Regulation of apolipoprotein secretion by biliary lipids in newborn swine intestinal epithelial cells

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Regulation of apolipoprotein secretion by biliary lipids in newborn swine intestinal epithelial cells. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G353–G362, 1999.—Biliary lipids, composed of bile acids, cholesterol, and phosphatidylcholine, are a major source of luminal lipid in the small intestine. In the present study in a newborn swine intestinal epithelial cell line (IPEC-1), taurocholate and phosphatidylcholine were found to have no effect on apolipoprotein B (apo B) secretion but did significantly increase the basolateral secretion of apo A-I. This regulation of apo A-I secretion occurred at the pretranslational level for taurocholate and at the posttranslational level for phosphatidylcholine. The regulation of apo A-I secretion by phosphatidylcholine did not involve changes in apo A-I degradation and may involve mobilization of a preformed pool of apo A-I. Cholesterol, whether solubilized with taurocholate or phosphatidylcholine, had no effect on the secretion of either apo B or apo A-I. However, when taurocholate, phosphatidylcholine, and cholesterol were combined, apo B secretion was decreased, and the increase in apo A-I secretion noted with taurocholate and phosphatidylcholine alone was ablated. Another primary bile acid, taurochenodeoxycholate, was found to decrease apo B secretion but had no effect on apo A-I secretion. However, the significance of this effect is uncertain, since this bile acid caused significant cellular membrane injury, as evidenced by increased apical medium lactate dehydrogenase activity. Phosphatidylcholine, but not taurocholate, dramatically increased the basolateral secretion of radiolabeled phospholipid with a modest increase in cellular triglyceride radiolabeling. Furthermore, this effect of phosphatidylcholine on lipid synthesis did not require significant hydrolysis or uptake of the phosphatidylcholine molecule. Studies using radiolabeled taurocholate did not demonstrate active transport of taurocholate by these cells.

[3H]glycerol; mRNA; [35S]methionine; [14C]phosphatidylcholine; phospholipid; [3H]taurocholate

The digestion, uptake, processing, and packaging of lipid with apolipoproteins to form nascent lipoprotein particles by enterocytes for basolateral secretion are crucial events in the absorption and assimilation of luminal lipid in the small intestine. Although dietary lipid is a major nutrient in the neonatal mammal, the small intestine is also the site of absorption of endogenous lipids (26). One important source of endogenous luminal lipids is bile. Biliary lipids consist of bile acids, cholesterol, and phospholipid (predominantly phosphatidylcholine) and normally undergo a quantitatively important enterohepatic circulation (24). Biliary phosphatidylcholine, in particular, appears to play an important role in the mucosal phase of lipid absorption by providing the surface coat for the nascent chylomicron particle (21, 26). Studies in the adult rat have demonstrated that biliary phosphatidylcholine is preferentially used for this purpose over dietary phosphatidylcholine (18, 22), and the presence of additional luminal phosphatidylcholine enhances triglyceride absorption in the rat (2). The effects of biliary lipids on intestinal apolipoprotein synthesis and secretion have been studied in vivo in the intact adult rat (10–13) and in vitro in the human colon carcinoma cell line Caco-2 (14, 19). However, studies in a neonatal model system are lacking.

We have previously described (3–7, 25, 29) the regulation of intestinal apolipoprotein expression in the newborn swine, a model very similar to the human infant with regard to intestinal development and lipoprotein metabolism. In addition to these in vivo studies, we have also characterized (15, 27) the specific effects of the uptake of various fatty acids on apolipoprotein secretion and lipid synthesis in vitro in a cell line (IPEC-1) derived from a newborn unsuckled piglet. These cells differentiate when plated in serum-free medium on collagen-coated filters in Transwell culture plates. The cells become polarized with an apical microvillus membrane and take up fatty acids from the apical culture medium. The fatty acids are reesterified, packaged into lipoprotein particles, and secreted into the basolateral medium (15). Apical uptake of fatty acids is also associated with increased basolateral secretion of apolipoprotein B (apo B) and apo A-I by IPEC-1 cells (15, 27). These cells also switch from exclusive production of apo B-100 in the undifferentiated state to production of apo B-48 with differentiation, accompanied by the appearance of apo B mRNA editing (15). Overall, these cells may be a more physiological model for the immature enterocyte compared with Caco-2 cells, which were derived from a human colon carcinoma (17).

Modulation of apolipoprotein secretion and lipid synthesis by the enterocyte in response to biliary lipid is a potentially important regulatory mechanism in the neonatal mammal. However, at present such regulation is poorly understood in the newborn. The aim of the present study was to determine the effects of incubation of major bile constituents (taurocholate, chole-
terol, and phosphatidylcholine) on the secretion of apo B and apo A-I, as well as the synthesis and secretion of triglyceride and phospholipid, by IPEC-1 cells.

**MATERIALS AND METHODS**

Materials, [1,2,3-H]glycercyl (200 mCi/mmol), trans-[^35]S)methionine (600 mCi/mmol), [dioleoyl-1-14C]phosphatidylcholine (120 mCi/mmol), and [24-14C]taurocholic acid (46.3 mCi/mmol) were purchased from DuPont NEN (Boston, MA). Cholesterol, taurocholate, taurochondoxycholate, egg yolk phosphatidylcholine, essentially fatty acid-free BSA, Triton X-100, phenylmethylsulfonyl fluoride (PMSF), and benzamidine were purchased from Sigma Chemical (St. Louis, MO). IgG-SORB was obtained from the Enzyme Center (Malden, MA), and protein A bound to Sepharose was purchased from Pharmacia Biotechnology (Piscataway, NJ).

Cell culture. The derivation of the IPEC-1 cell line has been described previously (15). Cells from passages 37 to 119 were used in these studies. Undifferentiated IPEC-1 cells were maintained in serial passage in plastic culture flasks (75 cm²; Corning Glassworks, Corning, NY) in growth medium (GM) composed of DMEM/Ham's F-12 medium ( Gibco BRL, Grand Island, NY) supplemented with 5% fetal bovine serum (FBS) (GIBCO BRL), insulin (5 µg/ml), transferrin (5 µg/ml), selenium (5 nmol) (ITS Premix, Collaborative Research, Bedford, MA), epidermal growth factor (5 µg/l) (Collaborative Research), penicillin (50 µg/ml), and streptomycin (4 µg/ml) (GIBCO BRL). To induce differentiation, we harvested undifferentiated cells by trypsinization and plated 2 x 10⁶ cells/well on 24.5-mm-diameter collagen-coated filters (3.0 µm pore size) in Transwell-COL six-well culture plates (Costar, Cambridge, MA). Cells were maintained in serum-containing GM for 48 h, then switched to the same medium containing 10⁻⁷ M dexamethasone (Sigma Chemical) but without FBS. Medium was then changed every 2 days. We have previously shown (15) that after 10 days IPEC-1 cells exhibit enterocytic features, including polarization with well-defined microvilli facing the apical medium. Cellular membrane integrity was assessed by measurement of apical medium lactate dehydrogenase (LDH) activity (Sigma Chemical).

Incubation of cells with lipids. At 10 days postplating on Transwell filters in serum-free medium, fresh serum-free medium was added to both the apical and basolateral compartments. Cells were incubated with various combinations of taurocholate, taurochondoxycholate, cholesterol, and phosphatidylcholine in the medium for 24 h. Cholesterol and phosphatidylcholine were dispersed in the culture medium by first evaporating the chloroform in which these lipids were dissolved under an N₂ stream in a sterile glass tube. Culture medium containing 1 mM taurocholate (for cholesterol dispersion) or medium alone (for phosphatidylcholine) was added, followed by sonication on ice for 1 min at the maximum setting with the microprobe of an ultrasonic dismembranator (Fisher, Pittsburgh, PA). Cells were incubated for 24 h followed by harvest of culture medium and cells. After experimental incubations, cells were rinsed and disrupted in ice-cold PBS containing 1% Triton X-100, 1 mM PMSF, and 1 mM benzamidine, using an ultrasonic dismembranator (Fisher). Cell homogenates were stored at −80°C. Culture medium samples containing the same concentrations of PMSF and benzamidine were also stored at −80°C.

Apo B and A-I quantitation by ELISA. Apo B and Apo A-I protein in cell homogenates and culture medium was quantified by competitive ELISA assays using rabbit anti-swine apo B and Apo A-I polyclonal antibodies, respectively, as previously described (5). Standard antigens consisted of swine plasma low-density lipoprotein (apo B) and high-density lipoprotein (apo A-I). All samples were run in duplicate, and variability between duplicates was < 5%. Secretion of apolipoprotein mass was expressed as nanograms per microgram of cell protein per 24 h, and cell apolipoprotein content was expressed as nanograms per microgram of cell protein.

Measurement of apo A-I synthesis and degradation. Radiolabeling of apo A-I with [35S]methionine in IPEC-1 cells was carried out in two separate experiments. To assess the effects of taurocholate and phosphatidylcholine on apo A-I synthesis, we added fresh medium to differentiated cells at 10 days postplating with 2.0 mM taurocholate, 1.0 mM phosphatidylcholine, or no additive (control) in the apical medium. Cells were incubated for 23 h, followed by the addition of fresh methionine-free medium containing the same additives. One hour later, [35S]methionine (0.5 mCi/well) was added to the apical medium. Cells and basolateral medium were harvested after a 15-min incubation for apo A-I immunoprecipitation as described below. Synthesis was expressed as apo A-I dpm in cell homogenate per microgram of cell protein after the 15-min incubation. During this short radiolabeling period, there was negligible appearance of labeled apo A-I in the basolateral culture medium.

To assess the effects of phosphatidylcholine treatment on apo A-I residence time as an index of degradation, we carried out a pulse-chase experiment in differentiated cells 10 days postplating incubated with and without 1.0 mM phosphatidylcholine added to the apical culture medium. After incubation for 20.5 h, fresh methionine-free medium was added to both the apical and basolateral compartments maintaining the same additives in the apical medium. One hour later, [35S]methionine (1.0 mCi/well) was added to the apical medium. After a 20-min pulse, the apical medium was removed, and the filter inserts were transferred to new plates with fresh serum-free medium containing 10 mCi nonradioabeled methionine with the same additives present during the pulse period. At 0, 0.5, and 2 h into the chase period, cells and basolateral medium were harvested.

After radiolabeling experiments were completed as described above, cells were rinsed and disrupted in ice-cold PBS containing 1% Triton X-100, 1 mM PMSF, and 1 mM benzamidine. An ultrasonic dismembranator (Fisher) was used to harvest cell homogenates at −80°C. Culture medium samples containing the same concentrations of PMSF and benzamidine were also stored at −80°C. Apo A-I immunoprecipitation was carried out as described below.

Apolipoprotein immunoprecipitation. After [35S]methionine radiolabeling, cell homogenates and culture medium were subjected to immunoprecipitation, using a technique adapted from Murthy et al. (20) with rabbit anti-swine apo A-I polyclonal antibodies. The basolateral medium and cell homogenate collected from each well were preclarified by incubation with 0.2 ml of IgG-SORB (10% solution wt/vol) for 1 h at 4°C with constant agitation. The samples were then centrifuged, and the supernatant was collected. Rabbit polyclonal antibodies to swine apo A-I were purified by ammonium sulfate precipitation from serum and added to the supernatant. The amount of antibody added was determined to be in excess by immunoprecipitation of samples. Samples were incubated for 18 h at 4°C with gentle agitation. The antigen-antibody complexes were harvested by the addition of 50 µl of protein A-Sepharose (10%, wt/vol) and incubation for 2 h followed by centrifugation to harvest the pellet. The pellet was washed six times with immunoprecipitation buffer (10 mM NaH₂PO₄, 5 mM Na₂EDTA, 100 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% Triton X-100, 1 mM PMSF, and 1 mM benzamidine) followed by suspension of the pellet in 50 µl.
of Laemmli reducing buffer. Samples were heated at 95°C for 5 min and centrifuged, and the supernatant was subjected to SDS-PAGE using a 15% acrylamide gel under reducing conditions, according to the method of Laemmli (16). After electrophoresis, gels were dried at 80°C for 4 h. Autoradiograms were performed by exposing the gels to Kodak X-Omat AR film for 3–5 days. Apolipoprotein bands were identified by comparison to coelectrophoresed molecular weight standards (GIBCO BRL). After autoradiography, gel bands containing immunoprecipitated apolipoproteins were sliced out, solubilized, and subjected to liquid scintillation counting.

Determination of apo-A-I mRNA levels by slot blot hybridization. Total RNA was extracted from IPEC-1 cells by the method of Chomczynski and Sacchi (9). Intactness of each RNA preparation was verified by agarose gel electrophoresis and visualization of ribosomal RNA subunits. Ten micrograms of RNA were applied to nitrocellulose filters using a slot blot apparatus (Hoefer, San Francisco, CA). Filters were serially hybridized with a radiolabeled swine apo A-I cDNA (25) and an 18S ribosomal subunit cDNA (generously provided by Dr. Nicholas Davidson, University of Chicago, Chicago, IL). Hybridization signals were quantitated using a Bio-Rad model GS-525 molecular imager system and associated software (Hercules, CA). Apo A-I mRNA abundance was expressed as apolipoprotein mRNA-to-18S ribosomal subunit RNA signal intensity ratio.

Triglyceride and phospholipid radiolabeling with [3H]glycerol and determination of fate of [14C]phosphatidylcholine added to apical medium. To determine the effects of taurocholate and phosphatidylcholine on triglyceride and phospholipid synthesis, we incubated cells for 24 h with [3H]glycerol (12 µCi/well) and taurocholate (2.0 mM) and/or phosphatidylcholine (1.0 mM). To determine the fate of phosphatidylcholine added to the apical medium, [14C]phosphatidylcholine (0.5 µCi/well) and unlabeled phosphatidylcholine (1.0 mM) were added to the apical medium and incubated for 24 h. After experimental incubations, cells and medium were collected, processed, and stored as described above. Total lipid in the cells and medium was extracted as previously described (28). Extracts were applied to silica gel G plates and subjected to TLC using a mixture of petroleum ether, diethyl ether, and acetic acid (80:20:1, vol/vol/vol). Lipid bands were identified by exposure to iodine vapor and scraped off the plate for liquid scintillation counting. Bands corresponding to phospholipids, diglycerides, monoglycerides, cholesterol, free fatty acids, triglycerides, and cholesteryl esters were identified by comparison to cochromatographed standards. Cellular content of radioabeled lipid was expressed as specific lipid dpm per well, and secretion of radioabeled lipid was expressed as specific lipid dpm per well for 24 h.

Determination of fate of [14C]taurocholate added to apical medium. The ability of IPEC-1 cells to transport taurocholate was tested by measuring both apical to basolateral and basolateral to apical movement of radioabeled taurocholate against a concentration gradient (0.5–2.0 mM). [14C]Taurocholate was added to either apical or basolateral medium containing the lower cold taurocholate concentration to achieve a final concentration of 0.17 µCi/ml. Cells were incubated for 24 h, followed by harvest of apical and basolateral media and cells for scintillation counting.

Phospholipid and protein measurement. Total lipid was extracted from cell homogenates and basolateral medium as previously described (28), followed by phospholipid measurement by the method of Bartlett (1). Phospholipid mass was expressed as micrograms per well for cells and basolateral medium. Cell homogenate protein was determined by the Bradford method (8).

Statistical analysis. Data from multiple experimental groups were analyzed by one-way ANOVA, followed by the Fisher’s least significant differences test to compare specific groups. Data from two experimental groups were compared by Student’s t-test. Statistical significance was set at two-tailed P < 0.05.

RESULTS

Effect of bile acids, cholesterol, and phosphatidylcholine on apo B and A-I cellular mass and basolateral secretion in IPEC-1 cells. Figure 1A shows IPEC-1 cell apo B content after incubation with no added lipid (control), taurocholate alone, cholesterol solubilized with taurocholate, phosphatidylcholine alone, phosphatidylcholine plus taurocholate, and cholesterol solubilized with phosphatidylcholine. There were no significant differences among the six experimental groups of cells after statistical analysis by ANOVA. Figure 1B shows basolateral secretion of apo B for the six groups. Again, there were no significant differences. Figure 2A shows cell apo A-I content. As observed for apo B, there was no significant difference among the experimental groups. Statistical analysis by ANOVA showed no significant differences.
The increase in apo A-I secretion induced by taurocholate was attenuated when this bile acid was combined with both phosphatidylcholine and cholesterol. Taurochenodeoxycholate had no effect on apo A-I secretion. None of the experimental groups demonstrated a significant increase in LDH activity in apical medium compared with control, except in the case of taurochenodeoxycholate. Incubation with this bile acid resulted in a six-fold increase in LDH activity (data not shown), indicating significant cellular membrane injury. Figure 5 shows a dose-response curve for basolateral apo A-I secretion with increasing concentrations of phosphatidylcholine and taurocholate added to the apical medium. As shown, apo A-I secretion peaks at a phosphatidylcholine concentration of 1.0 mM and begins to decline at a concentration of 2.0 mM. Secretion peaks at a taurocholate concentration of 2.0 mM and begins to decline at a concentration of 4.0 mM. In subsequent experiments, phosphatidylcholine and taurocholate were added to the apical culture medium at concentrations of 1.0 and 2.0 mM, respectively.

Fig. 2. Effect of biliary lipids added to the apical medium on IPEC-1 cellular apo A-I content (A) and basolateral secretion (B). Incubations were carried out for 24 h. See Fig. 1 legend for description of groups. Bars represent means ± SE; n = 5–8 per group. Group means were analyzed by ANOVA. Bars with different superscripts are significantly different at P < 0.05.

Fig. 3. Effect of biliary lipids added to the apical medium on IPEC-1 cellular apo B content (A) and basolateral secretion (B). Incubations were carried out for 24 h. Control, no additives; TC, taurocholate at 1.0 mM. TC + PC + CH, taurocholate plus phosphatidylcholine and cholesterol at 1.0, 0.5, and 0.5 mM, respectively; TCDC, taurochenodeoxycholate at 1.0 mM. Bars represent means ± SE; n = 3–7 per group. Group means were analyzed by ANOVA. Bars with different superscripts are significantly different at P < 0.05.
Effect of taurocholate and phosphatidylcholine on apo A-I synthesis and mRNA levels in IPEC-1 cells. Figure 6 shows apo A-I synthesis by IPEC-1 cells after incubation with taurocholate and phosphatidylcholine. Compared with the control group, taurocholate incubation resulted in an increase in apo A-I synthesis, whereas phosphatidylcholine did not change apo A-I synthesis.

To determine whether the increased apo A-I mass secretion after incubation with phosphatidylcholine might be due to changes in apo A-I degradation, we performed a pulse-chase radiolabeling experiment with \[^{35}\text{S}\]methionine as shown in Fig. 7. Plots of the decline in specific apo A-I counts in cells and medium over the chase period show almost identical slopes, indicating no significant difference in apo A-I degradation with or without phosphatidylcholine. Therefore, the mechanism of the induction of apo A-I secretion by IPEC-1 cells with phosphatidylcholine incubation appears to be posttranslational and does not involve changes in apo A-I degradation. This appears to be similar to the mechanism of the stimulation of apo A-I secretion by oleic acid by these cells (15), possibly involving mobilization of a preformed intracellular pool of apo A-I with a slow turnover.

We next wished to determine whether the observed increases in apo A-I secretion and synthesis induced by
incubation with taurocholate were mediated by changes in apoA-I mRNA levels. Figure 8 shows apoA-I mRNA levels in IPEC-1 cells after incubation with either taurocholate or phosphatidylcholine, compared with control incubations. As expected, phosphatidylcholine incubation did not result in a change in apoA-I mRNA levels. However, taurocholate incubation resulted in a significant increase in apoA-I mRNA levels, paralleling changes in apoA-I secretion and synthesis. Therefore, taurocholate upregulates apoA-I synthesis and secretion at the pretranslational level in IPEC-1 cells.

Effect of taurocholate and phosphatidylcholine on triglyceride and phospholipid synthesis and secretion by IPEC-1 cells. Figure 9 shows the incorporation of [3H]glycerol into triglyceride and phospholipid in cell homogenate and basolateral medium after incubation with the combination of taurocholate and phosphatidylcholine for 24 h. As shown, secretion of radiolabeled phospholipid into basolateral medium was increased 13-fold after incubation with the combination of taurocholate and phosphatidylcholine. In the cell homogenate, there was a modest increase in triglyceride labeling with no change in phospholipid labeling. Figure 10 shows the incorporation of [3H]glycerol into triglyceride and phospholipid in cell homogenate and basolateral medium after separate incubations with either taurocholate or phosphatidylcholine for 24 h. As shown in Fig. 10, phosphatidylcholine incubation was associated with a 12-fold increase in basolateral secretion of radiolabeled phospholipid and a modest increase in cellular triglyceride labeling. Taurocholate had no effect. To determine whether the striking increase in the basolateral secretion of radiolabeled phospholipid after phosphatidylcholine incubation was associated with a similar increase in the secretion of phospholipid mass, we measured phospholipid mass in the cell homogenate and basolateral medium (Fig. 11). As shown, there was an approximate six-fold increase in basolateral secretion of phospholipid after phosphatidylcholine incubation without change in cellular phospholipid mass.

Uptake of radiolabeled phosphatidylcholine by IPEC-1 cells. To determine whether the observed effects on apoA-I secretion and phospholipid synthesis and secretion after phosphatidylcholine incubation resulted from cellular uptake of the phosphatidylcholine, IPEC-1 cells were incubated with [3H]phosphatidylcholine added to the apical medium for 24 h, followed by harvest of the apical and basolateral media and cell homogenate. Total lipid was extracted, and various lipid classes were separated by TLC and subjected to liquid scintillation counting. Of the total counts recovered, 96% remained in the apical medium, 1% was present in the cell homogenate, and 3% was recovered from the basolateral medium. As shown in Table 1, the majority of these counts were present in intact phospholipid in all three locations.
Fate of radiolabeled taurocholate after incubation with IPEC-1 cells. Figure 12 shows the results of an experiment designed to test whether IPEC-1 cells actively transport taurocholate from apical to basolateral medium against a concentration gradient. First, radiolabeled taurocholate was added to the apical medium at a concentration of 0.5 mM, and cold taurocholate was added to the basolateral medium at a concentration of 2.0 mM. After a 24-h incubation, 0.160 µmol of radiolabeled taurocholate appeared in the basolateral medium (Fig. 12). Next, labeled taurocholate was added to the basolateral medium at a concentration of 0.5 mM, and unlabeled taurocholate was added to the apical medium at a concentration of 2.0 mM. After incubation, 0.123 µmol of labeled taurocholate appeared in the apical medium (Fig. 12). These results were not statistically different, demonstrating no net transfer of taurocholate from apical to basolateral medium against a concentration gradient, except for a small amount apparently due to diffusion, possibly by a paracellular route. Less than 1% of radiolabel was recovered in the cell homogenate under both conditions, indicating negligible accumulation of taurocholate within the cells.

DISCUSSION

Biliary lipids, composed of bile acids, cholesterol, and phosphatidylcholine, are a major source of luminal lipid in the small intestine. In the present study in a newborn swine intestinal epithelial cell line (IPEC-1), taurocholate and phosphatidylcholine were found to have no effect on apo B secretion but did significantly increase the basolateral secretion of apo A-I. This regulation of apo A-I secretion occurred at the pretranslational level for taurocholate and at the posttranslational level for phosphatidylcholine. The regulation of apo A-I secretion by phosphatidylcholine did not involve changes in apo A-I degradation. Cholesterol, whether solubilized with taurocholate or phosphatidylcholine, had no effect on the secretion of either apo B or apo A-I. However, when taurocholate, phosphatidylcholine, and cholesterol were combined, apo B secretion was decreased, and the increase in apo A-I secretion noted with taurocholate and phosphatidylcholine alone was ablated. Another primary bile acid, taurochenodeoxycholate, was found to decrease apo B secretion, but had no effect on apo A-I secretion. However, we are uncertain of the significance of this effect, since this bile acid caused significant cellular membrane injury, as evidenced by markedly increased apical medium LDH activity. We also found that phosphatidylcholine, but not taurocholate, dramatically increased the basolateral secretion of radiolabeled phospholipid with a modest increase in cellular triglyceride radiolabeling. Furthermore, this effect of phosphatidylcholine on lipid synthesis did not require significant hydrolysis or uptake of the phosphatidylcholine molecule. Studies using radiolabeled taurocholate did not support the active transport of taurocholate by these cells.

The results of our studies in the IPEC-1 cell line differ in some respects from those of previous studies in the adult rat and the Caco-2 intestinal epithelial cell line. In vivo studies in the adult rat have demonstrated

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### Table 1. Distribution of [14C] in various lipid classes in apical and basolateral media and cell homogenate after 24-h incubation with [14C]phosphatidylcholine

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Apical</th>
<th>Cell</th>
<th>Basolateral</th>
</tr>
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<tbody>
<tr>
<td>PL</td>
<td>99.6</td>
<td>81.1</td>
<td>99.5</td>
</tr>
<tr>
<td>DG + MG</td>
<td>9.2</td>
<td>2.7</td>
<td>9.5</td>
</tr>
<tr>
<td>CH</td>
<td>0.2</td>
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<tr>
<td>FFA</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>TG</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
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<tr>
<td>CE</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
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<td>Total</td>
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<th>Lipid Class</th>
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<th>DG + MG</th>
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<td>Apical</td>
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<tr>
<td>Cell</td>
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<td>2.7</td>
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<td>3.6</td>
<td>10.1</td>
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<td>100</td>
</tr>
<tr>
<td>Basolateral</td>
<td>99.5</td>
<td>9.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>100</td>
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Values are means of data obtained from 4 experiments. PL, phospholipid; DG + MG, diglycerides + monoglycerides; CH, cholesterol; FFA, free fatty acids; TG, triglyceride; CE, cholesteryl ester.
that jejunal apo B synthesis falls after bile diversion, and this fall may be completely prevented by continuous replacement with taurocholate (12). In these same studies (12), ileal apo B synthesis was downregulated to an even greater degree by biliary diversion but was only partially restored by bile acid replacement. Further studies (10) in a chronic bile-diverted rat model demonstrated that the reinfusion of either taurocholate or taurocholate plus lysophosphatidylcholine caused a reexpression of apo B synthesis. However, since apo B expression may also be regulated at the posttranslational level (23), these changes in synthesis may not fully reflect the complete spectrum of regulation under these experimental conditions. Other studies by this same group have demonstrated downregulation of apo A-I synthesis in ileum, but not jejunum, by biliary diversion in the adult rat. However, normal apo A-I synthesis levels could not be restored by bile acid infusion, suggesting the importance of other bile constituents, such as phospholipid (11). In vitro studies in the human colon carcinoma cell line, Caco-2, have demonstrated regulation of apo A-I synthesis and secretion by bile acids and phosphatidylcholine without any effect on apo A-I synthesis or secretion (14, 19).

In adult rat studies, it was shown that cholesterol absorption was negatively correlated with jejunal apo B synthesis (10). Although in vivo data from the rat suggest that changes in intestinal cholesterol absorption may regulate apo B expression (13), it is very difficult to sort out cholesterol as an independent variable, since this sterol requires solubilization with molecules such as bile acids, which may exert an independent effect on apo B. Caco-2 cells incubated with cholesterol or 25-hydroxycholesterol did not demonstrate a change in apo B secretion compared with controls (14). Intestinal apo A-I synthesis was not influenced by either acute or chronic perturbations in intestinal cholesterol flux in the rat (13). Studies in Caco-2 cells have demonstrated no regulation of apo A-I synthesis or secretion by cholesterol (14).

The variance of our results with the findings of others in the adult rat or in Caco-2 cells may be due to several factors. The regulatory patterns described in the present study may be peculiar to the newborn piglet with species and/or developmental specificity. We have previously found major differences between the adult rat and newborn swine in in vivo studies of the effects of dietary lipid on intestinal apolipoprotein expression (3–5, 29). Biliary diversion in the newborn piglet results in a decrease in jejunal apo A-I mass and synthesis without effect on apo B expression (5). Although Caco-2 cells are of human origin, they are derived from a colon carcinoma and may not respond in a completely physiological manner (17). Although caution should also be applied to the interpretation of results obtained from the IPEC-1 cell line, these cells were originally derived from the small intestine of a normal newborn unsuckled piglet, and data derived from our in vivo studies to date in the newborn piglet have generally agreed with in vitro IPEC-1 cell data.

An unexpected finding in this study was the marked increase in secretion of radiolabeled phospholipid into the basolateral medium after incubation of IPEC-1 cells with phosphatidylcholine. This increase in secretion of radiolabeled phosphatidylcholine was accompanied by a parallel increase in the basolateral secretion of phospholipid mass. However, experiments with radiolabeled phosphatidylcholine added to the apical medium demonstrated very limited uptake (4%) of the radiolabel with the majority of radiolabel recovered from the cell homogenate and both apical and basolateral media remaining as intact and unhydrolyzed phospholipid. The mechanism and significance of the uptake of this limited amount of mainly intact phosphatidylcholine are not known. It is possible that the small amount recovered in the cell homogenate may have been nonspecifically bound to the cell membrane, despite extensive washing, and the limited quantity recovered in the basolateral medium may have entered this compartment via the paracellular route. An alternative, but unproven, explanation would involve the hydrolysis of phosphatidylcholine at the apical membrane by a membrane-bound phospholipase, uptake of the hydrolytic products, reesterification within the cell, and basolateral secretion of intact phosphatidylcholine. However, if uptake of the phosphatidylcholine does occur, it is a quantitatively minor process. Overall, these findings suggest that the intact phosphatidylcholine molecule may interact with the apical cell membrane in some fashion to transduce an intracellular signal to upregulate phospholipid synthesis in the smooth endoplasmic reticulum. Mathur et al. (19) demonstrated an increase in basolateral secretion of triacylglycerol accompanied by a modest increase in triacylglycerol synthesis after incubation of Caco-2 cells with phosphatidylcholine. However, in the study by Mathur et al. (19) phospholipid synthesis and secretion were not affected. Determination of the cellular mechanism of this signaling in IPEC-1 cells will be the focus of future studies. We were unable to test the effects of lysophosphatidylcholine in our IPEC-1 cell culture system, since even low concentrations (100 µM) caused excessive cellular injury as reflected by high culture medium LDH activity (data not shown). Regulation of intestinal apolipoprotein expression and lipid synthesis by intact undigesteptd phosphatidylcholine in the intestinal lumen may be important in the neonate with developmental deficiency of pancreatic phospholipase A2, which would lead to significant amounts of intact phosphatidylcholine in the small intestinal lumen.

The cellular mechanisms of the regulation of apo A-I secretion by taurocholate and phosphatidylcholine in IPEC-1 cells are not known at present. With regard to the regulation by taurocholate, our studies using radiolabeled taurocholate demonstrated limited bidirectional diffusion of the bile acid but did not provide evidence for active transport of taurocholate from apical to basolateral medium against a concentration gradient. Less than 1% of the radiolabeled taurocholate was recovered from cell homogenates. Although the
cells were washed thoroughly, it is impossible to accurately determine how much of the associated radiolabel was intracellular or bound to the cell surface. Therefore, it is not known whether taurocholate exerted its effect by interacting with the microvillus membrane or acting intracellularly. However, it is likely that taurocholate induced a cellular signal transduction pathway to the cell nucleus to increase apo A-I mRNA transcript stability or increase transcription of the apo A-I gene to result in increased mRNA levels. Field et al. (14) found that taurocholate inhibited apo B secretion in Caco-2 cells by increasing its rate of degradation. Because treatment with a calcium ionophore produced the same effect, these investigators (14) speculated that taurocholate might produce its effect on apo B secretion through the same mechanism by causing the release of intracellular calcium. Although this may represent a potential mechanism for the regulation of apo A-I secretion in IPEC-1 cells, in the studies in Caco-2 cells taurocholate had no effect on apo A-I secretion. Field et al. (14) also found that apo B secretion was increased by phosphatidylcholine, independent of its hydrolysis, through increasing apo B synthesis. In the present study, phosphatidylcholine, also independent of hydrolysis, increased apo A-I secretion but had no effect on apo B secretion. This effect of phosphatidylcholine was not accompanied by an increase in apo A-I synthesis or mRNA levels or a decrease in apo A-I degradation. We have previously reported a similar posttranslational mechanism responsible for the increase in both apo B and A-I secretion after incubation of IPEC-1 cells with oleic acid, possibly involving the mobilization of a preformed intracellular pool of apolipoprotein with a slow turnover to account for the lack of observable differences in radiolabeling (15). In the present study, it is possible that the increased apo A-I secretion by IPEC-1 cells after phosphatidylcholine incubation may be related to the accompanying increase in secretion of newly synthesized phospholipid and the two secretory processes may be somehow coupled.

In summary, taurocholate and phosphatidylcholine in physiological concentrations, both alone and in combination, induced basolateral secretion of apo A-I, but not apo B, by IPEC-1 newborn swine intestinal epithelial cells. The mechanism of this upregulation of apo A-I secretion appeared to be different for taurocholate (pretranslational) and phosphatidylcholine (posttranslational). Cholesterol, whether dispersed with taurocholate or phosphatidylcholine, had no effect on either apo B or A-I secretion. However, the combination of taurocholate, phosphatidylcholine, and cholesterol decreased apo B secretion but had no effect on apo A-I secretion. Phosphatidylcholine, but not taurocholate, stimulated the synthesis and basolateral secretion of phospholipid. Significant hydrolysis and/or uptake of phosphatidylcholine was not necessary for these effects on apo A-I and phospholipid secretion. The effects of taurocholate on apo A-I expression were not associated with demonstrable active taurocholate transport in IPEC-1 cells. We speculate that these effects of phosphatidylcholine and taurocholate, both major components of bile, may be important in the regulation of apo A-I and phospholipid synthesis and secretion in the newborn mammalian small intestine.

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