Effect of 3-thia fatty acids on the lipid composition of rat liver, lipoproteins, and heart

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Abstract To investigate the importance of factors influencing the fatty acid composition, lipid and lipoprotein metabolism in the rat, the effect of 3-thia fatty acids of chain-length ranging from octyl- to hexadecylthioacetic acid were studied. In liver, very low density lipoprotein (VLDL), and low density lipoprotein (LDL), the hypolipidemic 3-thia fatty acids, namely C12-S-acetic acid to C14-S-acetic acid increased the amount of monoenes, especially oleic acid (18:1n–9). In contrast, the content of polyunsaturated fatty acids in liver, VLDL, and LDL decreased, mostly attributed to a reduction of eicosapentaenoic acid (EPA, 20:5n–3). Noteworthy, the hypolipidemic 3-thia fatty acids reduced the amount of arachidonic acid (AA, 20:4n–6) in LDL and HDL. 3-Thia fatty acids accumulated in the liver. In heart, as in liver, 3-thia fatty acids replaced fatty acids of chain-length homologues. In contrast to liver, we were unable to detect any changes in 18:1n–9. However, the n–3 polyunsaturated fatty acid content increased, particularly 20:5n–3 and docosahexaenoic acid (DHA, 22:6n–3) leading to an increased n–3/n–6 ratio. In conclusion, this study demonstrates that hypolipidemic 3-thia fatty acids change the fatty acid composition of organs and lipoproteins. These changes are linked to the expression and activity of hepatic Δ^9-desaturase, fatty acid oxidation, and displacement of normal fatty acids by 3-thia fatty acids. The fatty acid composition is regulated differently in liver and heart after administration of hypolipidemic 3-thia fatty acids.—**Frøyland, L., L. Madsen, W. Sjursen, A. Garras, Ø. Lie, J. Songstad, A. C. Rustan, and R. K. Berge.** Effect of 3-thia fatty acids on the lipid composition of rat liver, lipoproteins, and heart. *J. Lipid Res.* 1997. **38:** 1522–1534.

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3-Thia fatty acids are fatty acid analogues in which a sulfur atom replaces the β-methylene group in the alkyl chain (1). In the liver, mitochondrial and peroxisomal fatty acid oxidation and triacylglycerol biosynthesis are the major competitors for the utilization of fatty acids as substrates (2, 3). 3-Thia fatty acids are both mitochondrial and peroxisomal proliferators and they increase the hepatic mitochondrial and peroxisomal fatty acid oxidation capacities (4, 5).

Growing evidence indicates that availability of triacylglycerols is a major driving force in the secretion of triacylglycerol-rich lipoproteins (VLDL) by the liver (6, 7). The different plasma lipoproteins (VLDL, LDL, and HDL) are connected in a complex metabolic interrelationship. 3-Thia fatty acids that profoundly affect β-oxidation, glycerolipid biosynthesis, and plasma lipoprotein homeostasis are likely to challenge the homeostasis of this metabolic network (8–13). Recently, we found that the fatty acid composition of different rat organs was changed after administration of tetradecylthioacetic acid (C14-S-acetic acid), indicating that 3-thia fatty acids may regulate the fatty acid composition in heart and liver by different mechanisms (14, 15).

In the present study we have, therefore, investigated in more detail the effects of 3-thia fatty acids of chain length from octyl- to hexadecylthioacetic acid (C8-S-acetic acid to C16-S-acetic acid) on factors influencing the fatty acid composition in liver, plasma lipoproteins, and heart.

EXPERIMENTAL PROCEDURES

Chemicals

Isotopes used in this experiment were purchased from New England Nuclear (Boston, MA) except for

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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TABLE 1. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on plasma triacylglycerol and phospholipid levels after 7 days of treatment

Treatment	Plasma	
	Triacylglycerol	Phospholipids
	mmol/l	
Control	0.8 ± 0.1	1.3 ± 0.2
C8-S-acetic acid	0.7 ± 0.3	1.3 ± 0.2
C11-S-acetic acid	0.7 ± 0.1	1.2 ± 0.2
C12-S-acetic acid	0.5 ± 0.2[a]	0.8 ± 0.2[a]
C13-S-acetic acid	0.4 ± 0.2[a]	0.6 ± 0.1[a]
C14-S-acetic acid	0.4 ± 0.2[a]	0.7 ± 0.2[a]
C15-S-acetic acid	0.8 ± 0.3	0.9 ± 0.2
C16-S-acetic acid	1.0 ± 0.2	1.1 ± 0.1

Results are expressed as means ± SD of five animals in each group.
[a]Significantly different from control; $P < 0.05$.

[^{32}P-α]-dCTP which was obtained from Radiochemical Centre, (Amersham, UK). Thioglycolic acid, potassium hydroxide, 1-bromooctane, and 1-bromoundecane to 1-bromohexadecane were obtained from Fluka Chemie, Buchs, Switzerland. All other chemicals were obtained from common commercial sources and were of reagent grade.

Preparation of 3-thia fatty acids

All the sulfur-containing fatty acid derivatives were synthesized as previously described (15).

Animals and treatments

Male Wistar rats weighing between 250–270 g were purchased from Møllegaard Breeding Laboratory, (Ejby, Denmark). They were housed in metal wire cages and maintained on a 12-h light/dark cycle, at a temperature of 20 ± 3°C. The rats were acclimatized for 1 week before the start of the experiment and had free access to water and R34-EWOS-ALAB grower rat maintenance chow (Ewos, Sweden) which contained the following fatty acids (mol%); 16:0 (21%), 16:1n–7 (2%), 18:0 (4%), 18:1n–9 (25%), 18:2n–6 (42%), and 18:3n–3 (6%).

The 3-thia fatty acids and palmitic acid (control) were suspended in 0.1% CM-cellulose. A dose of 150 mg/kg body weight per day was administered by oro-gastric intubation in a final volume of 1.0 ml for 1 week. In addition, two groups, C13-S- and C14-S-acetic acid, received a dose of 300 mg/kg body weight per day. The stock suspensions were kept at 0–4°C when not in use. The overall food consumption and weight gain were not

TABLE 2. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on very low density lipoprotein (VLDL) fatty acid composition (mol % of total lipid) after 7 days of treatment

Fatty acid	VLDL				
	Control	C8-S-Acetic Acid	C12-S-Acetic Acid	C13-S-Acetic Acid	C14-S-Acetic Acid
14:0	1.1 ± 0.7	0.8 ± 0.2	1.0 ± 0.2	1.4 ± 0.6	0.9 ± 0.1
15:0	0.6 ± 0.2	0.4 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.5 ± 0.1
16:0	25.4 ± 1.6	23.7 ± 0.3	25.2 ± 0.1	23.2 ± 0.2	25.1 ± 0.7
C8-S-acetic acid	ND	ND	ND	ND	ND
16:1n–7	1.8 ± 0.3	1.8 ± 0.2	1.6 ± 0.3	1.2 ± 0.3	1.4 ± 0.4
16:1n–9	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.5 ± 0.1
17:0	0.4 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
18:0	5.8 ± 0.7	5.3 ± 0.2	5.0 ± 0.3	7.4 ± 0.3	5.7 ± 0.2
18:1n–7	1.8 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.4	2.0 ± 0.2
18:1n–9	16.0 ± 1.1	16.7 ± 0.3	18.3 ± 0.2[a]	18.5 ± 1.9[a]	20.6 ± 1.5[a]
18:2n–6	24.5 ± 2.8	27.6 ± 1.8	24.2 ± 1.7	23.1 ± 1.6	22.3 ± 2.3
C12-S-acetic acid	ND	ND	0.7 ± 0.1[a]	ND	ND
18:3n–3	1.2 ± 0.2	1.2 ± 0.0	0.9 ± 0.0	0.8 ± 0.2	0.9 ± 0.2
C13-S-acetic acid	ND	ND	ND	2.1 ± 0.2[a]	ND
20:3n–6	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.0[a]	1.0 ± 0.1[a]	0.7 ± 0.2[a]
20:4n–3	ND	ND	ND	ND	0.8 ± 0.3[a]
C14-S-acetic acid	ND	ND	ND	ND	1.8 ± 0.4[a]
20:4n–6	5.1 ± 1.2	6.1 ± 0.4	5.3 ± 0.7	4.4 ± 0.5	6.1 ± 0.2
20:5n–3	2.7 ± 0.4	2.5 ± 0.5	1.7 ± 0.2[a]	0.8 ± 0.2[a]	0.8 ± 0.3[a]
C14:1n–9-S-acetic acid	ND	ND	ND	ND	ND
22:5n–3	1.4 ± 0.3	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	0.8 ± 0.2
22:6n–3	6.1 ± 2.2	7.5 ± 1.3	6.9 ± 1.1	4.2 ± 0.4[a]	3.8 ± 0.7[a]
Σ saturated	33.7 ± 3.7	30.7 ± 0.4	33.1 ± 0.8	34.7 ± 0.7	33.6 ± 1.1
Σ monounsat.	20.2 ± 0.8	20.7 ± 0.5	22.4 ± 0.2[a]	22.1 ± 2.7	24.5 ± 1.8[a]
Σ polyunsat.	41.8 ± 3.7	46.4 ± 0.2	40.8 ± 3.4	36.0 ± 1.5[a]	36.3 ± 1.4[a]
Σ n–3	11.4 ± 3.0	12.4 ± 1.9	10.8 ± 1.0	7.0 ± 1.0[a]	7.1 ± 1.6[a]
Σ n–6	30.0 ± 4.0	34.0 ± 2.1	30.1 ± 2.5	32.4 ± 1.5	29.3 ± 1.5
n–3/n–6	0.38 ± 0.05	0.36 ± 0.08	0.36 ± 0.00	0.22 ± 0.06[a]	0.24 ± 0.03[a]

Results are expressed as means ± SD (n = 4). ND, not detected.
[a]Significantly different from control; $P < 0.05$.

changed. At the end of the experiment, the overnight-fasted rats were anesthetized with Hypnorm Dormicum® (fluanisone-fentanylmidazolam, 0.2 ml · 100 g^{-1} body weight) and cardiac puncture was performed to obtain blood samples in EDTA vacutainers. Selected organs were removed immediately, weighed, and parts were either chilled on ice, frozen in liquid N_2, or freeze-clamped and frozen in liquid N_2. The protocol was approved by the Norwegian State Board of Biological Experiments with Living Animals.

Preparation of total liver homogenates, subcellular fractions, and enzyme activities

Total homogenates of the liver and subcellular fractions were prepared as described earlier (13). Mitochondrial 2,4-dienoyl-CoA reductase activity was determined in the mitochondrial fraction according to Kunau and Dommes (16) with some minor modifications. Briefly, the assay medium (1 ml) contained 0.01% Triton X-100, 50 mM KH_2PO_4 (pH 7.4), 0.1 mM NADPH, and 100 μg protein. The reaction was started by addition of 100 μM 2,4-dienoyl-CoA substrate. The assay was run in duplicate and performed under conditions where product formation was linear with respect to time and the amount of protein. Mitochondrial β-oxidation was measured in the post-nuclear fraction as acid soluble products using [1-^{14}C]palmitoyl-CoA or [1-^{14}C]palmitoyl-L-carnitine as substrates as previously described (13). Cytosolic PLA2 (cPLA2) enzyme activity was determined in the post-nuclear fraction according to Wijkander and Sundler (17). Fatty acyl-CoA oxidase was measured in the post-nuclear fraction as described by Small, Burdett, and Connock (18). Protein was determined using the Bio-Rad protein kit (Bio-Rad, Richmond, CA).

Hepatocyte isolation and culture conditions

Rat liver parenchymal cells were isolated according to Seglen (19). The hepatocytes were plated at a density of 2.0 × 10^6 dish (1–1.5 mg cell protein) in 2 ml of DMEM containing 20 mM HEPES, Ultroser G (2%), gentamicin (50 μg/ml) and either L-carnitine (0.5 mM) or L-aminocarnitine (50 μM). Cultures were maintained in a humidified incubator at 37°C in an atmosphere containing 5% CO_2. After overnight incubation, the medium was replaced with DMEM (2 ml/dish) containing

TABLE 3. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on low density lipoprotein (LDL) fatty acid composition (mol % of total lipid) after 7 days of treatment

	LDL				
Fatty acid	Control	C8-S-Acetic Acid	C12-S-Acetic Acid	C13-S-Acetic Acid	C14-S-Acetic Acid
14:0	1.9 ± 0.8	1.2 ± 0.1	1.5 ± 0.4	1.5 ± 0.5	1.3 ± 0.2
15:0	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.1
16:0	14.7 ± 1.0	13.4 ± 0.3	14.0 ± 0.4	15.6 ± 0.2	15.4 ± 0.7
C8-S-acetic acid	ND	ND	ND	ND	ND
16:1n–7	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.1 ± 0.3	2.3 ± 0.4
16:1n–9	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.8 ± 0.1
17:0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
18:0	5.5 ± 1.3	4.0 ± 0.2	4.3 ± 0.1	4.4 ± 0.3	4.1 ± 0.2
18:1n–7	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
18:1n–9	20.0 ± 0.1	22.1 ± 1.8	27.0 ± 0.6[a]	28.1 ± 1.9[a]	30.3 ± 1.8[a]
18:2n–6	17.1 ± 0.8	18.0 ± 0.8	16.2 ± 0.5	15.6 ± 2.6	15.3 ± 1.3
C12-S-acetic acid	ND	ND	0.3 ± 0.1[a]	ND	ND
18:3n–3	0.8 ± 0.1	0.9 ± 0.0	0.7 ± 0.0	0.7 ± 0.2	0.5 ± 0.2
C13-S-acetic acid	ND	ND	ND	0.6 ± 0.2[a]	ND
20:3n–6	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
20:4n–3	ND	ND	ND	0.3 ± 0.1[a]	0.7 ± 0.3[a]
C14-S-acetic acid	ND	ND	ND	ND	0.7 ± 0.2[a]
20:4n–6	14.7 ± 0.5	14.5 ± 0.4	13.2 ± 0.2[a]	9.8 ± 1.3[a]	11.6 ± 1.1[a]
20:5n–3	4.6 ± 0.1	3.8 ± 0.6	2.9 ± 0.1[a]	1.2 ± 0.2[a]	1.3 ± 0.3[a]
C14:1n–9-S-acetic acid	ND	ND	ND	ND	ND
22:5n–3	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2
22:6n–3	5.5 ± 0.3	5.2 ± 0.8	6.2 ± 1.1	4.3 ± 0.4[a]	3.8 ± 0.7[a]
Σ saturated	23.5 ± 4.7	19.9 ± 1.4	21.2 ± 0.9	23.7 ± 0.1	20.9 ± 2.4
Σ monounsat.	25.3 ± 1.2	29.2 ± 1.5[a]	32.1 ± 0.7[a]	33.4 ± 3.7[a]	32.9 ± 3.8[a]
Σ polyunsat.	44.1 ± 1.8	44.0 ± 0.8	41.7 ± 0.4	35.5 ± 2.5[a]	33.2 ± 4.4[a]
Σ n–3	11.8 ± 0.6	10.6 ± 1.8	11.2 ± 0.7	7.5 ± 1.0[a]	6.6 ± 2.2[a]
Σ n–6	32.2 ± 1.1	32.9 ± 1.9	30.0 ± 0.8	26.1 ± 2.5[a]	25.0 ± 3.5[a]
n–3/n–6	0.37 ± 0.02	0.32 ± 0.08	0.37 ± 0.04	0.29 ± 0.04[a]	0.26 ± 0.06[a]

Results are expressed as means ± SD (n = 4). ND, not detected.
[a]Significantly different from control; $P < 0.05$.

TABLE 4. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on high density lipoprotein (HDL) fatty acid composition (mol % of total lipid) after 7 days of treatment

	HDL				
Fatty Acid	Control	C8-S-Acetic Acid	C12-S-Acetic Acid	C13-S-Acetic Acid	C14-S-Acetic Acid
14:0	1.4 ± 0.6	1.2 ± 0.4	1.9 ± 0.6	1.5 ± 0.2	1.3 ± 0.2
15:0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.8 ± 0.3	0.6 ± 0.1
16:0	13.2 ± 1.1	14.5 ± 0.7	15.3 ± 1.2	15.6 ± 0.9	15.4 ± 1.7
C8-S-acetic acid	ND	ND	ND	ND	ND
16:1n–7	1.7 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	1.1 ± 0.3	1.6 ± 0.4
16:1n–9	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.1
17:0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
18:0	6.0 ± 1.1	6.3 ± 0.2	7.0 ± 0.8	6.9 ± 0.6	6.8 ± 1.3
18:1n–7	0.9 ± 0.2	1.3 ± 0.4	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.2
18:1n–9	4.5 ± 1.3	4.9 ± 1.6	5.8 ± 0.8	5.4 ± 1.1	6.3 ± 1.7
18:2n–6	16.5 ± 2.5	13.6 ± 3.6	15.9 ± 1.3	15.9 ± 1.9	16.2 ± 1.4
C12-S-acetic acid	ND	ND	ND	ND	ND
18:3n–3	ND	ND	ND	ND	ND
C13-S-acetic acid	ND	ND	ND	ND	ND
20:3n–6	0.5 ± 0.1	0.4 ± 0.1	1.1 ± 0.2[a]	1.4 ± 0.3[a]	1.5 ± 0.4[a]
20:4n–3	ND	ND	ND	ND	ND
C14-S-acetic acid	ND	ND	ND	ND	ND
20:4n–6	51.0 ± 2.5	51.7 ± 3.6	45.9 ± 2.2[a]	46.2 ± 1.3[a]	45.9 ± 2.6[a]
20:5n–3	1.1 ± 0.4	0.9 ± 0.3	0.8 ± 0.1	0.7 ± 0.3	0.8 ± 0.2
C14:1n–9-S-acetic acid	ND	ND	ND	ND	ND
22:5n–3	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
22:6n–3	2.0 ± 0.3	2.4 ± 0.2	1.8 ± 0.4	1.9 ± 0.3	1.5 ± 0.6
Σ saturated	21.3 ± 2.6	22.7 ± 2.9	25.1 ± 3.1	25.2 ± 1.8	24.5 ± 1.7
Σ monounsat.	7.4 ± 1.4	8.0 ± 1.8	8.3 ± 0.1	7.7 ± 1.9	9.3 ± 2.8
Σ polyunsat.	71.3 ± 4.3	69.2 ± 5.6	65.6 ± 6.2	66.2 ± 4.7	66.0 ± 5.3
Σ n–3	3.3 ± 1.1	3.5 ± 0.7	2.7 ± 0.9	2.7 ± 0.6	2.4 ± 0.8
Σ n–6	68.0 ± 4.9	65.7 ± 3.2	62.9 ± 4.4	63.5 ± 5.1	63.6 ± 6.1
n–3/n–6	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Results are expressed as means ± SD (n = 4) (<0.01%); ND, not detected.
[a]Significantly different from control; $P < 0.05$.

fatty acids bound to BSA and components indicated in legend to Fig. 6. The molar ratio between fatty acids and BSA was 2.5:1.

Determination of fatty acid oxidation

The medium was centrifuged at 3500 rpm (2500 *g*); 250 µl cell-free medium was added to 100 µl 6% BSA and protein was precipitated with 1.0 ml ice-cold perchloric acid (1 M). The mixtures were shaken (vortex) and centrifuged at 2500 *g* for 10 min and samples (500 µl) of the supernatants were counted by liquid scintillation counter. Acid-soluble radioactivity in media was taken as measure of total oxidation. Control experiments revealed that CO_2 and retained cellular radioac-

TABLE 5. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on liver and heart triacylglycerol and phospholipid levels after 7 days of treatment

	Liver		Heart	
Treatment	Triacylglycerol	Phospholipids	Triacylglycerol	Phospholipids
	µmol/g		µmol/g	
Control	7.5 ± 1.6	11.0 ± 2.1	1.6 ± 0.8	1.7 ± 0.3
C8-S-acetic acid	9.1 ± 2.9	10.7 ± 1.2	1.7 ± 0.3	2.0 ± 0.5
C11-S-acetic acid	6.0 ± 0.9	10.0 ± 1.1	1.4 ± 0.5	2.1 ± 0.2
C12-S-acetic acid	8.4 ± 2.9	10.9 ± 1.4	1.6 ± 0.5	2.0 ± 0.6
C13-S-acetic acid	8.8 ± 3.7	10.7 ± 2.2	1.4 ± 0.5	2.8 ± 0.5[a]
C14-S-acetic acid	8.2 ± 2.9	11.2 ± 1.0	1.7 ± 0.8	2.8 ± 0.6[a]
C15-S-acetic acid	7.6 ± 2.7	11.3 ± 1.2	1.1 ± 0.2	1.9 ± 0.5
C16-S-acetic acid	8.6 ± 2.3	12.1 ± 1.0	1.0 ± 0.1	2.5 ± 0.4[a]

Results are expressed as means ± SD of 5 animals in each group.
[a]Significantly different from control; $P < 0.05$.

tivity accounted for < 10% of total activity. Samples taken from the different experimental media were precipitated as described above and used as background activities.

Lipid analysis

Plasma was prepared from the whole blood samples by centrifugation at 1000 *g* for 10 min. Triacylglycerols were determined according to Technicon Method no. SA4-0324L90 (Technicon Instruments, Tarrytown, NY) and phospholipids were measured by the method of bioMérieux, Marcy-l'Etoile, France.

Lipoprotein isolation

Lipoprotein quantitation was performed by ultracentrifugation (20). Briefly, plasma samples were centrifuged at a density 1.006 g/ml for 18 h at 35000 rpm in a Centrikon T-2060 ultracentrifuge (Contron Roche, Zürich, Switzerland) equipped with a 45.6 Ti rotor. The tubes were sliced, and the floating fraction, VLDL, was isolated. The infranatant fractions obtained after the initial ultracentrifugation procedure were subjected to sequential ultracentrifugation to obtain LDL ($1.025 < d < 1.063$ g/ml) and HDL ($1.063 < d < 1.21$ g/ml) fractions.

Determination of fatty acid composition

Total lipids were extracted from lipoproteins, liver, and heart as described by Lie, Lied, and Lambertsen (21). The lipid fractions were evaporated, saponified, and nondecanoic acid (19:0) was added as internal standard and the fatty acids were esterified in 12% BF_3 in methanol. The methyl esters were separated using a Carlo Erba 2900 gas-chromatograph ("cold on column" injection, 49°C/min to 160°C, 1°C/min to 190°C, and 4°C/min to 220°C), equipped with a 50 m CP-sil 88 (Chrompack) fused silica capillary column (id: 0.32 mm). The relative fatty acid distribution (% w/w of total fatty acid) was calculated from the peak areas obtained using a Maxima 820 Chromatography Workstation software, installed in an IBM-AT, connected to the gas chromatograph, and identification was ascertained by standard mixtures of methyl esters.

RNA purification

Total RNA was isolated from liver slices (immediately frozen in liquid N_2 and stored in −80°C after dissection) using the single-step method described by Chomezynski and Sacchi (22). The RNA concentrations were determined by measuring the UV absorbance at 260 nm. The degree of RNA degradation was tested on gel-electrophoresis using 1% agarose mini gel.

Preparation of hybridization probes

The different DNA probes were ^{32}P-labeled by random priming using the oligo-labeling technique (23, 24). The resulting specific activities were approximately 1×10^9 cpm/μg.

Hybridization analysis

RNA was transferred to nylon membranes using a Schleicher & Schuell slot-blot aperture. Hybridization reactions were performed in the presence of 45% formamide, 5 × SSC, 0.1 mg/ml heat-denaturated herring sperm DNA, 0.3% SDS, 20 mM sodium phosphate, pH 6.5, 10.25% dextran-sulphate and 1 × Denhart's at 42°C. Membranes were washed to high stringency (65°C, 0.2 × SSC, 0.1% sodium pyrophosphate, and 0.1% SDS) 3 × 20 min and exposed on Kodak X-OMAT AR diagnostic films at −80°C in the presence of intensifying screens. When the filters were to be rehybridized, the bound probe was first stripped off by incubation of the filters in 0.1% SDS for 7 min at 95°C.

Analysis of mRNA

Slot-blot hybridization analysis was used to detect mRNA. Three different amounts of RNA were probed for mRNA specific for Δ^9-desaturase, mitochondrial 2,4-dienoyl-CoA reductase and 28S rRNA. The 28S rRNA probe was the 1.4 kb Bam III fragment of the pA plasmid originally obtained from Dr. I. L. Gonzales. Mitochondrial 2,4-dienoyl-CoA reductase probe was 1.5 kb Sac I insert in pJA118A and Δ^9-desaturase probe was 358 b Bgl II-AvaI fragment from pDs3. Data were analyzed using the LKB Ultrogel laserdensitometer (Bromma, Sweden) or the hybridized membranes were analyzed by Instant Imager, Electronic Autoradiography (Packard). The values are given as mean of three slot-blot analyses from each animal. mRNA expression is given relative to the amount of RNA applied.

Statistical analysis and presentation of results

The results are presented as means ± standard deviation (SD) from five animals in each group. Statistical differences between treatment groups were evaluated by Student's *t* test.

RESULTS

Table 1 shows the effect of chain length of 3-thia fatty acids on plasma lipid levels. Administration of C12-S- and C13-S-acetic acid to rats resulted in a reduction of plasma lipids. C13-S-acetic acid exerted the strongest effect resulting in a reduction of triacylglycerols and

plasma phospholipids by 50% and 54%, respectively. The decrease in plasma triacylglycerol levels by the hypolipidemic 3-thia fatty acids was accompanied by a corresponding reduction in VLDL-triacylglycerols (data not shown). The data in **Table 2** and **Table 3** show that in the VLDL and LDL fractions 3-thia fatty acids with chain length from C12-S-acetic acid to C14-S-acetic acid could be detected. No 3-thia fatty acids could be observed in the HDL fraction (**Table 4**). Administration of these 3-thia fatty acids to rats significantly increased the amount of monoenes and especially oleic acid (18:1n–9) in the VLDL and LDL fractions compared to controls. Total (Σ) polyunsaturated fatty acids, eicosapentaenoic acid (20:5n–3), and docosahexaenoic acid (22:6n–3) were significantly decreased in the two lipoprotein fractions after administration of C13-S- and C14-S-acetic acid, thereby decreasing the ratio between n–3 and n–6 fatty acids compared to untreated rats (Tables 2 and 3). C12-S-acetic acid to C14-S-acetic acid increased the amount of dihomo-γ-linolenic acid (20:3n–6) and reduced the amount of arachidonic acid (20:4n–6) in both LDL and HDL fractions.

Hepatic triacylglycerol and phospholipid levels after administration were unchanged whereas an increased amount of phospholipids in the heart resulted (**Table 5**). The hepatic amount of 18:1n–9 was increased after animals were fed the hypolipidemic 3-thia fatty acids (**Fig. 1A**), but no alteration was observed with shorter or longer chain-length (C8-S-, C11-S-, C15-S, and C16-S-acetic acid) 3-thia fatty acids. The amount of linoleic acid (18:2n–6) in the liver, however, was reduced after administration of C14-S-acetic acid (Fig. 1B). In spite of detectable amounts of 3-thia fatty acids in both liver (except C8-S, C11-S-, and C16-S-acetic acid) and heart (except C11-S- and C16-S-acetic acid) and a tendency to a higher amount of C14-S-acetic acid in the heart than in the liver (Fig. 1C), no changes in 18:1n–9 and 18:2n–6 levels were observed in heart (Fig. 1A, B) after administration compared to untreated rats.

Increased hepatic Δ^9-desaturase mRNA levels were observed in C13-S- and C14-S-acetic acid-treated rats (**Fig. 2A, B**). **Figure 3** shows a positive correlation (r^2 = 0.80; $P < 0.0005$) between the hepatic level of 18:1n–9 and Δ^9-desaturase gene expression after 3-thia fatty acid treatment. In contrast, no Δ^9-desaturase mRNA expression could be detected in the heart (Fig. 2A, B).

The hepatic content of polyunsaturated fatty acids decreased after supplementation with hypolipidemic 3-thia fatty acids (data not shown), especially 20:5n–3, which was lowered with all 3-thia fatty acids (**Fig. 4A**). Hepatic C22:5n–3 was lowered after C14-S-acetic acid and C15-S-acetic acid treatments (Fig. 4B). In addition, 3-thia fatty acids with chain-lengths greater than C12 decreased the amount of C22:6n–3 (Fig. 4C).

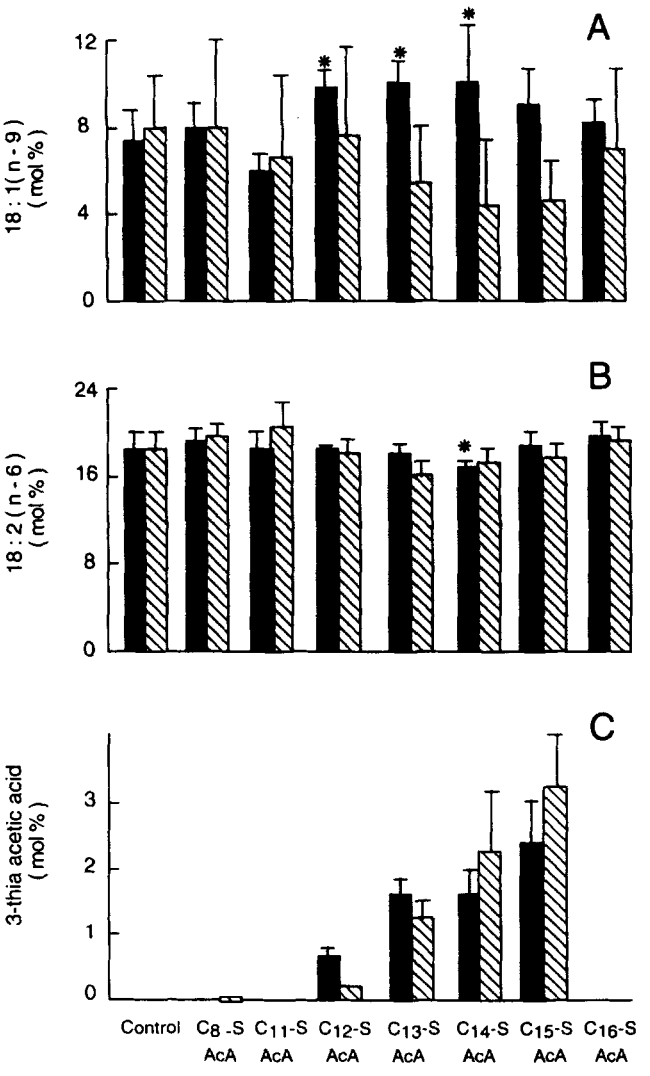

Fig. 1. Effect of 3-thia fatty acid treatment on the amount (mol %) of oleic acid (A), linoleic acid (B), and 3-thia fatty acids (C) in total homogenates of rat liver and heart. Liver (solid bars) and heart (hatched bars). The fatty acid composition was determined as described under Experimental Procedures. Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

Table 6 and Fig. 2C, D show that C11-S- to C15-S-acetic acid stimulated the gene expression and the activity of mitochondrial 2,4-dienoyl-CoA reductase in liver, a key enzyme involved in oxidation of unsaturated fatty acids. A negative correlation (r^2 = 0.94; $P < 0.006$) was found between the hepatic content of 20:5n–3 (Fig. 4A) and mitochondrial 2,4-dienoyl-CoA reductase activity (Table 6) with the most potent 3-thia fatty acids (C13-S- and C14-S-acetic acid). C14-S-acetic acid feeding led to an increased cPLA2 activity in a dose–response manner (Table 6). In contrast to liver, we were unable to detect mitochondrial 2,4-dienoyl-CoA reductase activity in heart (data not shown) whereas the mitochondrial

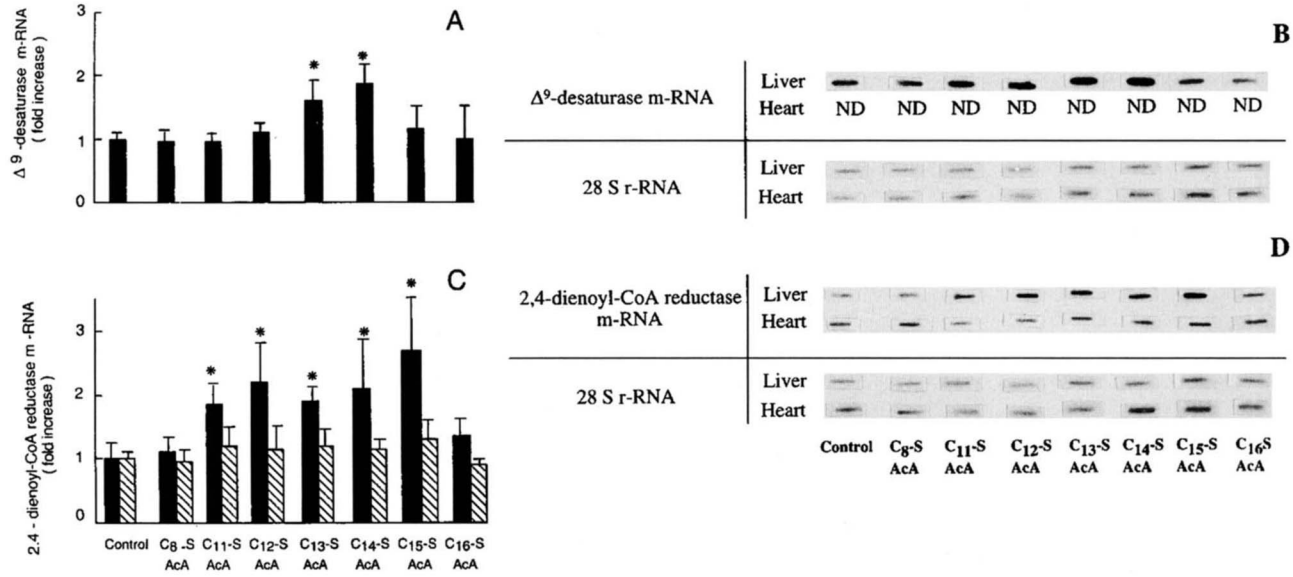

Fig. 2. Effect of 3-thia fatty acid treatment on the mRNA expression of Δ^9-desaturase (A,B) and mitochondrial 2,4-dienoyl-CoA reductase (C,D) in rat liver and heart. Liver (solid bars) and heart (hatched bars). The relative mRNA levels (panels A and C) were isolated and determined by densiometric scanning of the autoradiograms (see Experimental Procedures). The expression of mRNA was normalized to the corresponding 28S rRNA levels (i.e., the ratio of mRNA:28S rRNA); mean of control is set to 1.0. One representative slot-blot of a total of 12 is shown for Δ^9-desaturase (panel B) and mitochondrial 2,4-dienoyl-CoA reductase (panel D). Note, we were unable to detect Δ^9-desaturase mRNA expression in the heart (panels A and B). Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

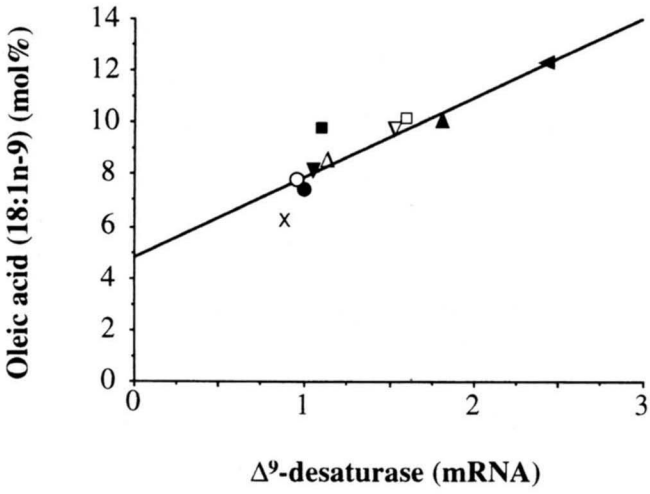

Fig. 3. Effect of 3-thia fatty acid treatment on the mRNA expression of Δ^9-desaturase and the amount (mol %) of oleic acid in total liver homogenates. All doses represent 150 mg/d per kg body weight unless otherwise is indicated. Control, (●); C8-S-acetate, (○); C11-S-acetate, (x); C12-S-acetate, (■); C13-S-acetate, (□); C13-S-acetate (300 mg/d per kg body weight) (▽); C14-S-acetate, (▲); C14-S-acetate (300 mg/d per kg body weight) (◀); C15-S-acetate, (△); and C16-S-acetate, (▼). Results represent means from five animals in each group ($r^2 = 0.80$).

2,4-dienoyl-CoA mRNA expression in heart remained unchanged by 3-thia fatty acid administration (Fig. 2C, D).

The data in Fig. 4D shows that the hepatic amount of 20:3n–6 was increased with chain length from C12-S-acetic acid to C15-S-acetic acid. The hepatic amount of Σ n–3 fatty acids was decreased in all 3-thia fatty acid-treated rats except for those treated with C8-S-acetic acid and C11-S-acetic acid (**Fig. 5A**). No changes of the Σ n–6 fatty acids resulted (Fig. 5B), but a decreased ratio between n–3 and n–6 fatty acids was observed in C13-S- and C14-S-acetic acid-treated rats compared to controls (Fig. 5C).

In cultured rat hepatocytes the oxidation of radiolabeled 18:1n–9, 20:5n–3, and 22:6n–3 was determined in the presence of L-carnitine or L-aminocarnitine, the latter being a potent inhibitor of carnitine palmitoyltransferase II (CPT II). **Figure 6** shows that 20:5n–3 was a better substrate for β-oxidation than 22:6n–3. Moreover, in the presence of L-aminocarnitine, oxidation of 18:1n–9 and 20:5n–3 was decreased when compared to cells grown in the presence of L-carnitine. This effect, however, was not observed for 22:6n–3 (Fig. 6).

In contrast to the liver, the Σ n–3 fatty acids in the heart was increased after treatment with C13-S-acetic acid, C14-S-acetic acid, and C15-S-acetic acid (Fig. 5A), resulting in an increased ratio between n–3 and n–6 fatty acids (Fig. 5C). The increase in n–3 fatty acids was

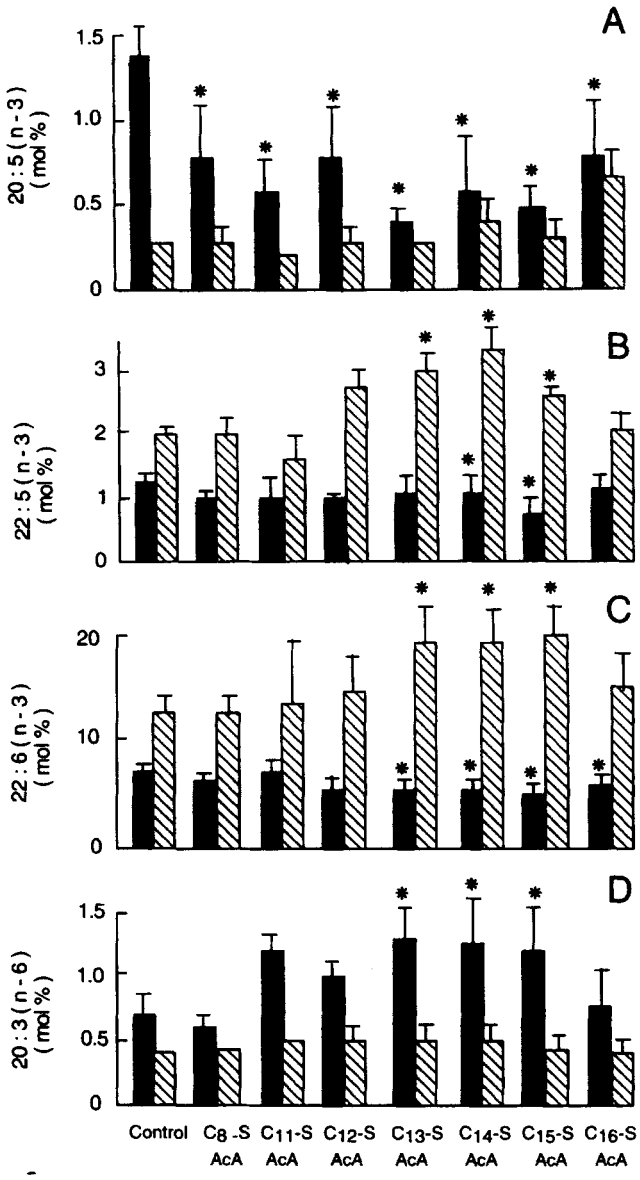

Fig. 4. Effect of 3-thia fatty acid treatment on the amount (mol %) of eicosapentaenoic acid (A), docosapentaenoic acid (B), docosahexaenoic acid (C), and dihomo-γ-linolenic acid (D) in total homogenates of rat liver and heart. Liver (solid bars) and heart (hatched bars). The fatty acid composition was determined as described under Experimental Procedures. Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

attributed to increased amounts of 20:5n–3, docosapentaenoic acid (22:5n–3), and 22:6n–3 (Fig. 4A-C).

In the case of saturated 3-thia fatty acids, **Fig. 7** shows that C13-S- and C15-S-acetic acids mainly replace homologues even-numbered fatty acids when incorporated into hepatic (Fig. 7A) and cardiac (Fig. 7B) lipids. Thus, in the case of palmitate (C16:0) only C13-S-acetic acid reduced the amount of C16:0 in the liver and heart whereas only C15-S-acetic acid reduced the amount of stearate in these tissues.

Mitochondrial β-oxidation, with both palmitoyl-CoA and palmitoyl-L-carnitine as substrates, was stimulated after 3-thia fatty acid treatment, except for C8-S-acetic acid. Maximal stimulation was observed with the hypolipidemic 3-thia fatty acids (**Table 7**).

Peroxisomal β-oxidation (measured as fatty acyl-CoA oxidase activity) and the fatty acyl-CoA oxidase gene expression were increased in the liver whereas in heart an increased activity and a tendency to decreased expression resulted after treatment with 3-thia fatty acids (**Fig. 8**). It is worth noting, however, that the plasma triacylglycerols (Table 1) correlated ($r^2 = 0.88$; $P < 0.02$) with the mitochondrial 2,4-dienoyl-CoA reductase activity in the liver (Table 6). Fatty acyl-CoA oxidase, Δ^9-desaturase, and mitochondrial 2,4-dienoyl-CoA reductase mRNA levels were determined from the same total RNA samples. **Figure 9** shows a representative Northern blot for mitochondrial 2,4-dienoyl-CoA reductase.

DISCUSSION

The hepatic content of polyunsaturated n–3 fatty acids decreased, especially eicosapentaenoic acid (20:5n–3), resulting in a lowered n–3/n–6 ratio after administration of the hypolipidemic 3-thia fatty acids. In heart, however, the amount of n–3 polyunsaturated fatty acids increased, particularly 20:5n–3 and docosahexaenoic acid (22:6n–3). This indicates that different factors influence the fatty acid composition in liver and heart in rats treated with hypolipidemic 3-thia fatty acids. 3-Thia fatty acids induce proliferation of mitochondria and peroxisomes in the liver (4, 13, 25). Under these conditions there was an increased rate of delivery of nonesterified fatty acids to the liver and a concomitant reduction of free fatty acids in the plasma (14). As 3-thia fatty acids did not increase the incorporation of 20:5n–3 into VLDL, it is unlikely that there is a selective increased secretion of 20:5n–3 from the liver to plasma and then to the heart. Conversion of 20:5n–3 to 22:5n–3 cannot explain the decreased content of 20:5n–3 as the hepatic docosapentaenoic acid (22:5n–3) content also decreased. These findings suggest that the decreased amount of hepatic n–3 fatty acids results from a selectively increased β-oxidation of 20:5n–3. The question arises as to the identity of the organelles, i.e., peroxisomes and/or mitochondria are involved in oxidation of 20:5n–3. It has been emphasized elsewhere that fatty acids of <C20 are oxidized by mitochondria (26) whereas a very-long-chain fatty acyl-CoA synthetase has been detected in peroxisomes and this

TABLE 6. Expression and activity of mitochondrial 2,4-dienoyl-CoA reductase and cytosolic phospholipase A2 (cPLA2) in liver of rats fed 3-thia fatty acids with different chain-lengths and palmitic acid (control) for 7 days

Treatment	Dose	2,4-Dienoyl-CoA Reductase		cPLA2
		mRNA	nmol/min/mg protein	
	mg/kg/d			%
Control	150	1.00 ± 0.17	1.0 ± 0.2	100 ± 17
C8-S-acetic acid	150	1.09 ± 0.16	ND	ND
C11-S-acetic acid	150	1.82 ± 0.33[a]	ND	ND
C12-S-acetic acid	150	2.18 ± 0.59[a]	ND	ND
C13-S-acetic acid	150	1.97 ± 0.17[a]	2.2 ± 0.5[a]	ND
	300	1.78 ± 0.83[a]	2.4 ± 0.2[a]	ND
C14-S-acetic acid	150	2.10 ± 0.67[a]	1.8 ± 0.3[a]	151 ± 25[a]
	300	2.73 ± 0.72[a]	2.6 ± 0.2[a]	211 ± 33[a]
C15-S-acetic acid	150	2.71 ± 0.88[a]	ND	ND
C16-S-acetic acid	150	1.33 ± 0.29	ND	ND

Results are expressed as means ± SD of 5 animals in each group. ND, not determined. The relative mRNA levels were determined by densiometric scanning of the autoradiograms (see Materials and Methods). The expression of mRNA was normalized to the corresponding 28S rRNA levels (i.e., the ratio of mRNA:28S rRNA), means of control are set to 1.00.

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

enzyme is most active towards very long-chain fatty acids (>C20) and is not present in mitochondria (27, 28). Indeed, in cultured rat hepatocytes incubated for 4 h, 20:5n–3 was oxidized almost 10 times the rate of 22:6n–3. Moreover, in contrast to 22:6n–3, the oxidation of 20:5n–3 and 18:1n–9 was L-aminocarnitine-sensitive (Fig. 6). This is also consistent with the report that pure 20:5n–3, and not 22:6n–3, increases the mitochondrial fatty acid oxidation and decreases plasma triacylglycerol levels (29–32). Moreover, it is well known that 2,4-dienoyl-CoA reductase is necessary to oxidize polyunsaturated fatty acids (33) and this enzyme is located in both mitochondria and peroxisomes (34, 35).

Hypolipidemic 3-thia fatty acids induced the mitochondrial 2,4-dienoyl-CoA reductase gene expression and activity in liver. This increase in mitochondrial 2,4-dienoyl-CoA reductase mRNA levels showed a positive correlation ($r^2 = 0.84$; $P < 0.01$) with the mitochondrial β-oxidation. In addition, there was a negative correlation ($r^2 = -0.67$; $P < 0.02$) between the mitochondrial 2,4-dienoyl-CoA reductase activity and plasma triacylglycerol levels with these 3-thia fatty acids.

In order to β-oxidize 20:5n–3 and 22:6n–3, these fatty acids must be available within the cell, and unsaturated fatty acids are mostly esterified in the *sn*-2 position of glycerophospholipids. Cytosolic phospholipase A2 (cPLA2) activity was determined in livers from C14-S-acetic acid-fed animals. The activity of this enzyme, which preferentially hydrolyzes fatty acids esterified at the *sn*-2 position of phospholipids in the cell membrane, increased in a dose–response manner (Table 6). Evidently this effect renders polyunsaturated fatty acids available for further metabolism, i.e., β-oxidation. Altogether, 3-thia fatty acids that cannot be β-oxidized themselves activate cPLA2 and seem to force 20:5n–3 to mitochondria, resulting in up-regulated mitochondrial 2,4-dienoyl-CoA reductase gene expression and activity concomitant to increased mitochondrial fatty acid oxidation capacity. According to this hypothesis one would expect that the amount of 20:5n–3 would not be decreased if there was no stimulated fatty acid oxidation. Indeed, in the heart, no changes in mitochondrial 2,4-dienoyl-CoA reductase gene expression (Fig. 2B) and mitochondrial fatty acid oxidation occurred (data not shown) resulting in an increased n–3/n–6 ratio. It is unlikely that the increase in n–3 fatty acids in the heart can be explained by an increased uptake from circulating lipoproteins as the n–3 content of both VLDL and LDL was significantly decreased. Moreover, the amount of VLDL-triacylglycerols was decreased (data not shown) and 3-thia fatty acid treatment is reported to decrease the VLDL secretion (36). In the heart as in liver, 3-thia fatty acids replace endogenous fatty acids with identical chain-lengths (Fig. 7), and in the heart the amount of phospholipids was increased (Table 1). It is therefore possible that the increased level of n–3 fatty acids in the heart, particularly 22:6n–3, might be diverted towards phospholipid synthesis and not β-oxidation. 22:6n–3 is preferentially incorporated into phospholipids and whether a diversion of 22:6n–3 to phospholipid synthesis will increase the rate of transfer of 22:6n–3 from the serum compartment into the heart should be considered. Moreover, we have previously reported that the amount of 22:6n–3 in adipose tissue was lowered concomitant with a reduction in circulating free fatty acids after C14-S-acetic acid treatment (14).

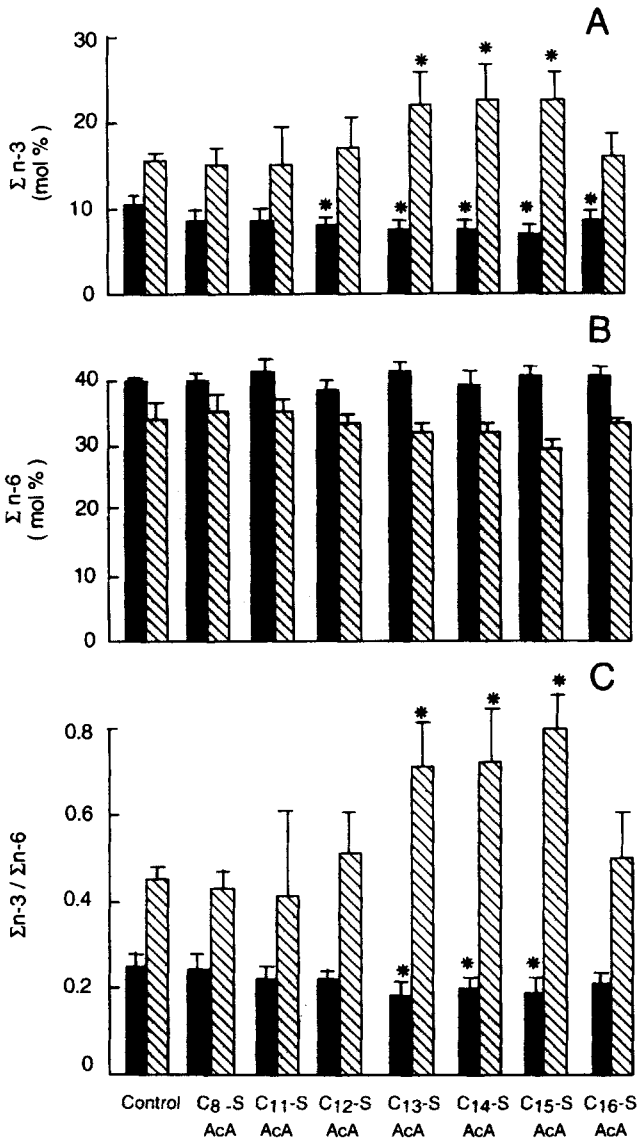

Fig. 5. Effect of 3-thia fatty acid treatment on the amount (mol %) of n–3 fatty acid (A), n–6 fatty acids (B), and the n–3/n–6 ratio (C) in total homogenates of rat liver and heart. Liver (solid bars) and heart (hatched bars). The fatty acid composition was determined as described under Experimental Procedures. Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

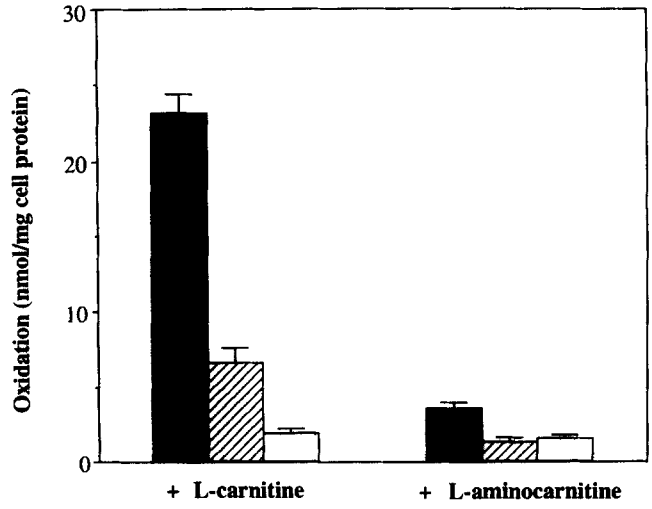

Fig. 6. Oxidation of [1-^{14}C]oleic acid (OA), [1-^{14}C]eicosapentaenoic acid (EPA), and [1-^{14}C]docosahexaenoic acid (DHA) by cultured rat hepatocytes. EPA (solid bars), OA (hatched bars), and DHA (open bars). The cells were plated and incubated in media containing L-carnitine (0.5 mM) or L-aminocarnitine (50 μM). After overnight incubation, media were removed and new medium containing L-carnitine or L-aminocarnitine and labeled fatty acids (100 μM, 0.25 μCi/ml) were added and the cells were incubated for 4 h. Acid-soluble activity was determined as described under Experimental Procedures. Data are expressed as mean of two separate experiments.

Thus, an uptake of circulating polyunsaturated n–3 fatty acids released by adipose tissue could attribute to the increased levels of 20:5n–3 and 22:6n–3 in the heart. The decreased amount of hepatic 22:6n–3 may be partly due to an induced peroxisomal fatty acid oxidation as the fatty acyl-CoA oxidase gene expression and activity increased (Fig. 8). We have recently obtained evidence that 22:6n–3, and not 20:5n–3, is a peroxisome proliferator (37).

The hypolipidemic 3-thia fatty acids increased the amount of 18:1n–9 in the liver and the 18:1n–9 level correlated to the Δ^9-desaturase gene expression. The accumulation of 18:1n–9 after 3-thia fatty acid treatment is in agreement with a previous finding (14) and we have been recently reported that 3-thia fatty acids upregulate hepatic Δ^9-desaturase gene expression and activity in a time- and dose-dependent manner (15). Thus, an increased hepatic content of 18:1n–9 will lead to an accumulation in both the liver and in the plasma lipoproteins. This was particularly evident in the LDL particles (Table 3). This is an interesting finding as a decreased amount of polyenes and increased amount of monoenes will render the circulating LDL particles less susceptible to oxidative modifications. Thus, the hypolipidemic 3-thia fatty acids may induce an in vivo antioxidant effect resulting in a less atherogenic plasma lipoprotein profile.

In conclusion, increased mitochondrial fatty acid oxidation of 20:5n–3 with a concomitant induction of Δ^9-desaturase gene expression seem to regulate the hepatic and lipoprotein fatty acid compositions in rats fed 3-thia fatty acid. In contrast, none of these effects were found in the heart and the increased n–3 fatty acid content may be a result of an uptake of circulating n–3 fatty acids released by adipose tissue and partially dependent on displacement of endogenous fatty acids by 3-thia fatty acids.

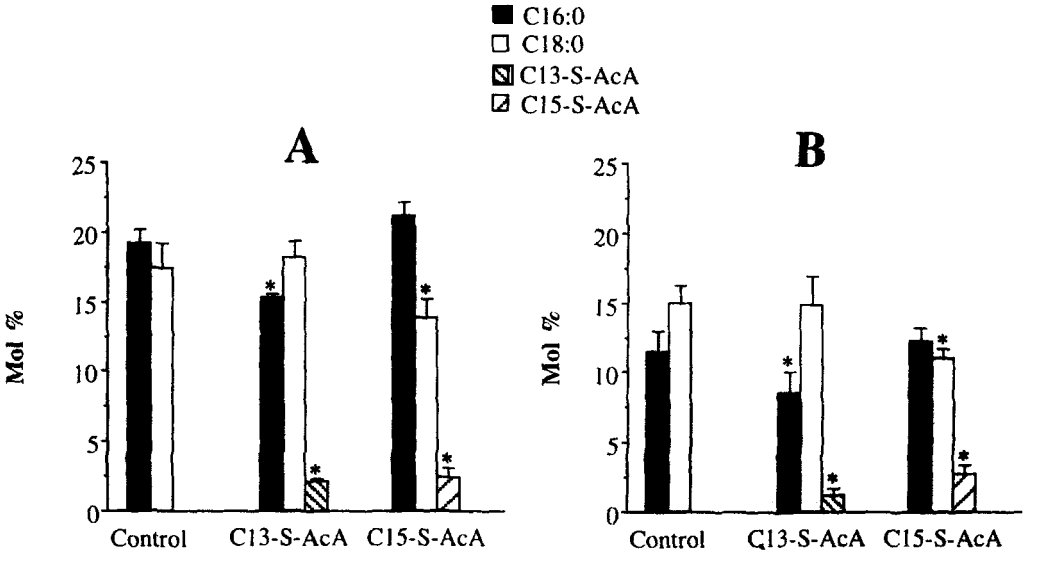

Fig. 7. Incorporation of 3-thia fatty acids affects the amount (mol %) of their homologues fatty acids in the liver (A) and heart (B). Liver (solid bars) and heart (hatched bars). The fatty acid composition was determined as described under Experimental Procedures. Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

TABLE 7. Effects of 3-thia fatty acids with different chain-length and palmitic acid (control) on hepatic fatty acid oxidation capacity with palmitoyl-L-carnitine and palmitoyl-CoA as substrates after 7 days of treatment

	Liver	
Treatment	Palmitoyl-L-Carnitine	Palmitoyl-CoA
	% of control	
C8-S-acetic acid	78	86
C11-S-acetic acid	179[a]	112
C12-S-acetic acid	223[a]	141[a]
C13-S-acetic acid	238[a]	145[a]
C14-S-acetic acid	305[a]	263[a]
C15-S-acetic acid	175[a]	99
C16-S-acetic acid	ND	ND

The values represent means of 5 animals in each group. ND, not determined.

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

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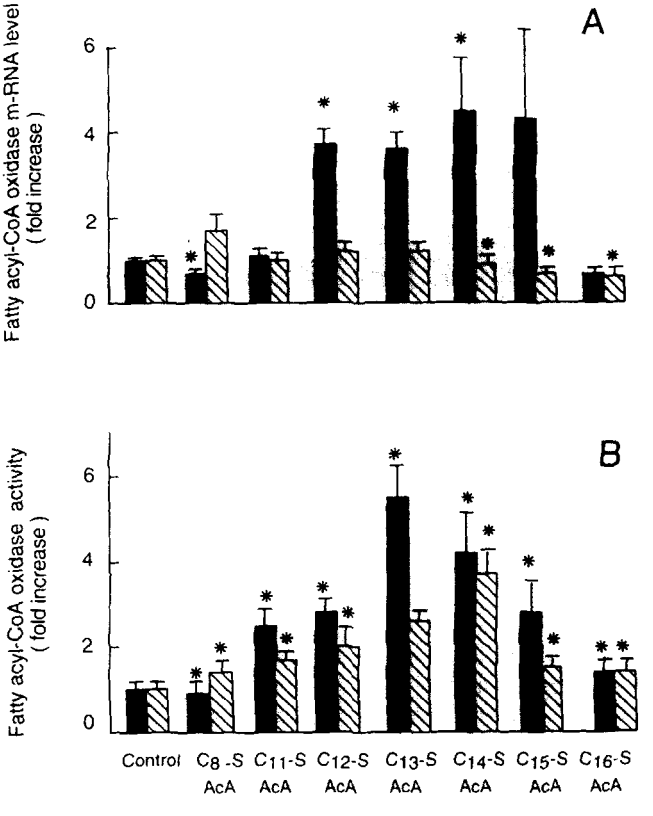

Fig. 8. Effect of 3-thia fatty acids on the mRNA expression (A) and activity of fatty acyl-CoA oxidase (B) in rat liver and heart. Liver (solid bars) and heart (hatched bars). The fatty acid composition was determined as described under Experimental Procedures. Results are expressed as means ± SD of five animals (*, $P < 0.05$ compared to controls).

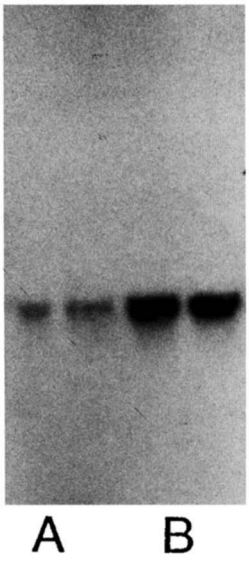

Fig. 9. Northern blots of mitochondrial 2,4-dienoyl-CoA reductase from controls (A) and C14-S-acetic acid-treated animals (B). Ten μg of total RNA was subjected to electrophoresis, transferred to nylon membrane, and hybridized to rat mitochondrial 2,4-dienoyl-CoA reductase cDNA.

REFERENCES

1. 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.
2. Asiedu, D., A. Aarsland, J. Skorve, A. M. Svardal, and R. K. Berge. 1990. Fatty acid metabolism in livers of rats treated with hypolipidemic sulphur-substituted fatty acid analogues. *Biochim. Biophys. Acta.* **1044:** 211–221.
3. Asiedu, D., J. Skorve, A. Demoz, N. Willumsen, and R. K. Berge. 1992. Relationship between translocation of long-chain acyl-CoA hydrolase, phosphatidate phosphohydrolase and CTP:phosphocholine cytidyltransferase and the synthesis of triglycerides and phosphatidylcholine in rat liver. *Lipids.* **27:** 241–247.
4. Berge, R. K., A. Aarsland, H. Kryvi, N. Aarsaether, and J. Bremer. 1989. Alkylthioacetic acid (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.
5. 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.
6. Gibbons, G. F. 1990. Assembly and secretion of hepatic very low density lipoprotein. *Biochem J.* **268:** 1–13.
7. Borén, J., M. Wettesten, S. Rustaeus, M. Andersson, and S. O. Olofsson. 1993. The assembly and secretion of apoB-100-containing lipoproteins. *Biochem. Soc. Trans.* **21:** 487–493.
8. Berge, R. K., and E. Hvattum. 1994. Impact of cytochrome P450 system on lipoprotein metabolism. Effect of abnormal fatty acids (3-thia fatty acids). *Pharmacol. Ther.* **61:** 345–383.
9. Hvattum, E., H. J. Grav, and J. Bremer. 1993. Hormonal and substrate regulation of 3-thia fatty acid metabolism in Morris 7800 C1 hepatoma cells. *Biochem. J.* **294:** 917–921.
10. Grav, H. J., D. K. Asiedu, and R. K. Berge. 1994. Gas chromatographic measurement of 3- and 4-thia fatty acids incorporated into various classes of rat liver lipids during feeding experiments. *J. Chromatogr. B.* **658:** 1–10.
11. Asiedu, D. K., A. Al-Shurbaji, A. C. Rustan, I. Björkhem, L. Berglund, and R. K. Berge. 1995. Hepatic fatty acid metabolism as a determinant of plasma and liver triacylglycerol levels. Studies on tetradecylthioacetic and tetradecylthiopropionic acids. *Eur. J. Biochem.* **227:** 715–722.
12. Al-Shurbaji, A., J. Skorve, R. K. Berge, M. Rudling, I. Björkhem, and L. Berglund. 1993. Effect of 3-thiadicarboxylic acid on lipid metabolism in experimental nephrosis. *Arterioscler. Thromb.* **13:** 1580–1586.
13. Frøyland, L., D. K. Asiedu, H. Vaagenes, A. Garras, Ø. Lie, G. K. Totland, and R. K. Berge. 1995. Tetradecylthioacetic acid incorporated into very low density lipoprotein: changes in the fatty acid composition and reduced plasma lipids in cholesterol-fed hamsters. *J. Lipid Res.* **36:** 2529–2540.
14. Asiedu, D. K., L. Frøyland, H. Vaagenes, Ø. Lie, A. Demoz, and R. K. Berge. 1996. Long-term effect of tetradecylthioacetic acid: a study on plasma lipid profile and fatty acid composition and oxidation in different rat organs. *Biochim. Biophys. Acta.* **1300:** 86–96.
15. Madsen, L., L. Frøyland, H. J. Grav, and R. K. Berge. 1997. 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. *J. Lipid Res.* **38:** 554–563.
16. Kunau, W. H., and P. Dommes. 1978. Degradation of unsaturated fatty acids. Identification of intermediates in the degradation of *cis*-4-decenoyl-CoA by extracts of beef-liver mitochondria. *Eur. J. Biochem.* **91:** 533–544.
17. Wijkander, J., and R. Sundler. 1991. An 100-kDa arachidonate-mobilizing phospholipase A2 in mouse spleen and the macrophage cell line J774. Purification, substrate interaction and phosphorylation by protein kinase C. *Eur. J. Biochem.* **202:** 873–880.
18. Small, G. M., K. Burdett, and M. J. Connock. 1985. A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase. *Biochem. J.* **227:** 205–210.
19. Seglen, P. 1973. Preparation of rat liver cells. 3. Enzymatic requirements for tissue dispersion. *Exp. Cell. Res.* **82:** 391–398.
20. Lopes-Virella, M. F., P. Stone, S. Ellis, and J. A. Colwell. 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* **23:** 882–884.
21. Lie, Ø., E. Lied, and G. Lambertsen. 1987. Lipid digestion in cod. *Comp. Biochem. Physiol.* **88B:** 697–700.
22. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162:** 156–159.
23. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.
24. Feinberg, A. P., and B. Vogelstein. 1984. A technique for

radiolabeling DNA restriction endonuclease fragments to high specific activity. Addendum. *Anal. Biochem.* **137:** 266–267.
25. Frøyland, L., K. Helland, G. K. Totland, H. Kryvi, and R. K. Berge. 1996. A hypolipidemic peroxisome proliferating fatty acid induces polydispersity of rat liver mitochondria. *Biol. Cell.* **87:** 105–112.
26. Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. De Schepper. 1979. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate treated rats. *J. Biol. Chem.* **254:** 4585–4595.
27. Singh, H., and A. Poulos. 1988. Distinct long chain and very long chain fatty acyl CoA synthetases in rat liver peroxisomes and microsomes. *Arch. Biochem. Biophys.* **266:** 486–495.
28. Lazo, O., M. Contreras, Y. Yoshida, A. K. Singh, W. Stanley, M. Weise, and Y. Singh. 1990. Cellular oxidation of lignoceric acid is regulated by the subcellular localization of lignoceroyl-CoA ligases. *J. Lipid Res.* **31:** 583–595.
29. Aarsland, A., M. Lundquist, B. Børretsen, and R. K. Berge. 1990. On the effect of peroxisomal β-oxidation and carnitine palmitoyltransferase activity by eicosapentaenoic acid in liver and heart from rats. *Lipids.* **25:** 546–548.
30. Willumsen, N., S. Hexeberg, J. Skorve, M. Lundquist, and R. K. Berge. 1993. The hypotriglyceridemic effect of eicosapentaenoic acid is reflected in increased mitochondrial fatty acid oxidation and diminished lipogenesis. *Lipids.* **28:** 683–690.
31. Rambjør, G. S., A. I. Wålen, S. L. Windsor, and W. S. Harris. 1996. Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oils in humans. *Lipids.* **31:** S-45–S-49.
32. Frøyland, L., H. Vaagenes, D. K. Asiedu, A. Garras, Ø. Lie, and R. K. Berge. 1996. Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholesterol and changed the fatty acid composition in rat blood and organs. *Lipids.* **31:** 169–178.
33. Schulz, H., and W-H. Kunau. 1987. Beta oxidation of unsaturated fatty acids: a revised pathway. *Trends. Biochem. Sci.* **12:** 403–406.
34. Hiltunen, J. K., H. Osmundsen, and J. Bremer. 1983. Beta-oxidation of polyunsaturated fatty acids having double bonds at even-numbered positions in isolated rat liver mitochondria. *Biochim. Biophys. Acta.* **752:** 223–232.
35. Hiltunen, J. K., T. Karki, I. E. Hassinen, and H. Osmundsen. 1986. Beta-oxidation of polyunsaturated fatty acids by rat liver peroxisomes. A role for 2,4-dienoyl-coenzyme A reductase in peroxisomal beta-oxidation. *J. Biol. Chem.* **261:** 16484–16493.
36. Skorve, J., A. Al-Shurbaji, D. Asiedu, I. Björkhem, L. Berglund, and R. K. Berge. 1993. On the mechanism of the hypolipidemic effect of sulfur-substituted hexadecanedioic acid (3-thiadicarboxylic acid) in normolipidemic rats. *J. Lipid Res.* **34:** 1177–1185.
37. Frøyland, L., L. Madsen, H. Vaagenes, G. K. Totland, J. Auwerx, H. Kryvi, B. Staels, and R. K. Berge. 1997. Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J. Lipid Res.* **38:** In press.