Regulation of fatty acid oxidation and triglyceride and phospholipid metabolism by hypolipidemic sulfur-substituted fatty acid analogues

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Abstract The mechanisms behind the hypotriglyceridemic effect of 1,10-bis(carboxymethylthio)decane (3-thiadicarboxylic acid) and tetradecylthioacetic acid and the development of fatty liver caused by 3-tetradecylthiopropionic acid (Aarsland et al. 1989. *J. Lipid Res.* **30:** 1711–1718.) were studied in the rat. Repeated administration of S-substituted non-β-oxidizable fatty acid analogues to normolipidemic rats resulted in a time-dependent decrease in plasma triglycerides, phospholipids, and free fatty acids. This was accompanied by an acute reduction in the liver content of triglycerides and an increase in the hepatic concentration of phospholipids. Mitochondrial fatty acid oxidation was stimulated, whereas lipogenesis was inhibited. The activity of phosphatidate phosphohydrolase decreased while the activity of CTP:phosphocholine cytidylyltransferase increased. These results suggest that the observed triglyceride-lowering effect was due to increased mitochondrial fatty acid oxidation accompanied by a reduction in the availability of the substrate i.e., free fatty acid, along with an enzymatic inhibition (phosphatidate phosphohydrolase). Administration of 3-tetradecylthiopropionic acid led to a drastic increase in the hepatic triglyceride content. Levels of plasma triglyceride phospholipid and free fatty acid also increased. Phosphatidate phosphohydrolase activity was stimulated whereas CTP:phosphocholine cytidylyltransferase was inhibited. Mitochondrial fatty acid oxidation was decreased. These data indicate that the development of fatty liver as an effect of 3-tetradecylpropionic acid is probably due to accelerated triglyceride biosynthesis, which is mediated by an increase in the availability of fatty acid along with stimulation of phosphatidate phosphohydrolase. The results of the present study speak strongly in favor of the hypothesis that phosphatidate phosphohydrolase is a major rate-limiting enzyme in triglyceride biosynthesis. Furthermore, they point out that the biosynthesis of triglycerides and phospholipids might be coordinately regulated. Such regulation is possibly mediated via phosphatidate phosphohydrolase and CTP:phosphocholine cytidylyltransferase. Whether the increase in hepatic phospholipids via increased CDP-pathway accounts for an increase of lipid components for proliferation of peroxisomes (3-thiadicarboxylic acid and tetradecylacetic acid) should be considered. —**Skorve, J., D. Asiedu, A. C. Rustan, C. A. Drevon, A. Al-Shurbaji, and R. K. Berge.** Regulation of fatty acid oxidation and triglyceride and phospholipid metabolism by hypolipidemic sulfur-substituted fatty acid analogues. *J. Lipid Res.* 1990. **31:** 1627–1635.

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It has recently been shown that 3-thiadicarboxylic acid and tetradecylthioacetic acid are lipid-lowering agents with both hypotriglyceridemic and hypocholesterolemic properties (1). The mechanism by which these non-β-oxidizable fatty acid analogues reduce plasma triglyceride levels is not fully elucidated. Both the mitochondrial and peroxisomal β-oxidation of fatty acids have been reported to be stimulated by these compounds (2), while lipogenesis and the incorporation of fatty acids into triglycerides were found to be decreased (3). The hypotriglyceridemia, however, was dissociated from induction of peroxisomal β-oxidation and peroxisome proliferation (4).

Possible effects on the enzymes involved in triglyceride biosynthesis have not been studied in detail. It was of particular interest to investigate whether an inhibition of phosphatidate phosphohydrolase, a key enzyme in triglyceride-biosynthesis, might contribute to the triglyceride-lowering effect observed.

3-Tetradecylthiopropionic acid is another S-substituted fatty acid analogue which, in contrast to the above mentioned compounds, can undergo one cycle of β-oxidation. Treatment of normolipidemic rats with 3-tetradecylthio-

Abbreviations: BCMTD, 1.10-bis(carboxymethylthio)decane (3-thiadicarboxylic acid); CMTTD, 1-(carboxymethylthio)tetradecane (tetradecylthioacetic acid); CETTD, 1-(carboxyethylthio)tetradecane (3-tetradecylthiopropionic acid); FFA, free fatty acids; VLDL, very low density lipoprotein.

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propionic acid increased plasma triglyceride levels and produced fatty liver (1, 5). How these effects are mediated is still largely unknown. In the present study possible mechanisms behind these effects were investigated.

Finally, the effects of both hyper- and hypotriglyceridemic fatty acid analogues on phospholipid biosynthesis and its relation to the synthesis of triglycerides were also studied.

MATERIALS AND METHODS

Chemical and drugs

[1-^{14}C]Palmitoyl-CoA with specific activity of 50 mCi/mmol was purchased from the Radiochemical Centre, Amersham, England. 1,10-bis(Carboxymethylthio)decane (BCMTD), 1-(carboxymethylthio)tetradecane (CMTTD), and 1-(carboxyethylthio)tetradecane (CETTD) were prepared as described earlier (6, 7). All other chemicals were obtained from common commercial sources and were of reagent grade.

Animals and treatments

Male Wistar rats from Möllegaard Breeding Laboratory, Ejby, Denmark, weighing 170–180 g, were housed individually in metal wire cages maintained at 12 h light-dark cycles and a constant temperature of 20 ± 3°C. The animals were acclimatized for at least 1 week under these conditions before the start of the experiment. BCMTD, CMTTD, and CETTD were suspended in 0.5% sodium carboxymethyl cellulose. In some experiments, the individual agents were administered by gastric intubation in a volume of 1 ml once a day for 5 days and the animals were killed at the start of the sixth day after 12 h of fasting as described below. In the time-study, a daily dose of 150 mg/kg body weight of BCMTD, CMTTD, or CETTD suspended in 0.5% carboxymethyl cellulose was administered by gavage in a total volume of 1 ml. The control animals received only carboxymethyl cellulose. All animals had free access to water and food (1, 5). In some experiments the rats were fed ad libitum with the different fatty acid analogues, palmitic acid and hexadecanedioic acid.

Body weights of the rats were measured daily. At the end of the experiments, the rats were fasted overnight and weighed. Under light halothane anesthesia, cardiac puncture was performed to obtain blood samples (in EDTA). The livers were removed and immediately chilled on ice and weighed. Plasma was prepared from the blood samples by centrifugation at 1000 *g* for 10 min.

Preparation and treatment of subcellular fractions

Livers from individual rats were homogenized in ice-cold sucrose medium (0.25 M sucrose in 10 mM HEPES buffer, pH 7.4, and 1 mM EDTA) and the resulting nuclear plus postnuclear fraction was taken as the total homogenate (1, 5). For further analytical differential centrifugation experiments, postnuclear fractions from three animals were pooled before microsome- and cytosol-enriched fractions were isolated (1, 2). Variation in the response from animal to animal was estimated separately for selected enzymes and lipids in the group of control animals.

Analytical methods

Protein was assayed by Bio-Rad protein assay kit (Bio-Rad, Richmond, CA).

Enzymatic activities of phosphatidate phosphohydrolase (8), CTP:phosphocholine cytidylyltransferase (9), glycerophosphate acyltransferase (1), and fatty acid synthetase (10) were determined as earlier described.

Lipid analyses were carried out by the Montest cholesterol enzymatic kit, Boehringer Mannheim, Germany; the Monotest phospholipids enzymatic kit, Boehringer Mannheim, Germany; and the Biopak Triglyceride enzymatic kit, Biotrol, Paris, France.

Plasma free fatty acids were determined by an enzymatic colorimetric method (WAKO NEFA C) (11).

RESULTS

Serum and liver lipids

All animals treated with the sulfur-substituted fatty acid analogues at various doses and as a function of time appeared healthy, had normal body weight, and behaved like normal animals.

In keeping with previous observations (4), plasma triglycerides, plasma cholesterol, and hepatic triglycerides were significantly lowered after administration of 3-thiadicarboxylic acid and tetradecylthioacetic acid (**Table 1**). The hypolipidemic effect (**Fig. 1**) and reduced concentration of hepatic triglyceride (**Fig. 2**) were already established after 1–2 days. The hypotriglyceridemia was mainly due to decreased triglyceride content of VLDL (Berge et al., unpublished observations). Repeated administration of 3-tetradecylthiopropionic acid, however increased the concentration of plasma and hepatic triglyceride (Table 1) within 1–2 days (Fig. 1, Fig. 2).

Tetradecylthioacetic acid and the 3-dicarboxylic acid caused a reduction of plasma phospholipids (Table 1) during the first 2 days of treatment. At this time a 50% reduction of plasma phospholipids was observed in animals given tetradecylthioacetic acid. In rats fed 3-thiadecarboxylic acid and tetradecylthioacetic acid, the hepatic content of phospholipid, however, increased about 30% in a dose-related manner (**Table 2**) within 1–3 days of treatment (Fig. 2). Administration of 3-tetradecylthiopropionic acid resulted in an increase of the plasma phospholipid level by 30 to 40% (Table 1) during the in-

TABLE 1. Changes of plasma and liver lipids in rats given sulfur-substituted fatty acid analogues

Group	Dose	Plasma			Liver		
		Triglyceride	Cholesterol	Phospholipid	Triglyceride	Cholesterol	Phospholipid
	%		*mmol/l*			*μmol/g*	
Control		1.19 ± 0.20	1.98 ± 0.26	1.47 ± 0.16	8.1 ± 1.1	4.5 ± 0.4	16.5 ± 1.1
CMTTD[a]	0.1	0.82 ± 0.15*	1.34 ± 0.38*	1.08 ± 0.10*	7.9 ± 1.5	5.1 ± 1.1	17.2 ± 0.7
CMTTD	0.3	0.67 ± 0.10*	0.83 ± 0.14*	0.84 ± 0.12*	6.0 ± 1.3*	3.5 ± 0.4*	20.7 ± 0.8*
CETTD[b]	0.3	2.45 ± 0.49*	1.80 ± 0.28*	1.95 ± 0.08*	43.7 ± 5.0*	4.3 ± 0.2	23.8 ± 1.5*
BCMTD[c]	0.3	0.58 ± 0.14*	0.70 ± 0.09*	0.67 ± 0.08*	5.5 ± 0.4*	2.4 ± 0.2*	21.3 ± 1.1*

The plasma and hepatic values represent means ± SD of 12 control animals and groups of 3 rats in each experimental group.*, $P < 0.02$ for difference between control and treated animals.

[a]CMTTD, $CH_3(CH_2)_{13}$-S-CH_2-COOH, tetradecylthioacetic acid

[b]CETTD, $CH_3(CH_2)_{13}$-S-CH_2-CH_2-COOH, 3-tetradecylthiopropionic acid.

[c]BCMTD, COOH-CH_2-S-$(CH_2)_{10}$-S-CH_2-COOH, 3-thiadicarboxylic acid.

itial 1–3 days of treatment (Fig. 1). The hepatic content of phospholipids was significantly increased after 3-tetradecylthiopropionic acid treatment for 5 days at a dose of 400 mg/day per kg body weight (Table 2). Administration of 150 mg/day per kg body weight of tetradecylthiopropionic acid to rats kept on a standard pellet diet, however, marginally affected the hepatic phospholipid content during the initial 1–2 days of treatment (Fig. 2).

In rats given palmitic acid or hexadecanedioic acid, no changes of the plasma phospholipids (data not shown) and liver phospholipids were observed (Table 2).

It is noteworthy that treatment of rats with tetradecylthioacetic acid resulted in an acute reduction of the plasma free fatty acid (FFA) level (Fig. 1). In contrast, 3-tetradecylthiopropionic acid administration increased the plasma content of FFA (Fig. 1).

Glycerophosphate acyltransferase, phosphatidate phosphohydrolase, and CTP:phosphocholine cytidylyltransferase activities

Of interest was the possibility that the three sulfur-substituted fatty acid analogues might interfere with triglyceride and phospholipid biosynthesis in the liver via the influence of key enzymes involved in these pathways. In control rats, the glycerophosphate acyltransferase activity was enriched in the metochondrial and microsomal fractions (**Fig. 3**). When tetradecylthioacetic acid (data not shown) and 3-tetradecylthiopropionic acid were given (Fig. 3B), the mitochondrial glycerophosphate acyltransferase activity was unchanged within hours of feeding, whereas this enzyme activity was increased in 3-thiadicarboxylic acid-adapted animals (Fig. 3C). After 3 days of feeding the mitochondrial glycerophosphate acyltransferase activity was increased in rats given tetradecylthioacetic acid (data not shown) whereas the activity tended to decrease in rats exposed to 3-tetradecylthiopropionic acid (Fig. 3B).

The microsomal glycerophosphate acyltransferase activity was increased in rats given tetradecylthioacetic acid or 3-dicarboxylic acid within hours of feeding (Figs. 3A and C). Subsequently, in tetradecylthioacetic acid-treated

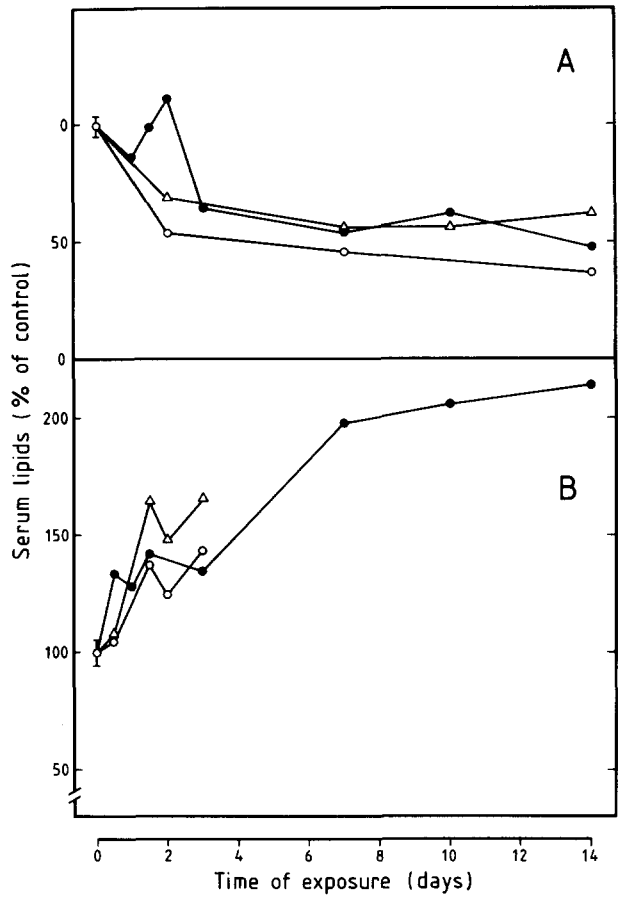

Fig. 1. Time-course of serum triglycerides (●), phospholipids (○), and free fatty acids (△) in rats given tetradecylthioacetic acid (A) and 3-tetradecylthiopropionic acid (B). Serum lipids of the experimental groups (n = 3) are presented relative to those of control animals (0, 7, and 14 days) = 100% (n = 12). In control animals the serum triglycerides, phospholipids, and free fatty acids were 0.85 ± 0.20 mmol/l, 1.79 ± 0.21 mmol/l, and 0.25 ± 0.01 mmol/l, respectively.

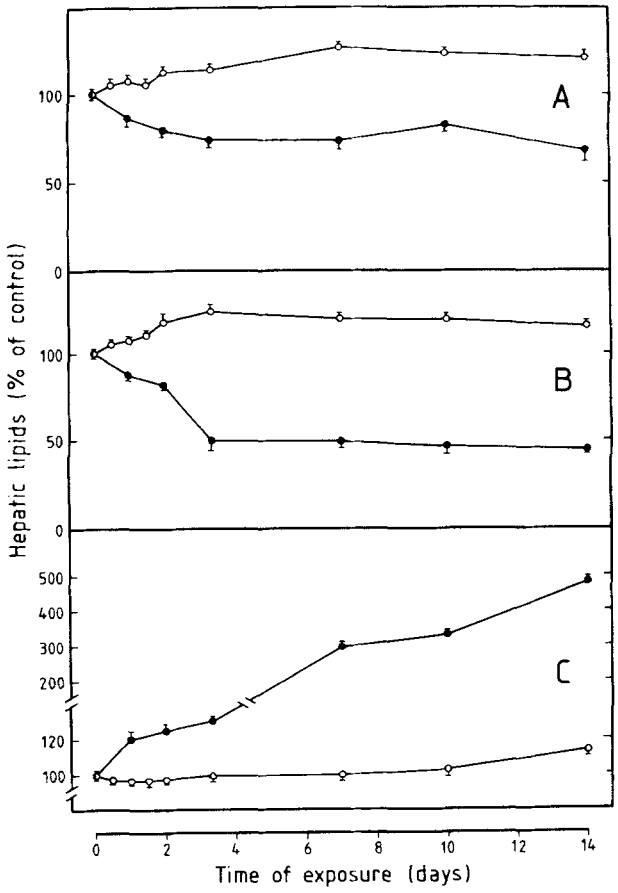

Fig. 2. Time-course of hepatic triglycerides (●), and phospholipids (○) in rats given tetradecylthioacetic acid (A), 3-thiadicarboxylic acid (B), and 3-tetradecylthiopropionic acid (C). Data are presented as described in the legend to Fig. 1, and in control animals the hepatic triglyceride and phospholipid levels were 8.6 ± 0.2 μmol/g liver and 14.2 ± 0.3 μmol/g liver, respectively.

rats, the microsomal enzyme activity fell almost to a normal value (2 days), but showed a new maximum within 7 days of feeding (Fig. 3A). In contrast to feeding with 3-thiadicarboxylic acid and tetradecylthioacetic acid, the microsomal glycerophosphate acyltransferase activity of 3-tetradecylthiopropionic acid-treated animals was decreased by 30% within 36 h of feeding (Fig. 3B).

It was observed that administration of tetradecylthioacetic acid and especially 3-thiadicarboxylic acid caused a decrease in both the microsomal and cytosolic phosphatidate phosphohydrolase activities, although to a lesser extent in the microsomal fraction (**Fig. 4**). Thus, no translocation of this enzyme from the cytosolic compartment to the microsomal compartment (12, 13) was observed after repeated administration of hypolipidemic peroxisome-proliferating fatty acid analogues (4). Repeated administration of 3-tetradecylthiopropionic acid caused an increase in phosphatidate phosphohydrolase activity both in cytosolic and microsomal fractions (Fig. 4) during the first 3 days of treatment.

Repeated administration of 3-thiadicarboxylic acid and tetradecylthioacetic acid to rats caused a dose-related increase of microsomal and cytosolic CTP:phosphocholine cytidylyltransferase activities (**Table 3**) during the 3 initial days of treatment (**Fig. 5**). 3-Thiadicarboxylic acid was considerably more potent than tetradecylthioacetic acid in increasing the cytidylyltransferase activity. At a dose of 150 mg/day per kg body weight, the microsomal cytidylyltransferase was stimulated more than 2-fold, whereas the cytosolic enzyme activity was increased about 1.5-fold after 10 days of feeding (Fig. 5).

Administration of an increasing amount of 3-tetradecylthiopropionic acid only marginally affected the microsomal cytidylyltransferase activity, whereas the cytosolic activity tended to decrease (Table 3). This decrease (about 40%) was established during the initial 1–3 days after start of treatment (Fig. 5).

Cyanide-sensitive and -insensitive fatty acid oxidation

Tetradecylthioacetic acid treatment increased the mitochondrial as well as peroxisomal oxidation of fatty acids (2, 5). This is especially seen with palmitoyl-CoA as

TABLE 2. Dose-dependent changes of hepatic phospholipids in rats treated for 5 days with sulfur-substituted fatty acid analogues and "normal" fatty acids

Dose (mg per day per kg body weight)	Phospholipids (μmol/g liver)					
	Nontreated	CMTTD	BCMTD	CETTD	Palmitic Acid	Hexadecanedioic Acid
0	16.5 ± 1.1					
75		20.5 ± 1.8*	18.6 ± 0.3*			16.4 ± 1.1
150		20.8 ± 0.4*	20.1 ± 0.8*	17.5 ± 0.7		16.2 ± 0.8
250		21.4 ± 1.6*	21.5 ± 1.1*			
400				20.1 ± 2.2*	17.8 ± 1.1	
500		20.9 ± 1.6*	20.6 ± 2.3*		17.5 ± 0.8	
750				20.6 ± 2.3*		16.6 ± 0.5
1000					16.7 ± 0.5	

The tabulated values are means ± SD of 12 control animals and 3 rats in each experimental group.*, $P < 0.02$ for difference between control and treated rats.

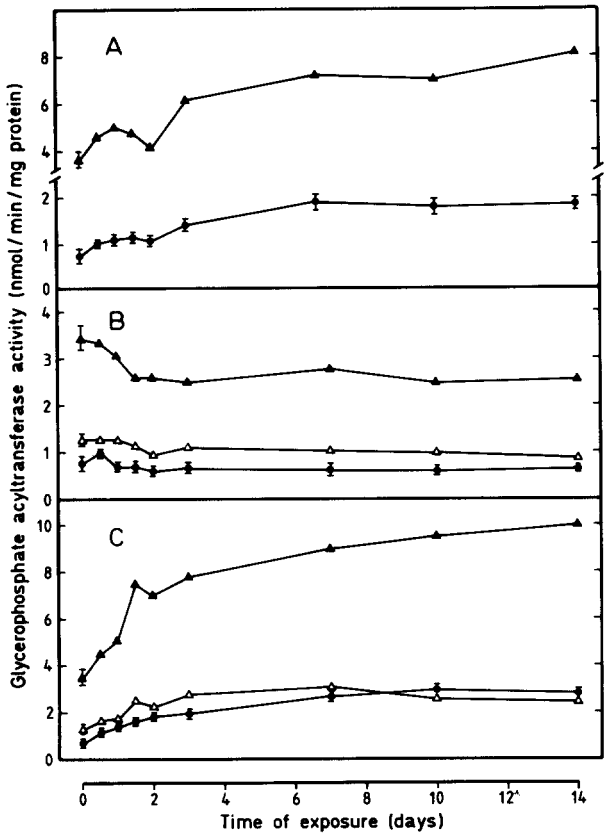

Fig. 3. The effect of tetradecylthioacetic acid (A), 3-tetradecylthiopropionic acid (B), and 3-thiadicarboxylic acid (C) on glycerophosphate acyltransferase activity as a function of time in total liver homogenates (●), the microsomal fraction (▲), and the mitochondrial fraction (△). No significant changes were observed in the control animals of the 14-day period (5 to 10% between 0, 7, and 14 days). The tabulated values represent the means ± SD for twelve animals of three control groups and means of six animals for two experimental groups.

substrate (**Fig. 6B**), where oxidation of palmitoyl-CoA was rapidly increased to its maximum after only 12 h of feeding tetradecylthioacetic acid. In the presence of KCN, an increased cyanide-insensitive oxidation of palmitoyl-CoA was revealed after 3 days of tetradecylthioacetic feeding (Fig. 6C).

As inhibition of fatty acid oxidation may be an important factor for induction of fatty liver, we investigated how 3-tetradecylthiopropionic acid alters fatty acid oxidation in isolated mitochondria. **Table 4** shows that in animals fed 3-tetradecylthiopropionic acid, oxidation of [1-^{14}C]palmitoyl-L-carnitine in the absence of KCN was decreased 60–70% after 10 days of treatment. Total oxidation of [1-^{14}C]palmitoyl-CoA was marginally affected during the first day of 3-tetradecylthiopropionic acid treatment, but dramatically decreased palmitoyl-CoA oxidation was observed after 10 days treatment. In the presence of KCN, the palmitoyl-CoA oxidation remained unaffected during 2 days of treatment, but after 3 to 10 days of feeding, an increased cyanide-insensitive oxidation of palmitoyl-CoA was observed, possibly due to increased peroxisomal β-oxidation (2).

Fatty acid synthetase activity

As diminished lipogenesis may contribute to reduced triglyceride formation and thereby reduction of VLDL-triglycerides (3), we examined whether fatty acid synthetase activity was changed after feeding of sulfur-containing fatty acid. **Table 5** shows that administration of 3-thiadicarboxylic acid, tetradecylthioacetic acid, and 3-tetradecylthiopropionic acid decreased the fatty acid synthetase activity by 20–70%.

DISCUSSION

This study demonstrates that administration of 3-thiadicarboxylic and tetradecylthioacetic acids decreases the hepatic and plasma content of triglycerides, whereas induction of fatty liver and hyperlipidemia were observed with 3-tetradecylthiopropionic acid (Fig. 1 and Fig. 2). Furthermore, it strongly suggests that these effects are

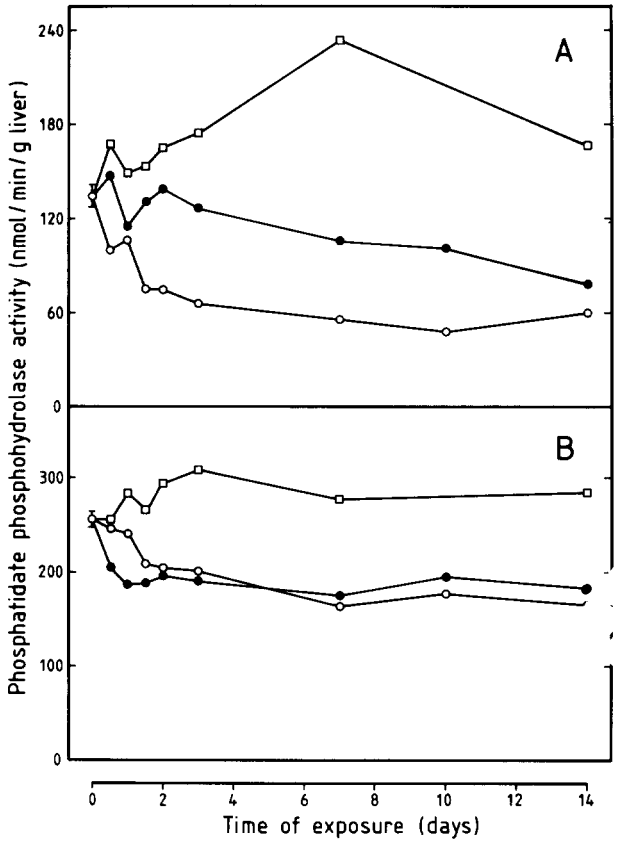

Fig. 4. Time-course of phosphatidate phosphohydrolase in cytosolic (A), and microsomal (B) fractions of rats treated with tetradecylthioacetic acid (●), 3-tetradecylthiopropionic acid (□), and 3-thiadicarboxylic acid (○). Data represent the means ± SD of twelve control animals and means of three rats in each experimental group.

TABLE 3. Dose-dependent changes of CTP:phosphocholine cytidylyltransferase activity in rats treated for 5 days with sulfur-substituted fatty acid analogues

Dose (mg per day per kg body weight)	CTP:Phosphocholine Cytidylyltransferase Activity					
	CMTTD		BCMTD		CETTD	
	S	P	S	P	S	P
0	100	100	100	100	100	100
75	105 ± 4	111 ± 6	110 ± 5	120 ± 10*	94 ± 5	101 ± 6
150	112 ± 6*	125 ± 10*	126 ± 10*	140 ± 8*	87 ± 8*	95 ± 4
250	125 ± 10*	130 ± 12*	140 ± 20	165 ± 15*		
500	140 ± 15*	150 ± 14*			73 ± 10*	96 ± 6

The specific activities of control animals = 100% and the results are expressed as in Table 1. In control animals, the specific cytidylyltransferase activity in the cytosomal fraction (S) and microsomal fraction (P) was 0.35 ± 0.08 and 0.85 ± 0.10 nmol/mg protein, respectively.*, $P < 0.05$ between control and treated animals.

partially mediated via phosphatidate phosphohydrolase, an enzyme that has previously been shown to vary in response to different stimuli affecting the rate of triglyceride biosynthesis (14). In cultured rat hepatocytes the activity of diacylglycerol acyltransferase is inhibited by eicosapentaenoic acid, and it has been suggested that this enzyme activity might contribute to a lowering of plasma triglycerides (15). However, in a previous study we reported that this enzyme was only marginally affected by the non-β-oxidizable fatty acid analogues (2). Glycerophosphoate acyltransferase may have considerable potential for regulation of triglycerides as it catalyzes the initial esterification step in triglyceride synthesis. As shown in Fig. 3 the activity of glycerophosphate acyltransferase does not seem to change in parallel with the rate of triglyceride biosynthesis. Taken together, these data strongly speak in favor of the hypothesis that phosphatidate phosphohydrolase is rate-limiting in triglyceride biosynthesis.

On the other hand it should be considered that the availability of fatty acids is a prerequisite for triglyceride synthesis. As the presence of tetradecylthioacetic acid and 3-thiadicarboxylic acid promotes increased mitochondrial fatty acid oxidation (Fig. 6), it is possible that the initial effect of the non-β-oxidizable sulfur-substituted fatty acid analogues is associated with reduction of fatty acid availability via increased mitochondrial fatty acid oxidation. It is noteworthy that induction of peroxisomal β-oxidation was not a prerequisite for the hypotriglyceridemia (4).

This study has addressed the question of a coordinate regulation of the synthesis of triglyceride and phospholipids. Of interest was the observation that when phosphatidate phosphohydrolase activity was stimulated (3-tetradecylthiopropionic acid) (Fig. 4) the activity of CTP:phosphocholine cytidylyltransferase was inhibited (Fig. 5). Similarly, an inhibition of phosphatidate phosphohydrolase activity (3-thiadicarboxylic acid and tetradecylthioacetic acid) was accompanied by a stimulation of CTP:phosphocholine cytidylyltransferase (Fig. 5). It is, thus, tempting to suggest that a coordinate regulation of triglyceride and phospholipid biosynthesis may exist. An inhibition of phosphatidate phosphohydrolase, coupled with a stimulation of the cytidylyltransferase, would accelerate phospholipid biosynthesis at the expense of retarded synthesis of triglycerides.

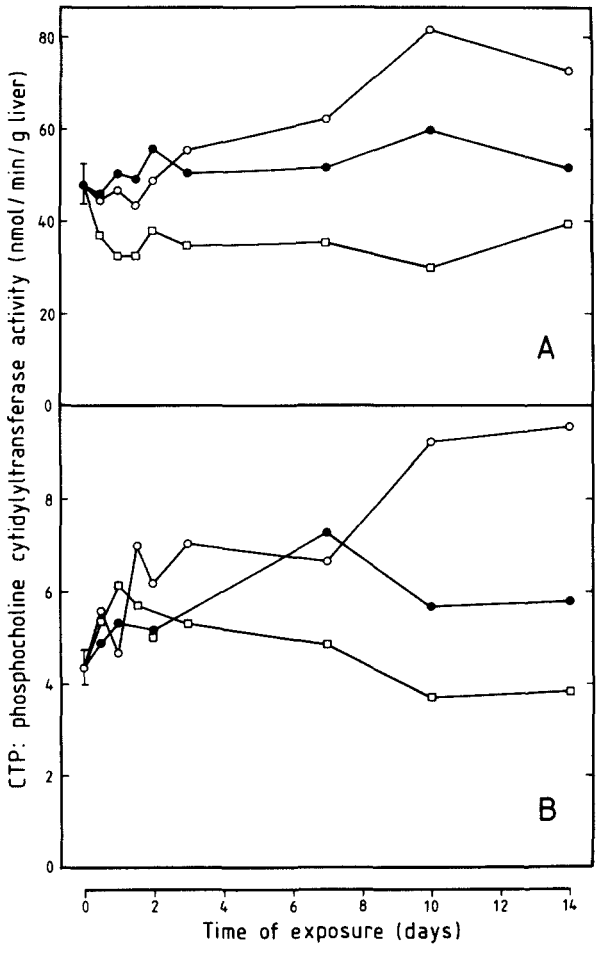

Fig. 5. Time-course of CTP:phosphocholine cytidylyltransferase activity in cytosolic (A), and microsomal (B) fractions of rats treated with tetradecylthioacetic acid (●), 3-tetradecylthiopropionic acid (□), and 3-thiadicarboxylic acid (○). The tabulated values are the means ± SD of twelve control animals and means of three rats in each experimental group.

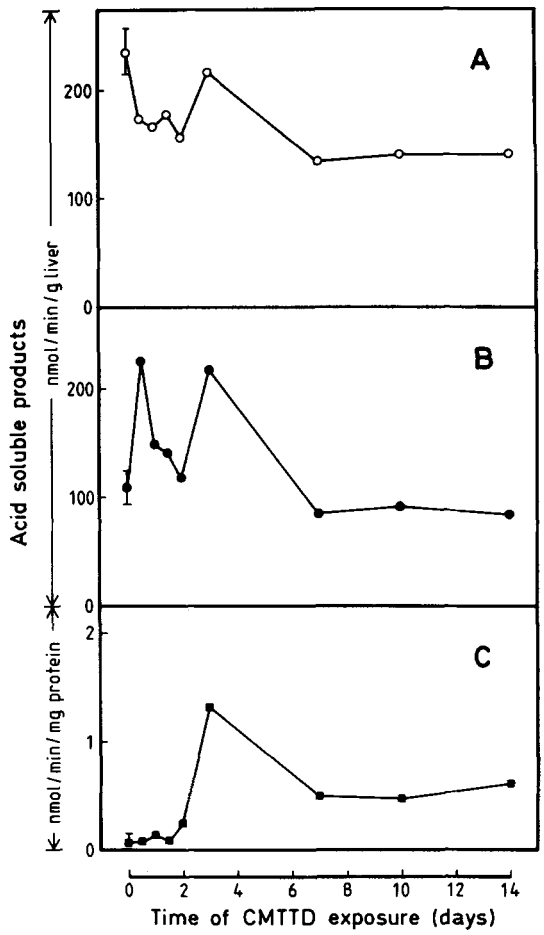

Fig. 6. Effect of tetradecylthioacetic acid exposure on [1-^{14}C]palmitoyl carnitine oxidation (A), and [1-^{14}C]palmitoyl-CoA oxidation (B) in liver mitochondria in the absence of KCN; (C), [1-^{14}C]palmitoyl-CoA oxidation in the presence of KCN. Data represent the means ± SD (control animals of 0, 7, and 14 days) and means (treated animals).

The modest increase in the phospholipid content of the liver observed in 3-thiadicarboxylic acid- and tetradecylthioacetic acid-treated rats was associated with a decrease in plasma phospholipid levels. This finding might be explained by the proliferation of liver peroxisomes (5), a process that is phospholipid-dependent. It is noteworthy that CTP:phosphocholine cytdiylyltransferase activity seems to correlate with the rate of phospholipid biosynthesis in 3-thiadicarboxylic acid- and tetradecylthioacetic acid-treated animals. This is consistent with a rate-limiting role of the enzyme activity. However, this was not the case in the 3-tetradecylthiopropionic acid-treated group. A high dose of this agent administered over an extended period of time resulted in an increase in hepatic and plasma phospholipid levels despite an inhibition in the enzyme activity (Table 3, Fig. 5). The explanation for the increased synthesis of phospholipids is not clear. However, it is evident that the cytidylyltransferase activity was not rate-limiting in this particular situation.

TABLE 4. β-Oxidation of [1-^{14}C]palmitoyl-L-carnitine and [1-^{14}C]palmitoyl-CoA in liver mitochondria of rats after 3-tetradecylthiopropionic acid treatment[a]

	β-Oxidation	
Time of Exposure	Palmitoyl-L-Carnitine	Palmitoyl-CoA
days		
0	230 ± 20	120 ± 10
1.0	160 ± 15*	130 ± 20
1.5	150 ± 20*	90 ± 20*
2.0	120 ± 10*	80 ± 10*
5.0	100 ± 15*	70 ± 10*
7.0	80 ± 15*	65 ± 10*
10.0	75 ± 10*	40 ± 10*

Acid-soluble metabolites were measured and the activity is expressed as nmol of substrate oxidized per min per mg of mitochondria protein. Values are reported as means ± SD for three to six rats. *, $P < 0.02$ between control and treated animals.

[a]Animals received 150 mg per day per kg body weight.

It is likely that the modest increase in hepatic phospholipids via increased CDP-pathway accounts for an increase of lipid components for proliferation of intracellular organelles, i.e., peroxisomes (5).

It has been reported that rats fed a choline-deficient diet secrete a reduced amount of circulating phospholipids, triglycerides, and apoproteins in their plasma lipoproteins (16). Furthermore, specific pools of phospholipids are used for lipoprotein secretion by rat hepatocytes (17). As 60–75% of the phospholipid content in the lipoprotein particles is phosphatidylcholine (17), it is conceivable that the synthesis of phospholipids via the CDP-choline pathway may influence the assembly and secretion of VLDL in sulfur-substituted fatty acid-fed animals.

Concentration of fatty acids has been shown to regulate activity of phosphatidate phosphohydrolase (12, 13) and CTP:phosphocholine cytidylyltransferase (16, 18). Repeated administration of 3-thiadicarboxylic acid and

TABLE 5. Time-dependent changes of fatty acid synthetase activity in cytosolic fractions in rats given sulfur-substituted fatty acid analogues[a]

	Fatty Acid Synthetase (% of control)		
Days of Treatment	CMTTD	BCMTD	CETTD
0	100	100	100
1	80 ± 10	77 ± 8	54 ± 6
2	81 ± 8	37	nd
7	52 ± 10	36 ± 7	36 ± 12
14	45 ± 8	30 ± 5	56 ± 8

The enzyme activity is calculated relative to that of pellet-fed controls (≈ 100%) and the results are expressed as in Table 1. In control animals (0, 7, and 14 days) the specific fatty acid synthetase activity in cytosol was 2.5 ± 0.2 nmol per min per mg protein; nd, not determined.

[a]Animals received 150 mg per day per kg body weight.

tetradecylthioacetic acid increased the long-chain acyl-CoA level within the first days of feeding, whereas 3-tetradecylthiopropionic acid increased the long-chain acyl-CoA content gradually as a function of time of exposure (2). No translocation of either phosphatidate phosphohydrolase or CTP:phosphocholine cytidylyltransferase was observed in animals treated with the sulfur-substituted fatty acid analogues (Fig. 4 and Fig. 5). Moreover, the phosphatidate phosphohydrolase activity was inhibited in 3-thiadicarboxylic- and tetradecylthioacetic acid-treated rats despite the increase in acyl-CoA content (2). The reason for this discrepancy is unclear at present. However, it suggests that other mechanisms for the enzymatic stimulation and inhibition might be operative.

Altogether, it would seem that triglyceride formation is reduced with non-β-oxidizable fatty acid analogues due to increased fatty acid oxidation, diminished lipogenesis, and reduced esterification at critical sites of initial VLDL synthesis. Whether the increase in mitochondrial fatty acid oxidation in rat liver after feeding of tetradecylthioacetic acid is the cause of, rather than the consequence of, the hypertriglyceridemia and hypercholesterolemia is not known. But a possible interpretation is that some fatty acid derivatives, for unknown reasons, induce proliferation of mitochondria (5) accompanied by increased β-oxidation (Fig. 6) and thereby reduce fatty acid availability for triglyceride synthesis and secretion. This, in turn, also decreases plasma cholesterol levels. The triglyceride-lowering effect of 3-thiadicarboxylic acid and tetradecylthioacetic acid was accompanied by a marked reduction of free fatty acid level, which could be caused by a decrease in the release of fatty acids from adipose tissue or increased β-oxidation.

Administration of 3-tetradecylthiopropionic acid led to an inhibition of mitochondrial and total oxidation of fatty acids (Table 4). In addition, plasma free fatty acid levels increased, indicating an effect on adipose tissue lipolysis. As a result, the influx of fatty acids into the pathway of triglyceride biosynthesis exceeded the capacity of the liver to transport triglycerides. Consequently, fatty liver developed. These results emphasize the importance of the availability of the substrate, i.e., fatty acids, as a major determinant of the rate of triglyceride biosynthesis.

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REFERENCES

1. Berge, R. K., A. Aarsland, H. Kryvi, J. Bremer, and N. Aarsaether. 1989. Alkylthioacetic acids (3-thia fatty acids)—a new group of non-β-oxidizable peroxisome-inducing fatty acid analogues. II. Dose-response studies on hepatic peroxisomal and mitochondrial changes and long-chain fatty acid metabolizing enzymes in rats. *Biochem. Pharmacol.* **38:** 3969–3979.
2. Asiedu, D., A. Aarsland, J. Skorve, A. Svardal, and R. K. Berge. 1990. Fatty acid metabolism in liver of rats treated with hypolipidemic sulphur-substituted fatty acid analogues. *Biochim. Biophys. Acta.* **1044:** 211–221.
3. Skrede, S., M. Narce, S. Bergseth, and J. Bremer. 1989. The effects of alkylthioacetic acids (3-thia fatty aicds) on fatty acid metabolism in isolated hepatocytes. *Biochim. Biophys. Acta.* **1005:** 296–302.
4. Aarsland, A., N. Aarsaether, J. Bremer, and R. K. Berge. 1989. Alkylthioacetic acids (3-thia fatty acids) as non-β-oxidizable fatty acid analogues: a new group of hypolipidemic drugs. III. Dissociation of cholesterol- and triglyceride-lowering effects and the induction of peroxisomal β-oxidation. *J. Lipid Res.* **30:** 1711–1718.
5. Berge, R. K., A. Aarsland, H. Kryvi, J. Bremer, and N. Aarsaether. 1989. Alkylthioacetic acid (3-thia fatty acids)—A new group of non-β-oxidizable, peroxisome-inducing fatty acid analogues. I. A study on the structural requirements for proliferation of peroxisomes and mitochondria in rat liver. *Biochim. Biophys. Acta.* **1004:** 345–356.
6. 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.
7. Aarsland, A., R. K. Berge, J. Bremer, and N. Aarsaether. 1990. The hypolipidemic peroxisome-proliferating drug, bis(carboxymethylthio)-1.10 decane, a dicarboxylic metabolite of tiadenol, is activated to an acylcoenzyme A thioester. *Biochim. Biophys. Acta.* **1033:** 176–183.
8. Mavis, R. D., J. N. Finkelstein, and B. P. Hall. 1978. Pulmonary surfactant synthesis. A highly active microsomal phosphatidate phosphohydrolase in the lung. *J. Lipid Res.* **19:** 467–477.
9. Sleight, R., and C. Kent. 1983. Regulation of phosphatidylcholine biosynthesis in mammalian cells. *J. Biol. Chem.* **258:** 831–835.
10. Roncari, D. A. 1981. Fatty acid synthetase from human liver. *Methods Enzymol.* **71:** 73–79.
11. Shimizu, S., K. Yasui, Y. Tani, and H. Yamada. 1979. Acyl-CoA oxidase from *Candida tropicalis*. *Biochem. Biophys. Res. Commun.* **91:** 108–113.
12. Cascales, C., E. H. Mangiapane, and D. N. Brindley. 1984. Oleic acid promotes the activation and translocation of phosphatidate phosphohydrolase from cytosol to particulate fractions of isolated rat hepatocytes. *Biochem. J.* **219:** 911–916.
13. Martin-Sanz, P., R. Hopewell, and D. N. Brindley. 1984. Long-chain fatty acids and their acyl-CoA esters cause the translocation of phosphatidate phosphodydrolase from the cytosolic to the microsomal fraction of rat liver *FEBS Lett.* **175:** 284–288.
14. Bell, R. M., and R. M. Coleman. 1980. Enzymes of glycerolipid synthesis in eucaryotes. *Annu. Rev. Biochem.* **49:** 459–487.

15. Rustan, A. C., J. Ø. Nossen, E. N. Christiansen, and C. A. Drevon. 1988. Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acylcoenzyme A:1,2-diacylglycerol acyltransferase. *J. Biol. Chem.* **263:** 8126–8132.
16. Pelech, S. L., and D. E. Vance. 1984. Regulation of phosphatidyl choline biosynthesis. *Biochim. Biophys. Acta.* **779:** 217–251.
17. Vance, J. E., and D. E. Vance. 1986. Specific pools of phospholipids are used for lipoprotein secretion by cultured rat hepatocytes. *J. Biol. Chem.* **261:** 4486–4491.
18. Pelech, S. L., P. H. Pritchard, D. N. Brindley, and D. E. Vance. 1983. Fatty acids promote translocation of CTP: phosphocholine cytidyltransferase to the endoplasmic reticulum and stimulate rat hepatic phosphatidylcholine synthesis. *J. Biol. Chem.* **258:** 6782–6788.