested on gel-electrophoresis using a 1% agarose mini-gel, followed by staining with ethidium bromide. 28S- and 18S-RNA were then easily visualized under ultraviolet light. A Schleicher & Schuell apparatus was used to transfer RNA to nylon-filter for hybridization as earlier described (15). Three different RNA concentrations were applied. Hybridization reactions were performed as described in (16). 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 extracted from plastids by restriction enzymes. Purified fragments were then ^{32}P-labeled using the oligolabeling technique (17, 18), resulting in specific activities ranging from 0.8–5 × 10^9 cpm/mg. The probes were purified fragments of cloned rat genes: Δ^9-desaturase, 358 bp BgIII-AvaI fragment from pDs3, was kindly provided by Dr. Stefan Alexson, Karolinska Institutet, Stockholm, Sweden. Fatty acyl-CoA oxidase was from rat 1.4 kb PST1 insert in pMJ125 (19) and 28S rRNA; 1.4 kb Bam HI insert in pA (Dr. I. L. Gonzalez, Department of Human Genetics, Philadelphia, PA, personal communication).

Statistical analysis and presentation of data

The data are presented as mean ± standard deviation (SD) from 4 to 9 animals and were evaluated by a two-sample variance Student's t test (two-tailed distribution). The level of statistical significance was set at $P < 0.05$.

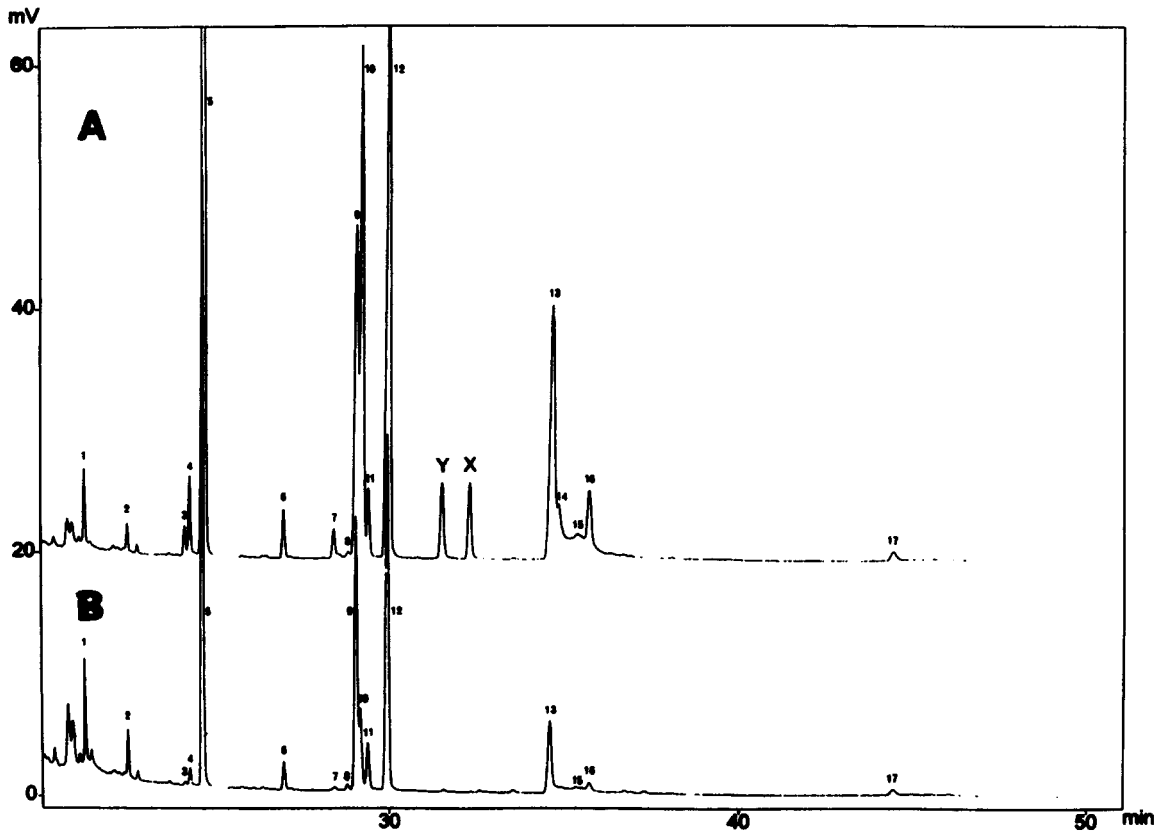

Fig. 1. Gas chromatography of fatty acid picolinyl esters derived from phospholipids. Phospholipids were isolated as described in Experimental Procedures from the livers of rats treated with 150 mg/day per kg body weight tetradecylthioacetic acid (A) or palmitic acid (B) for 12 weeks.

RESULTS

In a recent report, we demonstrated that the fatty acid composition in total liver homogenates changes after long-term administration of tetradecylthioacetic acid (C_{14}-S-acetic acid) (20). Analysis of the fatty acid composition in the phospholipid fraction (**Fig. 1**) showed that two new components appeared (X and Y). Using GS–MS, one component was identified as C_{14}-S-acetic acid itself (**Fig. 2**) and the second component as Δ^9-desaturated C_{14}-S-acetic acid (**Fig. 3**). **Table 1** shows that Δ^9-desaturated C_{14}-S-acetic acid was only detected in phospholipid and cholesterolester species after C_{14}-S-acetic acid treatment. In contrast, C_{14}-S-acetic acid was detected in all the different hepatic lipid fractions, but mainly in the phospholipids. Moreover, the Δ^9-desaturated product of stearic acid, 18:1 n–9, was increased by 9.5-fold in the phospholipid fraction. The 18:1 n–9/18:0 ratio increased approximately 5-fold in the phospholipid species after C_{14}-S-acetic acid administration, due to an increased level of 18:1 n–9 (Table 1).

The mole % of 18:1 n–9 increased in a time-dependent manner in both liver and VLDL (**Fig. 4 A and B**). Also, the hepatic content of Δ^9-desaturated C_{14}-S-acetic acid increased in a time-dependent manner (data not shown) whereas the mol% of C_{14}-S-acetic acid itself actually decreased (Fig. 4 C and D). Treatment with C_{13}-S-acetic acid and the dithiadicarboxylic acid (TD) for 1 week, increased the amount of 18:1 n–9 in liver and VLDL (**Table 2**). C_{13}-S-acetic acid was detected in both liver and VLDL, but TD was undetectable (Table 2).

As 18:1 n–9 is the Δ^9-desaturated product of stearic acid, it was likely that administration of 3-thia fatty acids increased this enzyme activity. Indeed, after 1 week of treatment with C_{14}-S-acetic acid, the Δ^9-desaturase activity was increased 2-fold (**Table 3**). In contrast, the Δ^9-desaturase activity was unchanged in TD treated rats (Table 3).

Both C_{14}-S-acetic acid and the stronger peroxisome proliferator, TD, are reported to activate the peroxisome proliferator-activated receptor (PPAR) (21) which leads to an increased transcription of a number of genes, including fatty acyl-CoA oxidase (22). The fatty acyl-CoA oxidase activity increased in a dose-dependent manner, after administration of both dicarboxylic and monocarboxylic 3-thia fatty acid analogues (**Table 4**). The different 3-thia fatty acids also significantly increased the mRNA levels of fatty acyl-CoA oxidase

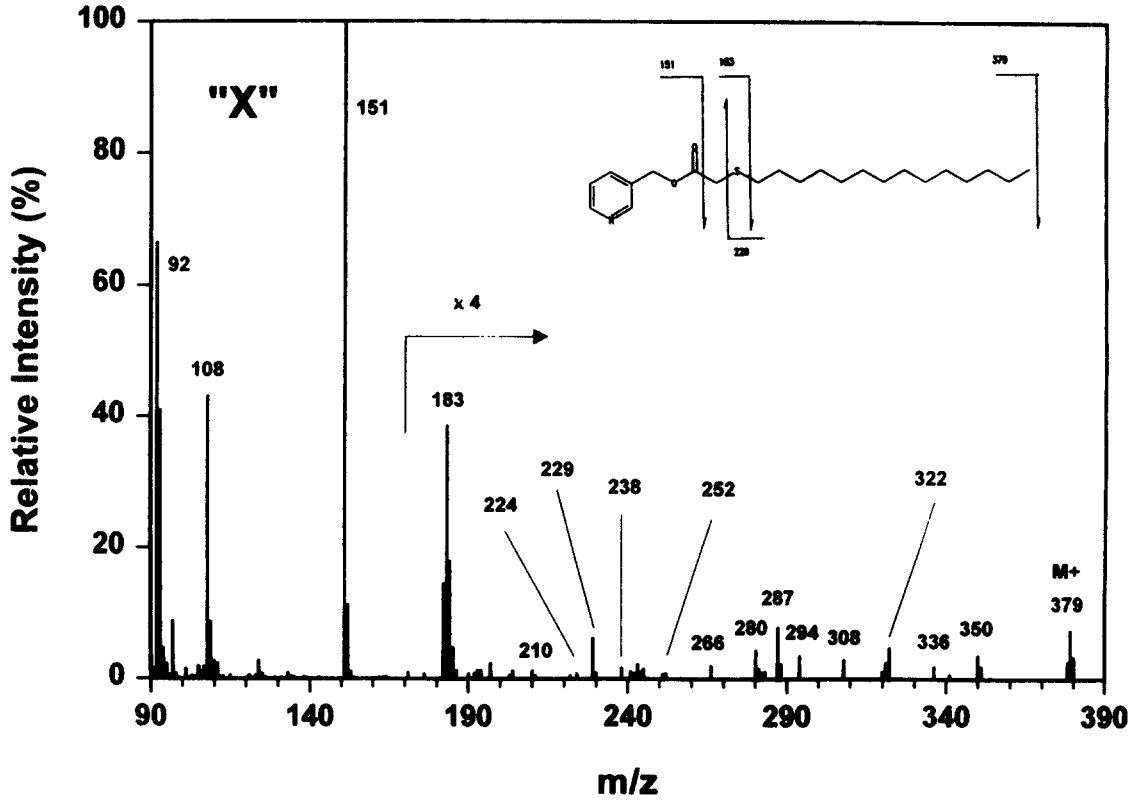

Fig. 2. Mass spectrum of the picolinyl ester of tetradecylthioacetic acid. Phospholipids from the livers of rats treated with tetradecylthioacetic acid at a dose of 150 mg/day per kg body weight for 12 weeks were isolated and analyzed with gas chromatography and mass spectrography. The insert indicates the presumed origin of major diagnostic ions.

(**Fig. 5 A**). However, only monocarboxylic 3-thia fatty acids increase the mRNA level of Δ^9-desaturase (Fig. 5 B). Moreover, after 1 week of C_{14}-S-acetic acid treatment, the mRNA levels of Δ^9-desaturase were increased 2-fold (**Fig. 6 A**), whereas the mRNA levels of fatty acyl-CoA oxidase were increased 5-fold (Fig. 6 B). Further treatment for 12 weeks increased the mRNA levels of Δ^9-desaturase 7-fold, whereas fatty acyl-CoA oxidase mRNA levels were marginally increased compared to 1 week (Fig. 6 A and B).

DISCUSSION

The hypolipidemic effect of tetradecylthioacetic acid and 1,10 bis(carboxymethylthio)decane (TD) is well known (1, 23) and in a recent study it was shown that tridecylthioacetic acid also possesses hypolipidemic properties (L. Frøyland, L. Madsen, A. Garras, Ø. Lie, J. Songstad, A. C. Rustan, and R. K. Berge, unpublished results).

The most prominent effect on glycerol metabolism after C_{14}-S-acetic acid administration was an increase in the incorporation of C_{14}-S-acetic acid itself and microsomal Δ^9-desaturated C_{14}-S-acetic acid into phospholipids (Table 3). This strongly suggests that specific C_{14}-S-acetyl-CoA and Δ^9-desaturated C_{14}-S-acetyl-CoA esters can be formed in vivo. Δ^9-Desaturated C_{14}-S-acetic acid was also found to be incorporated into cholesteryl esters, which further strengthens the hypothesis that this analogue is activated to its CoA ester in vivo.

Administration of 3-thia fatty acids caused a marked change in total fatty acid composition of hepatic lipids (Fig. 1). The increase in oleic acid (18:1 n–9) and Δ^9-desaturated C_{14}-S-acetic acid by C_{14}-S-acetic acid treatment (Table 2) may reflect high Δ^9-desaturase activity (Table 3) and that C_{14}-S-acetic acid is a good substrate for this enzyme. However, no correlation was found between the Δ^9-desaturase gene expression and the increased amount of 18:1 n–9 after 3-thia fatty acid administration, i.e., treatment with the dithiadicarboxylic acid stimulated incorporation of 18:1 n–9 into glycerolipids similar to C_{14}-S-acetic acid (Table 2), but only a marginal increase in Δ^9-desaturase mRNA levels and activity was observed by dithiadicarboxylic acid (Fig. 1

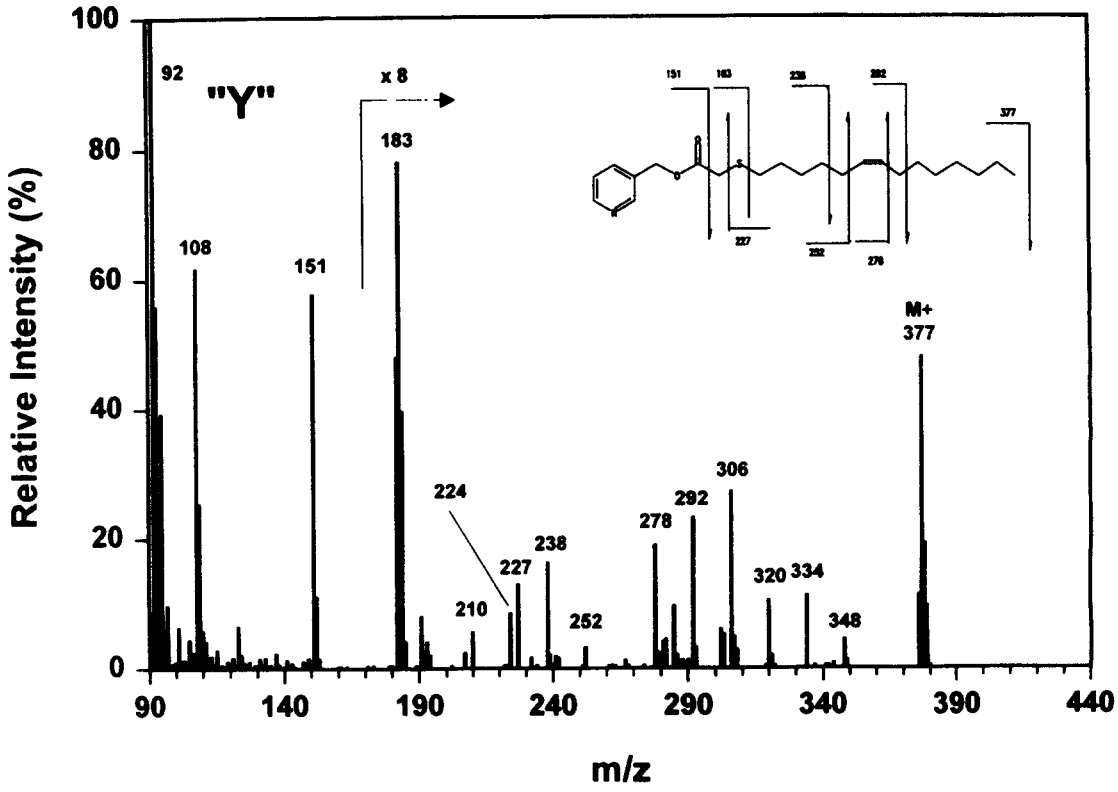

Fig. 3. Mass spectrum of the picolinyl ester of a desaturated metabolite of tetradecylthioacetic acid. Phospholipids from the livers of rats treated with tetradecylthioacetic acid at a dose of 150 mg/day per kg body weight for 12 weeks were isolated and analyzed with gas chromatography and mass spectrography. The insert indicates the presumed origin of major diagnostic ions.

B and Table 3). However, the Δ^9-desaturase mRNA levels were highly correlated with the amount of oleic acid in rats treated with different chain length monocarboxylic 3-thia fatty acid (L. Frøyland, L. Madsen, A. Garras, Ø. Lie, J. Songstad, A. C. Rustan, and R. K. Berge, unpublished results). This indicates that the dicarboxylic and monocarboxylic 3-thia fatty acids modulate the lipid metabolism through different mechanisms. As monocarboxylated 3-thia fatty acids seem to behave as ordinary saturated fatty acids in glycerolipid processes (5), replacement of ordinary fatty acid is probably also a mechanism leading to changed fatty acid composition.

The availability of fatty acids as substrates is crucial in determining not only the rate of triacylglycerol synthesis but also that of fatty acid oxidation. The results from this study demonstrated that administration of monocarboxylic, and especially dicarboxylic sulfur-substituted fatty acid analogues resulted in the induction of rat liver fatty acyl-CoA oxidase activity and mRNA levels (Table 4 and Fig. 5 A). Thus, the increased fatty acyl-

TABLE 1. Gas chromatographic measurement of acyl groups in liver lipid fractions of rats fed (150 mg/kg/day) palmitic acid (A) or C_{14}-S-acetic acid (B) for 12 weeks

	A				B			
Fatty Acyl Group	FFA	PL	TG	CE	FFA	PL	TG	CE
16:0	125 ± 5	9023 ± 691	2093 ± 244	17 ± 2	67 ± 4	16590 ± 480	1610 ± 110	280 ± 16
18:0	99 ± 6	6462 ± 641	358 ± 37	42 ± 10	76 ± 5	12348 ± 811	183 ± 5	244 ± 12
18:1 (n–9)	21 ± 2	748 ± 470	1050 ± 73	20 ± 2	51 ± 4	7114 ± 635	375 ± 41	435 ± 84
18:2 (n–6)	72 ± 6	3643 ± 380	1728 ± 84	26 ± 4	65 ± 5	4307 ± 162	230 ± 24	145 ± 6
C_{14}-S-acetic acid	ND	ND	ND	ND	5 ± 1	738 ± 7	19 ± 2	19 ± 2
C_{14}:1 (n–9)-S-acetic acid	ND	ND	ND	ND	ND	714 ± 103	ND	17 ± 3

The values represent nmol fatty acid/g liver in free fatty acid (FFA), phospholipid (PL), triacylglycerol (TG), and cholesteryl ester (CE) fractions. Results are expressed as means ± SD of four animals in each group, each measurement in duplicate; ND, not detected.

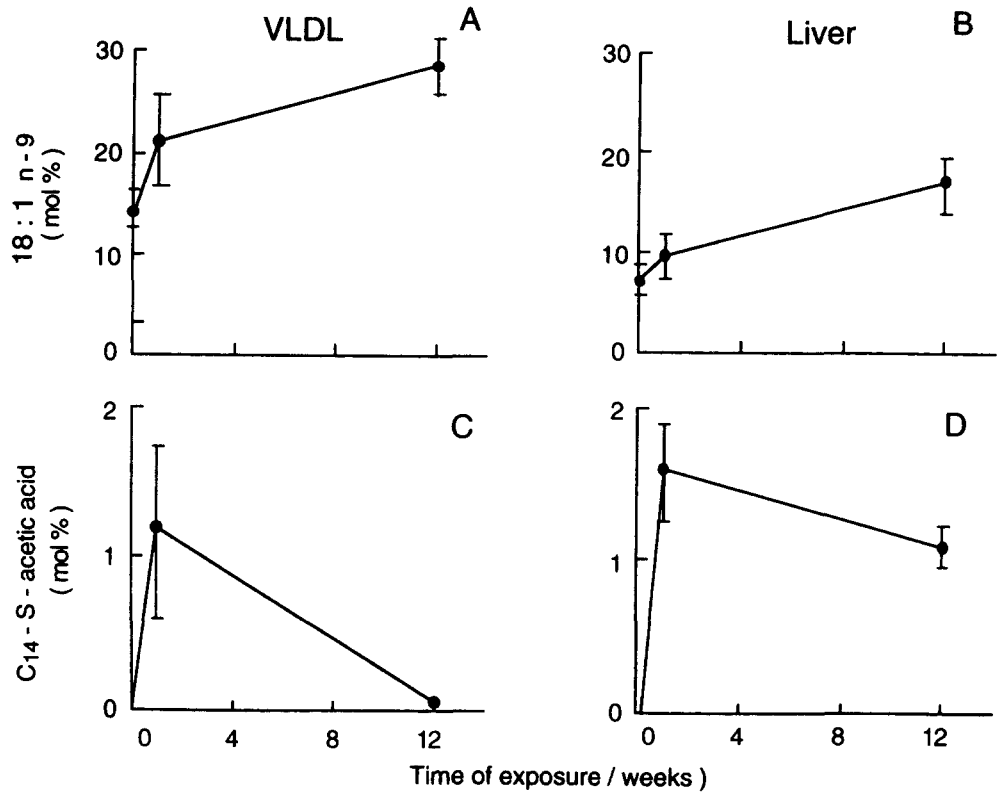

Fig. 4. Tetradecylthioacetic acid is incorporated in VLDL and hepatic lipids and increases the amount of oleic acid. Rats were treated with 150 mg/day per kg body weight C_{14}-S-acetic acid for 1 and 12 weeks. The amount of oleic acid (18:1 n–9) in VLDL (A) and liver (B) and the amount of C_{14}-S-acetic acid incorporated in VLDL (C) and liver (D) are shown. The values are given as mol% of total lipids.

CoA oxidase activity is apparently due to increased mRNA levels. The molecular mechanism involved in gene regulation by fatty acids remains unknown, but it has been suggested that they act through nuclear receptors of the steroid thyroid superfamily, the peroxisome proliferator-activated receptors (PPARs). The fatty acyl-CoA oxidase gene contains a peroxisome proliferator-response element (PPRE), through which the gene expression is mediated, during peroxisome proliferation (22). As the 3-thia fatty acids are reported to activate

TABLE 2. Accumulation of different 3-thia fatty acids and their effect on the level of oleic acid (18:1 n–9) in liver and VLDL

	Palmitic Acid mg/day/kg BW	C_{13}-S-Acetic Acid mg/day/kg BW		C_{14}-S-Acetic Acid mg/day/kg BW		TD mg/day/kg BW	
	150	150	300	150	300	150	300
	mol %	*mol %*		*mol %*		*mol %*	
Liver							
18:1 n–9	7.4 ± 1.5	10.0 ± 1.2[a]	9.9 ± 1.3[a]	10.0 ± 2.3[a]	12.0 ± 1.6[a]	8.8 ± 0.8[a]	9.5 ± 0.8[a]
C_{13}-S-acetic acid		1.6 ± 0.4[a]	2.6 ± 0.8[a]				
C_{14}-S-acetic acid				1.6 ± 0.3[a]	2.1 ± 0.2[a]		
Δ^9-desaturated C_{14}-S-acetic acid				0.7 ± 0.1[a]	0.9 ± 0.3[a]		
TD						ND	ND
VLDL							
18:1 n–9	16.0 ± 1.1	18.5 ± 1.9[a]	19.6 ± 1.2[a]	20.6 ± 1.5[a]	21.4 ± 1.4	21.8 ± 1.1[a]	24.9 ± 1.6[a]
C_{13}-S-acetic acid		2.1 ± 0.2[a]	2.2 ± 0.3[a]				
C_{14}-S-acetic acid				1.2 ± 0.4[a]	2.3 ± 0.5[a]		
Δ^9-desaturated C_{14}-S-acetic acid				ND	ND		
TD						ND	ND

Results represent mol % of total lipids and values are expressed as means ± SD of 5 animals in each group; ND, not detected; BW, body weight.

[a]Significantly different from palmitic acid, $P < 0.05$.

TABLE 3. Effect of different 3-thia fatty acids at a dose of 300 mg/day/kg body weight on hepatic Δ^9-desaturase activity

Treatment	Δ^9-Desaturase Activity
	pmol/min/mg
Palmitic acid	150 ± 20
C_{14}-S-acetic acid	312 ± 17[a]
TD	105 ± 27

The values represent means ± SD for 5 animals in each group.
[a]Significantly different from palmitic acid (control): $P < 0.05$.

PPAR (21, 24), it is likely that the fatty acyl-CoA oxidase gene expression is stimulated through this mechanism.

To our knowledge, the results from this study demonstrate for the first time that 3-thia fatty acid treatment results in the induction of rat liver Δ^9-desaturase mRNA levels (Figs. 5 B and 6 A and D). This effect confirms and extends previous findings that peroxisome proliferators are able to induce Δ^9-desaturase activity, e.g., clofibrate is reported to increase the Δ^9-desaturase mRNA levels and enzyme activity in mouse liver (25). A PPRE element was recently identified in the 5′-flanking region of the Δ^9-desaturase gene (26). However, if PPARs prove to mediate the effects of 3-thia fatty acids on Δ^9-desaturase expression, then one would expect that Δ^9-desaturase expression would be regulated by dietary factors, e.g., marine n–3 fatty acids. However, this was not the case (L. Madsen, H. Vaagenes, and R. K. Berge, unpublished data).

TD is an especially potent peroxisome proliferator (27). However, this hypolipidemic peroxisome-proliferating fatty acid analogue showed only marginal effect on the Δ^9-desaturase gene expression compared to monocarboxylic 3-thia fatty acid (Fig. 5 B). Moreover, C_{14}-S-acetic acid induced mRNA levels of Δ^9-desaturase in a time-dependent manner, whereas the fatty acyl-CoA oxidase mRNA levels remained almost constant in rats treated for 1 and 12 weeks (Fig. 6 B and D). Thus, our results suggest that the up-regulation of Δ^9-desaturase

TABLE 4. Effect of different 3-thia fatty acids at different doses on hepatic fatty acyl-CoA oxidase activity

Treatment	Dose	Fatty Acyl-CoA Oxidase Activity
	mg/day/kg body weight	*pmol/min/mg protein*
Palmitic acid	150	14.2 ± 2.1
C_{13}-S-acetic acid	150	81.1 ± 15.0[a]
	300	131.0 ± 35.0[a]
C_{14}-S-acetic acid	150	57.0 ± 20.2[a]
	300	163.7 ± 24.2[a]
TD	150	104.9 ± 26.5[a]
	300	154.5 ± 3.5[a]

Values represent means ± SD from 5 animals in each group.
[a]Significantly different from palmitic acid (control): $P < 0.05$.

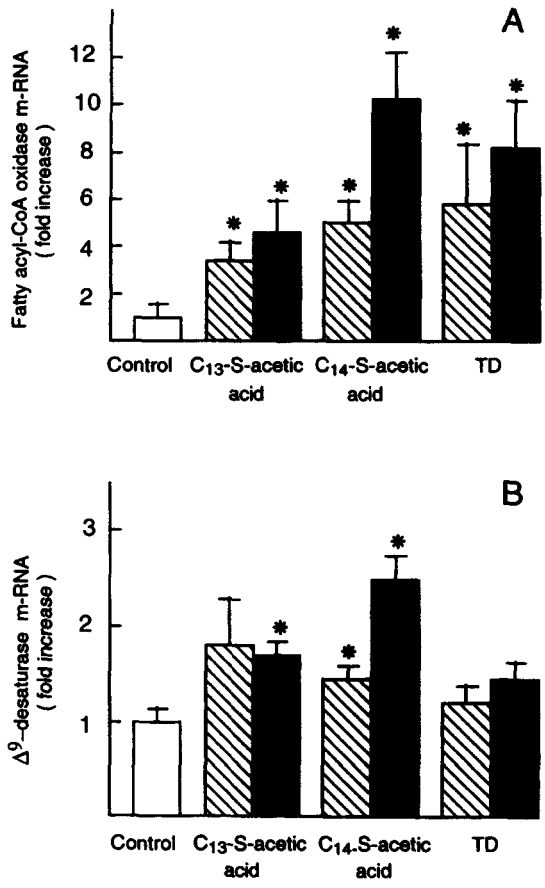

Fig. 5. Dose-dependent effect of 3-thia fatty acids on the mRNA level of fatty acyl-CoA oxidase and Δ^9-desaturase. One representative slot-blot of fatty acyl-CoA oxidase (A), Δ^9-desaturase (B) mRNA levels and 28S rRNA after 7 days of treatment with 150 (hatched bars) and 300 mg/day per kg body weight (solid bars) TD, C_{13}- and C_{14}-S-acetic acid. Total RNA was isolated from the liver of at least 4 animals in each experimental group. The relative mRNA levels were determined by densiometric scanning of autoradiograms and the expression of fatty acyl-CoA oxidase and of Δ^9-desaturase mRNA were normalized to the corresponding 28S rRNA levels. Mean of controls were set to 1.0. Values are presented as mean ± SD. *Significantly different from controls ($P < 0.05$).

gene expression occurs by a distinct mechanism and may be independent of the induction of peroxisomal enzymes and not related to peroxisome proliferation.

Activation of several enzymes and protein factors, e.g., protein kinase C (PKC) and PPAR, may depend on the cellular fatty acid content and molecular species. Administration of C_{14}-S acetic acid caused liver to incorporate ten times more oleic acid into phospholipids than control (Table 3). This suggests that this 3-thia acetic acid increases phospholipid synthesis and shifts the phospholipid membrane from a tightly packing form to a looser packing form. Consequently, this could affect the general mobility and activity of a number of membrane proteins, including protein receptors (28).

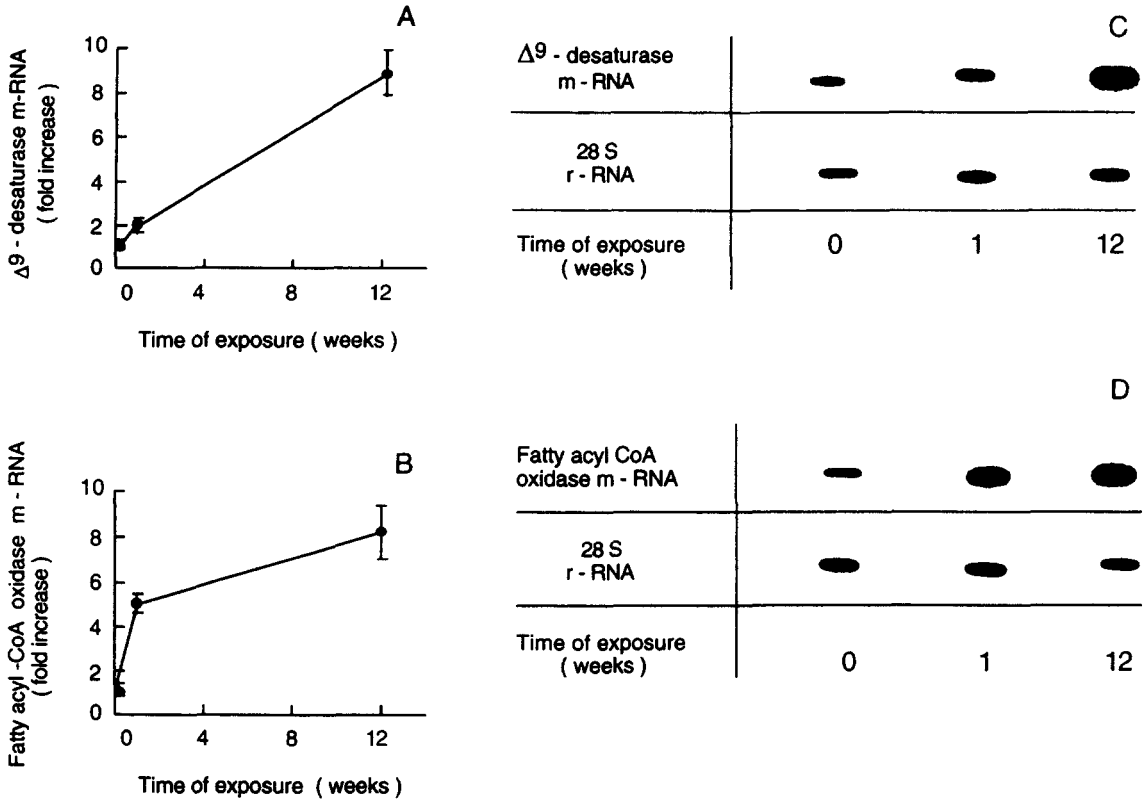

Fig. 6. Time-dependent effects of 3-thia fatty acids on the mRNA level of fatty acyl-CoA oxidase and Δ^9-desaturase. Total RNA was isolated from at least 4 animals after 1 and 12 weeks with 150 mg/day per kg body weight C_{14}-S-acetic acid treatment. The relative mRNA levels were determined by densiometric scanning of autoradiograms and the expression of Δ^9-desaturase (A) and of fatty acyl-CoA oxidase (B) mRNA were normalized to the corresponding 28S rRNA levels. Mean of controls were set to 1.0. Values are presented as mean ± SD. One representative blot for Δ^9-desaturase (C), fatty acyl-CoA oxidase (D) and 28S rRNA from each experimental group is shown. *Significantly different from controls ($P < 0.05$).

In addition, proper ligand binding is dependent on receptor dimerization which also may be dependent on membrane architecture. Thus, as 3-thia fatty acids generate accumulation of phospholipids with higher content of monounsaturated fatty acids, the membrane would lose its defined structure and may change the effectiveness of ligand receptor interaction and receptor dimerization.

The liver secretes lipids into circulation, such as lipoproteins, and the acyl moieties of these are used as components of lipids in other tissues. It is therefore possible that changes induced by 3-thia fatty acids in hepatic lipids might be reflected in the lipid levels in VLDL. The changes in fatty acid composition observed in VLDL was almost similar to those found in liver after 3-thia fatty acids, except for the Δ^9-desaturated metabolite of these analogues. Whether this is a degradation step in the metabolism and secretion of 3-thia fatty acids, additional to sulfur oxygenation, ω-hydroxylation and peroxisomal β-oxidation from the ω-end, (27) should be considered.

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