Overexpression of Apolipoprotein A-IV Enhances Lipid Transport in Newborn Swine Intestinal Epithelial Cells*

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Apolipoprotein A-IV (apoA-IV) has myriad functions, including roles as a post-prandial satiety factor and lipid antioxidant. ApoA-IV is expressed in mammalian small intestine and is up-regulated in response to lipid absorption. In newborn swine jejunum, a high fat diet acutely induces a 7-fold increase in apoA-IV expression. To determine whether apoA-IV plays a role in the transport of absorbed lipid, swine apoA-IV was overexpressed in a newborn swine enterocyte cell line, IPEC-1, followed by analysis of the expression of genes related to lipoprotein assembly and lipid transport, as well as quantitation of lipid synthesis and secretion. A full-length swine apoA-IV cDNA was cloned, sequenced, and inserted into a Vp and Rep gene-deficient adenovirus associated viral vector, containing the cytomegalovirus immediate early promoter/enhancer and neomycin resistance gene, and was used to transfect IPEC-1 cells. Control cells were transfected with the same vector minus the apoA-IV insert. Using neomycin selection, apoA-IV-overexpressing (+AIV) and control (−AIV) clones were isolated for further study. Both undifferentiated (−D) and differentiated (+D) +AIV cells expressed 40- to 50-fold higher levels of apoA-IV mRNA and both intracellular and secreted apoA-IV protein compared with −AIV cells. Expression of other genes was not affected by apoA-IV overexpression in a manner that would contribute to enhanced lipid secretion. +D +AIV cells secreted 4.9-fold more labeled triacylglycerol (TG), 4.6-fold more labeled cholesteryl ester (CE), and 2-fold more labeled phospholipid (PL) as lipoproteins, mostly in the chylomicron/very low density lipoprotein (VLDL) density range. ApoA-IV overexpression in IPEC-1 cells enhances basolateral TG, CE, and PL secretion in chylomicron/VLDL particles. This enhancement is not associated with up-regulation of other genes involved in lipid transport. ApoA-IV may play a role in facilitating enterocyte lipid transport, particularly in the neonate receiving a diet of high fat breast milk.

Apolipoprotein A-IV is a peptide expressed in the mammalian small intestine (1–3). It appears to have myriad functions, including roles as a post-prandial satiety signal (4, 5), lipoprotein anti-oxidant (6), participant in reverse cholesterol translocation (7, 8), and a major factor in the prevention of atherosclerosis (9, 10). Of all genes related to intestinal lipid transport, the apoA-IV gene is the most responsive to intestinal lipid flux (11). In the enterocyte, apoA-IV is incorporated into nascent chylomicrons at an early stage of biogenesis in the ER and is secreted with the chylomicron at the basolateral membrane (12). After secretion, most apoA-IV dissociates from the chylomicron surface and is present in the circulation as the free protein (13).

We have previously shown that apoA-IV is expressed in the small intestine of neonatal swine (14). In animals given a high triacylglycerol intraduodenal infusion for 24 h, jejunal apoA-IV expression increases 7-fold at the pre-translational level in comparison to control animals given a low triacylglycerol infusion (14, 15). This observation, coupled with the fact that this responsiveness decreases as the animals are weaned from a diet of high fat breast milk, suggests that apoA-IV may play a role in facilitating intestinal lipid absorption. Additional evidence for a role for apoA-IV in lipid absorption was recently provided with the observation that fractional cholesterol absorption was reduced in humans with the A-IV-2 allele, which encodes a Q360H substitution in apoA-IV, while receiving a high cholesterol, high polyunsaturated fat diet (16).

To test the hypothesis that the provision of excess apoA-IV will enhance enterocyte secretion of triacylglycerol and phospholipid in response to fatty acid absorption, we used an adenovirus-associated viral (AAV) expression vector to develop a stably transduced newborn swine intestinal epithelial cell line that synthesizes and secretes excess swine apoA-IV. We used the IPEC-1 cell line for this purpose, because we have extensively characterized the regulation of lipoprotein synthesis and secretion and apolipoprotein expression by lipid in these cells (17–20). The IPEC-1 cell line was derived from an unsuckled newborn piglet using selective subculture techniques. These cells are induced to differentiate when plated on collagen-coated permeable membranes in serum-free medium and have been shown to take up fatty acids through the apical membrane; esterify the fatty acids into triacylglycerol, cholesteryl ester, and phospholipid; package the lipid into lipoproteins with apolipoproteins; and secrete lipoproteins via the basolateral membrane (17). These cells synthesize and secrete relatively abundant amounts of apoB and apoA-I. However, we have made the observation that these cells have a very low lipid secretion efficiency and express very low levels of apoA-IV mRNA and protein, compared with normal enterocytes.

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1 The abbreviations used are: ER, endoplasmic reticulum; AAV, adenovirus-associated viral; MTF, microsomal triglyceride transfer protein; HNF-4, hepatic nuclear factor-4; OA, oleic acid; nt, nucleotides; ITR, inverted terminal repeat; LDH, lactate dehydrogenase; RT, reverse transcriptase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.
In the present studies, we wished to specifically test the hypothesis that increased availability of apoA-IV improves the lipid secretion efficiency of IPEC-1 cells. Lipid synthesis and secretion in response to incubation with radiolabeled oleic acid were studied in differentiated cells overexpressing apoA-IV, as well as in cells transfected with the control vector. In addition, both cell lines were characterized with regard to expression of apoA-I, -A-IV, -B, and -C-III, as well as microsomal triglyceride transfer protein (MTP) large subunit and hepatic nuclear factor-4 (HNF-4), under varying conditions of differentiation and fatty acid treatment, to rule out changes in expression of other genes involved in lipoprotein assembly and secretion, which might alter lipid secretion.

**EXPERIMENTAL PROCEDURES**

Materials—[1-14C]Oleate (50 mCi/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Unlabeled oleic acid (C18:1n-9, OA) and essentially fatty acid-free bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO).

Cloning of a Swine Full-length ApoA-IV cDNA—The following PCR primers were generated from the published human (21) and swine (GenBank™ accession number AJ222966) apoA-IV cDNA sequences: Forward, 5′-AGG TGA GCT GCC TGGGCCCCTTTTAAGGAGACGCTGCGACCAGGTCAACACC (nt 1 to 144); reverse, 5′-GAT AGT ACG TGC CAC TG-3′ (antisense, nt +1256 to +1275).

Total RNA was extracted from domestic pig intestinal cells and IPEC-1 cells, and 2.5 μg was used for cDNA synthesis. In the PCR reaction, one-tenth of the generated cDNA was used for high fidelity PCR. After 12 cycles, a 1.35-kb PCR product was purified and cloned into the pTA vector from CLONTECH (Palo Alto, CA) to generate pTA/AIV. Plasmid DNA sequences were confirmed by restriction enzyme digestion and automated sequencing.

**FIG. 1.** Apolipoprotein A-IV cDNA and protein sequences. A, portion of apoA-IV cDNA sequence with differences between the full-length cDNA sequence derived from mRNA from swine intestinal epithelial cells and IPEC-1 cells (top) and the previously reported swine sequence (middle) (GenBank™ accession AJ222966). The human sequence is also shown (bottom) (GenBank™ accession NM000482) (21). B, the corresponding amino acid sequences coded by the cDNA sequences.

**FIG. 2.** Map of the AAV expression vectors used for stable transfection of IPEC-1 cells. A, pSL/Neo control vector. B, pSL/AIV/Neo vector containing the full-length swine apoA-IV insert. The details of the construction of these vectors are provided under “Experimental Procedures.” AmpR, ampicillin resistance gene; BGH, bovine growth hormone polyadenylation signal; IRES, internal ribosome entry site; ITRs, inverted terminal repeats; IVS, synthetic intron; Neo, neomycin resistance gene; PcmvIE, human cytomegalovirus immediate early promoter/enhancer.
Expression of ApoA-IV in IPEC-1 Cells—The derivation of the IPEC-1 cell line has been described previously (17). Cells from passages 25 to 80 were used in these studies, and all cell cultures were carried out at 37 °C in an atmosphere containing 5% CO2. Undifferentiated IPEC-1 cells were maintained in serial passage in plastic culture flasks (75 cm2 in diameter) for 5 passages. Corn oil (BTC Biosciences, Bedford, MA), epidermal growth factor (5 µg/filiter) (BD Biosciences), penicillin (50 µg/ml), and streptomycin (4 µg/ml) (Invitrogen) were used in the initial differentiation, undifferentiated cells were harvested by trypsinization, and 2 × 106 cells/well were plated on 24.5-mm diameter collagen-coated filters (3.0-µm pore size) in Transwell culture plates (Costar, Corning, NY). Cells were maintained in serum-containing growth medium for 48 h and then switched to the same medium containing 10% dexamethasone (Sigma) but without fetal bovine serum. Medium was then changed every 2 days. We have previously shown that after 10 days IPEC-1 cells exhibit enterocyte features, including polarization with well-defined microvilli facing the apical membrane (17). Cellular membrane integrity was assessed by measurement of apical membrane lactate dehydrogenase (LDH) activity (Sigma).

Undifferentiated IPEC-1 cells were transfected with 0.2 µg of pSL/AIV-Neo and pSL/AIV-IRES-LCMV-2000 (Invitrogen, Carlsbad, CA) in six-well culture plates. Forty-eight hours later, 1 × 106 cells were harvested for preparation of total RNA and whole cell extracts. The cells in the remaining wells were used for G418 (neomycin analogue, Invitrogen) selection. After 3 weeks of G418 selection and clone expansion, pSL/AIV/Neo and pSL/AIV-IRES-LCMV-2000 IPEC-1 cell clones were characterized before, during, and after differentiation with regard to: 1) apoA-I, A-IV, B, and C-III mRNA expression; 2) MTP large subunit and MTP large subunit RNA analysis; and 3) apoA-IV and A-I antibody levels were determined as previously described (14, 24). The antisera were subjected to ammonium sulfate precipitation and used directly as a 1:1000 dilution for Western blot analysis.

IPEC-1 cells from Transwell filters were lysed in 0.5 ml of radiomucine precipitation assay buffer with protease inhibitors (Complete, Roche Diagnostics, Indianapolis, IN) for whole cell extraction. One milliliter of basolateral culture medium was collected and added to protease inhibitors on ice and concentrated 4/1 (Centriplus YM-10, Millipore, Austin, TX). Twenty micrograms of lysate or medium protein was electrofocused on an 8% SDS-PAGE gel followed by transfer to nitrocelulose filters. Western blotting was conducted using the ECL Western blot kit according to the manufacturer’s protocol (Amerssh Biosciences, Piscataway, NJ).

Incubation of Cells with Oleic Acid—Undifferentiated (on plastic in serum-containing medium), partially differentiated (5 days of post-plating on Transwell filters in serum-free medium), or maximally differentiated (10 days of post-plating Transwell filters in serum-free medium) were prepared, and fresh medium was added to the culture flask (undifferentiated cells) or both the apical and basolateral Transwell compartments (differentiated cells). The apical medium contained oleic acid complexed with albumin (41 molar ratio) at a concentration of 0.8 µm (25). This fatty acid concentration is in the physiologic range, and above this concentration the basolateral secretory of triacylglycerol begins to plateau in IPEC-1 cells (17). Cells were incubated for 24 h followed by harvest of culture medium and cells. For studies of protein and mRNA, cell lysates were prepared and processed as described above. In lipid radiolabeling experiments in maximally differentiated cells, [14C]oleate (8.5 µCi/well) complexed with albumin was added to the apical medium at a fatty acid concentration of 0.8 µm. At incubation, labeled cells were rinsed and disrupted in ice-cold phosphate-buffered saline containing 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine using an ultrasonic dismembranator (Branson, Danbury, CT). Cell homogenates were stored at –80 °C. Culture medium samples containing the same concentrations of phenylmethylsulfonyl fluoride and benzamidine were also stored at –80 °C.

Lipid Radiolabeling with [14C]Oleate—First, a pulse-chase experiment was performed by incubating differentiated cells with [14C]oleate complexed with albumin at a fatty acid concentration of 0.8 µm in the apical medium for 30 min, followed by change of medium and continued chase with 0.8 µm unlabeled oleic acid in the apical medium compartment. Collections of total basolateral medium were made at 0, 3, 6, 12, and 24 h for lipid extraction and determination of triacylglycerol and phospholipid radiolabeling.

Next, differentiated cells were incubated for 24 h with [14C]oleate complexed with albumin at a fatty acid concentration of 0.8 µm in the apical medium for 24 h, followed by harvest of cells and basolateral medium for lipid extraction and determination of triacylglycerol, cholesterol ester, and phospholipid radiolabeling. Basolateral medium was subjected to sequential ultracentrifugation, which isolated the lipoprotein fractions. 

Isolation of Basolateral Medium Lipoprotein Fractions—After incubation of cells with fatty acids, basolateral culture medium was subjected to sequential density ultracentrifugation using a Beckman SW 41 rotor (Beckman Instruments, Palo Alto, CA) at 17 °C (18). The density classes separated were chylomicron plus very low density lipoprotein (VLDL) (d ≤ 1.006 g/ml), low density lipoprotein (LDL) (1.006 g/ml ≤ d ≤ 1.063 g/ml), and high density lipoprotein (HDL) (1.063 g/ml ≤ d ≤ 1.21 g/ml).

Lipid Extraction and Thin-layer Chromatography—Radiolabeled cell homogenates, basolateral medium, and lipoprotein fractions were subjected to lipid extraction as previously described (18). Extracts were applied to Silica Gel G plates and subjected to thin-layer chromatography using petroleum ether-diethyl ether-acetic acid (80:20:1, v/v/v). Lipid bands were identified by exposure to iodine vapor and scraped off the plate for liquid scintillation counting. Bands corresponding to phosho...
lipid, cholesteryl ester, and triacylglycerol were identified by comparison to co-chromatographed standards. Cellular content of radiolabeled lipid was expressed as specific lipid dpm/well, and secretion of radiolabeled lipid, cholesteryl ester, and triacylglycerol were identified by comparison for each sample for Western blotting as described under "Experimental Procedures."

**RESULTS**

**IPEC-1 Cell Clones Overexpressing ApoA-IV**—As shown in Fig. 3A, semi-quantitative RT-PCR analysis of the mRNA from IPEC-1 pSL/AIV/Neo and pSL/Neo undifferentiated clones demonstrated the predicted 492-bp apoA-IV PCR product. The pSL/AIV/Neo clone had markedly higher levels of apoA-IV mRNA, as compared with that of the pSL/Neo clone. Fig. 3B shows an apoA-IV Western blot of cell lysates from a pSL/Neo clone and five pSL/AIV/Neo clones demonstrating a band of ~42 kDa. This apparent molecular mass is the same as we have reported previously for swine apoA-IV in studies of apoA-IV isolated from piglet mesenteric lymph and immunoprecipitated from newborn swine small intestine (14). Note the markedly higher levels of apoA-IV protein in the pSL/AIV/Neo clones.

**Expression of ApoA-I, B, and C-III, MTP Large Subunit and HNF-4 in IPEC-1 Cells Overexpressing ApoA-IV**—To ensure that any changes observed in lipid transport in pSL/AIV/Neo cells were not due to the up-regulation of other genes involved in cell differentiation or lipid absorption and metabolism by the transfection and/or clone selection process, the mRNA expression of the genes for apoA-I, C-III, B, MTP large subunit and HNF-4 was analyzed by semi-quantitative RT-PCR. Undifferentiated, partially differentiated (day 5 on Transwell filters in serum-free medium), and maximally differentiated (day 10 on Transwell filters in serum-free medium) pSL/Neo and pSL/AIV/Neo cells were studied with and without incubation with 0.8 mM oleic acid added to the apical medium compartment for 24 h.

As shown in Fig. 4, both undifferentiated and differentiated pSL/AIV/Neo cells expressed 40- to 50-fold higher levels of apoA-IV mRNA than pSL/Neo cells under corresponding conditions. ApoA-I mRNA levels appeared to be modestly suppressed by the presence of the apoA-IV insert, except in the intermediate differentiated cells, where the pSL/AIV/Neo cells appeared to have higher levels. ApoB mRNA expression was slightly higher in undifferentiated pSL/AIV/Neo cells but otherwise remained unchanged under all conditions. ApoC-III expression was suppressed in both undifferentiated and differentiated pSL/AIV/Neo cells, and the increase in apoC-III expression with oleic acid incubation in differentiated pSL/AIV/Neo cells was absent in the pSL/AIV/Neo cells. This observation was particularly striking in undifferentiated cells. The induction of MTP large subunit expression with oleic acid incubation in differentiated pSL/AIV/Neo cells was absent in the differentiated pSL/AIV/Neo cells. Expression of HNF-4 was detectable in undifferentiated and partially differentiated cells, but low in differentiated cells. We have previously found this pattern of HNF-4 expression during differentiation in native IPEC-1 cells. Because HNF-4 is a major regulator of apoA-IV transcription and basal gene expression, this low level of HNF-4 expression may contribute to the low level of apoA-IV expression in differentiated IPEC-1 cells. The presence or absence of apoA-IV overexpression or oleic acid incubation appeared to have no significant influence on HNF-4 expression. In summary, increased expression of these genes, above that normally associated with fatty acid treatment, was not present in overexpressing cells.

**ApoA-I and -A-IV Protein in IPEC-1 Cells Overexpressing ApoA-IV**—Fig. 5 shows the levels of apoA-I and -A-IV protein in...
lysates from undifferentiated and maximally differentiated pSL/Neo and pSL/AIV/Neo cells with and without 0.8 mM oleic acid incubation. Interestingly, apoA-I protein was expressed in cells at a higher level in the undifferentiated state for both pSL/Neo and pSL/AIV/Neo cells, as compared with differentiated cells. Oleic acid incubation appeared to modestly increase cellular apoA-I protein levels in both pSL/Neo and pSL/AIV/Neo cells, both undifferentiated and differentiated. ApoA-IV protein levels in pSL/Neo cells clearly increased with oleic acid incubation in undifferentiated cells. However, apoA-IV protein levels were very low in differentiated pSL/Neo cells without a clear change after oleic acid treatment. As expected, high levels of apoA-IV protein were present in both undifferentiated and differentiated pSL/AIV/Neo cells with no major change with incubation with oleic acid. In the basolateral culture medium from differentiated cells, pSL/AIV/Neo cells secreted high levels of apoA-IV protein with and without oleic acid treatment. Although basolateral medium levels of apoA-IV protein were very low for pSL/Neo cells, there did appear to be a slight increase with oleic acid incubation.

**Culture Medium LDH Activity and Cellular Alkaline Phosphatase Activity**—Fig. 6A shows medium LDH activity as a marker for cellular injury. Overexpression of apoA-IV did not result in any significant change in activity with or without oleic acid incubation, as compared with the control cells. Fig. 6B shows cellular alkaline phosphatase activity as a marker for differentiation. There were no differences in activity between pSL/Neo and pSL/AIV/Neo cells at 5 and 10 days after plating in Transwell culture plates. Each data point represents the mean of measurements from two culture wells.

**Synthesis and Secretion of Lipid and Lipoproteins by IPEC-1 Cells Overexpressing ApoA-IV**—A pulse-chase experiment was performed after incubation of pSL/Neo and pSL/AIV/Neo cells with 0.8 mM [14C]oleate in the apical medium compartment for 30 min, followed by a chase with 0.8 mM unlabeled oleate. Basolateral medium was collected at timed intervals from separate culture wells for lipid extraction and measurement of labeled triacylglycerol and phospholipid. Fig. 7 shows the results of this experiment for triacylglycerol (Fig. 7A) and phospholipid (Fig. 7B). Over the initial 12 h of the chase period, secretion of labeled lipid was nearly linear for both pSL/Neo
and pSL/AIV/Neo cells for both lipid classes. Secretion began to plateau from 12 to 24 h for both groups of cells. However, secretion of both labeled triacylglycerol and phospholipid was consistently higher in the pSL/AIV/Neo cells over the entire chase period.

To determine the influence of apoA-IV overexpression on the basolateral secretion of lipid in specific lipoprotein classes, differentiated pSL/Neo and pSL/AIV/Neo cells were incubated for 24 h with 0.8 mM oleic acid and [14C]oleate added to the apical medium compartment, followed by analysis of radioactivity in triacylglycerol, cholesteryl ester and phospholipid in cell homogenates and lipoproteins in basolateral culture medium. Fig. 8 shows the amounts of radiolabeled triacylglycerol, cholesteryl ester, and phospholipid incorporated into cellular lipids over a 24-h period. There were no major differences in lipid radiolabeling in either pSL/Neo or pSL/AIV/Neo cells. The somewhat less total labeled lipid in the pSL/AIV/Neo cells may reflect the more efficient basolateral secretion. Fig. 9 shows the incorporation of radiolabel into basolateral medium lipoproteins. After incubation with [14C]oleate, apoA-IV overexpression resulted in 4.9-fold increased labeled triacylglycerol, 4.6-fold increased labeled cholesteryl ester, and 2-fold increased labeled phospholipid secretion, as compared with control cells. The majority of this increased labeled lipid was secreted in particles in the chylomicron/VLDL density range as shown in Fig. 10A. No striking differences in the content of labeled lipid were noted in the particles of LDL (Fig. 10B) or HDL (Fig. 10C) density. The d > 1.21 g/ml lipoprotein-free fraction of the basolateral medium from both cell lines contained labeled phospholipid, as we have described previously in native IPEC-1 cells (17), but no labeled cholesteryl ester or triacylglycerol.

**DISCUSSION**

In the present studies, we have demonstrated that an AAV expression vector can be used to develop stably transfected clones of newborn swine intestinal epithelial cells expressing high levels of apoA-IV, present both intracellularly and secreted into the culture medium. These cells can be induced to

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**FIG. 7.** Effect of apoA-IV overexpression on basolateral lipid secretion in IPEC-1 cells. Differentiated pSL/Neo and pSL/AIV/Neo cells were pulsed for 30 min with 0.8 mM [14C]oleate complexed with albumin added to the apical medium compartment, followed by a chase with 0.8 mM unlabeled oleic acid. Basolateral medium from separate culture wells was then harvested at 0, 3, 6, 12, and 24 h, followed by lipid extraction, thin-layer chromatography, and scintillation counting to determine triacylglycerol (A) and phospholipid (B) radiolabeling, expressed as dpm/well. Points represent mean ± S.E. from three separate culture wells. Values for pSL/AIV/Neo cells were significantly different from those of pSL/Neo cells at: *, p < 0.05 using Student’s unpaired t test.

**FIG. 8.** Effect of apoA-IV overexpression on cellular lipid synthesis in IPEC-1 cells. Differentiated pSL/Neo and pSL/AIV/Neo cells were incubated for 24 h with 0.8 mM [14C]oleate complexed with albumin added to the apical medium compartment. Cells were then harvested, followed by lipid extraction, thin-layer chromatography, and scintillation counting to determine triacylglycerol, cholesteryl ester, and phospholipid radiolabeling, expressed as dpm/well. Bars represent the mean of measurements from two separate experiments that did not vary more than 10%.
differentiate and synthesize and basolaterally secrete complex lipids when incubated with oleic acid added to the apical culture medium. Furthermore, the basolateral secretion of newly synthesized triacylglycerol, cholesteryl ester, and phospholipid in chylomicron/VLDL particles was enhanced in IPEC-1 cells overexpressing apoA-IV.

Analysis of the expression of other genes relevant to lipid absorption and metabolism in the pSL/Neo and pSL/AIV/Neo cells under conditions of varying states of differentiation and fatty acid absorption did not reveal changes related to the overexpression of apoA-IV that would explain the observed differences in radiolabeled lipid secretion. ApoB is absolutely required for triacylglycerol-rich lipoprotein assembly in the ER and is an integral surface component of these lipoproteins during the trafficking and secretory processes, as well as during peripheral metabolism (27). Under all conditions, semiquantitative RT-PCR analysis demonstrated no change in apoB mRNA expression, except for slightly higher mRNA levels in undifferentiated pSL/Neo cells. In general, in both liver and small intestine, apoB expression is physiologically regulated at the pre-translational level by a degradative pathway from which apoB may be rescued by lipidation (25, 27). We did not study apoB protein levels, and it is conceivable that the excess apoA-IV protein in the ER might interfere with apoB degradation, leading to more apoB available for lipoprotein assembly. This issue is currently under investigation.

ApoA-I and -C-III are expressed in the small intestine, and their genes form a cluster with that of apoA-IV on the same chromosome (21). Although these apolipoproteins are not known to play a role in lipoprotein assembly and secretion, their expression is regulated by lipid absorption in newborn swine enterocytes (15, 28). In previous studies, we have shown that apoA-I and -C-III are regulated at the translational and pre-translational levels, respectively (15, 28). The major finding in the present study was that the increase in apoC-III mRNA levels induced by oleic acid incubation in the pSL/Neo cells in both the differentiated and, most strikingly, undifferentiated states was abrogated in the pSL/AIV/Neo cells. Whether this observation is related to some type of feedback regulation of apoC-III transcription or transcript stability remains to be determined. ApoA-I mRNA levels appeared to be modestly suppressed by the presence of the apoA-IV insert, except in the intermediate differentiated cells, where the pSL/AIV/Neo cells appeared to have higher levels.

Microsomal triglyceride transfer protein (MTP) is a heterodimeric protein complex, consisting of a large subunit (97 kDa), which possesses lipid transfer activity, and a smaller subunit identical to protein disulfide isomerase (55 kDa) (29). This protein complex has been recently found to function in the small intestine and liver to transport ER membrane-bound lipid, primarily newly synthesized triacylglycerol, to newly

![Fig. 9. Effect of apoA-IV overexpression on basolateral secretion of lipoprotein-associated lipid in IPEC-1 cells. Differentiated pSL/Neo and pSL/AIV/Neo cells were incubated for 24 h with 0.8 mM [14C]oleate complexed with albumin added to the apical medium compartment. Basolateral medium was then harvested and subjected to isolation of the total lipoprotein fraction by density ultracentrifugation, followed by lipid extraction, thin-layer chromatography, and scintillation counting to determine triacylglycerol, cholesteryl ester, and phospholipid radiolabeling, expressed as dpm/well. For each cell line, four culture wells were pooled for each experiment. Bars represent the mean of measurements from two separate experiments that did not vary more than 10%.](image1)

![Fig. 10. Effect of apoA-IV overexpression on basolateral secretion of chylomicron/VLDL (A), LDL (B), and HDL (C)-associated lipid in IPEC-1 cells. Differentiated pSL/Neo and pSL/AIV/Neo cells were incubated for 24 h with 0.8 mM [14C]oleate complexed with albumin added to the apical medium compartment. Basolateral medium was then harvested and subjected to isolation of each lipoprotein fraction by sequential density ultracentrifugation as described under "Experimental Procedures," followed by lipid extraction, thin-layer chromatography, and scintillation counting to determine triacylglycerol, cholesteryl ester, and phospholipid radiolabeling, expressed as dpm/well. For each cell line, four culture wells were pooled for each experiment. Bars represent the mean of measurements from two separate experiments that did not vary more than 10%.](image2)
translated apoB in the ER lumen as the first step in triacylglycerol-rich lipoprotein biogenesis (29, 30). In the small intestine, MTP may also facilitate the further lipiddation of nascent chylomicrons beyond the first apoB rescue step (31). In the present studies, there was in general no induction of MTP expression by apoA-IV overexpression. In fact, the up-regulation of MTP large subunit mRNA by oleic acid that we have described previously (32) was absent in the differentiated pSL/AIV/Neo cells. Therefore, an induction of MTP large subunit gene expression would not explain the increased lipid secretion in the pSL/AIV/Neo cells compared with the pSL/Neo cells.

The overexpression of apoA-IV did not appear to have any effect on the differentiation of the pSL/AIV/Neo cells, as reflected by the expression of HNF-4. This member of the nuclear hormone receptor superfamily is an important modulator of both development/differentiation and apoA-I, -AIV, and -C-III gene transcription (33, 34). In native IPEC-1 cells, we have found that HNF-4 expression declines with differentiation.2 This relative deficiency of apoHNF-4 may contribute to the low apoA-IV expression in native differentiated IPEC-1 cells. In the present studies, there were no differences in HNF-4 mRNA levels in either the pSL/Neo or pSL/AIV/Neo cells with expression highest in undifferentiated through day 5 of differentiation and lowest levels in maximally differentiated cells. As another marker of differentiation, we measured alkaline phosphatase activity and found no differences in apoA-IV overexpressing or control cells at 5 and 10 days after plating on Transwell filters.

The increased cellular content of apoA-IV is apparently not toxic to the IPEC-1 cells. Culture medium LDH activity as a marker for cell injury was not different between the two cell lines after maximal differentiation, either with or without oleic acid incubation.

The enhanced basolateral secretion of newly synthesized triacylglycerol, cholesteryl ester, and phospholipid by differentiated pSL/AIV/Neo cells, as compared with the pSL/Neo control cells, was the most interesting finding of this study. The majority of the increased lipid secretion in the cells overexpressing apoA-IV was in the chylomicron/VLDL density range. This increased secretion was not due to major differences in cellular synthesis of these lipids. It should be noted that, although the IPEC-1 cell line has been a useful in vitro model for studying immature enterocyte absorptive physiology, a major limitation is the fact that lipid secretion efficiency is very low. Intracellular synthesis and accumulation of triacylglycerol and phospholipid readily occurs, but these newly synthesized lipids are poorly secreted. Additionally, the relatively low expression of apoA-IV in native IPEC-1 cells may be a limiting factor in lipid secretion. Therefore, an effect of apoA-IV overexpression on lipoprotein assembly and/or secretion may have been responsible for the enhanced basolateral lipid secretion.

Several lines of evidence suggest that apoA-IV may play a role in intestinal lipid transport. ApoA-IV has been shown to be a component of apoB-containing, triacylglycerol-poor chylomicron precursors in the enterocyte ER in the rat (12). ApoA-IV expression in mammalian small intestine is highly regulated by lipid absorption (11). In the newborn piglet, a model for the human infant dependent on a diet of high fat breast milk, jejunal apoA-IV expression is up-regulated ~7-fold at the transcriptional level by a high fat duodenal infusion over a 24-h period (14, 15). Recently, fractional cholesterol absorption was shown to be reduced in humans, receiving a high cholesterol, high polyunsaturated fat diet, with the A-IV-2 allele, which encodes a Q360H substitution in apoA-IV (16). Because cholesterol absorption occurs via chylomicron assembly and secretion, the authors of that study (16) speculate that the mutant apoA-IV-2 isoprotein has a higher surface activity and may impede the influx of free cholesterol onto the surface of nascent chylomicrons as they are expanding during lipidation. All of these observations taken together suggest a role for apoA-IV in intestinal chylomicron assembly and/or secretion.

We speculate that the availability of additional apoA-IV in the ER during chylomicron assembly, either through increased synthesis (as in the newborn piglet) or overexpression by genetic manipulation (as in the present studies), may provide additional functional advantage (as in hepatic VLDL). These domains can modify their surface conformation in response to changes in particle size (35). Therefore, in small intestine, apoA-IV may not serve an obligatory role in chylomicron synthesis, as does apoB, but rather an accessory role, especially during conditions of high lipid flux. This hypothesis may not be inconsistent with findings in transgenic mouse models with overexpression of human apoA-IV or targeted disruption of the apoA-IV gene in which lipid absorption was reported as unaffected (36, 37). These studies were not performed under conditions of sustained lipid absorption approaching the rate-limiting capacity of the enterocyte. Also, the apoA-IV-deficient transgenic animals may develop or induce alternative pathways to augment lipid absorption. The suckling rat has been shown to inefficiently transport lipid in lymph chylomicrons, and indirect evidence suggests that the portal venous route may be important in this species during the suckling period (38). In contrast, we have found lymphatic transport to be an important lipid absorptive route in the suckling swine (24), a species whose intestinal development more closely resembles the human (39, 40). Studies are currently underway to characterize and compare the intracellular and secreted lipoprotein particles from pSL/Neo or pSL/AIV/Neo cells.

In summary, newborn swine intestinal epithelial cells may be stably transfected with an AAV expression vector to achieve sustained overexpression of apoA-IV. ApoA-IV overexpression in IPEC-1 cells enhances basolateral triacylglycerol, cholesteryl ester, and phospholipid secretion, primarily in particles in the chylomicron/VLDL density range. This enhancement is not associated with up-regulation of other genes involved in lipid transport. We speculate that apoA-IV may play a role in facilitating enterocyte lipid transport under conditions of high lipid flux such as the newborn receiving a diet of high fat breast milk.

**REFERENCES**

1. Haddad, I. A., Ordovas, J. M., Fitzpatrick, T., and Karathanasis, S. K. (1986) J. Biol. Chem. 261, 13268–13277
2. Apfelbaum, T. F., Davidson, N. O., and Glickman, R. M. (1987) Am. J. Physiol. 253, G662–G666
3. Elshourbagy, N. A., Walker, D. W., Paik, Y. K., Boguski, M. S., Freeman, M., Gordon, J. I., and Taylor, J. M. (1987) J. Biol. Chem. 262, 7973–7981
4. Fujimoto, K., Cardelli, J. A., and Tso, P. (1992) Am. J. Physiol. 262, G1002–G1006
5. Fujimoto, K., Fukagawa, K., Sakata, T., and Tso, P. (1993) J. Clin. Invest., 91, 1830–1833
6. Qin, X., Swertfeger, D. K., Zheng, S., Hui, D. Y., and Tso, P. (1998) Am. J. Physiol. 274, H1836–H1840
7. Dvorin, E., Gorder, L. N., Benson, D. M., and Gotto, A. M., Jr. (1986) J. Biol. Chem. 261, 15714–15718
8. Stein, O., Stein, Y., Lefevre, M., and Roheim, P. S. (1986) Biochim. Biophys. Acta 878, 7–13
9. Cohen, R. D., Castellani, L. W., Qiao, J.-H., Lenten, B. J. v., Lusis, A. J., and Reue, K. (1997) J. Clin. Investig. 99, 1906–1916
10. Duverger, N., Tremp, G., Gaillaud, J.-M., Emmanuel, F., Castro, G., Fruchtach, J.-C., Steinmetz, A., and Benelle, P. (1996) Science 273, 906–908
11. Kalogeris, T. J., Rodriguez, M. D., and Tso, P. (1998) J. Lipid Res. 31, 497–505
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15. Black, D. D., Wang, H., Hunter, F., and Zhan, R. (1996) Biochem. Biophys. Res. Commun. 221, 619–624
16. Weinberg, R. B., Geissinger, B. W., Kasala, K., Hockey, K. J., Terry, J. G., Easter, L., and Cruise, J. R. (2000) J. Lipid Res. 41, 2035–2041
17. Gonzalez-Vallina, R., Wang, H., Zhan, R., Berschneider, H. M., Lee, R. M., Davidson, N. O., and Black, D. D. (1996) Am. J. Physiol. 271, G249–G259
18. Wang, H., Berschneider, H. M., Du, J., and Black, D. D. (1997) Am. J. Physiol. 272, G535–G542
19. Wang, H., Roberson, R., Du, J., Eshun, J. K., Berschneider, H. M., and Black, D. D. (1999) Am. J. Physiol. 276, G353–G362
20. Wang, H., Lu, S., Du, J., Yao, Y., Berschneider, H. M., and Black, D. D. (2000) Am. J. Physiol. 279, G325–G331
21. Karathanasis, S. K. (1985) Proc. Natl. Acad. Sci., U. S. A. 82, 6374–6378
22. Samulski, R. J., Chang, L. S., and Shenk, T. (1989) J. Virol. 63, 3822–3828
23. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
24. Black, D. D., and Davidson, N. O. (1989) J. Lipid Res. 30, 207–218
25. Murthy, S., Albright, E., Mathur, S. N., Davidson, N. O., and Field, F. J. (1992) Arterioscler. Thrombosis 12, 691–700
26. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
27. Davis, R. A. (1997) Biochim. Biophys. Acta 1345, 136–150
28. Black, D. D., and Rohwer-Nutter, P. L. (1991) Pediatr. Res. 29, 32–38
29. Wetterau, J. R., Lin, M. C. M., and Jamil, H. (1997) Biochim. Biophys. Acta 1345, 136–150
30. Gordon, D. A., Wetterau, J. R., and Gregg, R. E. (1995) Trends Cell Biol. 5, 317–321
31. Cartwright, I. J., Plonne, D., and Higgins, J. A. (2000) J. Lipid Res. 41, 1728–1739
32. Lu, S., Huffman, M., Mansbach, C. M., Cheng, X., and Black, D. D. (2002) J. Lipid Res. 43, in press
33. Chen, W. S., Manova, K., Weinstein, D. C., Duncan, S. A., Plump, A. S., Prezioso, V. R., Bachvarova, R. F., and J. E. Darnell, J. (1994) Genes Dev. 8, 2466–2477
34. Kardassis, D., Lacotripe, M., Talianidis, I., and Zannis, V. (1996) Hypertension 37, 1080–1088
35. Chauhan, V., Wang, X., Ramsamy, T., Milne, R. W., and Sparks, D. L. (1998) Biochemistry 37, 3735–3742
36. Aalto-Setala, K., Bisgaier, C. L., Ho, A., Kieft, K. A., Trober, M. G., Kayden, H. J., Ramakrishnan, R., Walsh, A., Essenburg, A. D., and Breslow, J. L. (1994) J. Clin. Invest. 93, 1776–1786
37. Weinstock, P. H., Bisgaier, C. L., Hayek, T., Aalto-Setala, Sehayek, E., Wu, L., Shultzle, P., Merkel, M., Essenburg, A. D., and Breslow, J. L. (1997) J. Lipid Res. 38, 1782–1794
38. Ee, L. C., Zheng, S., Yao, L., and Tso, P. (2000) Am. J. Physiol. 279, G225–G231
39. Corring, T., Durand, G., and Henry, Y. (1982) World Rev. Nutr. Diet 39, 124–130
40. Henning, S. J. (1987) in Physiology of the Gastrointestinal Tract (Johnson, L. R., ed) 2nd Ed., pp. 285–300, 2 vols., Raven Press, New York