Phospholipase A_2 relieves phosphatidylcholine inhibition of micellar cholesterol absorption and transport by human intestinal cell line Caco-2[1]

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Abstract **Cholesterol absorption from bile acid micelles is suppressed by phosphatidylcholine (PC) in the micelles. The effects of micellar phospholipid composition on absorption, metabolism, and secretion of lipids were examined in Caco-2 cells incubated with micelles composed of taurocholic acid, cholesterol, oleic acid, monooleoylglycerol, and phospholipid. Significant amounts of all micelle lipids were absorbed from micelles lacking phospholipid. Cholesterol absorption was accompanied by cholesterol esterification and secretion. Micellar oleic acid was also absorbed and reesterified primarily into triacylglycerol which was also secreted. Lipid absorption and secretion from micelles containing lysophosphatidylcholine (LPC) were similar to that obtained with phospholipid-free micelles. LPC was also extensively absorbed. In contrast, incubations with PC-containing micelles resulted in large reductions in the absorption, esterification, and secretion of cholesterol without significant decreases in oleic acid absorption, conversion to acylated lipids, or triacylglycerol secretion. A relatively small reduction in monoacylglycerol absorption from PC-containing micelles was detected. Retinol absorption was not affected by micellar PC. Substitution of LPC for half or more of the PC reversed the PC-dependent decrease in cholesterol absorption. Pancreatic phospholipase A_2 ($pPLA_2$) enhanced cholesterol absorption from PC-containing micelles. The $pPLA_2$-dependent increase in cholesterol absorption was inhibited by the $pPLA_2$ inhibitor FPL 67047XX. The results indicate micellized cholesterol absorption by enterocytes is uniquely dependent on the elimination of micellar phosphatidylcholine and thus directly dependent on the lipolytic action of pancreatic phospholipase A_2 ($pPLA_2$). Consequently, $pPLA_2$ inhibitors may be a new and novel class of cholesterol absorption inhibitors for therapeutic use.**—Homan, R., and K. L. Hamelehle. **Phospholipase A_2 relieves phosphatidylcholine inhibition of micellar cholesterol absorption and transport by human intestinal cell line Caco-2.** ***J. Lipid Res.*** **1998.** 39: **1197–1209.**

Supplementary key words enterocyte • micelle • bile acid • phospholipid • monoacylglycerol • triacylglycerol • fatty acid • lysophosphatidylcholine • retinol • FPL 67047XX

The aqueous solubility of cholesterol is extremely low (1), yet almost half of the 1 to 1.5 grams of combined biliary and dietary cholesterol that enter the intestinal lumen of the adult human each day (2) are absorbed through the aqueous interface separating intestinal contents from intestinal epithelium. The diffusion of cholesterol from the lipid-rich phases of the intestinal contents to the epithelium is directly dependent on emulsification and micellar solubilization by the biliary lipids and the detergent-like products of dietary lipid lipolysis (3, 4). Phosphatidylcholine is one such emulsifier that is essential for this solubilization process, particularly in the bile (5). Biliary cholesterol, which constitutes the major portion of cholesterol entering the intestine (2) and thus the primary source of absorbed cholesterol, cannot be effectively solubilized in bile without biliary phosphatidylcholine (5). But, contrary to the predominant idea that lipid absorption is directly dependent on lipid dispersion and solubilization, there are indications that phosphatidylcholine may actually suppress cholesterol absorption despite the promotion of cholesterol solubilization. Much of the evidence for such a dichotomy has been obtained in in vitro studies examining the diffusion of cholesterol from micellar preparations to intestinal segments. These studies have shown that addition of phosphatidylcholine to the micelles greatly restricts cholesterol uptake by the segments (6–11). This suppression of cholesterol absorption by phosphatidylcholine has also been observed in perfused intestines (12), with brush-border membranes (13) and with isolated enterocytes (14). The dominant hypothesis to emerge from these many studies suggests that cholesterol absorption is reduced by a phosphatidylcholine-dependent shift of the lipid–water partitioning of cholesterol towards the micellar phase (9–11).

Abbreviations: $pPLA_2$, pancreatic phospholipase A_2; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; HBS, HEPES-buffered saline (150 mm NaCl, 5 mm HEPES, 1 mm EDTA, pH 7.4).

[1]Presented in part at the XII International Symposium on Drugs Affecting Lipid Metabolism, November 7–10, 1995, Houston, TX.

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Although there are numerous examples of restricted cholesterol absorption in the presence of phosphatidylcholine in vitro, the fact that lysophosphatidylcholine, the product of phosphatidylcholine lipolysis in the gut, does not inhibit micellar cholesterol absorption in vitro (8, 11) and the knowledge that pancreatic phospholipase A_2 ($pPLA_2$) is quite effective in hydrolyzing phosphatidylcholine to lysophosphatidylcholine and fatty acid in the intestinal contents (15) makes it uncertain whether phosphatidylcholine could actually interfere with cholesterol absorption in the gut. However, observations from animal and human studies suggest that phosphatidylcholine may indeed interfere with cholesterol absorption in vivo. For example, administration of phosphatidylcholine (12, 16) or a nonhydrolyzable diether phosphatidylcholine analog (17) to rats resulted in reduced cholesterol absorption. Similarly, in human subjects it was observed that dietary supplementation with phosphatidylcholine resulted in decreased cholesterol absorption (18, 19). The same was observed in a more direct analysis involving subjects that were intraduodenally infused with a cholesterol-rich lipid emulsion containing phosphatidylcholine (20).

Previous studies have focused on the association between aqueous phase lipid content and the translocation of cholesterol to intestinal epithelium. The relationship between phospholipid content of the extracellular medium and cellular lipid metabolism and secretion remains largely unexamined. The experimental inaccessibility of the intestinal epithelium in vivo and the limited viability of enterocytes in intestinal segments in vitro have prevented more detailed investigation of the potential effects of micellar phosphatidylcholine on lipid metabolism within the enterocytes of the intestinal epithelium. Therefore, we have used Caco-2 cells as a tissue culture model of the human intestinal epithelium to compare the effects of phosphatidylcholine and lysophosphatidylcholine on lipid absorption and the subsequent reesterification and secretion of absorbed lipids. Caco-2 cells are derived from a human colon adenocarcinoma and spontaneously form polarized cell monolayers in culture that exhibit many of the morphological and functional characteristics of normal enterocytes including the production and polarized secretion of lipoproteins synthesized from absorbed lipids (21, 22).

MATERIALS AND METHODS

Materials

[9,10-^{3}H]oleic acid and 1-[1-^{14}C]palmitoyl lysophosphatidylcholine were purchased from New England Nuclear (Boston, MA). [1,2α-^{3}H]cholesterol, [4-^{14}C]cholesterol, and 1-palmitoyl-2-[1-^{14}C]oleoyl phosphatidylcholine were obtained from Amersham (Arlington Heights, IL). Radiochemical purity was confirmed by high-performance liquid chromatographic analysis (see below). 1-Palmitoyl-2-oleoyl phosphatidylcholine and 1-palmitoyl lysophosphatidylcholine were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), cholesterol, retinol, 1-monooleoyl-*rac*-glycerol, oleic acid, sodium taurocholate, δ-tocopherol, bovine serum albumin (essentially fatty acid-free) (BSA), porcine pancreas phospholipase A_2, and fetal bovine serum were obtained from Sigma Chemical Co. (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l glucose, 25 mm HEPES, glutamine-free) was purchased from Life Technologies, Inc. (Grand Island, NY).

Cell culture

Caco-2 cells (American Type Culture Collection, Rockville, MD) were grown in T-75 flasks (Corning Glassworks, Corning, NY) containing DMEM supplemented with 20% fetal bovine serum. Cells were kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The growth medium was replenished every 48 h. Cells were reseeded when the cell monolayers became 60–80% confluent. For experiments, cells were seeded at 1×10^5 cells/well in 6-well multiwell plates or in Transwell® filter chambers (polycarbonate membrane, 0.4 μm pore size; Costar, Cambridge, MA) at 1×10^6 cells/well and grown under the same conditions as those described above with the exception that 50 μg/ml gentamycin was included in the growth medium and cells were cultured for 20 to 22 days to obtain maximum differentiation. Serum-free DMEM was used in the outer well of culture plates with filter chambers.

Lipid preparation and gel filtration

Micelles composed of 5 mm sodium taurocholate, 500 μm oleic acid, 300 μm monooleoylglycerol, 200 μm phospholipid, and 100 μm cholesterol were prepared by combining the appropriate volumes of 100 mm lipid stock solutions in a sterile bottle. Stock solutions included sodium taurocholate in ethanol (95%), phospholipids in chloroform–methanol 2:1, oleic acid and retinol in absolute ethanol, and the remaining lipids in chloroform. When radiolabeled lipids were included in the micelles, the final specific activities were 3 μCi/μmol [4-^{14}C]cholesterol, 1 μCi/μmol [9,10-^{3}H]oleic acid, 1.6 μCi/μmol 1-palmitoyl-2-[1-^{14}C]oleoyl phosphatidylcholine or 1.5 μCi/μmol 1-[1-^{14}C]palmitoyl lysophosphatidylcholine. The solvents were removed from the combined lipid solutions by vacuum in a vacuum desiccator connected to a water aspirator. DMEM containing 500 μm BSA was added to the dried lipids at a volume corresponding to 20% of the final volume. The remaining 80% of the volume was made up with DMEM. The resultant solutions were optically clear.

The size homogeneity of micelle preparations was determined by gel filtration chromatography of micelle samples prepared without radiolabeled lipids in 150 mm NaCl, 1 mm HEPES (pH 7.5) and 0.02% NaN_3 at lipid concentrations 2.5-fold greater than the concentrations used in cell incubations. The micelle preparations were chromatographed at room temperature through a column of Sepharose 4B-CL (1.5 cm diameter, 23 cm high) with elution buffer (5 mm sodium taurocholate, 150 mm NaCl, 1 mm HEPES (pH 7.5), 0.02% NaN_3) at a rate of 2.65 ml/h. Fractions were collected in 20-min increments (0.9 ml/fraction). The lipid content of each fraction was determined by HPLC as described below. The void volume was determined by elution of phosphatidylcholine vesicles that were prepared by extrusion through a polycarbonate filter of 0.1 μm pore size with a Mini-Extruder® (Avanti Polar Lipids, Inc., Alabaster, AL). Total column volume was determined by the elution of the fluorescent dye 8-hydroxy-1,3,6-pyrenetrisulfonic acid prepared at 0.5 mm in elution buffer.

Triacylglycerol-rich lipid emulsions were prepared by solvent injection. To prepare 20 mL of emulsion in DMEM, aliquots of chloroform stock solutions equivalent to 6 μmol trioleoylglycerol, 4 μmol 1-palmitoyl-2-oleoyl phosphatidylcholine, 0.4 μmol cholesterol, and 0.1 μmol cholesteryl oleate were combined with 10 μCi 1-palmitoyl-2-[1-^{14}C]oleoyl phosphatidylcholine and 50 μCi [1,2α-^{3}H]cholesterol. The solvents were removed by evapora-

tion under a stream of nitrogen followed by vacuum desiccation before redissolution in 0.4 mL dioxane. The dioxane solution was injected through a 22-ga needle into 2 mL of HEPES buffered saline (HBS, 150 mm NaCl, 5 mm HEPES, 1 mm EDTA, pH 7.4) that was vigorously agitated in a bath sonicator. Dioxane was removed from the resultant lipid emulsion by three cycles of dialysis (3500 MW cutoff) against HBS followed by one cycle against DMEM. The dialysate was combined with 2 ml of 50 mm sodium taurocholate in DMEM and diluted to a final volume of 20 mL with DMEM.

Lipid extraction and analysis

To isolate cell lipids, the cell culture plates were placed on ice, the incubation media were removed, and the plates were washed once with ice-cold 0.3% (w/v) BSA in HBS followed by two washes with ice-cold HBS. The cells were scraped from the culture plates in HBS and transferred to screw-cap test tubes. The cells were pelleted by centrifugation at 250 *g* for 15 min. The supernatants were discarded and the cell pellets were disrupted under nitrogen in 1 ml of HBS by bath sonication. An aliquot of each cell homogenate was removed for determination of protein content by the method of Lowry et al. (23) (BSA standard). Lipids were extracted from the remaining cell homogenate at room temperature by the method of Slayback, Cheung, and Beyer (24) with 3 ml of ethyl acetate–acetone 2:1 containing 0.01% (w/v) butylated hydroxytoluene. Media lipids were extracted by the same method with three volumes of extraction solvent for each volume of medium. All extracts were dried under a stream of N_2 followed by vacuum desiccation before resolubilization in isooctane–tetrahydrofuran 9:1 for HPLC analysis. An aliquot of each resolubilized lipid extract was taken for measurement of ^{14}C and ^{3}H content by liquid scintillation counting.

The radiolabeled lipid composition of cell lipid extracts was determined by a modification of the high-performance liquid chromatography (HPLC) procedure reported by Christie (25). The same mobile phase gradient program as that reported by Christie was used, but methylene chloride was substituted for chloroform in solvent B and 5 mm acetic acid, adjusted to pH 7.5 with ethanolamine, was included in the aqueous portion of solvent C. Samples were chromatographed on a 10 cm × 0.46 cm Spherisorb 5 μm silica column (Phase Separations, Norwalk, CT) heated to 40°C (Eppendorf column heater; Baxter, McGaw Park, IL) and linked to an HPLC system from Thermal Separation Products (Fremont, CA) consisting of an SP8800 pump, SP8880 autosampler, and SP4500 data interface controlled by Spectra STATION software. A Spectra FOCUS absorbance detector set at 325 nm wavelength was used to detect retinol and retinyl esters.

The elution of ^{3}H- and ^{14}C-labeled lipids was detected with a dual-channel radiochemical detector (Model A-140; Packard Instrument Co., Meriden, CT). Correction parameters for HPLC mobile phase quenching of scintillant and spillover were obtained in blank HPLC runs where the scintillant supplied to the detector contained 3000–5000 cpm/ml of either ^{3}H-labeled or ^{14}C-labeled oleic acid. The resultant detector outputs were used to calculate the relative distribution of isotope signal between channel A and channel B as a function of HPLC run time. Mobile phase quenching was calculated for each isotope as the total detector signal (sum of channel A and channel B) at run time *t* divided by the total detector signal at $t = 0$ min. As ^{3}H spillover into channel B was undetectable, the contribution of each isotope to the total detector output at run time *t* was calculated as follows (26):

$$H_t = A_t - \frac{n_t B_t}{m_t} \times \frac{1}{Q_t} \qquad \textit{Eq. 1)}$$

$$C_t = \frac{B_t}{m_t} \times \frac{1}{q_t} \qquad \textit{Eq. 2)}$$

H_t and C_t represent the total detector response to ^{3}H and ^{14}C, respectively, at run time *t*. A_t and B_t are the observed detector outputs for channels A and B, respectively. n_t and m_t are the relative responses of the detector to ^{14}C in channels A and B, respectively, as calculated from blank runs with ^{14}C-doped scintillant. Q_t and q_t are the run time-dependent quenching factors for ^{3}H and ^{14}C, respectively. As very little change in correction factors occurred over the time intervals in which individual chromatographic peaks eluted, Eq. 1 and Eq. 2 were applied to the areas of the chromatographic peaks detected in channels A and B using the peak retention time for the value of *t*.

The radiolabeled lipids extracted from basolateral media were quantitated by thin-layer chromatography. The lipid extracts were transferred in chloroform–methanol 2:1 to silica plates (Silica Gel G, 0.25 mm × 20 cm × 20 cm; Analtech, Newark, DE) that were subsequently developed in hexanes–diethyl ether–acetic acid 80:15:1. Lipids were visualized by iodine vapor staining and scraped into scintillation vials for determination of radiolabel content by liquid scintillation counting.

The masses of the lipids contained in lipid extracts were quantitated by HPLC with evaporative light-scattering detection, as previously described (27). The method of Roda et al. (28) was used to determine taurocholic acid content by HPLC with the same detector.

Phospholipase A_2 preparation

$pPLA_2$ was obtained as a suspension in 3.2 m $(NH_4)_2SO_4$ at a rated activity of 700–800 U/mg. $pPLA_2$ was pelleted from the $(NH_4)_2SO_4$ suspension by centrifugation at a relative centrifugal force of 7000. The supernatant was discarded and the pellet was redissolved in HBS containing 5 mm $CaCl_2$ to obtain a $pPLA_2$ concentration that was 200-fold greater than the final concentration used in the media.

RESULTS

Characterization of mixed-lipid micelles

A lipid mixture was chosen for this study that: *1*) contained lipid hydrolysis products found in intestinal contents during normal digestion, *2*) formed homogeneous bile acid, mixed-lipid micelles, and *3*) was not toxic to Caco-2 cells over extended incubation periods. A suitable lipid mixture was found to be composed of 500 μm oleic acid, 300 μm monooleoylglycerol, 100 μm cholesterol with or without 200 μm of either lysophosphatidylcholine or phosphatidylcholine in cell culture medium containing 5 mm taurocholic acid. This lipid composition is similar to the bile acid, mixed-lipid compositions that Staggers et al. (29) indicated would form a single micellar phase. The micellar size and homogeneity of the lipid mixtures was confirmed by gel-filtration chromatography. As shown in **Fig. 1**, all the lipids in phospholipid-free micelle preparations, or in micelles prepared with the phospholipids, eluted in a single peak in a size range expected for bile acid, mixed-lipid micelles (30). The apparent particle size was larger than simple taurocholate micelles and smaller

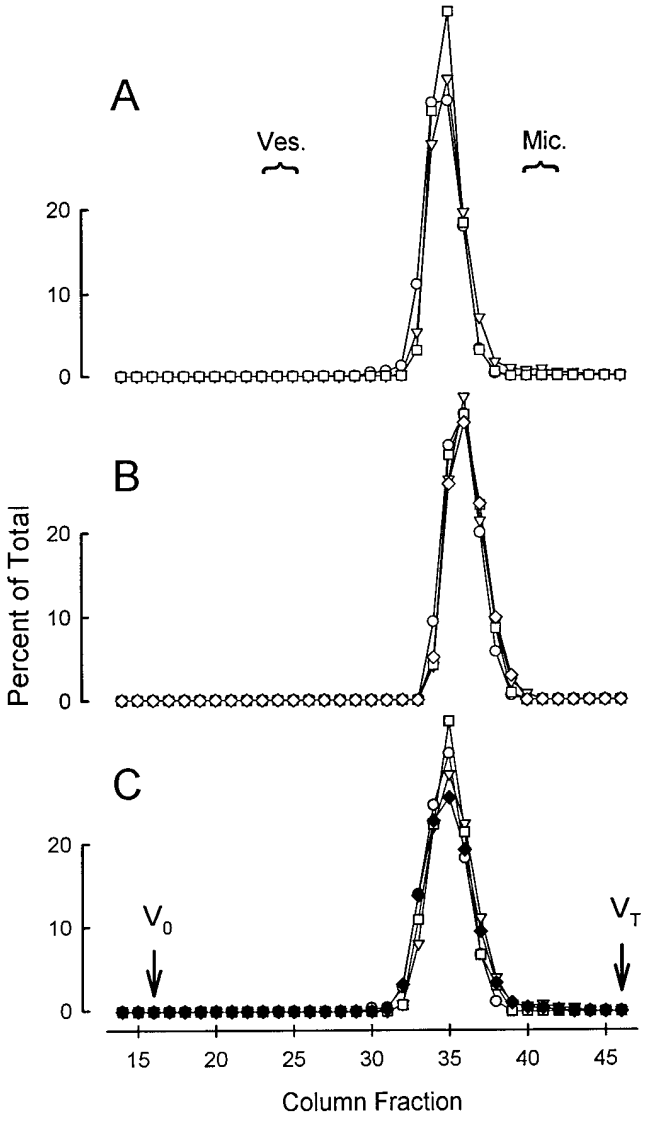

Fig. 1. Sepharose® CL-4B chromatography of the micelle preparations incubated with Caco-2 cells. To obtain lipid concentrations in the column fraction with the greatest lipid content that best approximated the actual media concentrations used, the lipid concentrations in the 0.5-ml samples applied to the column were 2.5-fold greater than the corresponding media concentrations. All samples were prepared in buffer (150 mm NaCl, 1 mm HEPES, 0.02% NaN_3, pH 7.4) containing 12.5 mm sodium taurocholate. All samples contained 1.25 mm oleic acid, 0.75 mm monooleoylglycerol, 0.25 mm cholesterol without phospholipid (A) or with 0.5 mm lysophosphatidylcholine (B) or with 0.5 mm phosphatidylcholine (C). The elution buffer consisted of 5 mm sodium taurocholate in buffer. The column fractions in which phospholipid vesicles (Ves) and pure taurocholate micelles (Mic) eluted are indicated. V_0 and V_t indicate the column void and total volumes, respectively. The results are plotted in percent format as the ratio of the mass of lipid in each fraction over the total mass of that lipid recovered in all fractions. The lipids were oleic acid (▽), monooleoylglycerol (□), cholesterol (○), lysophosphatidylcholine (◇), and phosphatidylcholine (◆).

than unilamellar vesicles. Long term (24 h) incubations of Caco-2 cell monolayers with micelles did not affect monolayer or cell morphology, as observed by light microscopy. Compared to filter chambers without cells, filters supporting confluent cell monolayers remained impermeable to [^{14}C]cholesterol and [^{3}H]oleic acid that were added to the apical medium as micellar components.

Caco-2 lipid absorption versus micellar phospholipid content

The effects of micellar phospholipid content on micellar lipid absorption by Caco-2 cells were initially examined by incubating cells with micelles prepared according to the proportions given in **Table 1** (0 h). After 6 h of incubation, the amounts of each lipid type remaining in the media and the cellular lipid contents were determined. As the results in Table 1 indicate, the medium concentrations of all micelle lipids were reduced in the presence of cells. While the amounts of micellar monooleoylglycerol and oleic acid removed from the medium by cells were not significantly affected by the micellar phospholipid content, the amount of cholesterol absorbed was significantly lower in incubations with micelles containing phosphatidylcholine than in incubations with phospholipid-free micelles or micelles containing lysophosphatidylcholine.

The reductions in micellar lipids were accompanied by increases in cell lipid masses. In all cases, the cellular triacylglycerol content increased more than 2-fold as a result of exposure to the micelles. Cellular phosphatidylcholine content also increased in all incubations, but to a lesser extent than that for triacylglycerol. The results for cholesterol and cholesteryl ester accumulation were less uniform. Significant accumulation of free cholesterol was only detected in cells incubated with phospholipid-free micelles or with micelles prepared with lysophosphatidylcholine. The results were similar for cellular cholesteryl ester content which increased more than 2-fold in the incubations with phospholipid-free micelles or with those containing lysophosphatidylcholine but increased only 20% in cells that presented micelles with phosphatidylcholine. These results mirror the changes in micellar lipids and indicate that cholesterol uptake and esterification are uniquely restricted when phosphatidylcholine is included in the micelles.

Further evidence of the consequences of micellar phospholipid content on the intracellular metabolism and transport of micelle-derived lipids was obtained by exposing cells to micelles prepared as before but also containing [^{14}C]cholesterol and [^{3}H]oleic acid. The micelles were prepared without phospholipid or with 200 μm phospholipid consisting of phosphatidylcholine and lysophosphatidylcholine in various molar ratios. Analysis of the amounts and types of radiolabeled lipids present in the cells after 6 h of incubation revealed that, as before, the phospholipid composition of the medium had profound effects on the cellular uptake and esterification of micellar cholesterol (**Figs. 2A**, **2B**). The maximum amounts of cellular free [^{14}C]cholesterol (Fig. 2A) and [^{14}C]cholesteryl ester (Fig. 2B) were detected in cells incubated with either phospholipid-free micelles or with micelles containing 100% lysophosphatidylcholine. In both cases, approximately 15% of the cell-associated [^{14}C]cholesterol was esterified. When 25% or more of the phospholipid in the

TABLE 1. Effect of micelle phospholipid composition on lipid content of apical media and Caco-2 cells

		Micellar Phospholipid					
		None		Lysophosphatidylcholine		Phosphatidylcholine	
Lipid	0 h	6 h	Δ	6 h	Δ	6 h	Δ
Apical medium (μm)							
Ch	100	65 ± 1	−35%	66 ± 1	−35%	95 ± 2[c]	−5%
MO	300	37 ± 1	−88%	36 ± 2	−88%	82 ± 1[c]	−73%
FA	500	357 ± 8	−29%	331 ± 7	−34%	370 ± 6	−26%
PC	(200)[a]	—	—	—	—	170 ± 15	−15%
LPC	(200)[a]	—	—	66 ± 6	−67%	—	—
Cell (μg/mg protein)							
CE	13.1 ± 0.3	31.0 ± 0.2[d]	137%	26.4 ± 0.4[d]	102%	15.7 ± 0.3[b]	20%
Ch	35.7 ± 0.6	41.5 ± 1.6[b]	16%	40.9 ± 0.4[c]	15%	35.7 ± 0.8	0%
TG	86.3 ± 1.0	195.0 ± 2.4[d]	126%	214.4 ± 0.4[d]	149%	202.0 ± 1.7[d]	134%
PC	89.6 ± 1.7	101.3 ± 0.4[c]	13%	120.1 ± 3.8[c]	34%	99.6 ± 1.0[b]	11%

Caco-2 cells were incubated 6 h in DMEM containing 100 μm bovine serum albumin and micelles composed of 5 mm sodium taurocholate and the apical medium lipids indicated at 0 h. Cell and apical medium lipids were extracted for analysis of lipid content by HPLC as described in Materials and Methods. The values at 6 h are the average ± SEM for three wells. The percent changes relative to 0 h (Δ) are shown. Ch, cholesterol; MO, monooleoylglycerol; FA, fatty acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; CE, cholesteryl ester; TG, triacylglycerol.

[a]Phospholipid included in micelles only where indicated.

[b]$P < 0.05$, [c]$P < 0.005$, and [d]$P < 0.001$ vs. None.

apical medium consisted of phosphatidylcholine, cellular absorption and esterification of [^{14}C]cholesterol were significantly reduced (Figs. 2A, 2B). Relative to phospholipid-free incubations, incubations with phospholipid mixtures containing 50% or more phosphatidylcholine resulted in reductions of cell-associated free [^{14}C]cholesterol and [^{14}C]cholesteryl ester of greater than 60% and 92%, respectively.

In contrast to the results for micelle-derived [^{14}C]cholesterol, uptake and conversion of [^{3}H]oleic acid to acylated lipid products were largely unaffected by micellar phospholipid content (Figs. 2D, 2E). Only the conversion of absorbed [^{3}H]oleic acid to cholesteryl [^{3}H]oleate (Fig. 2C) was dependent on micellar phospholipid content. Significant reductions in cholesteryl [^{3}H]oleate synthesis were observed in incubations with micelles containing 50 mol% or more phosphatidylcholine. Because the bulk (89%) of the cell-associated [^{3}H]oleic acid label was incorporated into triacylglycerol and phospholipids while the maximum cholesteryl [^{3}H]oleate accumulation, which occurred in incubations with phospholipid-free micelles, accounted for only 7% of the total cell-associated [^{3}H]oleate label, no significant differences were detected in total cell-associated [^{3}H]oleic acid label as a function of micelle composition.

The dependence of cholesterol absorption and esterification on micellar phospholipid content was reflected in the cellular secretion of lipids into the basolateral medium. Relative to incubations with phospholipid-free micelles, the secretion of [^{14}C]cholesteryl ester and cholesteryl [^{3}H]oleate from the basolateral side of Caco-2 cell monolayers was significantly reduced when the micellar phospholipids consisted of 25% or more phosphatidylcholine (**Figs. 3A**, **3B**). In contrast, the secretion of [^{3}H]oleate-labeled triacylglycerol was independent of micelle phospholipid content in the apical medium (Fig. 3C).

Retinol absorption

Retinol, like cholesterol, is a fatty alcohol that is absorbed in the intestine via a pathway involving esterification of absorbed retinol within the enterocytes followed by packaging of the retinyl esters into chylomicrons and secretion into the circulation (31). The similarities between cholesterol and retinol in absorption pathways suggests retinol absorption may also be dependent on micelle phospholipid content. To examine this, Caco-2 cells were incubated for 6 h with micelles containing 10 μm retinol in the absence or presence of phosphatidylcholine or lysophosphatidylcholine. As the results in **Fig. 4** demonstrate, the micelle phospholipid content had no significant effect on total retinol absorption or on retinol esterification. Thus, compared to retinol, the phospholipid-dependent absorption of cholesterol is unique.

Phosphatidylcholine and lysophosphatidylcholine absorption

To determine whether the contrasting effects of phosphatidylcholine and lysophosphatidylcholine on cholesterol absorption and esterification were due to differences in phospholipid uptake and metabolism, medium containing micelles prepared with either 1-palmitoyl-2-[1-^{14}C] oleoyl phosphatidylcholine or 1-[1-^{14}C]palmitoyl-lysophosphatidylcholine was applied to the apical surface of Caco-2 cell monolayers. The radiolabeled lipid species detected in the cells after 6 h of incubation are shown in **Table 2**. The results indicate that phosphatidylcholine absorption was much less than lysophosphatidylcholine absorption. Approximately 1% of the phosphatidylcholine label introduced to the cells in the apical medium associated with the cells after 6 h of incubation, whereas more than 33% of the lysophosphatidylcholine label entered the cells in the same time period. Moreover, greater than 98% of the cell-associated lysophosphatidylcholine label was converted to other cellular acylated lipids while only 23% of the relatively small portion of label associated with cells in incubations with phosphatidylcholine was converted to another acylated lipid product, which was detected entirely as triacylglycerol. These data indicate that cholesterol absorption is independent of phospholipid uptake as

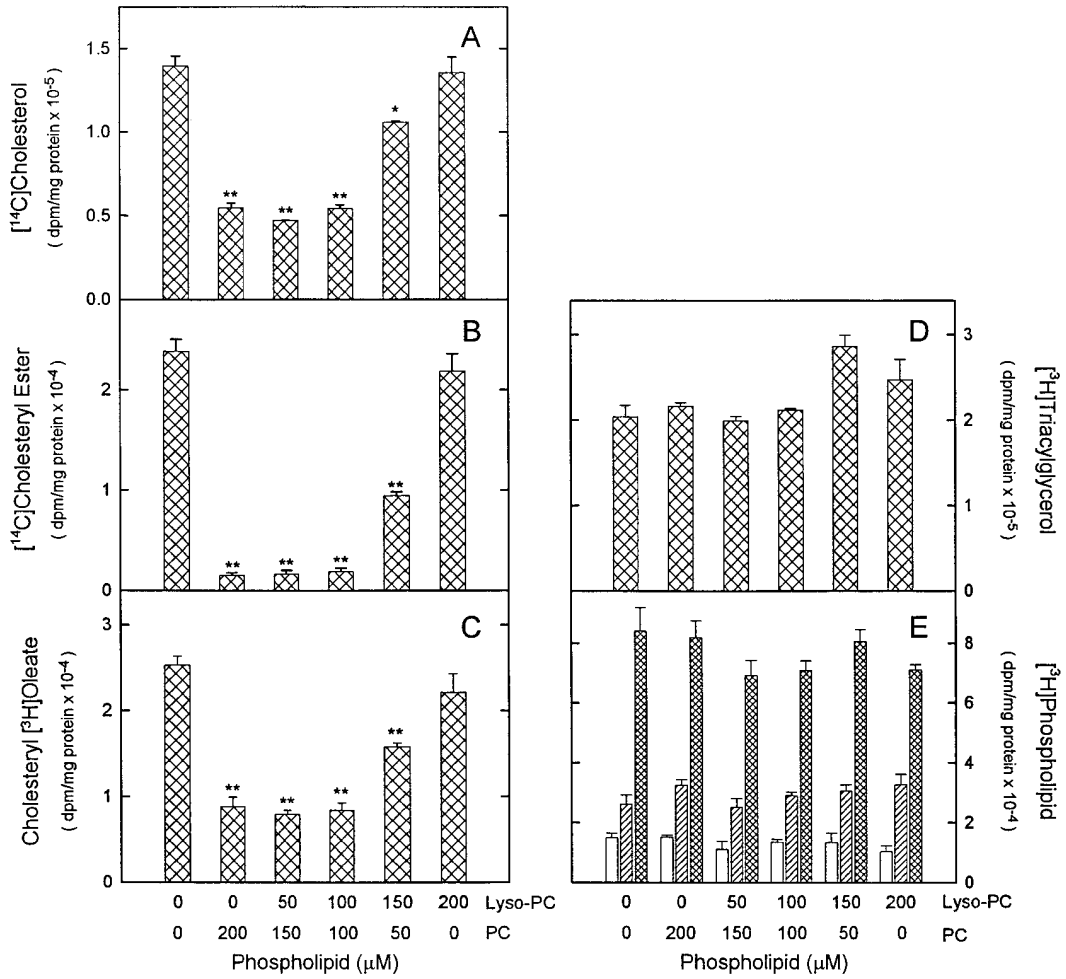

Fig. 2. Effect of micellar phospholipid content on micellar [^{14}C]cholesterol and [^{3}H]oleic acid uptake and conversion to acylated lipids by Caco-2 cells. Micropore filter inserts containing 21-day-old Caco-2 cell monolayers were washed with DMEM and transferred to cell culture wells containing DMEM to bathe the basolateral cell surface (i.e., insert bottom). DMEM solutions containing 100 μm bovine serum albumin, 5 mm sodium taurocholate, 300 μm monooleoylglycerol, 500 μm [^{3}H]oleic acid, 100 μm [^{14}C]cholesterol and the indicated amounts of lysophosphatidylcholine (Lyso-PC) and phosphatidylcholine (PC) were placed inside the filter inserts to cover the apical cell surface. The radiolabeled lipid compositions of the cell monolayers were determined after 6 h incubation as described in Materials and Methods. The [^{3}H]phospholipids (E) detected were phosphatidylinositol (open bar), phosphatidylethanolamine (hatched bar), and phosphatidylcholine (cross-hatched bar). The data represent the mean ± SEM of three wells. *$P < 0.05$, **$P < 0.001$ versus incubations without phospholipid.

there were no significant differences in cholesterol uptake and esterification between cells incubated under phospholipid-free conditions and cells that accumulated phospholipid when given lysophosphatidylcholine. The results support the conclusion that retention of phosphatidylcholine in the medium is the dominant factor in restricted cholesterol uptake from phosphatidylcholine-containing micelles.

The retention of phosphatidylcholine in the medium may have modified the micelle properties in a manner that retarded cholesterol absorption. While the initial lipid compositions of the incubation media resulted in the formation of uniform, mixed-lipid micelles, the aggregation state of medium lipids could be altered during cell incubations by the disproportional depletion of medium lipids stemming from the unequal rates of lipid uptake. In the case of phosphatidylcholine this effect led to the enrichment of medium lipids with phosphatidylcholine (Table 1). To examine the effects of compositional changes on lipid aggregation state in the apical medium, aqueous suspensions of lipids were prepared according to the lipid proportions found in media at 6 h incubation (Table 1) and analyzed by gel filtration chromatography. The results (**Fig. 5**) indicated a less uniform distribution of lipid aggregates at the 6 h time-point than at the start of incubation. This was particularly evident for the lipid mixture containing phosphatidylcholine (Fig. 5C). In that case, two distinct lipid phases were detected of which one phase was micellar in size and consisted of all four lipid components while the other phase appeared as larger particles composed of only cholesterol and phosphatidylcholine. Cholesterol comprised 67 mol% of the total lipid content of the larger particles.

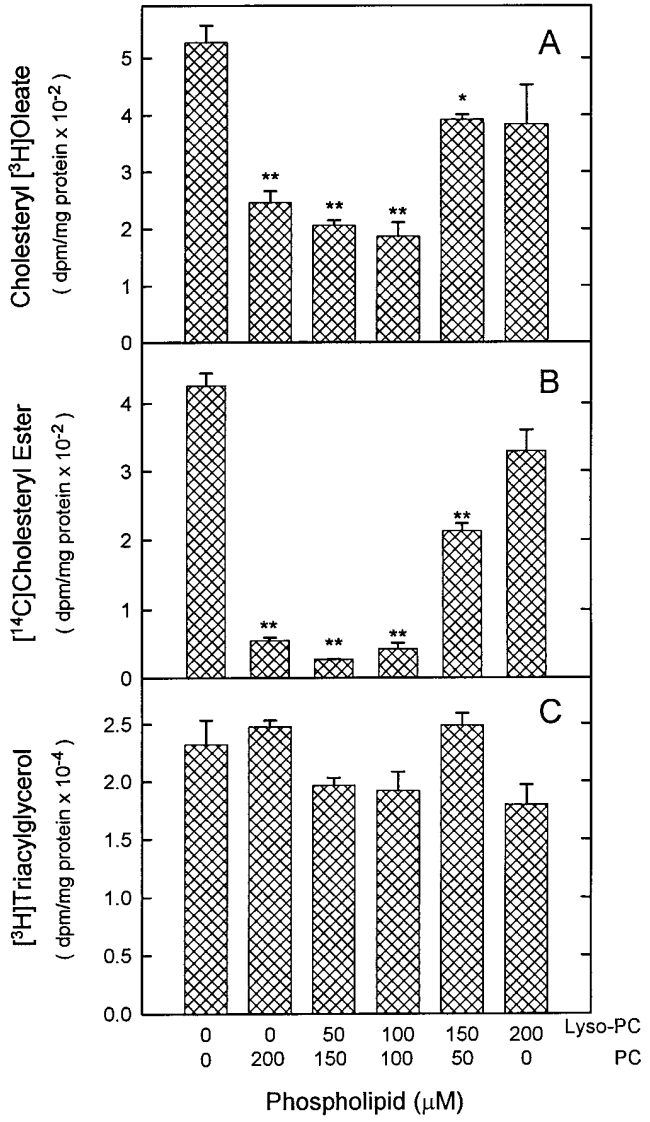

Fig. 3. Effect of micellar phospholipid content on basolateral secretion of acylated lipids synthesized by Caco-2 cells from micellar [^{14}C]cholesterol and [^{3}H]oleic acid absorbed at the apical cell surface. Caco-2 cell monolayers were incubated as described in Fig. 2. The radiolabeled lipid contents of the basolateral media were determined after 6 h incubation as described in Materials and Methods. The data represent the mean ± SEM of three wells. *$P < 0.05$, **$P < 0.001$ versus incubations without phospholipid.

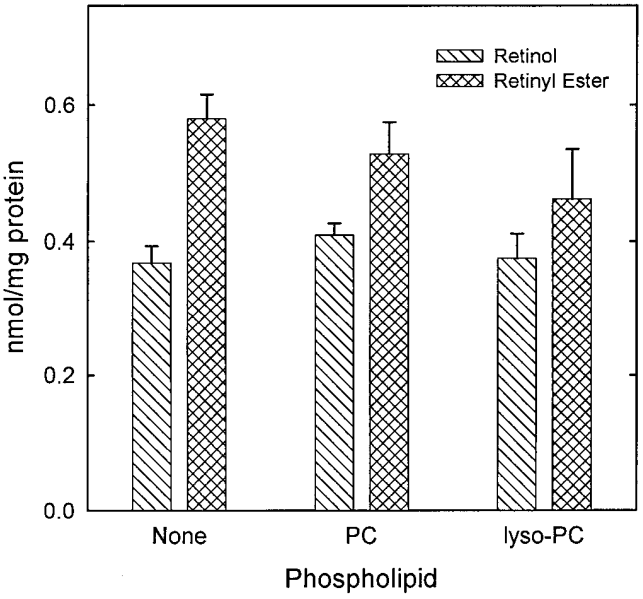

Fig. 4. Effect of micellar phospholipid content on micellar retinol uptake and conversion to retinyl esters by Caco-2 cells. Caco-2 cell monolayers, grown in cell culture plates, were incubated in DMEM containing 100 μm bovine serum albumin and micelles composed of 5 mm sodium taurocholate, 300 μm monooleoylglycerol, 500 μm oleic acid, 100 μm cholesterol, 10 μm retinol, and 200 μm of the indicated phospholipid. The amounts of esterified and unesterified retinol in the lipids extracted from the monolayers were determined as described in Materials and Methods.

Phospholipase A_2-dependent cholesterol absorption

The data indicate that micellar phosphatidylcholine restricts cholesterol diffusion from the micelles to the cells. To further test this observation, experiments were performed to examine the effect of micellar phosphatidylcholine hydrolysis by $pPLA_2$ on cholesterol absorption by Caco-2 cells. The time-courses of Caco-2 cell absorption and metabolism of [^{14}C]cholesterol and [^{3}H]oleic acid from micelles containing phosphatidylcholine were determined in the absence or presence of $pPLA_2$. For comparison, incubations were also performed with phospholipid-free micelles and with micelles containing lysophosphatidylcholine. As observed previously (Fig. 2), the absorption and esterification of [^{14}C]cholesterol (**Figs. 6A**, **6B**) and [^{3}H]oleic acid conversion to cholesteryl [^{3}H]oleate (Fig. 6C) were greatly reduced in Caco-2 cells exposed to micelles containing phosphatidylcholine, in comparison to cells incubated with phospholipid-free micelles or with micelles containing lysophosphatidylcholine. The restricted absorption and esterification of cholesterol from micelles containing phosphatidylcholine was significantly enhanced by the introduction of $pPLA_2$ into the incubations (Figs. 6A, 6B, 6C). The increased absorption due to $pPLA_2$ was most evident at incubation times of 6 h or greater.

While micellar phosphatidylcholine did not significantly affect the incorporation of [^{3}H]oleic acid into phosphatidylcholine and triacylglycerol, it was evident at 12 h of incubation that [^{3}H]oleic acid incorporation into cellular phosphatidylcholine was reduced almost 30% in incubations with lysophosphatidylcholine-containing micelles or in the incubations with $pPLA_2$. This may be a result of decreased fatty acid incorporation into phosphatidylcholine due to cellular uptake of lysophosphatidylcholine, which would be present in both cases. It is unlikely that this effect resulted from decreased [^{3}H]oleic acid absorption as [^{3}H]oleic acid conversion to triacylglycerol, which accounted for approximately 75% of the [^{3}H]oleic acid label in the cell, was not decreased.

Oil–water emulsions are another significant reservoir of free cholesterol in the intestinal contents (32). To determine whether phosphatidylcholine could also restrict cholesterol absorption from such emulsions, Caco-2 monolay-

TABLE 2. Comparison of Caco-2 cell absorption and metabolism of 1-palmitoyl-2-[^{14}C]oleoyl phosphatidylcholine and 1-[^{14}C]palmitoyl lysophosphatidylcholine

^{14}C-Labeled Cell Lipids	^{14}C-labeled Fatty Acid Distribution in Cells			
	Phosphatidylcholine Incubation		Lysophosphatidylcholine Incubation	
	nmol/mg protein	*%*	*nmol/mg protein*	*%*
Cholesteryl ester	—[a]		2.6	2.2
Triacylglycerol	0.7	22.6	42.8	35.9
Diacylglycerol	—		1.2	1.0
Fatty acid	—		4.6	3.8
Phosphatidylethanolamine	—		4.2	3.5
Phosphatidylinositol	—		2.9	2.4
Phosphatidylcholine	2.4	77.4	59.9	50.2
Lysophosphatidylcholine	—		1.2	1.0
Total	3.1		119.3	

Caco-2 cells were incubated for 6 h in Dulbecco's modified Eagle's medium containing 5 mm taurocholic acid, 500 μm oleic acid, 300 μm monooleoylglycerol, 100 μm cholesterol, 200 μm of either 1-palmitoyl-2-[1-^{14}C]oleoyl phosphatidylcholine or 1-[1- ^{14}C]palmitoyl lysophosphatidylcholine, and 100 μm bovine serum albumin. After 6 h incubation, the cells were isolated for lipid extraction and composition analysis by HPLC, as described in Experimental Procedures.

[a]Not detected.

ers were incubated 6 h with a lipid emulsion composed of 5 mm sodium taurocholate, 300 μm trioleoylglycerol, 5 μm cholesteryl oleate, 20 μm [^{3}H]cholesterol, and 200 μm 1-palmitoyl-2-[^{14}C]oleoyl phosphatidylcholine in the absence or presence of $pPLA_2$ (**Fig. 7**). Free [^{3}H]cholesterol was detected in the cells in incubations without $pPLA_2$, but no esterification of the cell-associated [^{3}H]cholesterol could be detected. The cells also accumulated a relatively small quantity of ^{14}C-labeled lipids, which consisted of [^{14}C] phosphatidylcholine (78.5%) and [^{14}C]triacylglycerol (21.5%). Inclusion of $pPLA_2$ in the incubation greatly enhanced the cellular accumulation of both radiolabels. These included a 2-fold increase in the amount of cell-associated [^{3}H]cholesterol accompanied by the appearance of cellular [^{3}H]cholesteryl ester and a 33-fold increase in the cellular content of ^{14}C-labeled lipids, the majority of which were detected as [^{14}C]triacylglycerol (57.6%) and [^{14}C]phosphatidylcholine (28.5%). The increases in cell-associated radiolabels were paralleled by an 82% reduction in the [^{14}C]phosphatidylcholine content of the apical medium.

To further establish that the $pPLA_2$-dependent increase in cholesterol absorption was directly due to phosphatidylcholine hydrolysis, incubations similar to the previous ones were performed with FPL 67047XX, a potent inhibitor of $pPLA_2$ (33). Addition of FPL 67047XX to the incubations with $pPLA_2$ and emulsion reversed the $pPLA_2$-dependent increases in lipid absorption by amounts that were directly proportional to the inhibitor concentration (Fig. 7). The greatest decrease occurred in cellular [^{3}H]cholesteryl ester content which was reduced 95% at 1 μm inhibitor, compared to inhibitor-free incubations with phospholipase. Relative to incubations with emulsion and $pPLA_2$, treatment of cells with 50 μm FPL 67047XX, the highest concentration tested, resulted in the reduction of [^{3}H]cholesteryl ester to below detection limits, a 63% decrease in cell-associated [^{3}H]cholesterol and a 94% drop in ^{14}C-labeled cell lipids. The ^{14}C-labeled lipids remaining in the cells consisted of 64% [^{14}C]phosphatidylcholine and 36% [^{14}C]triacylglycerol. The reductions in cell-associated lipids at 50 μm FPL 67047XX were accompanied by retention of 97% of the [^{14}C]phosphatidylcholine in the apical medium, relative to incubations with uninhibited $pPLA_2$.

Although these data strongly indicate that FPL 67047XX inhibited $pPLA_2$, the large reduction in cellular [^{3}H]cholesteryl ester synthesis with FPL 67047XX treatment could also be due to direct inhibition of acyl-coenzyme A:cholesterol acyltransferase (ACAT), the microsomal enzyme responsible for cholesteryl ester synthesis. Thus, FPL 67047XX inhibition of ACAT in rat liver microsomes was examined (34) but no inhibition of ACAT was observed at FPL 67047XX concentrations up to 50 μm, which was the highest concentration tested (data not shown).

DISCUSSION

Prior efforts to reveal the origin of the phosphatidylcholine-dependent suppression of cholesterol absorption by intestinal epithelium focused on cholesterol absorption by isolated intestinal segments placed in aqueous mixtures of lipids and bile salts that were intended to be micellar in form (7–11). Micellized lipids were selected as the vehicle for cholesterol delivery as mixed-lipid micelles are considered to be the principal lipid phase in the intestinal lumen from which absorbed cholesterol originates (3). A similar strategy was pursued in the current study, but with Caco-2 cells rather than intestinal segments as the Caco-2 cell line is more stable and amenable to manipulation than intestinal segments. The micellized lipid preparations used in this study were composed of bile salt, phospholipid, cholesterol, and glycerolipid hydrolysis products characteristic of the mixed-lipid micelles found in intestinal contents (32). The proportions and concentrations of the various lipid components were selected to ensure that

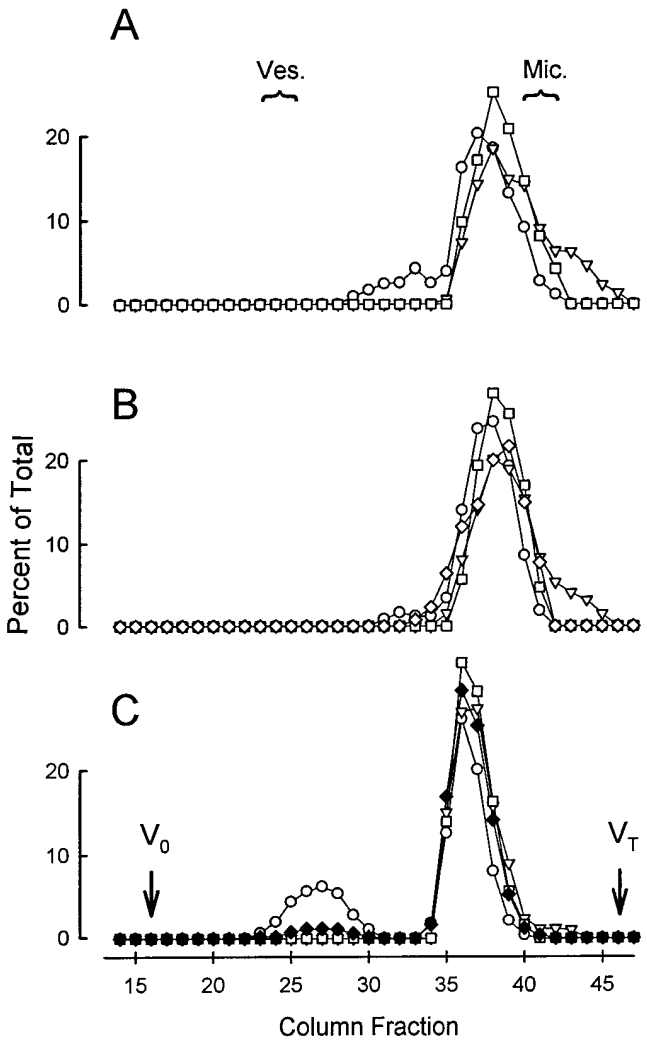

Fig. 5. Sepharose® CL-4B chromatography of bile acid–lipid mixtures corresponding to the media lipid compositions (Table 1) at the apical surface of Caco-2 cell monolayers after 6 h of incubation. All samples were prepared, chromatographed, and analyzed as described in Fig. 1 and contained 0.89 mm oleic acid, 0.09 mm monooleoylglycerol, 0.16 mm cholesterol without phospholipid (A) or with 0.17 mm lysophosphatidylcholine or with 0.43 mm phosphatidylcholine (C). The symbols indicate oleic acid (▽), monooleoylglycerol (□), cholesterol (○), lysophosphatidylcholine (◇), and phosphatidylcholine (◆).

a single micellar phase was created, which was confirmed by gel filtration chromatography (Fig. 1).

Although lipids aggregate to form several distinct phases in the intestinal contents (32), it is well established that the lipid aggregates as such are not absorbed. Instead, the more water-soluble lipid constituents, consisting primarily of acylated lipid hydrolysis products, dissociate from the lipid aggregates and into the aqueous phase as monomers which subsequently diffuse into the brush border membrane for absorption (3). Such a monomer absorption mechanism was evident in the current study as the various micelle lipid components were absorbed to different extents (Table 1). Further evidence of the independent uptake of micelle lipid constituents was obtained when the introduction of phosphatidylcholine into the micelles greatly reduced cholesterol absorption without changing the absorption of oleic acid (Figs. 2, 6) or retinol (Fig. 4) and diminishing monooleoylglycerol absorption to only a minor extent (Table 1).

The selective reduction in cholesterol absorption from micelles containing phosphatidylcholine was accompanied by reduced cellular synthesis and secretion of cholesteryl esters without any significant changes in the cellular conversion of micelle-derived fatty acid to other acylated lipid types or in the secretion of triacylglycerol. This peculiar effect of micellar phosphatidylcholine on cholesterol uptake and metabolism by Caco-2 cells may stem from perturbations in the capacity of micelles to deliver cholesterol to the cells or it may result from a direct impact of micellar phosphatidylcholine on the capacity of the cells to synthesize and secrete cholesteryl ester. Evidence for the later is suggested by results from a previous study that showed exposure of Caco-2 cells to phosphatidylcholine alone in the presence of taurocholate and [^{3}H]oleic acid resulted in diminished cholesteryl [^{3}H]oleate synthesis and secretion (35). It is unlikely that the interference by micellized phosphatidylcholine resulted from phosphatidylcholine entry into the cells as the effects were observed under conditions known to yield negligible uptake of micellized phosphatidylcholine (36). It is possible that the reduced cholesterol secretion and esterification resulted from transfer of cellular cholesterol to the micelles. Such a process was not evident in the current study as there was a net transfer of cholesterol from the cholesterol-loaded micelles to the cells.

The restricted cholesterol absorption associated with micellar phosphatidylcholine in this study did not result from a lack of phospholipid delivery to the cells as lysophosphatidylcholine was extensively absorbed and converted to phosphatidylcholine within the cells without any significant change in cholesterol transport, compared to phospholipid-free incubations. The current results for lysophosphatidylcholine absorption and metabolism correspond to those reported earlier in a similar study (37), but, differ significantly with respect to the effects of lysophosphatidylcholine on cholesterol secretion. Results reported for the previous study indicated that pretreatment of Caco-2 monolayers with lysophosphatidylcholine alone significantly diminished cholesterol esterification and secretion while enhancing triacylglycerol synthesis and secretion. The divergent outcomes between the current study and the previous one may stem from the dissimilar methods by which cells were presented with lipids. In the former study, cells were treated sequentially with lysophosphatidylcholine followed by oleic acid, whereas in the current study cells were exposed to a more complex mixture of lipids simultaneously. In the current scheme of lipid addition to cells, which may be a closer approximation of enterocyte exposure to dietary lipids during normal lipid digestion, only micellar phosphatidylcholine interfered with cholesterol uptake and secretion.

The limited cholesterol absorption associated with micellar phosphatidylcholine may be a result of phosphati-

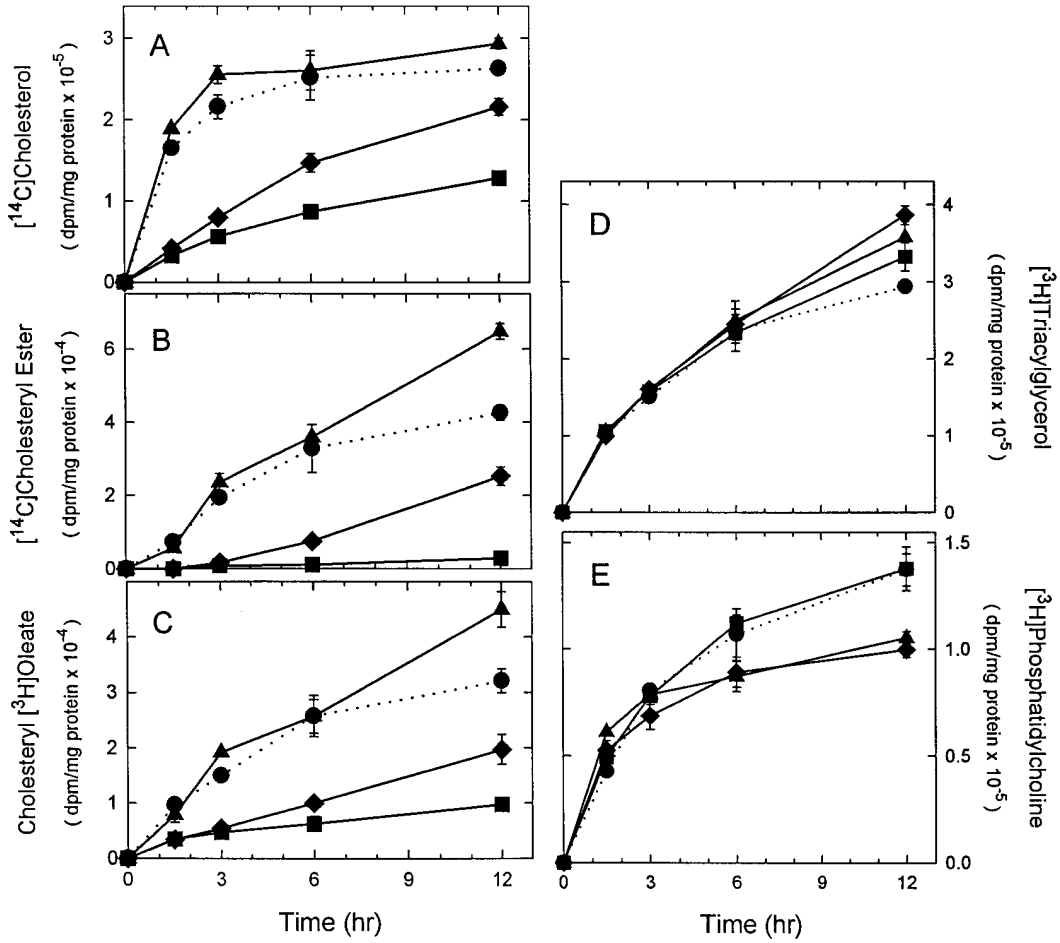

Fig. 6. Effect of micellar phospholipid content and phospholipase A_2 hydrolysis of micellar phosphatidylcholine on the time-course of micellar [^{14}C]cholesterol and [^{3}H]oleic acid uptake and conversion to acylated lipids by Caco-2 cells. Caco-2 cell monolayers, grown in cell culture plates, were incubated in DMEM containing 100 μm bovine serum albumin and micelles composed of 5 mm sodium taurocholate, 300 μm monooleoylglycerol, 500 μm [^{3}H]oleic acid, 100 μm [^{14}C]cholesterol, and either no phospholipid (●), 200 μm lysophosphatidylcholine (▲), or 200 μm phosphatidylcholine (■). A fourth group of cells were incubated in the presence of porcine pancreas phospholipase A_2 (4 U/ml) and micelles containing 200 μm phosphatidylcholine (◆). The radiolabeled lipid compositions of the cell monolayers were determined as described in Materials and Methods. The data represent the mean ± SEM of three wells. Error bars are not shown where the SEM is smaller than the symbol size.

dylcholine retention in the micellar phase. If cholesterol absorption occurs via cholesterol monomer diffusion through the aqueous phase then, as Rampone (6) first proposed, the unique suppression of cholesterol absorption by phosphatidylcholine may involve a shift in the partitioning of cholesterol from the aqueous phase to the micellar phase. This would result in a reduction of the amount of aqueous phase cholesterol available for diffusion to the brush-border membrane. The fact that phosphatidylcholine is also poorly absorbed from the micellar phase suggests that the associated decrease in cholesterol absorption is the result of a phosphatidylcholine-dependent change in the properties of the micellar phase. For example, the micellar retention of phosphatidylcholine may limit the decrease in micellar phase volume that otherwise occurs as a result of the depletion of the more water-soluble micelle constituents through absorption. The micelle volume preserved by phosphatidylcholine retention could then act as a sink for cholesterol. It has also been proposed that cholesterol absorption may be driven by the tendency of micelles to become supersaturated with cholesterol as the micelles are initially depleted of the more water soluble and, consequently, more rapidly absorbed lipids. Micellar retention of phosphatidylcholine in that case would delay the onset of cholesterol supersaturation (4, 11).

The enrichment of the micellar phase with phosphatidylcholine might also lead to phase separation of cholesterol into other types of lipid phases from which cholesterol desorption may be more restricted. Incubations with phosphatidylcholine-containing micelles in the current study did lead to the appearance of a new two-component lipid phase containing cholesterol and phosphatidylcholine (Fig. 5C). The structure of lipid aggregates in this cholesterol-rich phase was not determined but the elution profile of this aggregate was in the same size range as vesicles. A previous analysis of the phase behavior of lipid mixtures similar to those used in this study indicated that a

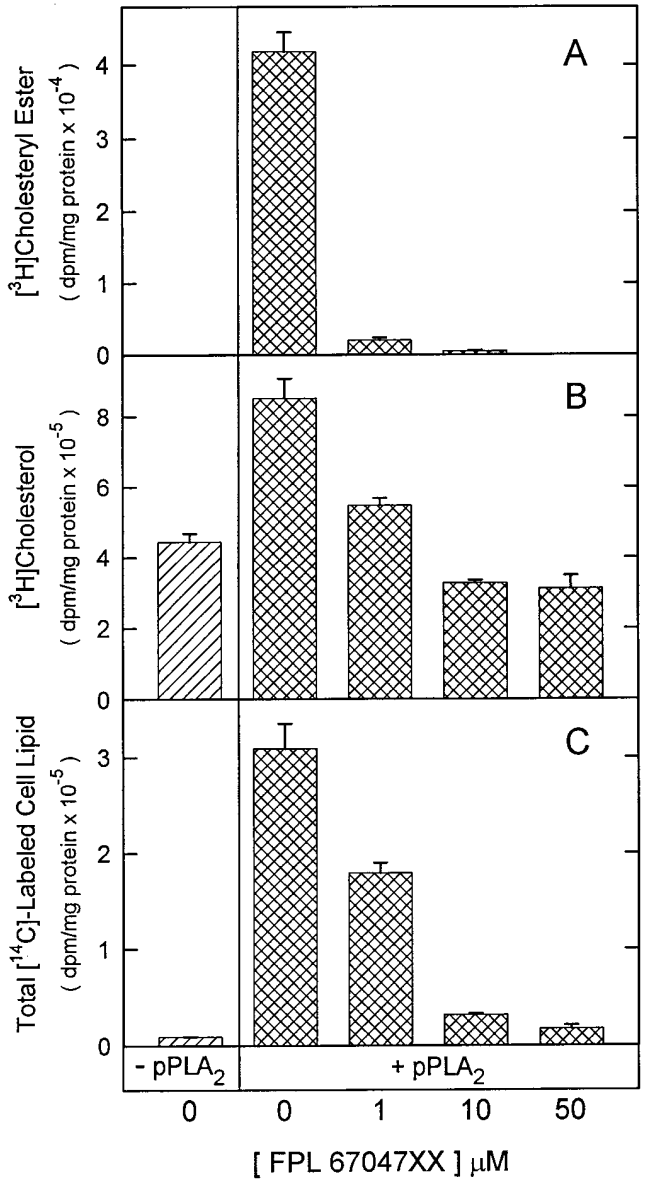

Fig. 7. FPL 67047XX inhibition of pancreatic phospholipase A_2 ($pPLA_2$)-dependent [^{3}H]cholesterol and [^{14}C]phosphatidylcholine absorption from triacylglycerol-rich emulsions. Caco-2 cell monolayers, grown in cell culture plates, were incubated 6 h in DMEM containing lipid dispersions composed of 5 mm sodium taurocholate, 300 μm trioleoylglycerol, 5 μm cholesteryl oleate, 20 μm [^{3}H] cholesterol, and 200 μm 1-palmitoyl-2-[^{14}C]oleoyl phosphatidylcholine. $pPLA_2$ (0.5 U/ml) was added at the start of the incubation period. FPL 67047XX was introduced from concentrated DMSO stock solutions such that the medium content of DMSO was 0.1%. Inhibitor-free incubations contained DMSO alone. After the incubation period, the radiolabeled lipid compositions of the cell monolayers were determined as described in Materials and Methods. The data represent the mean ± SEM of three wells.

homogeneous micellar phase existed under a comparatively limited range of lipid compositions and that relatively minor changes in the proportions of lipids led to situations where both lamellar and crystalline phases coexisted with the micellar phase (28). A similar demonstration of the narrow compositional boundaries within which a single homogeneous micellar phase is formed has been created for a less complex but related mixture of taurocholic acid, phosphatidylcholine, and cholesterol (5). Thus, it is possible that the compositional changes in aqueous lipid dispersions resulting from differential lipid absorption can lead to phase separation. In human intestinal contents both micellar and non-micellar phases coexist (32). The extent to which rates of cholesterol desorption might vary among the various types of phases is not known, but the demonstration in this study of the phosphatidylcholine-dependent reduction in cholesterol uptake from micelles as well as from a triglyceride-rich emulsion suggests that phosphatidylcholine can retard cholesterol desorption from both micellar and non-micellar lipid phases.

Investigations of cholesterol transfer kinetics among a variety of lipid phases in aqueous suspension have shown that cholesterol desorption from the lipid phase is the rate-limiting step in cholesterol exchange (38, 39). Such studies indicate that exposure of the large hydrophobic surface of cholesterol to the aqueous phase as cholesterol diffuses out of the host lipid matrix is the principal energy barrier to cholesterol exchange. Additionally, interaction forces with the host matrix lipids may influence cholesterol desorption as exchange studies have shown that cholesterol desorption rates are also dependent on the composition and structure of the lipid matrix in which cholesterol is incorporated. Detailed data on cholesterol exchange kinetics have only been obtained in non-micellar systems. The extent to which the data obtained in such systems apply to cholesterol desorption from bile acid micelles has not been determined but there are indications that host lipid matrix properties are important. For example, the scant cholesterol transfer obtained from phospholipid vesicles to intestinal segments is enhanced many fold when the bilayer structure of the host lipid matrix is transformed to micelles by the addition of bile acid (9). It remains to be determined whether the further enhancement in cholesterol absorption obtained by the replacement or elimination of micellar phosphatidylcholine results from lipid matrix changes, phase saturation effects, or both.

Unlike cholesterol, the absorption of oleic acid, retinol, and monooleoylglycerol were essentially unchanged by the introduction of phosphatidylcholine into the micelles. This result indicates that, unlike cholesterol, desorption of oleic acid, retinol, and monooleoylglycerol from the micellar lipid matrix is not rate-limiting for absorption. Such an outcome is consistent with the fact that lipid desorption rates are inversely correlated with the hydrophobicity of the transferring lipid (40). As a 27-carbon alcohol, cholesterol is significantly more hydrophobic than retinol, a 20-carbon alcohol, and cholesterol is also more hydrophobic than oleic acid or monooleoylglycerol. In fact, cholesterol may be the most hydrophobic of the lipids that are considered absorbable and may even be on the threshold of nonabsorbability. The proximity of cholesterol to an absorption threshold is indicated by the fact that β-sitosterol, a plant sterol structurally homologous to cholesterol but slightly more hydrophobic due to an addi-

tional ethyl group, is essentially nonabsorbable (41). Moreover, the observation that β-sitosterol desorption rates from phosphatidylcholine bilayers are more than 4-fold lower than the corresponding desorption rates for cholesterol (42) is a further indication of a link between lipid desorption rates and intestinal absorption.

The extent to which cholesterol absorption in vivo is restricted by the presence of phosphatidylcholine in the lumenal contents during normal digestion is not known. Because cholesterol absorption in man is typically less than 60% efficient (43), the possibility exists that the incomplete absorption may in some part be due to interference by phosphatidylcholine in the lumenal contents. Certainly, the 10 to 20 grams of phosphatidylcholine that are contributed to the intestinal contents from the bile alone (44) could have a significant impact on cholesterol absorption. Substantial quantities of phosphatidylcholine are detected in the lumenal contents during the early stages of digestion (32). However, it is also clear that lumenal phosphatidylcholine is effectively hydrolyzed by $pPLA_2$ as intestinal contents move along the small intestine (15). Thus, it remains a question whether sufficient quantities of biliary and dietary phosphatidylcholine remain intact in the lumenal contents to significantly interfere with cholesterol absorption. Several studies in humans do indicate, however, that cholesterol absorption can be reduced by supplying exogenous phosphatidylcholine to increase the amount of phosphatidylcholine entering the intestine (18–20).

If cholesterol absorption is dependent on the elimination of phosphatidylcholine from the intestinal contents then the function of $pPLA_2$ in lipid digestion must include facilitation of cholesterol absorption in addition to the promotion of phosphatidylcholine absorption through phosphatidylcholine lipolysis. This was recently demonstrated in a study that identified $pPLA_2$ as the active component in porcine pancreas extracts responsible for the facilitation of cholesterol uptake from phosphatidylcholine-containing micelles by Caco-2 cells (45). Similarly, in the current study, $pPLA_2$ enhanced Caco-2 cell absorption of cholesterol from both micelles (Fig. 6) and a lipid emulsion containing phosphatidylcholine (Fig. 7). In addition, the enhanced cholesterol uptake was inhibited by the $pPLA_2$ inhibitor FPL 67047XX, which not only indicates a dependence of cholesterol uptake on phosphatidylcholine hydrolysis but also demonstrates the potential of such inhibitors for probing the function of $pPLA_2$ in vivo. Indeed, we have tested the effect of FPL 67047XX on lipid absorption in rats and have found a specific reduction in cholesterol absorption with the administration of FPL 67047XX (43). These observations are also consistent with prior studies that showed that rats treated with lipid emulsions containing non-hydrolyzable diether phosphatidylcholine retained significantly more cholesterol in intestinal contents than rats given a corresponding diester phosphatidylcholine (17, 46).

The large body of evidence indicating a direct dependence of intestinal cholesterol absorption on the elimination of phosphatidylcholine from the intestinal contents adds a new facet to the commonly accepted function of $pPLA_2$ in phospholipid digestion and absorption. Moreover, the apparent requirement for $pPLA_2$ hydrolysis of phosphatidylcholine to shift the lipid partition balance from cholesterol dispersion and retention in phosphatidylcholine-rich lipid phases in the intestinal contents to cholesterol release and uptake by the enterocytes provides a potential means to regulate cholesterol absorption with $pPLA_2$ inhibitors for therapeutic benefit (43).

We would like to thank Dr. F. J. Field for suggestions concerning the culture of Caco-2 cells. We also thank Drs. B. R. Krause, B. K. Trivedi, and R. Newton for many helpful discussions, H. Roark for the synthesis of FPL 67047XX, and M. K. Anderson for assistance in lipid analysis.

Manuscript received 25 July 1997 and in revised form 22 January 1998.

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