Regulation of apolipoprotein secretion by long-chain polyunsaturated fatty acids in newborn swine enterocytes

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Wang, Heng, Song Lu, Jianhui Du, Ying Yao, Helen M. Berschneider, and Dennis D. Black. Regulation of apolipoprotein secretion by long-chain polyunsaturated fatty acids in newborn swine enterocytes. Am J Physiol Gastrointest Liver Physiol 280: G1137–G1144, 2001.—Long-chain polyunsaturated fatty acids (LC-PUFA) are important in the development of the immature nervous system, and adding these fatty acids to infant formula has been proposed. To determine the effect of n-3 LC-PUFA on apolipoprotein secretion and lipid synthesis in newborn swine enterocytes, differentiated IPEC-1 cells were incubated for 24 h with docosahexaenoic acid (DHA; 22:6) or eicosapentaenoic acid (EPA; 20:5) complexed with albumin at a fatty acid concentration of 0.8 mM or albumin alone (control) added to the apical medium. Oleic acid (OA; 18:1) was used a control for lipid-labeling studies. Both DHA and EPA reduced apolipoprotein (apo) B secretion by one-half, whereas EPA increased apo A-I secretion. The increased apo A-I secretion occurred primarily in the high-density lipoprotein fraction. These changes in apoprotein secretion were not accompanied by significant changes in synthesis. Modest decreases in apo B mRNA levels were observed for DHA and EPA, whereas there were no changes in apo A-I mRNA abundance. EPA reduced cellular triglyceride labeling by one-half, whereas EPA increased apo A-I secretion. The increased apo A-I secretion occurred primarily in the high-density lipoprotein fraction. If present in vivo, these effects should be considered before supplementing infant formula with these fatty acids.

docosahexaenoic acid; eicosapentaenoic acid; messenger ribonucleic acid; phospholipid; triglycerol

THE LONG-CHAIN POLYUNSATURATED fatty acids (LC-PUFA), arachidonic acid [AA; 20:4(n-6)], and docosahexaenoic acid [DHA; 22:6(n-3)] are important for mammalian central nervous system and retinal development (2, 3, 6–8, 15, 16). Humans do not synthesize n-3 or n-6 long-chain fatty acids. Therefore, AA and DHA may be synthesized from linoleic acid [18:2(n-6)] and linolenic acid [18:3(n-3)], respectively, which must be derived from the diet and are considered essential nutrients. However, human infants, especially preterm infants, may have limited ability to elongate and desaturate linoleic and linolenic acids to AA and DHA, thereby predisposing to a deficiency state, even with adequate stores of the precursor fatty acids (14). As a result, there is interest in supplementing infant formula with LC-PUFA. However, the effects of these fatty acids on immature enterocyte lipid and apolipoprotein synthesis and secretion are not known. There is particular concern regarding the n-3 LC-PUFA, DHA, and eicosapentaenoic acid [EPA; 20:5(n-3)], because these fatty acids have been shown (18, 20, 23, 29, 30, 34, 36, 37) to inhibit lipoprotein secretion from cultured enterocytes (Caco-2 cells) and hepatocytes. A study (13) in the lymph-fistula rat demonstrated impaired transport of DHA and EPA into mesenteric lymph relative to oleic acid (OA). However, direct evidence for impairment of triacylglycerol secretion from enterocytes in vivo is lacking, because enterocytes in vivo synthesize triglycerides via the mono-glyceride pathway, whereas Caco-2 cells produce triglycerides through the α-glycerophosphate pathway (19). These fatty acids have also been shown to reduce hepatic very low density lipoprotein (VLDL) secretion in humans (25, 26).

In the present study, we focused on two n-3 LC-PUFA: DHA and EPA. EPA is a precursor of DHA, and both are found in marine oils. DHA and EPA have both been found to lower serum triglyceride levels in adult human studies (12, 28). The aim of our study was to determine the effect of DHA and EPA on apolipoprotein and lipid synthesis and secretion in a newborn swine intestinal epithelial cell line (IPEC-1). This cell line was derived from a newborn unsuckled piglet, and we (11, 32, 33) have previously characterized the apical uptake, cellular processing, and basolateral secretion of several fatty acids and biliary lipids, as well as...
associated changes in apolipoprotein secretion. Although these cells do differentiate in serum-free medium to resemble enterocytes functionally and morphologically, several features suggest that the cells do not mature to ultimately resemble adult enterocytes but rather remain in a relatively immature state, more closely resembling late fetal or early neonatal enterocytes (11). For example, in undifferentiated IPEC-1 cells, there is no expression of apolipoprotein B (apo B) mRNA editing activity, and only apo B-100 is synthesized and secreted, as is the case in the early human and swine fetus (11). In differentiated cells cultured in serum-free medium on collagen-coated filters for 10–12 days, ~50% of the apo B mRNA is edited, and these cells synthesize and secrete both apo B-100 and B-48. In neonatal and adult swine, >90% of the apo B mRNA is edited in small intestine, and their enterocytes produce predominantly apo B-48 (11). In addition, even when maximally differentiated, these cells tend to be cuboidal and have glycogen inclusions, a feature of immature enterocytes (22). In the present study, lipid and apolipoprotein synthesis and secretion were characterized after incubation of IPEC-1 cells with DHA and EPA in the apical culture medium compartment.

MATERIALS AND METHODS

Materials. [1,2,3-3H]glycerol (0.2 Ci/mmol) was purchased from Dupont New England Nuclear (Boston, MA). DHA [C22:6(n-3)], EPA [C20:5(n-3)], OA [C18:1(n-9)], essentially fatty acid-free BSA, Triton X-100, phenylmethylsulfonyl fluoride (PMSF), and benzamidine were purchased from Sigma Chemical (St. Louis, MO).

Cell culture. The derivation of the IPEC-1 cell line has been described previously (11). Cells from passages 25 to 80 were used in these studies, and all cell culture was carried out at 37°C in an atmosphere containing 5% CO2. Undifferentiated IPEC-1 cells were maintained in serum-free medium in plastic culture flasks (75 cm2, Corning Glassworks, Corning, NY) in growth medium (GM): DMEM/F-12 medium (GIBCO BRL), supplemented with 5% fetal bovine serum (FBS) (GIBCO BRL, Grand Island, NY) in growth medium (GM): DMEM/F-12 medium (GIBCO BRL), supplemented with 5% fetal bovine serum (FBS) (GIBCO BRL), insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml) (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, undifferentiated cells were harvested by trypsinization, and 2 x 106 cells/well were plated on 24.5-mm diameter collagen-coated filters (3-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) but without FBS. Medium was then changed every two days. We have previously shown that after 10 days IPEC-1 cells exhibit enterocyte features, including polarization, with well-defined microvilli facing the apical medium (11). Cellular membrane integrity was assessed by measurement of apical medium lactate dehydrogenase (LDH) activity (Sigma Chemical).

Incubation of cells with fatty acids. At 10 days postplating on Transwell filters in serum-free medium, we added fresh serum-free medium to both the apical and basolateral compartments. The apical medium contained fatty acid complexed with albumin (4:1 molar ratio) at a concentration of 0.8 mM (23). This fatty acid concentration is in the physiological range, and above this concentration the basolateral secretion of triacylglycerol begins to plateau in IPEC-1 cells (11). Cells were incubated for 24 h followed by harvest of culture medium and cells. In lipid radiolabeling experiments, [3H]glycerol (12 μCi/well) was also added to the apical medium, concomitant with the addition of fatty acid and albumin. 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 dismembrator (Fisher, Pittsburgh, PA). Cell homogenates were stored at ~80°C. Culture medium samples containing the same concentrations of PMSF and benzamidine were also stored at ~80°C.

Triacylglycerol and phospholipid radiolabeling with [3H]glycerol. Cells were incubated for 24 h with [3H]glycerol and fatty acids complexed with albumin, and the cells and medium were collected and processed as described above. Total lipids in the cells and medium was extracted as previously described (32). Extracts were applied to silica gel G plates and subjected to TLC using petroleum ether-diethyl ether-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 and triacylglycerols were identified by comparison to cochromatographed standards. Cellular content of radiolabeled lipid was expressed as specific lipid dpm per well, and secretion of radiolabeled lipid was expressed as specific lipid dpm per well per 24 h.

Isolation of basolateral medium lipoprotein fractions and lipoprotein-free fraction. After incubation of cells with fatty acids, basolateral culture medium was subjected to sequential density ultracentrifugation using a Beckman SW 41 Ti rotor (Palo Alto, CA) at 17°C (32). The density classes separated were chylomicron (CM) plus VLDL [density (d) = 1.006 g/ml], low-density lipoprotein (LDL; 1.063 g/ml ≤ d ≤ 1.063 g/ml), and high-density lipoprotein (HDL; 1.063 g/ml ≤ d ≤ 1.21 g/ml). The d > 1.21 g/ml lipoprotein-free fraction was also collected. Lipoprotein fractions were subjected to apo A-I quantitation by ELISA, as well as lipid extraction followed by phospholipid measurement.

Apo B and A-I mass quantitation by ELISA. Apo B and A-I mass in cell homogenates and culture medium was quantitated by competitive ELISA using rabbit anti-swine apo B and A-I polyclonal antibodies, respectively, as previously described (4). Standard antigens consisted of swine plasma LDL (apo B) and HDL (apo A-I). All samples were run in duplicate, and variability between duplicates was <5%. Secretion of apolipoprotein mass was expressed as micrograms per milligram cell protein per 24 h, and cell apolipoprotein content was expressed as micrograms per milligram cell protein.

Measurement of apo B and A-I synthesis. Radiolabeling of apo B and A-I with [35S]methionine in IPEC-1 cells was carried out to assess the effect of DHA and EPA on apolipoprotein synthesis. Fresh medium was added to differentiated cells at 10 days postplating with 0.8 mM DHA or EPA complexed with albumin or albumin only (control), as described in previous experiments (11, 32), 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 B and A-I immunoprecipitation as described below. Synthesis was expressed as apolipoprotein dpm in cell homogenate per well after the 15-min incubation. We (11) have demonstrated previously the negligible appearance of labeled apo B or A-I...
in the basolateral culture medium during this short radiolabeling period.

After radiolabeling experiments were completed, cells were rinsed and disrupted in ice-cold PBS containing 1% Triton X-100, 1 mM PMSF, and 1 mM benzamidine by using an ultrasonic dismembranator (Fisher). Cell homogenates were stored at −80°C. Apo B and 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. (23) with rabbit anti-swine apo B and A-I polyclonal antibodies. The cell homogenates collected from each well were precleared 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 B and 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 reimmunoprecipitation 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 (Na₂)EDTA, 100 mM NaCl, 0.02% Na azide, 0.1% SDS, 1% Triton X-100, 1 mM PMSF, and 1 mM benzamidine] following by suspension of the pellet in 50 μl of Laemmli reducing buffer (17). Samples were heated at 95°C for 5 min and centrifuged, and the supernatant was subjected to SDS-PAGE using a 3% to 20% acrylamide gradient gel for 5 min and centrifugation to harvest the pellet. The pellet was washed six times with immunoprecipitation buffer [10 mM NaH₂PO₄, 5 mM (Na₂)EDTA, 100 mM NaCl, 0.02% Na 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 (17). Samples were heated at 95°C for 5 min and centrifuged, and the supernatant was subjected to SDS-PAGE using a 3% to 20% acrylamide gradient gel under reducing conditions according to the method of Laemmli (17). After electrophoresis, gels were dried at 80°C for 4 h. Autoradiography was 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 apo B-48, B-100, and A-I were sliced out, solubilized, and subjected to liquid scintillation counting.

**Apo B and A-I mRNA quantitation by RT-PCR.** Total RNA was extracted from cells after experimental incubations (9). Aliquots (2–10 μg) were treated with 0.5 U of DNase RQ1 (Promega, Madison, WI) at 37°C for 60 min in 50 μl 40 mM Tris·HCl, pH 7.5, 6.6 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, and 20 U RNase inhibitor (RNasin, Promega). The RNA was then sequentially extracted with phenol-chloroform and chloroform, precipitated with ethanol, washed once (with 70% ethanol), and resuspended in 20–40 μl H₂O. For reverse transcription, 5 μg total RNA were used. Reverse transcription was performed at 42°C for 15 min in a final volume of 20 μl in buffer containing 10 mM Tris·HCl, pH 8.3, 90 mM KCl, 1 mM MnCl₂, 200 μM of each dNTP, 0.5 μg oligo(dT)₁₅ as primer, and 15 U apical membrane vesicle RT (Promega). After reverse transcription, the single-strand cDNA was amplified using the Qiagen Taq PCR core kit (Santa Clarita, CA) with 1 μl cDNA and 100 pmol of each specific primer (B100F and B100R for apo B, AIF and AIR for apo A-I, and B2MGF and B2MGR for β₂-microglobulin) in a total volume of 50 μl. After incubation for 3 min at 95°C, PCR was performed for 18 cycles in a thermal cycler (Perkin-Elmer Cetus) as follows: 45 s at 94°C, 45 s at 55°C, and 45 s at 72°C. For each RNA sample, a negative control was run to check for DNA contamination by using AmpliTaq (Perkin-Elmer Cetus), leaving the sample on ice during reverse transcription. Additionally, each reaction contained a tube with all of the above buffers and enzymes but without RNA to exclude PCR product contamination. After RT-PCR, two-fifths of the reaction products were subjected to 1.5% agarose electrophoresis. Expected product sizes were confirmed as follows: β₂-microglobulin, 172 bp; apo B, 290 bp; and apo A-I, 371 bp. RT-PCR products were transferred to nitrocellulose filters (Trans-Blot, Bio-Rad, Hercules, CA) and hybridized with sequence-confirmed relevant PCR fragments. Quantitation was carried out using a GS-700 scanning densitometer (Bio-Rad). Results were expressed as a ratio of apolipoprotein to β₂-microglobulin arbitrary densitometric units.

**Oligonucleotides.** The following primers were used for PCR: B2MGF, 5′-GAA GAT GAA GGC GGA GCA GT-3′ (5′ at 192 nt); B2MGR, 5′-TGC CCG TTA GTG TGC TC-3′ (5′ at 363 nt); B100F, 5′-GCA GCT CCA CCA TTC AGT TC-3′ (5′ at 5912 nt); B100R, 5′-CTG TTC TAA GGC CAC AGT GC-3′ (5′ at 6201 nt); AIF, 5′-GAA CAA GTG GCA GGA GGA GA-3′ (5′ at 384); and AIR, 5′-GGA TGC TGA CCT GGT GGT TC-3′ (5′ at 751 nt).

**Protein and phospholipid measurement.** Cell homogenate protein was determined by the Bradford method (5). Phospholipid mass content in cell homogenates and culture medium lipoprotein fractions was determined by the Bartlett method (1) after lipid extraction.

**Statistical analysis.** Data in experimental groups were analyzed by one-way ANOVA followed by Fisher's least significant difference test to compare specific groups. Statistical significance was set at two-tailed P < 0.05.

## RESULTS

**Effect of DHA and EPA on apolipoprotein secretion.** Figure 1, top, shows IPEC-1 cellular apo B and A-I content in control cells and in cells incubated for 24 h with either DHA or EPA. There were no differences among experimental groups for either apo B or A-I. Basolateral secretion of apo B and A-I, as depicted in Fig. 1, bottom, was regulated by LC-PUFA. For apo B, both DHA and EPA reduced apo B mass secretion by approximately one-half. In the case of apo A-I, EPA treatment increased apo A-I secretion relative to the control group. Figure 2 shows that the additional apo A-I secreted in response to EPA treatment was secreted in the HDL range, making it extremely likely that this apo A-I is associated with lipid particles. Note that there is a significant amount of free apo A-I in the lipoprotein-free d > 1.21 density fraction, but this does not change with EPA treatment. DHA treatment did not result in a significant increase in apo A-I secretion relative to controls (Fig. 1). None of the fatty acids caused significant cellular injury as assessed by measurement of culture medium LDH activity (data not shown).

**Effect of DHA and EPA on apolipoprotein mRNA levels and synthesis.** Figure 3, top, shows apo B and A-I synthesis as assessed by [35S]methionine radiolabeling, followed by immunoprecipitation and SDS-PAGE. The use of SDS-PAGE allowed the resolution of both apo B-48 and apo B-100 immunoprecipitates. There were no statistically significant differences among the three experimental groups, although apo A-I synthesis tended to parallel apo A-I secretion for the three experimental groups.
Apo B and A-I mRNA levels were measured by RT-PCR after IPEC-1 cell incubation for 24 h with DHA and EPA. As shown in Fig. 3, bottom, there were modest, but statistically significant, decreases in apo B mRNA abundance after treatment with DHA (−16%) and EPA (−23%) relative to the control group. This finding suggests that the decrease in basolateral secretion of apo B after DHA and EPA exposure may be at least partially regulated at the pretranslational level. There were no statistically significant differences among the three experimental groups for apo A-I mRNA levels.

Effect of DHA and EPA on triacylglycerol and phospholipid synthesis and secretion. Figure 4, top, shows the incorporation of [3H]glycerol into cellular triacylglycerol and phospholipid after incubation with OA (control), DHA, and EPA. Cellular triacylglycerol labeling was significantly reduced by EPA to 55% of the control value, with DHA having no significant effect. Cellular phospholipid labeling was reduced 42% by DHA and 61% by EPA relative to the control group. In basolateral medium (Fig. 4, bottom), radiolabeled triacylglycerol secretion paralleled cellular radiolabeling with EPA, reducing secretion of labeled triacylglycerol by 75% relative to the OA control group. Incubation with DHA increased the secretion of labeled phospholipid approximately twofold, and EPA had no effect relative to the OA control group.

We performed an additional experiment to address a seemingly paradoxical observation, namely a decrease in phospholipid labeling in cell homogenate in the face of increased secretion of labeled phospholipid in cells treated with DHA relative to those incubated with OA. Figure 5 shows phospholipid mass in cell homogenates, medium lipoprotein fractions, and the medium lipoprotein-free fraction from control cells and cells treated with OA and DHA. Compared with control cells, both OA and DHA induce increased phospholipid mass in cell homogenate and the CM plus VLDL and HDL fractions of the basolateral medium. Contrary to our findings with radiolabeled phospholipid, there was no difference in cell homogenate total phospholipid content.
between OA- and DHA-treated cells. There was a modest increase in basolateral medium phospholipid mass in the lipoprotein-free fraction from DHA-treated cells compared with OA-treated cells. This change paralleled that observed for total basolateral medium phospholipid in the phospholipid-labeling experiments, although not to the same magnitude. Together, these findings suggest that phospholipid mass changes lag behind the changes observed for phospholipid radiolabeling. Furthermore, both radiolabeled phospholipid and phospholipid mass data suggest that DHA stimulates production of new phospholipid, which is promptly secreted into the lipoprotein-free basolateral medium fraction, compared with OA-treated cells and does not accumulate in the cell.

DISCUSSION

This study is the first to demonstrate that the n-3 LC-PUFA, EPA and DHA, regulate apolipoprotein secretion and lipid synthesis in newborn swine intestinal epithelial cells. Apo B secretion was reduced by a modest extent by both DHA and EPA. In contrast, apo A-I secretion, predominantly in the basolateral medium HDL fraction, was significantly increased by EPA. Cellular triacylglycerol labeling was reduced by EPA, and cellular phospholipid labeling was reduced by both DHA and EPA. Secretion of radiolabeled triacylglycerol was reduced by EPA, whereas secretion of labeled phospholipid was doubled by DHA.

Inhibition of apo B secretion by EPA has previously been described (23, 29, 30) in the human colon carcinoma cell line Caco-2. Murthy et al. (23) demonstrated that apo B synthesis and secretion were reduced in Caco-2 cells after EPA treatment for 48 h, relative to OA. Apo B mRNA levels were also reduced, suggesting
Fig. 5. Effect of DHA on basolateral secretion of phospholipid mass in lipoprotein fractions and cell homogenate (Homog). After incubation with fatty acids, cell homogenates and basolateral medium lipoprotein fractions were subjected to lipid extraction and phospholipid mass quantitation. Bars represent means of data from 2 wells.

pretranslational regulation by EPA. Ranheim et al. (29, 30) also observed reduced apo B secretion from Caco-2 cells with EPA incubation relative to OA after relatively shorter incubation times. No such studies have been performed in Caco-2 cells by using DHA. In addition to these observations in Caco-2 cells, n-3 fatty acids have been shown to reduce apo B secretion by cultured rat hepatocytes (18, 34), primary human hepatocytes (20), Hep G2 cells (36), and perfused rat liver (37). Furthermore, n-3 fatty acids appear to inhibit apo B secretion from hepatocytes by stimulating apo B degradation (34). In the present study in IPEC-1 cells, we found reduced secretion of apo B by both EPA and DHA relative to the fatty acid-free control. We also found modest, but significant, accompanying decreases in apo B mRNA levels, suggesting at least partial regulation at the pretranslational level. However, there were no significant differences in apo B synthesis after incubation with either DHA or EPA compared with controls. It is possible that we may have observed greater reduction in apo B mRNA levels and/or synthesis with longer incubation times.

With regard to the cellular mechanism of posttranslational downregulation of apo B secretion by DHA and EPA in IPEC-1 cells, there are four possibilities. First, although not specifically addressed in the present study, apo B degradation may be enhanced by DHA and EPA. Second, mobilization of a preformed, intracellular pool of apo B with slow turnover, as we (11) have previously described as a mechanism for the increase in apo A-I secretion induced by OA, which seems to involve the mobilization of a preformed intracellular pool of apo A-I with a relatively slow turnover. This mechanism may also mediate the increased basolateral secretion of apo A-I induced by EPA.

The suppression of triacylglycerol synthesis and secretion by EPA may potentially be explained by at least three mechanisms. First, EPA may inhibit triacylglycerol synthesis. Previous studies (27, 35) in rat hepatocytes suggested that n-3 fatty acids are poor substrates for triacylglycerol synthesis compared with OA. Other studies (21, 31) in hepatocytes have suggested that n-3 fatty acids inhibit the activities of diacylglycerol acyltransferase and phosphatidate phosphohydrolase, two enzymes in the triacylglycerol synthetic pathway. Murthy et al. (24) demonstrated that in Caco-2 cells, phospholipids of microsomes from cells incubated with EPA were enriched in this fatty acid. Furthermore, this microsomal lipid compositional change was associated with a decrease in microsomal triacylglycerol synthesis and diacylglycerol acyltransferase activities. Increased fatty acid oxidation is another potential mechanism to explain decreased availability of fatty acids for incorporation into intestinal lipoprotein triacylglycerol. In rat liver, EPA acts as a mitochondrial proliferator and enhances mitochondrial β-oxidation, whereas prolonged DHA feeding increases peroxisomal β-oxidation.
In these rat experiments (10), EPA was noted to be hypotriglycerideremic and DHA was not. However, it has been shown that both OA and EPA are minimally oxidized to CO₂ in Caco-2 cells (24). Thus it appears that in contrast to liver, intestinal epithelial cells shunt very limited amounts of fatty acids into oxidative pathways. Finally, decreased apical uptake of EPA relative to OA could explain the present findings. However, this is doubtful, because EPA has been shown to be taken up by Caco-2 cells at a somewhat higher rate than OA (30).

Both DHA and EPA reduced cellular phospholipid radiolabeling relative to OA. However, relative to OA, DHA doubled the basolateral secretion of labeled phospholipid and EPA had no effect. The mechanism of these effects of DHA and EPA on phospholipid synthesis and secretion in IPEC-1 cells is not clear. However, these observations may represent a depletion of the intracellular pool of phospholipid to maintain an equivalent secretion of labeled phospholipid, as in the case of EPA, or an enhanced secretion, as in the case of DHA, relative to OA. Compared with control cells, both OA and DHA induced increased phospholipid mass in cell homogenate and the CM plus VLDL and HDL fractions of the basolateral medium. Contrary to our findings with radiolabeled phospholipid, there was no difference in cell homogenate total phospholipid content between OA- and DHA-treated cells. There was a modest increase in basolateral medium phospholipid mass in the lipoprotein-free fraction from DHA-treated cells compared with OA-treated cells. This change paralleled that observed for total basolateral medium labeled phospholipid in the phospholipid-labeling experiments, although not to the same magnitude. Together, these findings suggest that phospholipid mass changes lag behind the changes observed for phospholipid radiolabeling. Furthermore, both radiolabeled phospholipid and phospholipid mass data suggest that DHA stimulates production of new phospholipid, which is promptly secreted into the lipoprotein-free basolateral medium fraction compared with OA-treated cells and does not accumulate in the cell. Detailed studies of the intracellular trafficking of phospholipid, as well as triacylglycerol, are needed to further elucidate these mechanisms.

In summary, the present studies demonstrate that apo B and A-I secretion, as well as triacylglycerol and phospholipid synthesis and secretion, are regulated by DHA and EPA in newborn swine intestinal epithelial cells. Although DHA decreases apo B secretion, it has only a modest effect on triglyceride synthesis and secretion and actually increases phospholipid secretion. A novel finding not previously reported in either cultured Caco-2 cells or hepatocytes is the induction of basolateral apo A-I secretion in the medium HDL fraction by EPA in IPEC-1 cells. Whether this might be beneficial to the neonate by increasing serum apo A-I and HDL levels and cholesterol transport is not known. Although EPA increases apo A-I secretion, it decreases both apo B secretion and triglyceride synthesis and secretion, which may be potentially deleterious. These findings may have important clinical implications for infants fed formulas supplemented with these fatty acids. Further studies may be needed before infant formulas are routinely supplemented with LC-PUFA, particularly from marine oil sources.

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