Stimulation of fatty acid oxidation by a 3-thia fatty acid reduces triacylglycerol secretion in cultured rat hepatocytes

Steinar Skrede,[1] Jon Bremer, Rolf K. Berge,* and Arild C. Rustan†

Institute of Medical Biochemistry, University of Oslo; Laboratory of Clinical Biochemistry,* University of Bergen; and Department of Pharmacology,† Institute of Pharmacy, University of Oslo, Norway

Abstract The present work shows that when mitochondrial β-oxidation is stimulated by the hypolipemic, non-β-oxidizable fatty acid analogue tetradecylthioacetic acid, there is a decrease in the secretion of triacylglycerol in cultured rat hepatocytes. In order to study the effects of tetradecylthioacetic acid in cells with different fatty acid oxidation rates, cells were grown without or with L-carnitine supplement or with addition of the β-oxidation inhibitor L-aminocarnitine. In cells grown without and with L-carnitine in the medium, the oxidation of [1-^{14}C]oleic acid was stimulated by tetradecylthioacetic acid, whereas it was not significantly changed by palmitic acid. In cells grown with L-aminocarnitine, oxidation of [1-^{14}C]oleic acid was almost abolished both in the absence and in presence of tetradecylthioacetic acid. The effect of tetradecylthioacetic acid and palmitic acid on incorporation of [1-^{14}C]oleic acid into triacylglycerol was similar under all conditions. In the presence of L-carnitine, secretion of oleic acid-labeled triacylglycerol was reduced significantly more by tetradecylthioacetic acid than by palmitic acid. The effects of tetradecylthioacetic acid and palmitic acid on secretion of oleic acid-labeled triacylglycerol were reversed in cells grown with L-aminocarnitine, where palmitic acid was the stronger inhibitor. These results were substantiated by determination of mass of triacylglycerol secreted. It is concluded that tetradecylthioacetic acid reduces secretion of triacylglycerol from rat hepatocytes mainly by acutely stimulating fatty acid oxidation.—**Skrede, S., J. Bremer, R. K. Berge, and A. C. Rustan.** Stimulation of fatty acid oxidation by a 3-thia fatty acid reduces triacylglycerol secretion in cultured rat hepatocytes. *J. Lipid Res.* 1994. **35:** 1395–1404.

Supplementary key words L-aminocarnitine • L-carnitine palmitoyltransferase • tetradecylthioacetic acid • very low density lipoprotein

Tetradecylthioacetic acid (TTA) is a fatty acid analogue in which a sulfur atom replaces the β-methylene group in the alkyl chain (a 3-thia fatty acid). TTA therefore cannot be β-oxidized (1). When fed to rats, TTA induces peroxisomal proliferation and activity of peroxisomal β-oxidation enzymes, total carnitine palmitoyltransferase (CPT), and several other enzymes involved in hepatic lipid metabolism (2, 3). The hypolipemia in rats given 3-thia fatty acids is well documented (2–4). After prolonged feeding the lipid-lowering effect of TTA is, at least in part, explained by increased mitochondrial and peroxisomal fatty acid oxidation along with reduced lipogenesis (5). In rats fed TTA the hypolipemia precedes the increase in peroxisomal fatty acid oxidation, which therefore cannot explain the triacylglycerol-lowering effect (6). One day after administration of a single dose of TTA to rats there is a twofold increase in mitochondrial β-oxidation in isolated hepatocytes and, paradoxically, an increased level of hepatic malonyl-CoA (7).

In cultured rat hepatocytes TTA induces severalfold the mRNAs and activities of acyl-CoA synthetase (8) and the peroxisomal β-oxidation enzymes (9–12). Also, in isolated rat hepatocytes, oxidation of palmitic acid is immediately stimulated by TTA (13), whereas de novo fatty acid synthesis (13) and cholesterologenesis (14) are inhibited.

The present work was undertaken to study whether the TTA-stimulated fatty acid oxidation in rat hepatocytes will influence fatty acid incorporation into synthesized and secreted triacylglycerol. To see whether a possible effect of TTA on triacylglycerol secretion depended on TTA's potential to increase fatty acid oxidation, fatty acid metabolism was studied in rat hepatocytes cultured under different conditions: without L-carnitine supplement, with a supplement of L-carnitine, known to accentuate effects of clofibrate analogues on hepatocyte lipid metabolism (15), or with the CPT II inhibitor L-aminocarnitine (16).

Oleic acid was chosen because it stimulates triacylglycerol and apolipoprotein B secretion from primary rat hepatocyte cultures (17, 18), perfused rat liver (19), and rat hepatoma cells (20).

Abbreviations: BSA, bovine serum albumin; CPT, carnitine palmitoyltransferase; DMEM, Dulbecco's Modified Eagle's medium; GPAT, glycerophosphate acyltransferase; TTA, tetradecylthioacetic acid; TLC, thin-layer chromatography; VLDL, very low density lipoproteins.

[1]To whom correspondence should be addressed.

As TTA behaves in many ways as a normal fatty acid and represents a tool to look for primary fatty acid effects in metabolism (7), all studies compared the effects of TTA to palmitic acid. Palmitic acid was chosen to be control for TTA because they are metabolized in a similar fashion, except for different oxidation pathways (13, 21).

EXPERIMENTAL

Materials

Tetradecylthioacetic acid (unlabeled and [1-^{14}C]TTA) was synthesized as described by Spydevold and Bremer (9). [1-^{14}C]palmitic acid (58 mCi/mmol) and [1-^{14}C]oleic acid (52 mCi/mmol) were purchased from Amersham Int., Buckinghamshire, U.K. L-Aminocarnitine (L-3-amino-4-trimethyl-aminobutyrate) was a kind gift from Sigma-Tau S.p.A., Rome, Italy. Dulbecco's Modified Eagle's (DME) medium (cat. no 10-331-22) and gentamicin (10 mg/ml) were obtained from Flow Lab., Irvine, Ayrshire, U.K. The DME medium was supplemented with $NaHCO_3$ (24 mM) from Sigma Chemical Co., St. Louis, MO. Ultroser G (a serum substitute) was obtained from Gibco, Life Technologies Inc., Gaithersburg, MD. Tissue culture equipment was supplied from Costar, Cambridge, MA. Silicagel F 1500 thin-layer chromatography plates were obtained from Scleicher and Schuell, Dassel, Germany. Collagenase (type I), 4-(hydroxyethyl)1-piperazine-ethanesulfonic acid (HEPES), essentially fatty acid-free BSA, and L-carnitine were obtained from Sigma Chemical Co. Triacylglycerol was determined using a commercial enzymatic kit obtained from bioMerieux, Marcy-l'Etoile, France.

Animals

Male Wistar rats (Møllegaard Breeding Center, Ejby, Denmark) of approx. 250–300 g were maintained in cages at 23°C in rooms with lights on from 7 AM to 7 PM. All rats had free access to water and a standard, commercial low-fat diet.

Preparation of hepatocytes and culture conditions

Hepatocytes were prepared from rats given barbiturate anesthesia at 9 AM. Rat liver parenchymal cells were isolated as described by Berry and Friend (22) with modifications according to Seglen (23), suspended, and plated immediately. Hepatocytes were plated overnight in DME medium containing HEPES (20 mM), Ultroser G (2%), gentamicin (50 μg/ml) at a cell density of approx. 2×10^6 cells/dish (1–1.5 mg cell protein) in a volume of 2 ml. Cells used for measurement of secreted triacylglycerol were cultured under identical conditions in 75-cm^2 culture flasks (in 12 ml DME medium). Additions to cell cultures are stated in the legends to figures and tables. The cells were incubated at 37°C in air/CO_2 (19:1) for 18–20 h; medium was removed and incubations were performed as detailed below.

Incubation of cultured hepatocytes with radioactive fatty acids

Cultured hepatocytes were incubated for 4 h with different concentrations of [1-^{14}C]oleic acid (0.5 mCi/mmol), [1-^{14}C]palmitic acid (0.5 mCi/mmol), or [1-^{14}C]TTA (0.5 mCi/mmol) in the presence of BSA in a fatty acid–BSA ratio of 2.5:1, and supplements of L-carnitine (0.5 mM) or L-aminocarnitine (50 μM) as stated in legends to tables and figures. As there was a 0.5–1 h delay in secretion of labeled triacylglycerol followed by a linear increase over several hours, an incubation time of 4 h was chosen (data not shown).

Termination of incubations

Incubations were terminated by cooling the culture dishes on ice. Incubation medium (2 ml) was removed and the cells were scraped off the culture dish by a rubber policeman into 2 ml phosphate-buffered saline (PBS) and washed once with PBS (2 ml). The cells were centrifuged (600 g_{av}/5 min) and the supernatant was discarded. The cells were resuspended in distilled water and frozen at −20°C. Protein content of each culture dish was determined in an aliquot of the resuspended cells after sonication, using BSA as standard (24).

Determination of fatty acid oxidation

Medium (250 μl) was precipitated with 1.0 ml $HClO_4$ (0.75 M). To secure complete precipitation of free fatty acids and lipids, BSA (0.45%) was added. The ice-cold acidic extract was centrifuged (1800 g_{av}/10 min) and 500 μl of the supernatant was assayed for radioactivity by liquid scintillation counting. Control experiments revealed that CO_2 production and retained cellular acid-soluble radioactivity accounted for ca. 10% of total oxidation products. Acid-soluble radioactivity in media, therefore, was taken as measure of total oxidation.

Lipid extraction and quantitation

Cellular lipids were extracted according to Folch, Lees, and Sloane Stanley (25). The organic phase was evaporated under N_2 and the extracted lipids were dissolved in n-hexane, and separated by thin-layer chromatography on silica plates developed in hexane–diethyl ether–glacial acetic acid 80:20:1. The bands were visualized by iodine vapor, cut into pieces, and counted. The incubation medium was centrifuged (600 g_{av}, 5 min) and to an aliquot of cell-free medium (1.0 ml) was added distilled water (1.0 ml), calf serum as lipid carrier (20 μl), and approximately 10 mg NaCl (s) before the lipids were extracted with n-butanol (1.0 ml) according to Bjerve, Daae, and Bremer (26). Radioactivity in an aliquot of the n-butanol phase (100 μl) was used to determine total

[1-^{14}C]fatty acid-labeled lipids in the medium. The remaining n-butanol phase was evaporated and the lipids were separated by TLC as described for cellular lipids.

Mass measurement of secreted triacylglycerol

Cells grown overnight in the presence of either L-carnitine (0.5 mM) or L-aminocarnitine (50 μM) were incubated for 6 h with new medium containing fatty acids and/or TTA in the presence of L-carnitine (0.5 mM) or L-aminocarnitine. The medium from each culture dish was removed and centrifuged and duplicate samples of medium (4 ml) devoid of cells were extracted twice with chloroform–methanol 2:1 (20 ml). The chloroform phases were combined and evaporated. The lipids were then redissolved in n-hexane (1 ml), transferred to new tubes, and again evaporated. Triacylglycerol was redissolved in methanol (150 μl) and assayed as described in the triacylglycerol enzymatic kit. Standard solutions of triolein in methanol (150 μl) were used to calculate triacylglycerol content in the samples.

Presentation of data

Results for radioactivity are presented as means ± SD (in nmol/mg protein per 4 h) for values obtained in cells from three to seven rats. In each experiment duplicate or triplicate cultures were analyzed to obtain a mean value. Results for mass measurement of secreted triacylglycerol are presented as means ± SD (as % of the control; cells incubated with BSA alone) for values obtained in cells from five rats. In each experiment duplicate cultures were analyzed to obtain a mean value. The data were evaluated statistically with the Student's *t*-test (unpaired, two-tailed). Differences between groups were considered to be statistically different when P was ≤ 0.05.

RESULTS

Comparison of oleic acid, palmitic acid, and TTA metabolism

Table 1 shows that in rat hepatocytes cultured without L-carnitine supplement, metabolism (sum of incorporation into lipids and oxidation) of [1-^{14}C]TTA, [1-^{14}C]oleic acid, and [1-^{14}C]palmitic acid was quantitatively not very different. At the end of the incubations, approximately 50% of the fatty acids and TTA added to the cells was still available in the medium. TTA was incorporated into synthesized triacylglycerol at a rate about two-thirds that of the normal fatty acids, while its secretion in triacylglycerol into the incubation medium was about one-half of the normal fatty acids at 0.5 mM. The secretion of triacylglycerol was linear in a concentration range of 25–500 μM with oleic acid and palmitic acid (not all data shown). TTA was the best substrate for phospholipid formation, while oleic acid was the poorest. At a concentration of 0.5 mM, palmitic acid was oxidized at twice the rate of oleic acid and 4 times the rate of TTA. Oleic acid and palmitic acid were oxidized 3 times faster when the fatty acid concentration was increased from 0.25 to 0.5 mM, while the oxidation of TTA did not increase.

Effect of TTA on total metabolism of [1-^{14}C]oleic acid

Figure 1 shows that oleic acid metabolism in cells grown with L-carnitine (Fig. 1B) was significantly higher than in cells grown with L-aminocarnitine (Fig. 1C). There was no significant difference in the effect of TTA and palmitic acid on oleic acid metabolism under the three culture conditions. At the end of incubations the uptake of [1-^{14}C]oleic acid (0.4 μmol added) ranged from approximately 0.13 μmol (when oleic acid was given in com-

TABLE 1. Metabolism of oleic acid (OA), tetradecylthioacetic acid (TTA), and palmitic acid (PA) in rat hepatocytes cultured without L-carnitine

	[^{14}C]OA		[^{14}C]TTA		[^{14}C]PA	
	0.25 mM	0.5 mM	0.25 mM	0.5 mM	0.25 mM	0.5 mM
Metabolized	104.7 ± 9.1[a] (5)	226.5 ± 54.9 (5)	114.6 ± 12.3 (5)[b]	178.4 ± 35.1 (4)[b]	139.1 ± 12.3 (5)	255.4 ± 22.9 (4)
Triacylglycerol						
Synthesized	66.1 ± 9.7[c] (6)	157.5 ± 57.7 (7)	41.5 ± 13.2 (6)[b]	100.7 ± 45.3 (5)	68.6 ± 2.5 (6)	139.3 ± 15.4 (5)
Secreted	14.0 ± 3.5 (6)	28.7 ± 5.5[c] (7)	8.9 ± 2.8 (6)	9.7 ± 5.5 (5)[b]	13.4 ± 2.7 (6)	23.9 ± 4.7 (5)
Phospholipid	16.5 ± 4.5[a,c] (6)	26.7 ± 6.4[a,c] (7)	52.6 ± 13.7 (6)[b]	75.5 ± 23.6 (5)[b]	35.3 ± 5.8 (6)	49.1 ± 7.5 (5)
Diacylglycerol	4.7 ± 2.2 (6)	15.3 ± 4.6[a,c] (7)	3.1 ± 1.8 (6)	6.1 ± 3.2 (5)	4.5 ± 2.0 (6)	7.2 ± 4.0 (5)
Cholesteryl ester	0.7 ± 0.5 (6)	1.9 ± 0.9 (7)	1.0 ± 0.6 (6)	1.3 ± 0.9 (5)	0.8 ± 0.5 (6)	0.9 ± 0.5 (5)
Oxidized	6.4 ± 2.7 (5)	21.9 ± 6.2[a,c] (5)	10.0 ± 3.9 (5)	9.0 ± 6.1 (4)[b]	13.2 ± 7.2 (5)	41.3 ± 10.0 (4)

Cells were incubated for 4 h with fatty acids in BSA (ratio 2.5:1). Oxidation and lipids were measured as described in Experimental. Results in nmol/mg protein per 4 h are presented as means ± SD. Number of rats in parentheses.

[a]OA different from PA.

[b]TTA different from PA.

[c]OA different from TTA.

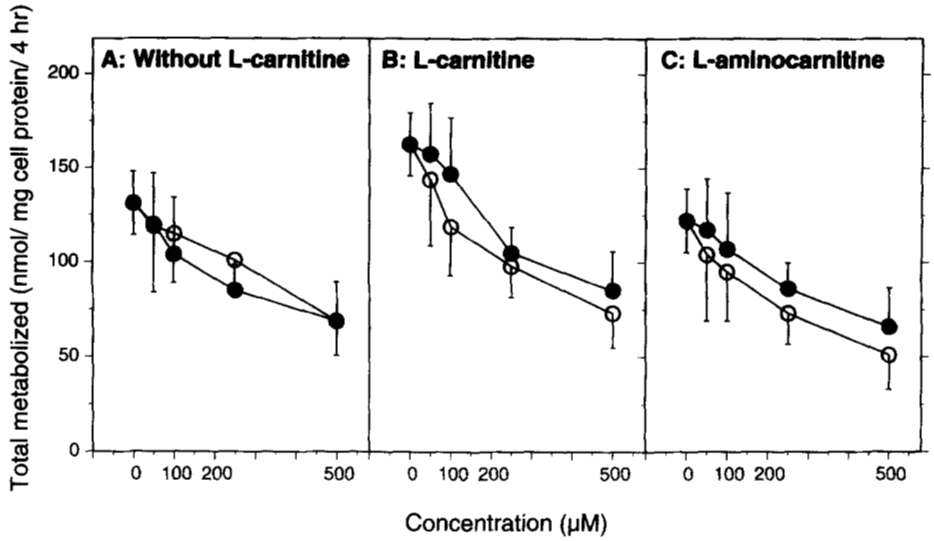

Fig. 1. Effect of tetradecylthioacetic acid (●) and palmitic acid (○) on total metabolized [1-^{14}C]oleic acid in hepatocytes plated and incubated in media; A, without addition of L-carnitine; B, supplemented with L-carnitine (0.5 mM) or; C, supplemented with L-aminocarnitine (50 μM). After ca. 20 h, plating media were removed and incubations of 4 h were performed with new media containing; A, no addition of L-carnitine; B, L-carnitine (0.5 mM) or; C, L-aminocarnitine (50 μM) and [1-^{14}C]oleic acid (0.2 mM, 0.5 mCi/mmol) without or with increasing concentrations of palmitic acid (○) or TTA (●). Ratio fatty acid/BSA was 2.5:1. Results are given as mean ± SD for values obtained in three to five independent experiments.

bination with 0.5 mM TTA or palmitic acid) to approximately 0.26 μmol (in incubations with 0.2 mM oleic acid alone).

Effect of TTA on oxidation of [1-^{14}C]oleic acid

Oxidation of oleic acid (0.2 mM) was 3 times higher in cells grown with L-carnitine (**Fig. 2B**) than in cells grown without addition of L-carnitine (Fig. 2A). TTA significantly stimulated oxidation of oleic acid in absence and presence of L-carnitine. Cells grown with L-aminocarnitine oxidized almost no oleic acid to acid-soluble products both in absence and in presence of TTA (Fig. 2C). Calculating oxidation as percentage of oleic acid oxidation alone (control value), the stimulatory effect of TTA in presence of L-carnitine differed from that of palmitic acid with a P-value <0.01 at all concentrations (Fig. 2B). Oxidation of oleic acid was not significantly changed in the presence of increasing concentrations of palmitic acid under all three culture conditions.

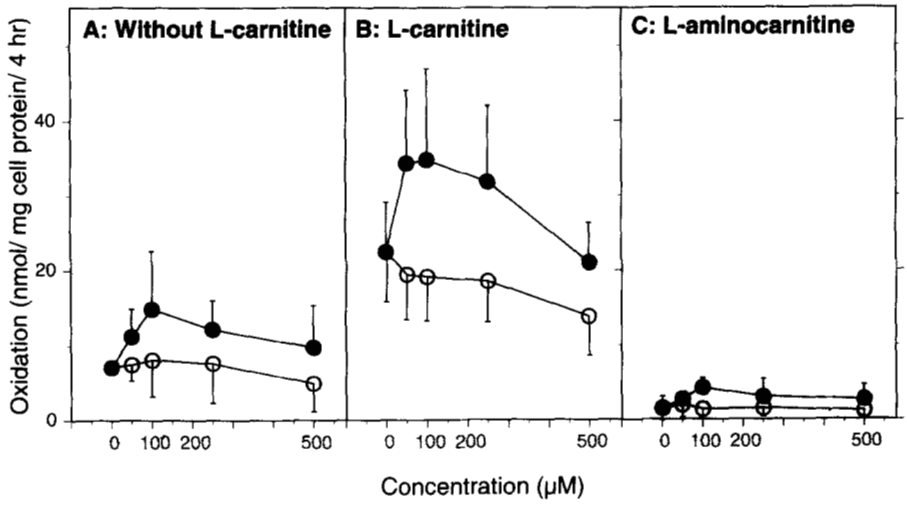

Fig. 2. Effect of tetradecylthioacetic acid (●) and palmitic acid (○) on oxidation of [1-^{14}C]oleic acid in cells plated and incubated for 4 h with [1-^{14}C]oleic acid (0.2 mM) without or with palmitic acid (○) or TTA (●), as described in Fig. 1. Acid-soluble radioactivity was determined as described in the Experimental section. Results are given as mean ± SD for values obtained in three to five independent experiments.

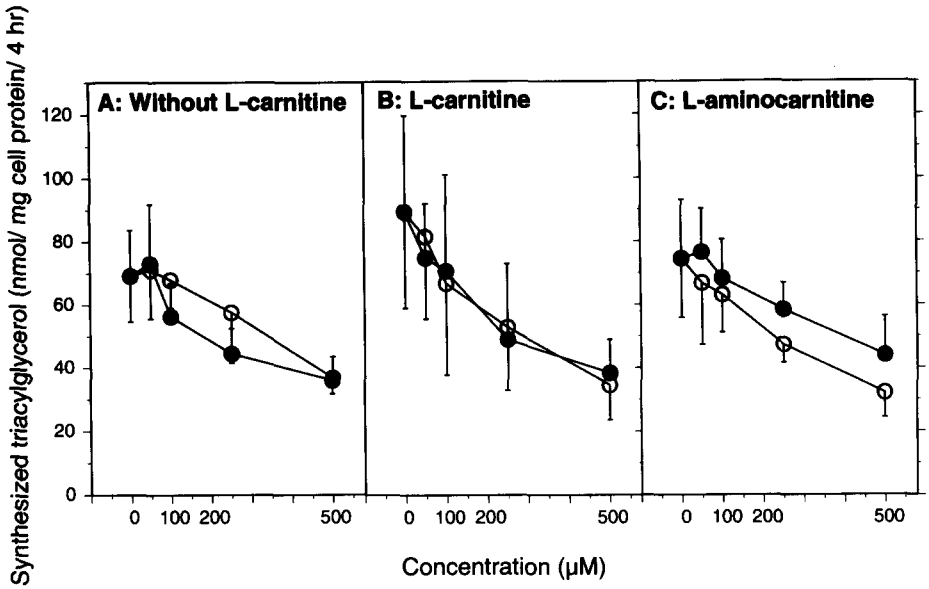

Fig. 3. Effect of tetradecylthioacetic acid (●) and palmitic acid (○) on incorporation of [1-^{14}C]oleic acid into synthesized triacylglycerol in hepatocytes plated and incubated for 4 h with [1-^{14}C]oleic acid (0.2 mM) without or with palmitic acid (○) or TTA (●), as described in Fig. 1. The results were calculated as the sum of triacylglycerol found in cells and in media. Results are given as mean ± SD for values obtained in three to five independent experiments.

Effect of TTA on synthesis of triacylglycerol from [1-^{14}C]oleic acid

Figure 3 shows that there were no significant differences in the effects of TTA and palmitic acid on the incorporation of labeled oleic acid into triacylglycerol in the three culture conditions. Furthermore, the synthesis of oleic acid-labeled triacylglycerol was reduced about equally by TTA and palmitic acid, irrespective of culture conditions (Fig. 3A–C). There were no statistically significant differences among any of the experiments.

Effect of TTA on secretion of [1-^{14}C]oleic acid-labeled triacylglycerol

Figure 4 shows that with oleic acid as the only added fatty acid, secretion of triacylglycerol was similar under

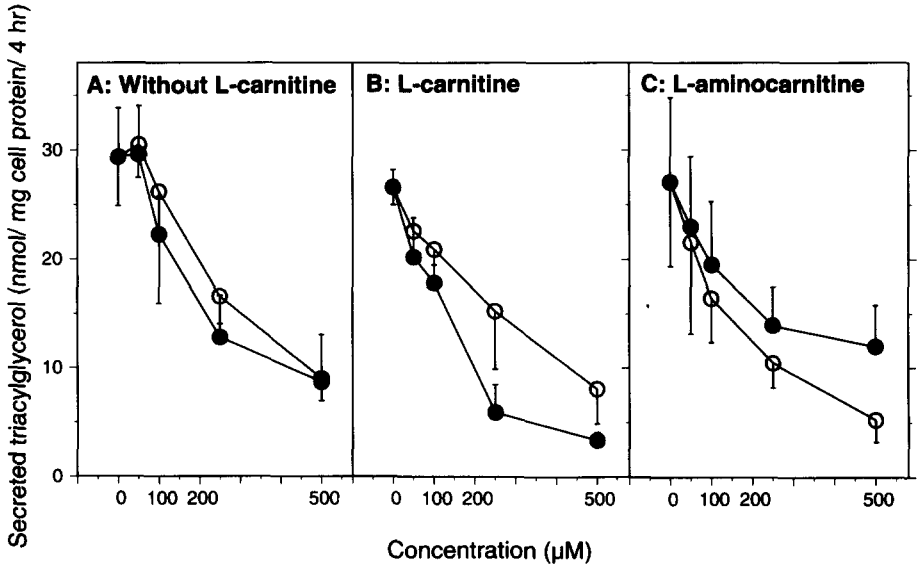

Fig. 4. Effect of tetradecylthioacetic acid (●) and palmitic acid (○) on secretion of triacylglycerol labeled with [1-^{14}C]oleic acid from cells plated and incubated with [1-^{14}C]oleic acid (0.2 mM) without or with palmitic acid (○) or TTA (●), as described in Fig. 1. Results are given as mean ± SD for values obtained in three to five independent experiments.

all three culture conditions. In cultures grown with L-carnitine, TTA (at 250–500 μM) caused a statistically significant lower secretion of oleic acid-labeled triacylglycerol than did palmitic acid (Fig. 4B). On the other hand, in cells grown with L-aminocarnitine, the relative effects of palmitic acid and TTA were reversed. At a concentration of 0.5 mM, palmitic acid reduced secretion of oleic acid-labeled triacylglycerol significantly more than did TTA (Fig. 4C). Furthermore, the reduction with TTA at concentrations of 250–500 μM was significantly greater in presence of L-carnitine than under the other conditions. Palmitic acid inhibited secretion of oleic acid-labeled triacylglycerol similarly under all culture conditions, except that at a concentration of 0.5 mM its inhibition was stronger in cells grown in presence of L-aminocarnitine when compared to cells grown without addition of L-carnitine.

Effects of TTA on the distribution of oleic acid between oxidation and triacylglycerol synthesis/secretion

Figure 5 shows synthesis and secretion of oleic acid-labeled triacylglycerol and oleic acid oxidation measured as percent of oleic acid uptake. The left panel in Fig. 5 shows effects of TTA on oleic acid metabolism; the right panel shows effects of palmitic acid. In cells grown with L-aminocarnitine it is striking that TTA and palmitic acid had almost identical effects on oleic acid metabolism. Incorporation of oleic acid into triacylglycerol was not significantly changed. Secretion of labeled triacylglycerol was moderately inhibited by palmitic acid. Palmitic acid at a concentration of 0.5 mM significantly inhibited secretion, whereas TTA did not change the secretory rate. In cells grown without or with L-carnitine, there was about a twofold increase in oxidation rate of oleic acid at concentrations of TTA >50 μM. In the presence of L-carnitine, the secretory rate of triacylglycerol was significantly reduced by TTA at all concentrations. In cells grown without L-carnitine, the effects of TTA on secretion were less pronounced, while in cells grown with L-aminocarnitine, TTA failed to change the secretory rate. At its highest concentration palmitic acid inhibited secretion of oleic acid-labeled triacylglycerol similarly in the presence of both L-carnitine and L-aminocarnitine.

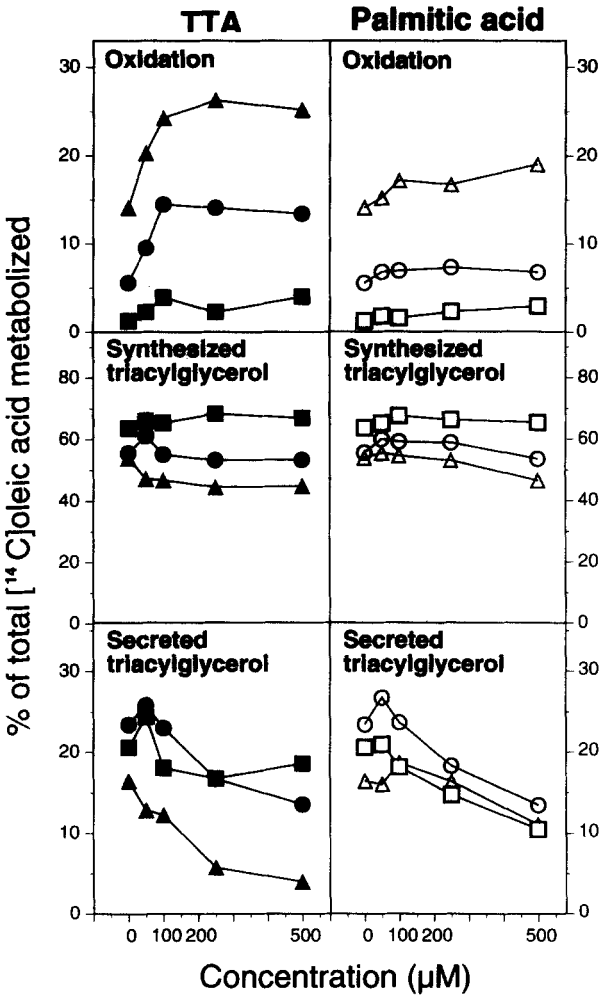

Fig. 5. Effect of tetradecylthioacetic acid and palmitic acid on [1-^{14}C]oleic acid metabolism in cells plated without addition of L-carnitine, supplemented with L-carnitine (0.5 mM) or L-aminocarnitine (50 μM). After ca. 20 h, plating media were removed and incubations of 4 h were performed with new media: (●, ○) no addition of L-carnitine; (▲, △) L-carnitine (0.5 mM); (■, □) L-aminocarnitine (50 μM) and [1-^{14}C]oleic acid (0.2 mM, 0.5 mCi/mmol) without or with increasing concentrations of TTA (closed symbols) or palmitic acid (open symbols). The ratio of fatty acid/BSA was 2.5:1. The results are given as % of total metabolized oleic acid and are means of three to five independent observations. The absolute values for oleic acid metabolism (in nmol/mg protein per 4 h) are given in Fig. 1.

Effects of TTA and oleic acid on [1-^{14}C]palmitic acid metabolism

Table 1 shows that palmitic acid was oxidized about twice as fast as oleic acid in hepatocytes cultured without L-carnitine supplement. Figure 2 shows that the oxidation of oleic acid was increased 3 times by L-carnitine. **Table 2** shows that palmitic acid oxidation was also stimulated to a similar extent and that TTA stimulated palmitic acid oxidation further, in agreement with earlier experiments (13), although the stimulation was not statistically significant. Oleic acid moderately inhibited palmitic acid oxidation, as expected. Conversion of palmitic acid to triacylglycerol was reduced equally by TTA and oleic acid, while TTA reduced secretion of labeled triacylglycerol significantly more than did oleic acid, as was the case with the secretion of oleic acid-labeled triacylglycerol. There were no differences in the effects of TTA and oleic acid on the total metabolism of palmitic acid, or on the conversion of palmitic acid to diacylglycerol and cholesteryl ester. Palmitic acid-labeling of phospholipids was expectedly (13, 21) strongly reduced by TTA. In cells grown with L-aminocarnitine there was no significant difference between the effects of oleic acid and TTA on palmitic acid

TABLE 2. Metabolism of [1-^{14}C]palmitic acid (PA) in absence or presence of oleic acid (OA) and tetradecylthioacetic acid (TTA) in rat hepatocytes cultured with L-carnitine

	[^{14}C]PA	[^{14}C]PA + OA	[^{14}C]PA + TTA
Oxidation	45.8 ± 14.8	35.8 ± 9.3[a]	56.2 ± 4.2
Triacylglycerol			
Synthesized	61.7 ± 9.3[b,c]	37.5 ± 4.3	32.6 ± 4.8
Secreted	14.1 ± 5.0[b,c]	6.9 ± 2.3[a]	3.4 ± 0.7
Phospholipid	42.3 ± 6.2[b,c]	27.8 ± 6.1[a]	16.8 ± 6.5
Diacylglycerol	3.4 ± 0.5[b]	1.7 ± 0.4	2.4 ± 1.3
Cholesteryl ester	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
Metabolized	165.5 ± 13.3[b,c]	113.6 ± 9.1	115.0 ± 13.6

Cells were plated overnight in medium supplemented with L-carnitine (0.5 mM), and incubated for 4 h with [1-^{14}C]palmitic acid (0.2 mM) without or with oleic acid (0.25 mM) and TTA (0.25 mM) in media supplemented with L-carnitine and BSA (ratio of fatty acid to BSA was 2.5:1). Results in nmol/mg protein per 4 h are presented as means ± SD of values obtained in cells from four rats.

[a]PA + OA different from PA + TTA.

[b]PA different from PA + OA.

[c]PA different from PA + TTA.

incorporation into synthesized triacylglycerol, whereas secretion of palmitic acid-labeled triacylglycerol was reduced more strongly by oleic acid than by TTA, as was the case with oleic acid-labeled triacylglycerol (data not shown).

Mass of secreted triacylglycerol

Figure 6 shows that oleic acid (0.2 mM), as well as oleic acid plus an equimolar concentration of palmitic acid, significantly stimulated the secretion of triacylglycerol compared to cells incubated with TTA (0.2 mM) and TTA in combination with an equimolar concentration of oleic acid. In cells grown with L-aminocarnitine, TTA failed to inhibit triacylglycerol secretion, as secretion from oleic acid plus TTA was equal to that from oleic acid plus palmitic acid (29.2 ± 6.6 vs. 33.1 ± 5.1 μg/ml protein per 6 h, n = 3).

DISCUSSION

In previous work we have shown that the hypolipemic compound tetradecylthioacetic acid (TTA) stimulates fatty acid oxidation in isolated rat hepatocytes (13). The present study was undertaken to determine whether TTA-stimulated hepatic β-oxidation influences triacylglycerol secretion from rat hepatocytes.

TTA metabolism resembles that of normal fatty acids (Table 1), except that it is metabolized by ω-oxidation (27). Fatty acids served as controls to help determine which effects of TTA on hepatic lipid metabolism were unique to the 3-thia fatty acid and which were caused by its resemblance to fatty acid. In experiments with labeled oleic acid, palmitic acid was the control for TTA because both palmitic acid and TTA are incorporated into triacylglycerol and phospholipids at similar rates in isolated hepatocytes (13). Also, TTA efficiently replaces palmitic acid in phospholipids in Morris 7800 C1 hepatoma cells (21). To see whether effects of TTA were valid with more than one fatty acid, experiments were performed with labeled palmitic acid. In these experiments oleic acid was

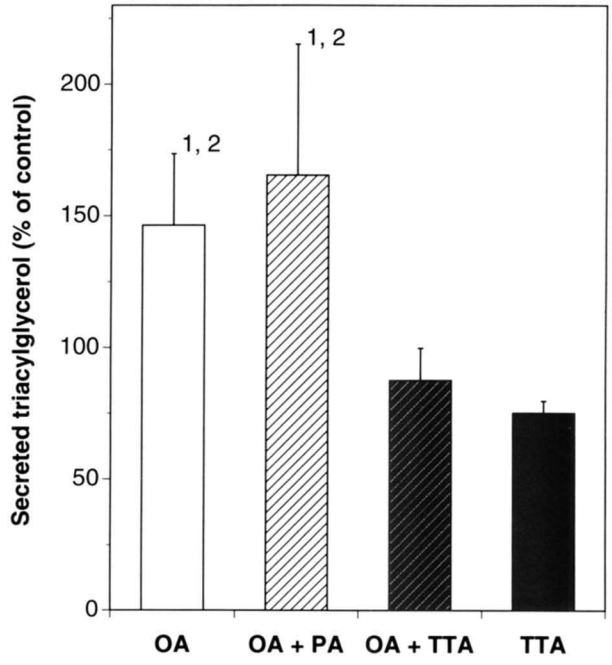

Fig. 6. Effect of tetradecylthioacetic acid and fatty acids on mass of triacylglycerol secreted into culture media. Cells were plated in media supplemented with L-carnitine (0.5 mM). After ca. 20 h plating media were removed and cells were incubated for 6 h with BSA (80 μM), oleic acid (0.2 mM) alone and in combination with palmitic acid (0.2 mM), or TTA (0.2 mM) alone and in combination with oleic acid (0.2 mM). Incubation media were supplemented with L-carnitine (0.5 mM) and BSA (ratio fatty acid/BSA 2.5:1). Triacylglycerol mass was measured as described in Experimental. Control cells incubated with BSA (80 μM) secreted 24.6 ± 5.8 μg triacylglycerol/mg protein per 6 h (obtained in cells prepared from five rats). Results in % of BSA control in each experiment are presented as means ± SD from cells from five different rats. [1]Results different from oleic acid + TTA and [2]results different from TTA.

the control for TTA. As expected, mutual inhibition or competition for oxidation, conversion to triacylglycerol and to secreted triacylglycerol was observed between oleic acid and palmitic acid with some variance in preference (Figs. 2–4 and Tables 1, 2). Similar results were obtained with 1-^{14}C-labeled fatty acids and TTA, (Fig. 4 and Table 2), and in incubations with [1-^{14}C]TTA in combination with oleic acid (data not shown). In the presence of L-aminocarnitine oxidation was eliminated, but palmitic acid and oleic acid still competed for esterification and secretion (Figs. 1C, 3C, and 4C), and in these cells TTA behaved as a normal fatty acid; it inhibited oleic acid uptake and esterification. Triacylglycerol secretion was also reduced, although somewhat less than by palmitic acid (Figs. 1C, 3C, and 4C). As TTA has a weaker effect than palmitic acid on oleic acid incorporation into secreted triacylglycerol, its effect on the secretory pathway for triacylglycerol as such seems to be insignificant.

When one looks at the distribution of [1-^{14}C]oleic acid between esterification and oxidation (Fig. 5), one sees that in cells with intact fatty acid oxidation, TTA-stimulated oleic acid oxidation was accompanied by a reduced export of oleic acid-labeled triacylglycerol, whereas TTA barely changed the incorporation of oleic acid into synthesized triacylglycerol. Secretion of triacylglycerol labeled with oleic acid was less reduced by palmitic acid, and this reduction was largely independent of oleic acid oxidation rate. Furthermore, in cells grown with L-carnitine, TTA stimulated β-oxidation of palmitic acid and reduced triacylglycerol secretion from palmitic acid more than the control, oleic acid (Table 2). Also, with palmitic acid as labeled substrate, there was a reversal in the TTA inhibition of triacylglycerol secretion when cells were grown with L-aminocarnitine. Thus, when TTA was added to cells, a possible direct relationship between oxidation rate and triacylglycerol secretory rate proved to be valid with two differently metabolized radiolabeled fatty acids. However, stimulation of fatty acid oxidation with L-carnitine alone (Figs. 2A and 2B) led to an increased uptake of oleic acid rather than a significant decrease in secretion of triacylglycerol (Figs. 1, 3, and 4).

The different effects of TTA and palmitic acid on triacylglycerol secretion were substantiated by mass determination of triacylglycerol secreted from hepatocytes cultured with L-carnitine and L-aminocarnitine. The reduced secretion of triacylglycerol from endogenous fatty acids with TTA (Fig. 6) may be explained by an increased oxidation of endogenous fatty acids, which become available by a continuous lipolysis of triacylglycerol (28).

At present, the regulation of hepatic CPT I is not fully understood (29). Fatty acids and fatty acid analogues or their CoA-esters may activate CPT I which may be desensitized towards malonyl-CoA (30), possibly by a long-chain acyl-CoA competition for the malonyl-CoA binding site at CPT I. In addition, both TTA and exogenous fatty acids inhibit fatty acid synthesis in rat hepatocytes (13). This may reduce the intracellular concentration of malonyl-CoA, the physiological inhibitor of CPT I (31), thereby relieving an inhibition of acylcarnitine formation. We have previously shown that hepatic malonyl-CoA is reduced acutely in rats fed a single dose of TTA (7).

Secretion of triacylglycerol in the form of VLDL is regulated by fatty acids (17–20, 32), by intracellular triacylglycerol level (33), and by the de novo synthesis of phosphatidylcholine, cholesteryl ester, and cholesterol (34–36). TTA may interfere with hepatic lipogenesis, as shown in vivo and in vitro (2–5, 13, 21). In the present study, however, there were no differences between effects of TTA and palmitic acid on synthesis rates of oleic acid-labeled triacylglycerol (which ultimately would affect triacylglycerol secretion (33), phospholipids, diacylglycerol, or cholesteryl ester (data not shown).

It is possible to accentuate triacylglycerol secretion from rat hepatocytes more than observed in the present study (32). However, this requires higher fatty acid concentrations and a higher ratio of fatty acid to BSA than used in the present work. The results in Table 1 and Fig. 6 demonstrate that within the range of fatty acid concentrations used throughout the present study, the effect of TTA on triacylglycerol secretion was studied under conditions where cellular capacity for conversion of fatty acids to triacylglycerol subsequently secreted was not saturated.

The present data suggest that two processes (β-oxidation and esterification into secreted triacylglycerol) compete for cellular acyl-CoA. In isolated rat liver mitochondria there is a competition for acyl-CoA between CPT and glycerophosphate acyltransferase (GPAT) (37). Although CPT and GPAT are inversely regulated in fasting and refeeding (37), a role for the latter enzyme in regulation of subsequent triacylglycerol secretion is, to the best of our knowledge, not known.

We conclude that TTA inhibits secretion of triacylglycerol from rat hepatocytes mainly by acutely stimulating fatty acid oxidation, thereby reducing fatty acid availability for synthesis of triacylglycerol destined for secretion. The increased fatty acid oxidation contributes to the rapid hypotriglyceridemia seen in rats treated with TTA.

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