Tetradecylthioacetic acid (a 3-thia fatty acid) decreases triacylglycerol secretion in CaCo-2 cells

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Abstract The effects of the hypolipidemic fatty acid analogue tetradecylthioacetic acid (TTA) on synthesis and secretion of lipoproteins in CaCo-2 cells were studied. Radiolabeled tetradecylthioacetic acid was absorbed and metabolized as efficiently as oleic acid, although a discrepancy in the metabolic fate, was evident. Whereas tetradecylthioacetic acid was incorporated into cell-associated triacylglycerol to the same extent as normal fatty acids (e.g., oleic acid and palmitic acid), the amount of triacylglycerol secreted from cells incubated with tetradecylthioacetic acid was 8 to 10 times lower than the amount secreted from cells incubated with palmitic acid and oleic acid, respectively. On the other hand, there was an enhanced incorporation of tetradecylthioacetic acid into cell-associated and secreted phospholipids. Despite incorporation of tetradecylthioacetic acid into cellular triacylglycerol, unlike oleic acid, tetradecylthioacetic acid did not stimulate production of triacylglycerol-rich particles. Ultracentrifugation of basolateral media from cells incubated with tetradecylthioacetic acid revealed low amounts of triacylglycerol in the triacylglycerol-rich fraction ($\varrho < 1.006$ g/ml), suggesting secretion of lipoproteins with a higher density than chylomicrons. However, the present study shows that the stimulated triacylglycerol secretion caused by oleic acid was inhibited in the presence of TTA. The decreased rate of triacylglycerol secretion from these cells was not accompanied by a stimulation of fatty acid oxidation. Based on these findings, we therefore suggest that tetradecylthioacetic acid mainly affects secretion of lipoproteins in CaCo-2 cells.—**Gedde-Dahl, A., T. Ranheim, C. A. Drevon, S. Skrede, R. K. Berge, and A. C. Rustan.** Tetradecylthioacetic acid (a 3-thia fatty acid) decreases triacylglycerol secretion in CaCo-2 cells. *J. Lipid Res.* 1995. **36:** 535–543.

Supplementary key words lipoprotein secretion • phospholipids • fatty acids • intestine

Tetradecylthioacetic acid (TTA) is a fatty acid analogue in which a sulfur atom substitutes the β-methylene group in the alkyl chain. The analogue closely resembles normal fatty acids (1), except that it is unable to be metabolized by β-oxidation (2). When fed to rats TTA induces mitochondrial and peroxisomal proliferation along with induction of several mitochondrial and peroxisomal enzymes (3, 4). Recently, it has been demonstrated that this 3-thia fatty acid is a potent activator of the peroxisome proliferator-activated receptor (PPAR) (5, 6), presumably mediating their peroxisome proliferating as well as their hypolipidemic actions. Repeated administration of TTA to normolipidemic rats reduces plasma lipids (7–9). At hypolipidemic doses, TTA only marginally affects peroxisomal enzyme activities, suggesting that the hypotriglyceridemic effect is dissociated from induction of peroxisomal β-oxidation and peroxisome proliferation (7). The triacylglycerol-lowering effect after prolonged feeding has been ascribed to increased mitochondrial fatty acid oxidation and inhibited liver lipogenesis, along with diminished biosynthesis of triacylglycerol (9). In rats fed a single dose of TTA, there is an increase in mitochondrial β-oxidation in isolated hepatocytes, accompanied by a reduced inhibition of fatty acid oxidation by malonyl-CoA (10). Asiedu et al. (8) also showed that the triacylglycerol-lowering effect after a single dose of TTA was initially due to increased mitochondrial β-oxidation in rat livers. In isolated rat hepatocytes, oxidation of palmitic acid is stimulated by TTA, whereas de novo fatty acid synthesis is inhibited (1). It has recently been shown that TTA reduces secretion of triacylglycerol from rat hepatocytes mainly by acutely stimulating mitochondrial fatty acid oxidation (11).

Previous studies concerning the effect of TTA on lipid metabolism refer mostly to experiments with intact animals, perfused liver, and isolated rat hepatocytes. Little knowledge exists about how this fatty acid analogue is absorbed from the intestine and whether any influence upon intestinal lipid metabolism contributes to the hypolipidemic effect.

Abbreviations: BSA, bovine serum albumin; PBS, phosphate-buffered saline; TTA, tetradecylthioacetic acid; OA, oleic acid; PA, palmitic acid; TG, triacylglycerol; DG, diacylglycerol; PL, phospholipid; FFA, free fatty acids; CE, cholesteryl ester; TLC, thin-layer chromatography.

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The purpose of this study was to examine the metabolism of TTA and its effect on synthesis and secretion of lipoproteins in the human intestinal CaCo-2 cell line. These cells have the capacity to secrete nascent lipoproteins from the basolateral side when grown to confluency on filters (12–14) and therefore constitute a suitable in vitro model for studying intestinal lipid metabolism.

MATERIALS AND METHODS

Chemicals

[1,2,3-^{3}H]glycerol (200 Ci/mol), [1,2-^{3}H]polyethylene glycol 900 (6 Ci/mol), D-[1-^{14}C]mannitol (45–55 Ci/mol), [1-^{14}C]oleic acid (58 Ci/mol), and [1-^{14}C]palmitic acid (57 Ci/mol) were obtained from DuPont, NEN Products, Boston, MA. Unlabeled and [1-^{14}C]tetradecylthioacetic acid (5.97 Ci/mol) were synthesized as described by Spydevold and Bremer (15). Bovine serum albumin (BSA), oleic acid, palmitic acid, and sodium taurocholate were purchased from Sigma Chemical Co., St. Louis, MO. Silica gel F 1500 thin-layer chromatography plates were purchased from Schleicher & Schuell, Dassel, Germany.

Fatty acid preparation

Sodium salt solutions of oleic acid and palmitic acid were prepared in distilled water, whereas tetradecylthioacetic acid was dissolved in 0.1 M NaOH. The micellar solutions of fatty acids and sodium taurocholate (12 mM) were prepared as described previously, and only optically clear micellar solutions were used (16).

Cell culture

CaCo-2 cells were obtained from American Type Culture Collection (Rockville, MD) at passage #17. Monolayer cultures were maintained at 37°C in air and 5% CO_2 in 75-cm^2 plastic flasks (Costar, Cambridge, MA) in Dulbecco's Modified Eagle's (DME) medium (4.5 g/l glucose and 3.7 g/l sodium bicarbonate) (Bio-Whittaker, Walkersville, MD) supplemented with 20% fetal calf serum (FCS) (Gibco, Paisley, UK), insulin (10 μg/ml) (Sigma), L-glutamine (2 mM), penicillin (50 IU/ml), streptomycin (50 μg/ml), and 1% nonessential amino acids (Bio-Whittaker). Grown under these conditions the doubling time of the CaCo-2 cells was approximately 70 h. Cell viability, as evaluated by trypan blue exclusion test, was always more than 90%. The cells were mycoplasma-negative as determined with Hoechst 33258 (17). The culture medium was changed every other day, and the day before an experiment.

For subculture the medium was removed, and the cells were detached from the culture flasks with 0.25% trypsin (Difco Laboratories, Detroit, MI) in a Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline (PBS), containing 0.2 g/l EDTA. Culture medium with FCS was added to stop trypsinization. Cells were suspended and seeded at approximately 4×10^4 cells/cm^2 in new flasks according to the method of Mohrmann et al. (18).

The cells were grown to confluency on collagen-treated cell culture filter inserts with a surface area of 4.7 cm^2 and 3.0 μm pore size (Transwell™-COL, Costar). This large pore size of filter membranes was used to allow chylomicron particles to diffuse freely through the membrane (14). The seeding density was 2×10^5 cells/cm^2 plated on the apical side of presoaked membrane filters. Culture medium was added to the upper (1.5 ml) and the lower (2.6 ml) wells. The growth of the cells and the degree of confluency were evaluated microscopically. Barrier properties of the cell monolayers were examined by following the transepithelial transport of the radiolabeled macromolecular markers, polyethylene glycol (mol wt 900) and mannitol (15). The monolayers were used 2 weeks after reaching confluency.

Measurement of cell-associated and secreted lipids

The culture medium was removed and the remaining cells were rinsed once with serum-free DME medium; unattached and damaged cells were thereby washed off. The cells were incubated with micellar fatty acid (concentrations and incubation times are indicated in legends to tables and figures) in serum-free DME medium. Either [1,2,3-^{3}H]glycerol (13 μCi/ml, 66 μM) or [1-^{14}C]fatty acid (1 μCi/ml) was used as radioactive precursor. Radioisotope and fatty acids were added to the upper chamber of the cell culture system, whereas the lower chamber contained serum-free DME medium only. After incubation, the medium was collected from the inside and outside of the tissue culture inserts and extracted as explained below. The cells were scraped off the filter membranes into PBS and centrifuged at 2000 rpm for 5 min. Samples were taken for protein determination, using BSA as a reference protein (19).

Lipid extraction and thin layer chromatography

Lipids from cells and media were extracted with chloroform–methanol 2:1 (v/v). The homogenized cell fraction was mixed with 20 volumes of chloroform–methanol 2:1 (v/v) (20). Four volumes of a 0.9% sodium chloride solution of pH 2 were added and the mixture was allowed to separate into two phases. The organic phase was dried under a stream of nitrogen at 40°C. To samples of media, devoid of cellular debris, were added 4 volumes of chloroform–methanol 2:1 (v/v) and 2% serum as unlabeled carrier for the lipids. The water phase was reextracted once with 4 volumes of chloroform–methanol 2:1 (v/v), and the combined organic phases were further treated in the same way as for the cells.

The residual lipid extract was redissolved in 200 μl hexane and separated by thin-layer chromatography (TLC),

using hexane–diethyl ether–acetic acid 80:20:1 (v/v/v) as developing solvent. The various lipid bands were finally identified by iodine vapor, scraped into 8 ml liquid scintillation fluid, and counted in a scintillation spectrometer (TRI-CARB 1900 TR, Packard Instrument, Downers Grove, IL).

Cellular uptake and degradation of labeled fatty acids

The cells and media contained negligible amounts of acid-soluble radioactivity (<3% of the total radioactivity added). Thus, cellular uptake was measured as acid-precipitable radioactivity in the cells. Medium (0.5 ml) and cell homogenate (0.1 ml) were precipitated with equal volumes of $HClO_4$ (1 M). BSA (0.5 mM) was added as a co-precipitant. The mixtures were centrifuged at 3500 rpm for 15 min and samples from the supernatants (acid-soluble activity) were counted by liquid scintillation spectrometry. The precipitates were resuspended, washed once with 1 ml $HClO_4$ (1 M), and resolubilized in 1.0 ml of saline containing sodium dodecyl sulfate (SDS) (70 mM) and Triton X-100 (10%) before counting. A set of no-cell controls was analyzed together with the experimental samples.

After the cells had been incubated with labeled fatty acids, the cells were scraped off the filter membranes into PBS and centrifuged. To achieve good recovery, samples of the supernatant were analyzed after centrifugation. After 5 h incubation, almost 50% of the added radioactivity was recovered in the supernatant of the cells. As previously suggested, this radioactivity may be associated with the unstirred waterlayer (UWL) adjacent to the cell monolayers (15). In addition, chylomicrons still associated with the cells and not yet secreted into the basolateral medium, will be in the PBS and therefore contribute to the large amount of radioactivity observed in the supernatant.

Lipoprotein isolation and characterization

CaCo-2 cells were incubated with tetradecylthioacetic acid or oleic acid for 5 h in serum-free DME medium with added [^{3}H]glycerol (13 μCi/ml, 66 μM). Basolateral medium was removed and cellular debris was eliminated by centrifugation at 2000 rpm for 5 min. Human plasma was added as a carrier, and lipoproteins were isolated by ultracentrifugation in a Sorvall TFT 45.6 fixed-angle rotor at 38000 rpm (21). Triacylglycerol-rich lipoproteins were isolated without altering the density of the medium (ϱ < 1.006 g/ml for 20 h), whereas the total lipoprotein fraction was isolated by increasing the density of the medium to ϱ < 1.21 g/ml by NaBr and centrifuging for 48 h. The top fractions were collected by tube-slicing and further examined by analysis of lipid composition as described above. The remaining volume was made homogeneous before samples were analyzed together with the top fractions.

Mass measurements of cellular lipids by gas–liquid chromatography (GLC)

The fatty acid composition of triacylglycerol and phospholipids in CaCo-2 cells was determined as fatty acid methyl esters on a gas chromatograph (Shimadzu GC-14A, Kyoto, Japan), equipped with a polar capillary column (SGE BPX70, 0.33 mm internal diameter, 25 m length) and using helium as the carrier gas. The temperature was programmed to rise from 40 to 220°C. The procedure for the transesterification reaction is described by Mason and Waller (22). Glycerolipid spots (separated by TLC and visualized by fluorescein) were scraped into vials and 0.5 ml benzene, 1 ml methanolic-HCl (3 N) (Supelco, Supelco Park, Bellefonte, PA), and 200 μl 2,2-dimethoxypropane (Supelco) were added and the vials were stored overnight at room temperature. The mixtures were then neutralized with 2.0 ml $NaHCO_3$ (0.7 M) and extracted with 2 × 2 ml of hexane. Triheptadecanoylglycerol and

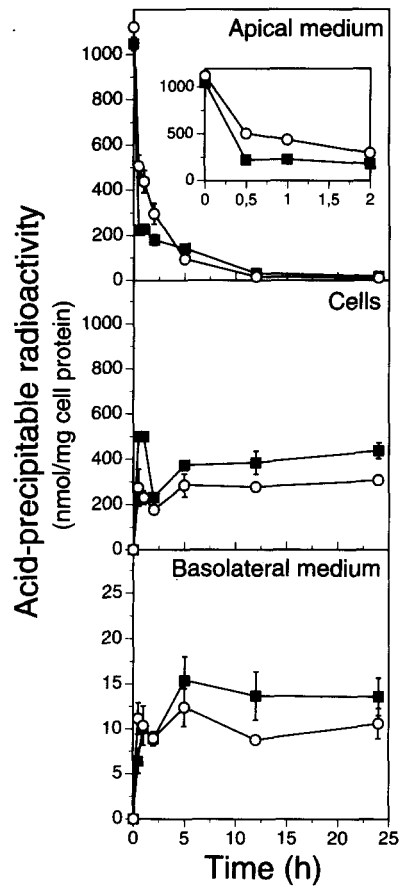

Fig. 1. Acid-precipitable products in CaCo-2 cells incubated with [1-^{14}C]tetradecylthioacetic acid and [1-^{14}C]oleic acid. Cells, cultured for 2 weeks after confluency on filter membranes, were incubated up to 24 h in serum-free DME medium containing tetradecylthioacetic acid (■) or oleic acid (○) (1.7 Ci/mol, 0.6 mM). Insert: acid-precipitable products in apical medium plotted with an expanded time scale from 0–2 h. Data, given as nmol/mg cell protein, are means ± SD of triplicate samples of one representative experiment.

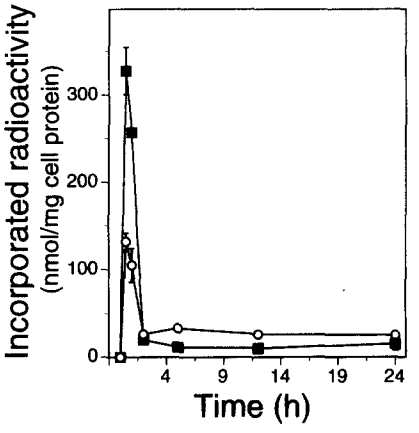

Fig. 2. Content of labeled free fatty acids in CaCo-2 cells after incubation with [1-^{14}C]tetradecylthioacetic acid and [1-^{14}C]oleic acid. Cells, cultured for 2 weeks after confluency on filter membranes, were incubated up to 24 h in serum-free DME medium containing tetradecylthioacetic acid (■) or oleic acid (○) (1.7 Ci/mol, 0.6 mM). Labeled free fatty acids were measured as described in Materials and Methods. Data, given as nmol/mg cell protein, are means ± SD of triplicate samples of one representative experiment.

diheptadecanoylphosphatidylcholine were used as internal standards.

Presentation of data

All values are reported as means ± SD of at least triplicate samples obtained from indicated number of experiments, unless otherwise stated. Statistical analyses were performed with the Mann-Whitney non-parametric test (two-tailed). A *P*-value less than 0.05 was considered significant.

RESULTS

Comparison of oleic acid, palmitic acid, and TTA metabolism

A micellar solution of either [1-^{14}C]TTA or [1-^{14}C]OA was added to CaCo-2 monolayers and acid-precipitable products in cells and media were measured. Labeled TTA disappeared somewhat faster than labeled oleic acid from the apical media during the initial 2 h of incubation (**Fig. 1,** insert). During this period of time twice as much acid-precipitable radioactivity was recovered in cells incubated with TTA as compared to oleic acid (Fig. 1, Cells). The intracellular pool of labeled free fatty acids was greater in the presence of TTA than oleic acid during the initial 2 h of incubation (**Fig. 2**). After 12 h, almost the entire pool of labeled fatty acids had disappeared from the apical medium, whereas less than 30% was recovered from the cells (Fig. 1, Apical medium and Cells). Approximately 1% of the added radioactivity was recovered as acid-precipitable products in the basolateral medium after 5 h (Fig. 1, Basolateral medium).

Incubation of CaCo-2 cells with labeled fatty acid (0.6 mM) for 5 h showed a difference in the metabolic fate between TTA and the normal fatty acids (**Table 1**). Palmitic acid was a poorer substrate for both triacylglycerol and phospholipid formation than oleic acid. Whereas [1-^{14}C]TTA was incorporated into cell-associated triacylglycerol to the same extent as [1-^{14}C]OA, the amount of TTA-labeled triacylglycerol in the basolateral medium was ten and eight times lower than that of OA-labeled and PA-labeled triacylglycerol, respectively. Incubation of CaCo-2 cells with increasing concentrations (up to

TABLE 1. Metabolism of [1-^{14}C]fatty acids in CaCo-2 cells

	[1-^{14}C]OA	[1-^{14}C]TTA	[1-^{14}C]PA
	nmol/mg cell protein		
Triacylglycerol			
Cell-associated	105.6 ± 34.3[b]	104.8 ± 55.6	75.3 ± 11.5
Secreted	6.3 ± 3.2[a]	0.6 ± 0.4[c]	4.6 ± 0.5
Phospholipids			
Cell-associated	68.8 ± 12.9[a,b]	114.4 ± 15.2[c]	32.4 ± 4.4
Secreted	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
Diacylglycerol			
Cell-associated	15.4 ± 5.2	12.2 ± 6.2	11.8 ± 1.9
Free fatty acids			
Cell-associated	66.3 ± 32.4[a,b]	115.9 ± 67.7[c]	228.1 ± 38.7
Secreted	0.6 ± 0.4[a,b]	4.7 ± 3.1	7.2 ± 5.3
Cholesteryl ester			
Cell-associated	2.7 ± 0.8[a]	2.0 ± 0.8	2.1 ± 0.6
Secreted	0.06 ± 0.03[b]	0.05 ± 0.03[c]	0.1 ± 0.02
Acid-soluble activity	31.0 ± 11.1[a]	4.3 ± 1.9[c]	23.9 ± 4.7

Cell monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated with labeled oleic acid (OA), tetradecylthioacetic acid (TTA), or palmitic acid (PA) (1.7 Ci/mol, 0.6 mM) for 5 h. Acid-soluble products and lipids from cells and basolateral media were measured as described in Materials and Methods. Data represent means ± SD obtained from more than three separate experiments.

[a]OA different from TTA.
[b]OA different from PA.
[c]TTA different from PA.

TABLE 2. Effect of tetradecylthioacetic acid and palmitic acid on metabolism of [1-^{14}C]oleic acid in CaCo-2 cells

	[1-^{14}C]OA	[1-^{14}C]OA + TTA	[1-^{14}C]OA + PA
		nmol/mg cell protein	
Triacylglycerol			
Cell-associated	105.6 ± 34.3	108.4 ± 33.9	98.1 ± 40.6
Secreted	6.3 ± 3.2[a]	3.5 ± 1.8[c]	6.0 ± 1.9
Phospholipids			
Cell-associated	68.8 ± 12.9	66.8 ± 11.1	67.0 ± 12.0
Secreted	0.2 ± 0.2[a]	0.3 ± 0.1[c]	0.1 ± 0.1
Diacylglycerol			
Cell-associated	15.4 ± 5.2[a]	20.0 ± 6.5	17.0 ± 4.6
Free fatty acids			
Cell-associated	66.3 ± 32.4	66.5 ± 31.6	75.7 ± 35.5
Secreted	0.6 ± 0.4[a]	1.8 ± 1.5	1.0 ± 1.1
Cholesteryl ester			
Cell-associated	2.7 ± 0.8[a,b]	1.2 ± 0.8	1.3 ± 0.4
Secreted	0.06 ± 0.03[a]	0.03 ± 0.03	0.05 ± 0.03
Acid-soluble activity	31.0 ± 11.1	24.1 ± 7.2	25.7 ± 9.6

Cell monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated for 5 h with serum-free DME medium containing [1-^{14}C]oleic acid (OA) (1.7 Ci/mol, 0.6 mM) alone or in combination with either tetradecylthioacetic acid (TTA) (0.6 mM) or palmitic acid (PA) (0.6 mM). Acid-soluble products and lipids from cells and basolateral media were measured as described in Materials and Methods. Data represent means ± SD obtained from more than four separate experiments.

[a]OA different from OA + TTA.

[b]OA different from OA + PA.

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

1 mM) of labeled fatty acids resulted in a markedly lower secretion of TTA-labeled triacylglycerol than oleic acid-labeled at all concentrations examined, whereas the cellular content of labeled triacylglycerol was similar (data not shown). Table 1 also shows that TTA was the best substrate for phospholipid formation. On the other hand, it was the poorest oxidizable fatty acid.

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

Incorporation of labeled oleic acid into cell-associated triacylglycerol and phospholipids was unchanged in the presence of TTA and palmitic acid when compared to labeled oleic acid alone (**Table 2**). However, secretion of oleic acid-labeled triacylglycerol to the basolateral medium was reduced by approximately 50% after addition of TTA, whereas co-administration of palmitic acid did not influence triacylglycerol secretion. Furthermore, labeled oleic acid was more efficiently incorporated into secreted phospholipids in the presence of TTA, as compared to palmitic acid. On the other hand, the amount of oleic acid-labeled cholesteryl ester in cells and medium was decreased after addition of TTA as well as palmitic acid. Oxidation of [1-^{14}C]oleic acid to acid-soluble products was unchanged when the cells were incubated in the presence of TTA and palmitic acid, as compared to labeled oleic acid alone.

Effects of tetradecylthioacetic acid and oleic acid on synthesis and secretion of glycerol-labeled lipids

CaCo-2 cells were incubated with [^{3}H]glycerol for 5 h with increasing concentrations of TTA or oleic acid (**Fig. 3**). While there was a dose-dependent increase in both cell-associated and secreted triacyl[^{3}H]glycerol after incubation with oleic acid, TTA did not stimulate triacylglycerol production, as compared to a fatty acid-free control (indicated as 0 mM fatty acid) (Fig. 3). Secretion of triacylglycerol from cells incubated with TTA was even reduced with increasing concentrations. At a fatty acid concentration of 0.6 mM, secreted triacylglycerol expressed as percentage of total synthesized triacylglycerol (cell-associated + secreted) was 0.7 ± 0.1 for TTA compared to 2.5 ± 0.8 in cells incubated in absence of fatty acid.

Table 3 shows the influence of TTA (0.6 mM) compared to oleic acid (0.6 mM) on formation of glycerol-labeled lipids after 5 and 24 h incubation. The amount of triacylglycerol in cells and basolateral media was markedly decreased after incubation with TTA, whereas the formation and secretion of phospholipids was enhanced. Cell-associated diacylglycerol was also reduced in cells incubated with TTA.

When the cells were incubated with equimolar concentrations of TTA and oleic acid (0.6 mM each),

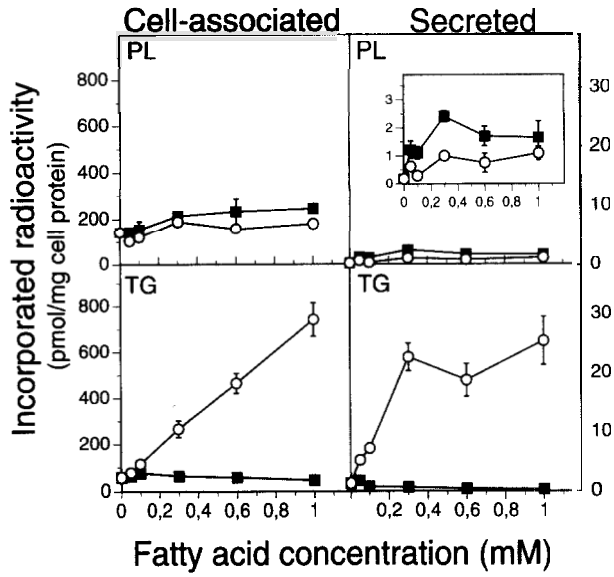

Fig. 3. Effect of fatty acid concentration on cell-associated and secreted [^{3}H]glycerophospholipids (PL) and triacyl[^{3}H]glycerol (TG) in CaCo-2 cells. Cells, cultured for 2 weeks after confluency on filter membranes, were incubated for 5 h with increasing concentrations of tetradecylthioacetic acid (■) or oleic (○) acid in serum-free DME medium containing [^{3}H]glycerol (200 Ci/mol, 66 μM). Cells and media from the basolateral chambers were collected and treated as described in Materials and Methods. Insert: secreted phospholipids shown in a diagram with reduced scale. Data, given as nmol/mg cell protein, represent means ± SD of three cultures.

cell-associated [^{3}H]phospholipids were increased by 20%, as compared to oleic acid alone, whereas both diacyl[^{3}H]glycerol and triacyl[^{3}H]glycerol formation were almost doubled. However, triacylglycerol secretion from cells exposed to TTA in combination with oleic acid was only 25% of the secretion from cells incubated with oleic acid alone (data not shown).

Distribution of [^{3}H]glycerolipids in lipoprotein fractions

Basolateral media from CaCo-2 cells supplemented with fatty acids were separated by ultracentrifugation into a triacylglycerol-rich fraction ($\varrho < 1.006$ g/ml) and a total lipoprotein fraction ($\varrho < 1.21$ g/ml) (**Table 4**). The $\varrho < 1.006$ g/ml fraction of the medium, which corresponds to chylomicrons, contained 80% of the total triacylglycerol secreted from cells incubated with oleic acid, whereas only 2% of the total triacylglycerol was recovered in this fraction after incubation with TTA. The $\varrho < 1.21$ g/ml fraction, which includes all lipoproteins, contained 91% and 63% of the total triacylglycerol after incubation with oleic acid and TTA, respectively. The chylomicron fraction contained only 2% of the total secreted phospholipids, and less than 70% was associated with lipoproteins, regardless of the fatty acid added to the incubation medium (Table 4).

TABLE 3. Effect of tetradecylthioacetic acid on incorporation of [^{3}H]glycerol into cell-associated and secreted glycerolipids

	Cell-Associated Lipids		Secreted Lipids	
	5 h	24 h	5 h	24 h
	% of oleic acid			
TG	18 ± 6	25 ± 27	4 ± 3	18 ± 14
PL	137 ± 33	146 ± 24	185 ± 57	249 ± 90
DG	64 ± 29	68 ± 16		

CaCo-2 monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated with tetradecylthioacetic acid (TTA) or oleic acid (0.6 mM) in serum-free DME medium containing [^{3}H]glycerol (200 Ci/mol, 66 μM). The cells were incubated for 5 and 24 h before the contents of [^{3}H]phospholipids (PL), diacyl[^{3}H]glycerol (DG), and triacyl[^{3}H]glycerol (TG) in cells and basolateral media were measured. Results, given for TTA as % of oleic acid, represent means ± SD from more than three separate experiments. TTA is significantly different from oleic acid in all data.

Mass measurements and fatty acid composition of cellular lipids

As shown in **Table 5,** TTA did not induce triacylglycerol production in CaCo-2 cells as was observed with oleic acid. On the other hand, the phospholipid formation was increased in cells incubated with TTA, as compared to a fatty acid-free control. In addition, the amounts of both triacylglycerol and phospholipids were increased after exposure to TTA in combination with oleic acid, when compared to oleic acid alone. The fatty acid composition of triacylglycerol and phospholipids is presented in **Table 6.** TTA was effectively incorporated into both lipid classes, and there was a reduction in 18:1 (n-9) in cells exposed to TTA, as compared to oleic acid.

TABLE 4. Effects of tetradecylthioacetic acid and oleic acid on distribution of [^{3}H]glycerophospholipids and triacyl[^{3}H]glycerol in lipoproteins secreted from CaCo-2 cells

	Triacylglycerol		Phospholipids	
Density	TTA	OA	TTA	OA
g/ml		*%*		
$\varrho < 1.006$	2 ± 1[a]	80 ± 4	2 ± 1	2 ± 1
$\varrho < 1.21$	63 ± 10	91 ± 6	67 ± 23	65 ± 25
$\varrho > 1.21$	37 ± 10	6 ± 2	33 ± 23	35 ± 25

Cell monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated with tetradecylthioacetic acid (TTA) or oleic acid (OA) (0.6 mM) for 5 h in serum-free DME medium containing [^{3}H]glycerol (200 Ci/mol, 66 μM). The basolateral media were collected and lipoproteins were isolated by ultracentrifugation at $\varrho < 1.006$ and $\varrho < 1.210$ g/ml. The amounts of labeled lipids were measured as described in Materials and Methods. Data represent means ± SD from six cultures.
[a]Percentage distribution of each lipid in each fraction.

TABLE 5. Mass measurements of cell-associated lipids

	Fatty Acid-Free Control	OA	TTA	OA + TTA
		μg/mg cell protein		
TG	24.5 ± 3.0	39.8 ± 3.3	22.3 ± 1.7	53.6 ± 9.4
PL	129.3 ± 15.6	139.8 ± 6.3	166.3 ± 14.1	167.6 ± 10.0

Cell monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated for 24 h with serum-free DME medium containing oleic acid (OA) (0.6 mM), tetradecylthioacetic acid (TTA) (0.6 mM), or a combination of the two fatty acids (0.6 mM each). Mass determination of cell-associated triacylglycerol (TG) and phospholipids (PL) was performed by gas–liquid chromatography. Data represent means ± SD of three cultures given as μg/mg cell protein.

DISCUSSION

The triacylglycerol-lowering effect of the sulfur-substituted fatty acid analogue tetradecylthioacetic acid in rats is well documented (7–9). The reduced serum level of triacylglycerol has mainly been attributed to a decreased output of triacylglycerol from the liver (1, 8, 11, 23). In the present work we demonstrate that tetradecylthioacetic acid acutely decreases triacylglycerol secretion in the enterocytic cell line CaCo-2.

TTA was absorbed and metabolized as efficiently as oleic acid (Fig. 1, Table 1). This confirms previous findings that TTA resembles normal fatty acids (1, 11), except that it is metabolized by ω-oxidation (24). The intracellular pool of labeled free fatty acids was greater in the presence of TTA than oleic acid during the first 2 h of incubation, indicating that TTA was initially metabolized slower than oleic acid (Fig. 2). This could be due to slow activation of TTA by acyl-CoA synthetase as previously shown in rat liver homogenates (25).

Despite similar incorporation of labeled TTA and normal fatty acids into cell-associated triacylglycerol, the secretion of triacylglycerol was markedly reduced after incubation with TTA (Table 1). This effect was dependent of the presence of the sulfur-substitute, as secretion of triacylglycerol from TTA-incubated cells was significantly lower than from cells incubated with palmitic acid, the corresponding saturated fatty acid. In addition, TTA significantly decreased secretion of oleic acid-labeled triacylglycerol as compared to palmitic acid (Table 2). TTA has recently been found to reduce triacylglycerol secretion in cultured rat hepatocytes by acutely stimulating mitochondrial fatty acid oxidation (11). This is in accordance with Asiedu et al. (8) who showed that the triacylglycerol-lowering effect after a single administration of TTA initially might be due to increased hepatic mitochondrial β-oxidation. However, in CaCo-2 cells, the decreased triacylglycerol secretion was not accompanied by a stimulation of fatty acid oxidation (Table 2).

As compared to a fatty acid-free control, TTA did not

TABLE 6. Fatty acid composition of cellular glycerolipids

	Triacylglycerol			Phospholipids		
Fatty acid	OA	TTA	OA + TTA	OA	TTA	OA + TTA
			% of total fatty acids			
12:0	–[a]	1.6 ± 0.1	–	–	–	–
14:0	1.6 ± 0.1	3.0 ± 0.2	1.5 ± 0.1	–	–	–
16:0	11.4 ± 0.3	26.6 ± 1.0	12.3 ± 1.2	15.2 ± 0.2	10.2 ± 0.2	8.9 ± 0.4
16:1 (n-7)	3.9 ± 0.1	3.8 ± 0.3	3.1 ± 0.1	4.8 ± 0.2	5.8 ± 0.2	4.5 ± 0.1
TTA	–	9.7 ± 0.7	12.0 ± 0.7	–	25.4 ± 0.5	20.0 ± 0.2
18:0	5.9 ± 0.3	14.1 ± 0.9	6.7 ± 0.4	15.9 ± 0.2	13.9 ± 0.1	12.1 ± 0.2
18:1 (n-7/n-9)	68.5 ± 0.5	33.1 ± 1.2	57.6 ± 1.4	46.9 ± 0.8	32.1 ± 0.3	44.3 ± 0.2
18:2 (n-6)	–	–	–	1.7 ± 0.04	1.8 ± 0.01	1.5 ± 0.04
20:4 (n-6)	–	–	–	7.0 ± 0.3	1.2 ± 0.02	1.1 ± 0.01
20:5 (n-3)	–	–	–	1.6 ± 0.04	1.7 ± 0.1	1.4 ± 0.02
22:1 (n-9/n-11)	–	2.2 ± 0.3	–	–	–	–
22:5 (n-3)	1.2 ± 0.1	1.4 ± 0.6	1.0 ± 0.2	6.2 ± 0.03	6.9 ± 0.1	5.8 ± 0.1
22:6 (n-3)	1.7 ± 0.1	1.0 ± 0.01	1.4 ± 0.2	–	–	–
Others	2.9	2.7	2.0	0.6	1.1	0.5

Cell monolayers, cultured for 2 weeks after confluency on filter membranes, were incubated for 24 h with serum-free DME medium containing oleic acid (OA) (0.6 mM), tetradecylthioacetic acid (TTA) (0.6 mM), or a combination of the two fatty acids (0.6 mM each). The fatty acid pattern of triacylglycerol and phospholipids was determined by gas–liquid chromatography. Data represent means ± SD of three cultures given as % of total fatty acids.

[a]Not detectable or trace amounts (less than 1%).

stimulate triacylglycerol synthesis, but increased phospholipid production in CaCo-2 cells (Fig. 3, Table 5). These findings may be caused by decreased activity of phosphatidate phosphohydrolase (PAP), as previously discussed as a reason for reduced production of triacylglycerol in rats fed TTA (9). It was suggested that the decreased activity of PAP found in rat liver after repeated TTA treatment, accompanied by a stimulation of the rate limiting enzyme in synthesis of phosphatidylcholine, CTP:phosphocholine cytidylyltransferase, may accelerate phospholipid synthesis at the expense of retarded triacylglycerol synthesis. In addition, production of phosphatidylinositol from phosphatidic acid may be increased in presence of TTA, leading to decreased substrate for diacylglycerol and hence triacylglycerol formation. This has been observed in preliminary studies with CaCo-2 cells (A. Gedde-Dahl, T. Ranheim, C. A. Drevon, and A. C. Rustan) and in rat liver (unpublished observations, R. K. Berge). However, it is reasonable to suggest that diacylglycerol acyltransferase (DGAT) could also be involved in the observed triacylglycerol-lowering effect, as the content of triacylglycerol is much more affected than the diacylglycerol (Table 3). The increased phospholipid content of rat liver after TTA supplementation has been explained previously by the proliferation of liver peroxisomes, a phospholipid-dependent process (9). Recently, it has been suggested that the increased hepatic level of phosphatidylcholine observed in TTA-treated rats is linked to peroxisome proliferation (26). Unpublished observations from our laboratory (A. Gedde-Dahl, T. Ranheim, C. A. Drevon, and A. C. Rustan) indicate an increased level of fatty acyl-CoA oxidase mRNA in CaCo-2 cells incubated with TTA for 5 h, demonstrating that proliferation of peroxisomes may already have been initiated.

The present study indicates secretion of triacylglycerol associated with lipoprotein particles with a higher density than chylomicrons after incubation with TTA (Table 4). This is in accordance with previous results with CaCo-2 cells incubated in absence of fatty acids. Under these conditions, lipoproteins secreted were in the high density and low density lipoprotein range (27, 28). HDL particles are composed mainly of phospholipids, the most abundant lipid secreted from TTA-treated cells (Table 3). However, secreted phospholipids are not likely lipoprotein-associated (12), supporting our observation that a major part of the secreted phospholipids was found in the $\varrho > 1.21$ g/ml fraction (Table 4).

In summary, we have demonstrated that TTA, like normal fatty acids, was taken up and metabolized in CaCo-2 cells, although it did not stimulate triacylglycerol-rich lipoprotein production. However, the present study shows that the stimulated triacylglycerol secretion caused by oleic acid was inhibited in presence of TTA. We conclude that this effect, which is not accompanied by an increased fatty acid oxidation, suggests that TTA mainly affect lipoprotein assembly and/or secretion in CaCo-2 cells. Thus, the triacylglycerol-lowering effect observed in animals treated with TTA may also involve decreased triacylglycerol secretion from the intestine.

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