Lysophosphatidylcholine increases the secretion of cholesteryl ester-poor triacylglycerol-rich lipoproteins by CaCo-2 cells

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To address the effect of lysophosphatidylcholine on triacylglycerol transport in intestine, CaCo-2 cells, grown on semi-permeable supports, were incubated with lysophosphatidylcholine solubilized in 1 mM taurocholate. [^{14}C]Palmitoyllysophosphatidylcholine was readily taken up and incorporated predominantly into cellular phospholipids, particularly phosphatidylcholine. Twenty-five percent of the label was found in triacylgycerols. Compared with labelled cellular phospholipids, labelled triacylglycerols were preferentially secreted. Lysophosphatidylcholine caused a profound decrease in cholesteryl ester synthesis and secretion, whereas cellular triacylglycerol mass and triacylglycerol synthesis and secretion were increased. The effect was more pronounced with oleoyllysophosphatidylcholine than with either palmitoyl- or stearyl-lysophosphatidylcholine. Lysophosphatidylcholine increased the secretion of immunoreactive and newly-synthesized apoprotein B (apoB) without altering the rate of apoB synthesis. Thus, luminal lysophosphatidylcholine and/or its uptake decreases cholesterol esterification and secretion, but increases triacylglycerol synthesis and secretion, triacylglycerol mass accumulation and the secretion of apoB by CaCo-2 cells.

INTRODUCTION

The amount of phosphatidylcholine which enters the lumen of the small intestine is considerable. Most luminal phosphatidylcholine is derived from bile with smaller amounts originating from the diet. Prior to the uptake of the phospholipid by the intestinal cell, it is first hydrolysed by pancreatic and cellular phospholipases to lysophosphatidylcholine [1]. Some luminal phosphatidylcholine invariably escapes hydrolysis but it is thought that only small amounts of the intact phosphatidylcholine molecule are actually taken up by the intestinal cell [2,3]. Uptake of lysophosphatidylcholine, however, occurs readily and appears to be complete [2]. Within the intestinal absorptive cell, acylation enzymes reesterify the lysophosphatidylcholine back to phosphatidylcholine [2]. The resynthesized phosphatidylcholine molecule can then be used for the assembly and secretion of lipoproteins or the restructuring of cellular membranes.

There is evidence to suggest that phosphatidylcholine is important for the absorption of luminal lipids by the intestine. Several animal studies have demonstrated increased triacylglycerol absorption in the presence of luminal phosphatidylcholine [4–7]. Since lysophosphatidylcholine is the form of the phospholipid which is absorbed, it has been suggested that it is this species of the phospholipid which increases the uptake of lipids and/or the secretion of triacylglycerol-rich lipoproteins. In the intact animal, however, the presence of both phosphatidylcholine and its hydrolytic product lysophosphatidylcholine, together with bile acids, monoglycerols, cholesterol and fatty acids, make it difficult, if not impossible, to sort out the effects of individual luminal lipids on intestinal lipid transport. Mechanisms for why lysophosphatidylcholine should enhance lipid absorption are unknown and information concerning lysophosphatidylcholine absorption and its effect on apoprotein B (apoB) secretion are lacking.

In this report, we demonstrate that the influx of lysophosphatidylcholine into CaCo-2 cells increases the synthesis and basolateral secretion of triacylglycerols without altering apoB synthesis. Immunoreactive and newly-synthesized apoB secretion are increased, but cholesteryl ester synthesis and secretion are markedly decreased.

METHODS AND MATERIALS

Trans-[^{35}S]methionine (1 100 Ci/mmol) was purchased from ICN Radiochemicals (Irvine, CA, U.S.A.). [1,2,3-^{3}H]glycerol, [9,10-^{3}H]oleic acid, [7-^{3}H]cholesterol, [cholesteryl-1,2,6,7-^{3}H]cholesteryl oleate, [oleoyl-1-^{14}C]oleoyl coenzyme A and L-1[palmitoyl-1-^{14}C]-lysopalmitoyl phosphatidylcholine were purchased from New England Nuclear (Boston, MA, U.S.A.). Lysophosphatidylcholine, palmitoyllysophosphatidylcholine, stearyllysophosphatidylcholine, oleoyllysophosphatidylcholine, cholesterol, taurocholate and fatty acid-poor BSA were from Sigma (St. Louis, MO, U.S.A.). ApoB and apoB monoclonal antibody (clone # 1607, immunoglobulin G26-fraction purified by column chromatography) and apoB sheep immunopurified polyclonal antibody conjugated to horseradish peroxidase were purchased from Biodesign International (Kennebunkport, ME, U.S.A.). Rabbit polyclonal antibody (IgG fraction) specific for human apoB was from Calbiochem (San Diego, CA, U.S.A.). Rabbit sera containing antibody to human apoA 1 were provided by Dr. Dennis Black, Department of Pediatrics, University of Chicago, IL, U.S.A. Protein A bound to Sepharose was from RepliGen (Cambridge, MA, U.S.A.). TMB Microwell Peroxidase Substrate System was purchased from Kirkgaard and Perry Labs Inc. (Gaithesburg, MD, U.S.A.). Ninety-six well Nunc-Immuno plates were from VWR Scientific (Batavia, IL, U.S.A.). All other reagents were reagent grade.

Cell culture

CaCo-2 cells were grown in T-75 flasks as described previously

Abbreviations used: apoB, apoprotein B; apoA, apoprotein A; ACAT, acyl-CoA:cholesterol acyltransferase; LPC, lysophosphatidylcholine.
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[8]. They were subcultured on polycarbonate micropore membranes (0.5 μm pore size) inserted in transwells (Costar, Cambridge, MA, U.S.A.). Unless otherwise specified, inserts of 6.5 mm diameter were used. Cells were initially plated at a density of 2×10^5 cells per filter and medium was changed every two days. Cells were used 14 days after plating.

Bile-acid solutions

Taurocholate was dissolved in 95% ethanol. Lysophosphatidylcholines were kept in a chloroform solution under nitrogen at 4 °C. On the day of the experiment, appropriate amounts of the ethanol and chloroform stock solutions were added together and dried under a stream of nitrogen. Medium-199 (M199) (Earle's Gibco, Grand Island, NY, U.S.A.) containing 10 mM Hepes, pH 7.4, was added to make the correct concentrations of the lipids and vortexed until clear.

Measurement of lipid synthesis and secretion

[^{3}H]-Oleic acid was mixed with unlabelled oleic acid and NaOH (2 mol of NaOH/mol of oleic acid). The mixture was dried under nitrogen and 0.025 ml of 95% ethanol was added. Water, 0.2 ml, was added followed by fatty acid-poor bovine serum albumin so that the ratio of oleic acid to albumin was 3:1 (mol/mol). This solution was stirred well. Dulbecco's Modified Eagle minimum essential medium (Gibco, Grand Island, NY, U.S.A.) was added to make the final concentration of oleic acid 25 μM with a specific activity of 5000 d.p.m./nmol.

After incubating with the various treatments, media were removed and the cells were washed with M199. Fresh M199, 0.6 ml, was added to the lower wells in contact with the basolateral surface of the cells. The media of the upper wells, in contact with the apical surface of the cells, contained the treatments and the labelled oleic acid–albumin solution. At the appropriate time intervals as described for each experiment, the media from the lower wells (basolateral media) were removed and centrifuged at 2000 rev./min. (600 *g*) for 15 min to remove any debris. The apical media were removed and the cells washed three times with M199. Lipids were extracted from the cells and the basolateral media with chloroform/methanol (2:1, v/v). The chloroform phase was taken to dryness under a stream of nitrogen. The residue was taken up in 1 ml of chloroform and 0.1 ml was taken for liquid scintillation counting to estimate the incorporation of oleate into total lipids. The remaining 0.9 ml of chloroform was again dried under a stream of nitrogen. The residue was taken up in 0.125 ml of chloroform and all of it was applied to Silica Gel G thin-layer plates. The plates were developed with hexane/diethyl ether/methanol/acetic acid (70:30:1:1, by volume) and the lipids were visualized with iodine vapour. The bands corresponding to cholesteryl esters, triacylglycerols and phospholipids were scraped into 4 ml of liquid scintillation fluid and counted. Recoveries did not differ significantly among experiments and ranged from 84–87%.

In other experiments, labelled glycerol was used to estimate triacylglycerol and phospholipid synthesis and secretion. Conditions were exactly as described above except that oleic acid was not labelled and 10 μCi of [^{3}H]glycerol (specific activity of 450 d.p.m./pmol) was added to the apical media. Following lipid extraction with chloroform/methanol, the aqueous phase was washed once with chloroform. The combined chloroform phases were washed three times with a 1:1 methanol/water solution, pH 3.0. The chloroform phase was then dried under nitrogen and the lipids separated using the same methodology described above except that only bands corresponding to triacylglycerols and phospholipids were scraped from the plates and counted.

Incorporation of lysophosphatidylcholine into cellular lipids

As final concentrations, [^{14}C]palmitoyllysophosphatidylcholine, 1 μM, specific activity 1600 d.p.m./pmol, was added to the apical medium in 1 mM taurocholate. At time intervals over 4 h, the apical and basolateral media were collected and lipids extracted as described above. The cells were washed three times with M199 and the lipids extracted with chloroform/methanol. In one set of cells, cellular neutral lipids were separated and the radioactivity incorporated was estimated. In another set of cells, the individual phospholipid classes were separated. For phospholipid separation, the elution system used was chloroform/methanol/acetic acid/water/acetone (40:25:2:2:4, v/v). Small amounts of lysophosphatidylcholine, sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine were added before lipid extraction to add mass and to verify their location on t.l.c. The bands were visualized by iodine vapour, scraped from the plates and counted.

ApoB and apoA 1 measurements

The estimation of apoprotein mass by e.l.i.s.a., estimation of apoprotein synthesis and degradation by pulse–chase, and immunoprecipitation of apoproteins were performed exactly as described previously [9]). Immunoblot for detecting apoB in the basolateral medium was performed by collecting the media of cells incubated for 16 h with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine. The proteins were separated on SDS/PAGE (5% stacking and 8% separating gel) by applying to a 1.5 mm thick slab gel, 60 μl of the medium mixed with 10 μl of 2.5 × Laemmli sample buffer. The proteins were transferred to an Immobilon-P membrane (Millipore Corp., Bedford, MA, U.S.A.) at 100 V for 1.5 h using a buffer containing 10% methanol and 25 mM Tris base/192 mM glycine, pH 8.0. The membrane was blocked at 37 °C for 30 min with BLOTTO [3% non-fat dry milk in a blotting buffer containing 50 mM Tris/HCl, pH 8.0/80 mM sodium chloride/2 mM calcium chloride/0.2% Polydet P-40 (Polysciences, Inc., Warrington, PA, U.S.A.) and 0.01% Antifoam A (Sigma)]. The membrane was then incubated for 1 h with 50 μg of anti-human apoB antibody from sheep complexed with horseradish peroxidase (Biodesign, Kennebunkport, ME, U.S.A.) in 10 ml of the blotting buffer. Excess antibody–peroxidase was removed by washing the membrane three times for 5 min each with 3% BLOTTO. This was followed with one wash of phosphate buffered saline. The peroxidase activity associated with the apoprotein bands was detected by rapid screen web (IBI Enzygraphic Web, International Biotechnologies Inc., Eastman Kodak, New Haven, CT, U.S.A.). The amount of apoB protein in the bands was quantified by densitometry using the Ambis Image Acquisition and Analysis System (Ambis Inc., San Diego, CA, U.S.A.).

Chemical analysis and enzyme activities

Protein was measured according to the method of Lowry et al. [10]. Triacylglycerol mass was measured as previously described [11]. Phospholipid mass was estimated by the method described by Chalvardjian and Rudnicki [12]. Acyl-CoA:cholesterol acyltransferase (ACAT) [13] and triacylglycerol synthetase [11] activities were estimated in total membranes as described previously [14]. The specific activity of [^{14}C]oleoyl CoA was 19000 d.p.m./nmol.

RESULTS

Uptake of lysophosphatidylcholine and its incorporation into lipids

To estimate the uptake of lysophosphatidylcholine and its incorporation into cellular lipids, 1 μM labelled palmitoyllysophosphatidylcholine solubilized in 1 mM taurocholate was added to the apical medium of CaCo-2 cells. The percent uptake was estimated by determining the amount of labelled lysophosphatidylcholine remaining in the apical medium over time. Results in Figure 1 show that 1 μM lysophosphatidylcholine was completely removed from the medium after 3 h and after 30 min approximately 50% of it was taken up. No free fatty acids were found in the apical medium during the incubation suggesting that little or no palmitoyllysophosphatidylcholine was being hydrolysed apically.

To determine the fate of lysophosphatidylcholine within the cell, lipids were extracted from the basolateral medium and cells.

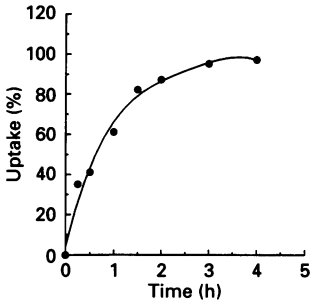

Figure 1 Uptake of lysophosphatidylcholine

CaCo-2 cells grown on semipermeable membranes were incubated with 1 mM taurocholate and 1 μM [^{14}C]palmitoyllysophosphatidylcholine (1600 d.p.m./pmol). At the times indicated, total lipids were extracted from the apical medium. The data points represent the loss of radioactivity within the total lipid fraction of the apical medium expressed as a percentage of the amount of labelled palmitoyllysophosphatidylcholine added. n = Mean of two transwells at each time-point from two separate experiments.

Table 1 Incorporation of [^{14}C]palmitoyllysophosphatidylcholine into cellular lipids

Cells grown on semipermeable membranes were incubated with 1 mM taurocholate containing 1 μM labelled palmitoyllysophosphatidylcholine. At the time points indicated in Figure 1, the cells were washed twice with M199 medium and the lipids were extracted from the cells and basolateral medium (lower wells). Neutral and polar lipids were separated by t.l.c. as described in Methods. Because the percentage of the labelled lysophosphatidylcholine incorporated into lipids was similar at each time point, the data were expressed as a percentage of the label incorporated into total lipids and phospholipids. The percentages represent the mean ± S.E.M. of 15 transwells from two separate experiments.

	Incorporated (% of total)
Cellular lipids	
Phospholipids	75 ± 1.9
Triacylglycerols	25 ± 1.9
Cholesteryl esters	0.6 ± 0.1
Cellular phospholipids	
Phosphatidylcholine	85 ± 1.3
Phosphatidylethanolamine	6.0 ± 0.8
Lysophosphatidylcholine	2.2 ± 0.2
Phosphatidylserine, phosphatidylinositol, sphingomyelin	6.6 ± 0.8

Within the cell, 75% of the labelled fatty acid of lysophosphatidylcholine remained in phospholipids with most of the remainder being incorporated into triacylglycerols (Table 1). Less than 1% of the label was found in cholesteryl esters. Thus, at minimum, 25% of absorbed lysophosphatidylcholine was hydrolysed with the released fatty acids being re-utilized for triacylglycerol synthesis.

The predominant fate of absorbed lysophosphatidylcholine was the resynthesis of phosphatidylcholine (Table 1). Eighty-five percent of the label found in phospholipids was in phosphatidylcholine. The next major class was phosphatidylethanolamine. Only 2.2% of the phospholipid label remained as lysophosphatidylcholine. It is likely that most of the label found in phosphatidylcholine was secondary to direct acylation of lysophosphatidylcholine. The contribution from reincorporation of hydrolysed labelled palmitic acid back into phosphatidylcholine cannot be determine from the data.

In data not shown, 4% of the intracellular triacylglycerol label was secreted into the basolateral medium after 4 h. In contrast, only 0.8% of the intracellular phospholipid label was secreted after 4 h. Triacylglycerol accounted for 57% of the total labelled lipids secreted basolaterally compared with 43% found in phospholipids. No detectable label was found in free fatty acids or cholesteryl esters in the basolateral medium.

Lipid synthesis and secretion

To determine the effect of lysophosphatidylcholine on the synthesis and secretion of lipids, cells were incubated for 16 h with medium alone, medium containing 1 mM taurocholate or medium containing 1 mM taurocholate and increasing concentrations of lysophosphatidylcholine. After the incubation, labelled oleic acid was added and the incorporation of label into cellular and basolateral lipids was estimated. Table 2 shows these results. Since taurocholate did not significantly alter the incorporation of labelled oleic acid into lipids compared with control, only data from cells incubated with taurocholate alone and taurocholate plus lysophosphatidylcholine are shown. The predominant effect of lysophosphatidylcholine on the incorporation of labelled oleic acid into cellular lipids was a profound, concentration-dependent decrease in the synthesis of cholesteryl esters. The incorporation of oleate into phospholipids and triacylglycerols was not altered except at 250 μM lysophosphatidylcholine, at which concentration the amount of label found within triacylglycerols was modestly decreased. In the basolateral medium, however, there was a concentration-dependent increase in the secretion of labelled phospholipids and triacylglycerols. Because of a decrease in the amount of labelled cholesteryl esters found within cells incubated with lysophosphatidylcholine, the amount of cholesteryl esters secreted by these cells was also decreased.

Figure 2 shows results of the effect of 1 mM taurocholate and 250 μM lysophosphatidylcholine on the incorporation of labelled oleate into cellular and basolateral lipids over 4 h. Again, a significant decrease in the synthesis and secretion of labelled cholesteryl esters was observed. Lysophosphatidylcholine had only modest effects on the synthesis of phospholipids and triacylglycerols. In contrast, the amount of labelled phospholipids and triacylglycerols which were secreted by cells incubated with lysophosphatidylcholine was significantly increased compared with controls.

When cells were incubated with lysophosphatidylcholine containing a specific fatty acid the effects on lipid synthesis and secretion were similar to those observed with egg lysophosphatidylcholine, with the exception of oleoyllysophosphatidylcholine

Table 2 Effect of lysophosphatidylcholine on [^{3}H]oleic acid incorporation into cellular and basolateral lipids

Cells cultured on semipermeable membranes were incubated 16 h with 1 mM taurocholate or taurocholate and increasing concentrations of lysophosphatidylcholine. Following the incubation, [^{3}H]oleic acid attached to albumin was added for 4 h and the incorporation of label into cellular- and basolateral-medium lipids was determined as described in Methods. The data represent the mean ± S.E.M. of four transwells at each concentration from two experiments. LPC, lysophosphatidylcholine

	(pmol incorporated/4 h per dish)			
	Total lipids	Phospholipids	Triacylglycerols	Cholesteryl esters
Cells				
Taurocholate	1418 ± 68	482 ± 20	404 ± 23	28 ± 1
+ 50 μM LPC	1477 ± 30	508 ± 14	413 ± 9	17 ± 1***
+ 100 μM LPC	1313 ± 27	472 ± 12	406 ± 9	7 ± 0.4***
+ 250 μM LPC	1262 ± 20	466 ± 9	322 ± 9*	1 ± 0.1***
Medium				
Taurocholate	12 ± 0.6	2.3 ± 0.1	4.8 ± 0.3	0.5 ± 0.0
+ 50 μM LPC	13 ± 0.4	2.8 ± 0.1*	5.9 ± 0.2*	0.4 ± 0.0**
+ 100 μM LPC	18 ± 0.6***	3.6 ± 0.1***	8.7 ± 0.3***	0.2 ± 0.0***
+ 250 μM LPC	28 ± 0.4***	4.9 ± 0.1***	15 ± 0.3***	0.1 ± 0.0***

* $P < 0.05$ versus taurocholate.
** $P < 0.01$ versus taurocholate.
*** $P < 0.001$ versus taurocholate.

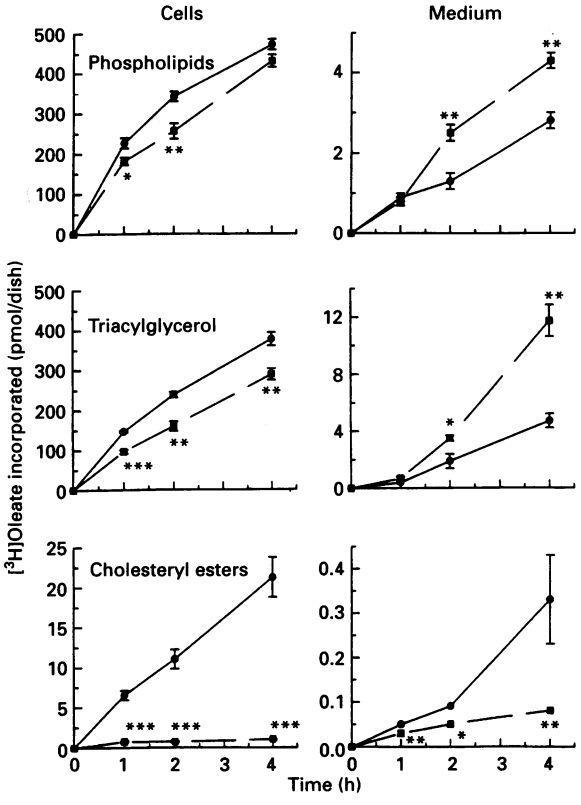

Figure 2 Effect of lysophosphatidylcholine on [^{3}H]oleic acid incorporation into cellular and basolateral lipids

Cells were grown on semipermeable membranes and incubated for 16 h with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine. Following the incubation, [^{3}H]oleic acid was added and the incorporation of label into cellular- and basolateral-medium lipids was determined as described in Methods. The data represent the mean ± S.E.M. from two separate experiments of 4–10 transwells at each time point. (●) Taurocholate; (■) taurocholate and lysophosphatidylcholine. * $P < 0.05$ versus taurocholate; ** $P < 0.01$ versus taurocholate; *** $P < 0.001$ versus taurocholate.

(Table 3). This species caused an increase in the incorporation of oleate into cellular triacylglycerols and cholesteryl esters compared with palmitate- and stearate-containing lysophosphatidylcholines.

To substantiate the changes observed in the rates of lipid synthesis and secretion, experiments were repeated using labelled glycerol as substrate to estimate phospholipid and triacylglycerol synthesis (Figure 3). In contrast to the results using labelled oleate, lysophosphatidylcholine caused a significant increase in the rate of incorporation of labelled glycerol into cellular phospholipids and triacylglycerols, whereas the secretion of labelled phospholipids was not altered. Confirming results with labelled oleic acid, however, lysophosphatidylcholine increased the basolateral secretion of labelled triacylglycerols.

The observed increase in the secretion of newly synthesized triacylglycerols by lysophosphatidylcholine might be explained by intracellular hydrolysis of the fatty acid contained in lysophosphatidylcholine which then drives triacylglycerol synthesis and secretion. To address this possibility, cells were incubated with 1 mM taurocholate, taurocholate and 250 μM lysophosphatidylcholine, or taurocholate and 250 μM palmitic acid. The incorporation of labelled glycerol into cellular and basolateral lipids was then estimated (Figure 4). Although palmitic acid did increase the incorporation of glycerol into cellular triacylglycerols compared with taurocholate alone, it was less than that observed in cells incubated with lysophosphatidylcholine. Moreover, in cells incubated with palmitic acid, the secretion of labelled triacylglycerols was similar to taurocholate alone, whereas in cells incubated with lysophosphatidylcholine, the secretion of newly synthesized triacylglycerols was markedly enhanced more so than in either control cells or cells incubated with the fatty acid. Thus, hydrolysis of the lysophosphatidylcholine fatty acid cannot explain the increase in triacylglycerol transport observed in cells incubated with lysophosphatidylcholine.

ACAT and triacylglycerol synthetase activities

To further address the effect of lysophosphatidylcholine on cholesteryl ester and triacylglycerol synthesis, ACAT and triacyl-

Table 3 Effect of fatty acid composition of lysosphosphatidylcholine on [^{3}H]oleic acid incorporation into cellular and basolateral lipids

Cells grown on semipermeable membranes were incubated 16 h with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine containing palmitate, stearate or oleate. Following the incubation, the incorporation of labelled oleic acid into cellular- and basolateral-medium lipids was determined. The data represent the mean ± S.E.M. of 4–6 transwells. LPC, lysophosphatidylcholine.

	(pmol incorporated/4 h per dish)		
	Phospholipids	Triacylglycerols	Cholesteryl esters
Cells			
Taurocholate	590 ± 18	386 ± 5	18 ± 1
+ palmitoyl-LPC	418 ± 21*	279 ± 11*	1.8 ± 0.2*
+ stearoyl-LPC	416 ± 11*	281 ± 6*	1.8 ± 0.1*
+ oleoyl-LPC	451 ± 14*	744 ± 12*	5.9 ± 0.2*
Medium			
Taurocholate	2.72 ± 0.02	1.66 ± 0.10	0.17 ± 0.00
+ palmitoyl-LPC	3.66 ± 0.06*	5.28 ± 0.22*	0.09 ± 0.00*
+ stearoyl-LPC	3.91 ± 0.10*	6.11 ± 0.51*	0.09 ± 0.00*
+ oleoyl-LPC	3.97 ± 0.09*	9.17 ± 0.18*	0.14 ± 0.00

* $P < 0.001$ versus taurocholate.

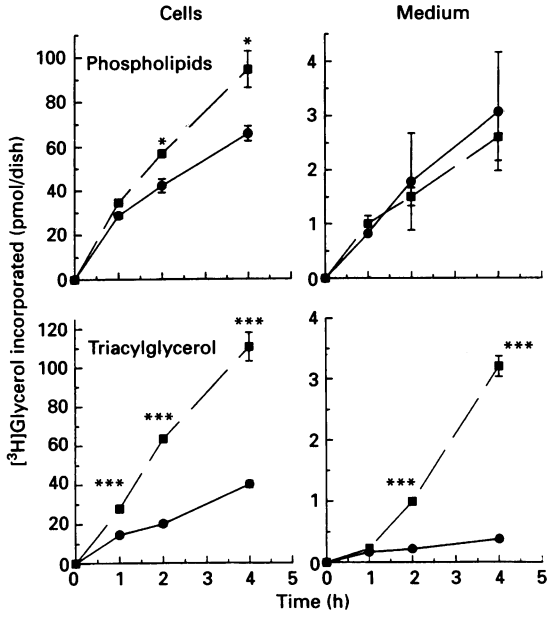

Figure 3 Effect of lysophosphatidylcholine on [^{3}H]glycerol incorporation into cellular and basolateral triacylglycerols and phospholipids

The cells were incubated as described in Figure 2. Following the incubation, the incorporation of [^{3}H]glycerol into cellular and basolateral lipids was determined as described in Methods. The data represent the mean ± S.E.M. of three transwells at each time-point. (●) Taurocholate; (■) taurocholate and 250 μM lysophosphatidylcholine. * $P < 0.05$ versus taurocholate; *** $P < 0.001$ versus taurocholate.

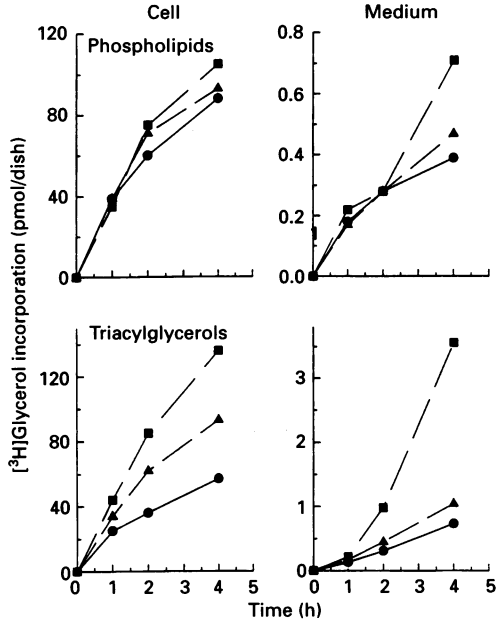

Figure 4 Effect of palmitic acid on [^{3}H]glycerol-incorporation into cellular and basolateral triacylgycerols and phospholipids

Cells grown on semipermeable membranes were incubated 16 h with 1 mM taurocholate, taurocholate and 250 μM lysophosphatidylcholine or taurocholate and 250 μM palmitic acid. Following the incubation the incorporation of [^{3}H]glycerol into cellular and basolateral triacylglycerols and phospholipids was estimated. The data represent the mean of two transwells at each time-point. (●) Taurocholate; (■) taurocholate and lysophosphatidylcholine; (▲) taurocholate and palmitic acid.

glycerol synthetase activities were determined in total membranes prepared from cells incubated with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine. To determine whether lysophosphatidylcholine decreased the rate of cholesterol esterification by inhibiting the ACAT enzyme directly or by causing a decrease in its supply of cholesterol, ACAT activity was estimated in the presence or absence of cholesterol added to the assay. The results shown in Table 4 demonstrate that membranes prepared from cells incubated with lysophosphatidylcholine contained significantly more triacylglycerol synthetase activity compared with controls. In the absence of added cholesterol, ACAT activities were substantially lower in membranes from cells incubated with lysophosphatidylcholine. In contrast, ACAT activities were similar in both membrane

Table 4 Effect of lysophosphatidylcholine on ACAT and triacylglycerol synthetase activities

Cells grown on semipermeable membranes were incubated for 16 h with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine. Following the incubation, the monolayers were washed, the cells scraped from the filters and total membranes prepared. Triacylglycerol synthetase activity and ACAT activity with and without added cholesterol to the assay were determined. The data represent the mean ± S.E.M. of six separate membrane preparations for each treatment with the assays performed in duplicate.

	(pmol/mg per 10 min)		
	ACAT	ACAT with cholesterol	Triacylglycerol synthetase
Taurocholate	442 ± 14	1472 ± 87	1280 ± 56
Taurocholate + lysophosphatidylcholine	134 ± 5*	1434 ± 128	2057 ± 81*

* $P < 0.001$ versus taurocholate.

preparations when exogenous cholesterol was added to the assay.

Triacylglycerol and phospholipid mass

The data so far suggest that lysophosphatidylcholine increased the synthesis and secretion of triacylglycerols by CaCo-2 cells. To address whether the uptake of lysophosphatidylcholine increased the secretion of triacylglycerol and/or phospholipid mass, cells were incubated for 16 h with taurocholate or taurocholate and 250 μM of lysophosphatidylcholine. In cells incubated with lysophosphatidylcholine, cellular triacylglycerol mass increased from 60 to 100 μg/mg cell protein ($P < 0.001$). Total membrane phospholipids were also increased in cells incubated with lysophosphatidylcholine, 368 ± 18 versus 309 ± 18 nmol/mg protein ($P < 0.05$). The basolateral-secretion of phospholipids was not altered, but the secretion of triacylglycerols increased from 4.2 μg/dish to 6.0, $P < 0.01$, in cells incubated with lysophosphatidylcholine compared with cells incubated with taurocholate alone.

ApoB mass

To determine if the increase in triacylglycerol transport by cells incubated with lysophosphatidylcholine was associated with an increase in the secretion of lipoprotein particles, apoB mass within cells and that secreted into the basolateral medium was estimated following a 16 h incubation with 1 mM taurocholate or taurocholate and 250 μM lysophosphatidylcholine. ApoB mass was 35% higher in cells incubated with 250 μM lysophosphatidylcholine compared with controls, 62 ± 2 ng/well versus 46 ± 1 ng/well, respectively ($P < 0.001$). The secretion of apoB mass by cells incubated with lysophosphatidylcholine was increased by 31%, 138 ± 7 ng/well versus 105 ± 5 ng/well, $P < 0.001$ ($n = 24$ from three separate experiments). Taurocholate decreased the secretion of apoB mass by approximately 40% compared with the amount secreted by cells incubated with medium alone ($P < 0.001$).

To further address and substantiate the effect of lysophosphatidylcholine on total apoB mass secretion, apoB 100 and apo-B 48 were estimated by immunoblots of basolateral media from cells incubated for 16 h with 1 mM taurocholate with or without 250 μM lysophosphatidylcholine (Figure 5). In support of what was observed using the more sensitive e.l.i.s.a. for estimating apoB mass, in four separate experiments lysophosphatidylcholine increased the total amount of apoB 100 and apoB 48 secreted.

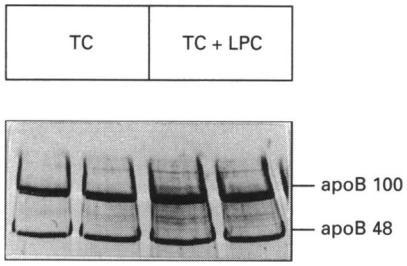

Figure 5 Immunoblot of apoB secreted into the basolateral medium

Cells grown on semipermeable membranes were incubated 16 h with 1 mM taurocholate with or without 250 μM lysophosphatidylcholine. Following the incubation, the basolateral media were collected and apoB was detected by standard immunoblotting techniques exactly as described in Methods. This figure represents one experiment of four separate experiments done in duplicate all showing similar results. TC, 1 mM taurocholate; TC + LPC, 1 mM taurocholate and 250 μM lysophosphatidylcholine.

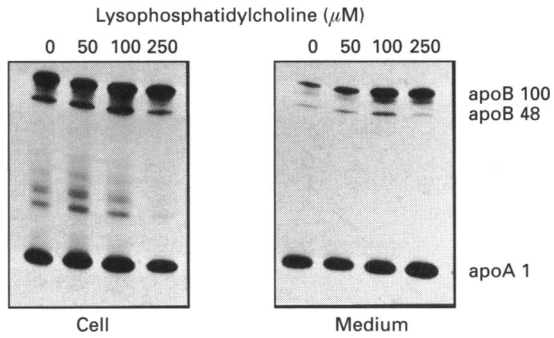

Figure 6 Effect of lysophosphatidylcholine on the synthesis and secretion of apoB

Cells grown on semipermeable membranes were incubated 16 h with 1 mM taurocholate or taurocholate and increasing concentrations of lysophosphatidylcholine. Following the incubation the cell monolayers were incubated with 100 μCi [^{35}S]methionine. At 4 h the cells and basolateral media were harvested, apoB and apoA 1 immunoprecipitated and the apoproteins separated by SDS/PAGE. The Figure is a representative autoradiogram from one of three separate experiments showing similar results.

ApoB synthesis and secretion

To address whether lysophosphatidylcholine increased the synthesis and/or secretion of newly synthesized apoB, CaCo-2 cells were incubated for 16 h with taurocholate or taurocholate and increasing concentrations of lysophosphatidylcholine. [^{35}S]Methionine was then added to the apical medium for 4 h and the incorporation of label into cellular and basolateral apoB and apoA 1 was estimated. Figure 6 shows a representative autoradiogram of a polyacrylamide gel following immunoprecipitation and separation of the apoproteins by electrophoresis. Lysophosphatidylcholine did not alter the incorporation of labelled methionine into cellular apoB or apoA 1. However, in a

concentration-dependent manner, the secretion of labelled apoB was increased by lysophosphatidylcholine without altering the secretion of labelled apoA 1.

DISCUSSION

Results from animal studies have suggested that luminal phosphatidylcholine plays a role in the absorption of lipids from the intestine [4]. In bile-diverted lymph-fistula rats, Bennett Clark [6] observed that triacylglycerol output was significantly enhanced when phosphatidylcholine was included in the duodenal infusion. Interestingly, the uptake of lipids from the lumen into the intestinal mucosa was not altered. Thus, compared with intestines of control animals, intestines of animals infused with phosphatidylcholine took up similar amounts of lipids from the lumen, but secreted more triacylglycerols into the lymph. Mansbach et al. [7] also observed that the addition of phosphatidylcholine to a duodenal perfusion in lymph-cannulated animals, significantly increased the lymphatic secretion of newly synthesized triacylglycerol and triacylglyercol mass. Others have made similar observations [5]. It was suggested that phosphatidylcholine may be limiting in triacylglycerol transport in the intestines of rats with biliary fistulae [7]. There is evidence however to suggest otherwise. Luminal phosphatidylcholine was found to enhance triacylglycerol transport into the lymph even in the presence of an intact biliary system; conditions in which phosphatidylcholine would not be limiting [6]. Moreover, the duodenal infusion of phosphatidylcholine into lymph-fistula animals did not increase the lymphatic transport of phosphatidylcholine [6]. If phosphatidylcholine were limiting, the opposite would be expected to occur. Our data do not specifically address the issue of phosphatidylcholine availability during triacylglycerol transport, however, unlike previous animal studies in which triacylglycerol absorption was enhanced, CaCo-2 cells secreted significantly more triacylglycerol in response to lysophosphatidylcholine in the absence of an increase in triacylglycerol absorption; conditions in which phosphatidycholine should not be limiting.

Since little phosphatidylcholine is absorbed intact by the intestine, it has been suggested, but not proven, that luminal phosphatidylcholine increases lipid transport by the uptake of its hydrolytic product, lysophosphatidylcholine [5]. Results from the present study show that the uptake of apical lysophosphatidylcholine increases the secretion of newly synthesized triacylglycerol and triacylglycerol mass. This would appear to be in response to a substantial increase in the rate of intracellular triacylglycerol synthesis and the accumulation of triacylglycerol mass. As shown in Figure 4, hydrolysis of the fatty acid of lysphosphatidylcholine and its reincorporation into triacylglycerols cannot explain the increase in synthesis and secretion of triacylglycerols. Moreover, only 25% of the fatty acid label in lysophosphatidylcholine was incorporated into cellular triacylglycerols. This would represent only 6.3 nmol of fatty acid utilized for triacylglycerol synthesis, an amount insufficient to account for the marked increase in triacylglycerol mass observed in cells incubated with 250 μM lysophosphatidylcholine. In contrast, the data demonstrate that 75% of [^{14}C]palmitoyllysophosphatidylcholine was incorporated into phospholipids suggesting that most lysophosphatidylcholine was being utilized for resynthesis of phosphatidylcholine. Although an increase in phospholipid synthesis from glycerol or oleic acid was not observed, phospholipids, particularly phosphatidylcholine, were being produced by reacylation of the absorbed lysophosphatidylcholine. An increase in cellular phosphatidylcholine mass, particularly of the endoplasmic reticulum (ER), may have contributed to the observed increase in triacylglycerol synthetase activity in membranes prepared from cells incubated with lysophosphatidylcholine. These data would support an earlier observation by O'Doherty et al. [15] demonstrating an increase in the activities of mono- and di-acylglycerol acyltransferases in intestinal microsomes enriched in phosphatidylcholine.

An increase in the secretion of triacylglycerol by cells incubated with lysophosphatidylcholine was associated with an increase in the secretion of lipoprotein particles as estimated by the amount of [^{35}S]-methionine labelled and immunoreactive apoB secreted. There is ongoing debate as to which lipid or lipids might regulate apoB secretion (see ref. [16] for review). The present data would argue against the importance of newly synthesized cholesteryl esters in apoB secretion as triacylglycerol and apoB secretion were increased in cells in which rates of cholesteryl ester synthesis were markedly decreased by lysophosphatidylcholine. Phosphatidylcholine synthesis has been shown to be required for triacylglycerol-rich lipoprotein secretion by hepatocytes from choline-deficient animals [17]. Since cells in the present study were not actively transporting triacylglycerols, there is no reason for phosphatidylcholine to be limiting. It is unlikely, therefore, that an increase in baseline cellular phosphatidylcholine would cause an increase in the secretion of triacylglycerol-rich lipoproteins. It has been postulated that oleic acid increases apoB secretion by causing a rapid association of apoB with newly synthesized triacylglycerols within the lumen of the endoplasmic reticulum [18]. This, in turn, leads to a decrease in the intracellular degradation of apoB and an increase in its secretion. Lysophosphatidylcholine may be acting similarly by making more newly synthesized triacylglycerol available to apoB.

In cells incubated with lysophosphatidylcholine, the rate of cholesterol esterification and the secretion of labelled cholesteryl esters were found to be significantly decreased. In lymph-fistula rats, Bennett Clark [6] observed that the inclusion of phosphatidylcholine in duodenal infusions caused a marked decrease in the lymphatic transport of cholesteryl esters. Inhibition of intestinal ACAT was implicated as a mechanism to explain this observation, although the activity was never measured. The present data substantiate and extend the observations made in the intact animal. The addition of lysophosphatidylcholine to the apical medium did decrease the rate of cholesterol esterification by decreasing the influx of cholesterol to the ACAT pool. By disrupting normal cholesterol trafficking, i.e. the recycling of plasma membrane cholesterol into the cell [19], luminal lysophosphatidylcholine or the uptake of lysophosphatidylcholine by the intestinal cell could lead to a decrease in cholesterol absorption while at the same time increase triacylglycerol-rich lipoprotein secretion.

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