Hepatocyte nuclear factor-4 mediates apolipoprotein A-IV transcriptional regulation by fatty acid in newborn swine enterocytes

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Leng S, Lu S, Yao Y, Kan Z, Morris GS, Stair BR, Cherny MA, Black DD. Hepatocyte nuclear factor-4 mediates apolipoprotein A-IV transcriptional regulation by fatty acid in newborn swine enterocytes. Am J Physiol Gastrointest Liver Physiol 293: G475–G483, 2007. First published June 7, 2007; doi:10.1152/ajpgi.00072.2007.—Hepatocyte nuclear factor-4α (HNF-4α) regulates transcription of several genes involved in lipid metabolism, including that of apolipoprotein (apo) A-IV, which is tightly regulated by lipid absorption and enhances enterocyte chylomicron secretion. Studies were performed to define the role of HNF-4α in the regulation of apo A-IV gene transcription by dietary fatty acid in neonatal swine small intestine. HNF-4α mRNA was expressed in liver > intestine > kidney in suckling, weaning, and weaned pigs. Jejunal HNF-4α mRNA and protein and apo A-IV and swine microsomal triglyceride transfer protein (MTP) large subunit mRNA expression were induced in parallel in 2-day-old swine by a 24-h high-fat intraduodenal infusion. In IPEC-1 cells, incubation with oleic acid (OA) resulted in coordinate induction of both HNF-4α, apo A-IV, and MTP mRNA, similar to that observed in vivo. When HNF-4α expression was driven by doxycycline by using the TET-On system in the absence of OA to observe the effect of HNF-4α directly on apo A-IV and MTP mRNA levels in the absence of other factors that might be concomitantly induced by fatty acid absorption, apo A-IV and MTP expression were increased. In luciferase reporter gene assays in IPEC-1 cells using apo A-IV/C-III intergenic region constructs, TET-On-regulated HNF-4α expression without OA increased luciferase activity, and incubation with OA did not further increase activity. These data suggest that acute induction of the apo A-IV and MTP genes by dietary lipid in newborn intestine occurs, at least in part, via ligand-independent transactivation by HNF-4α that is itself induced by a lipid-mediated mechanism.

apo B; IPEC-1 cells; lipid absorption; microsomal triglyceride transfer protein; neonate; oleic acid

Apolipoprotein A-IV is a lipid-binding polypeptide synthesized in the small intestine and is tightly regulated by lipid absorption (1, 5, 22). Although myriad extraintestinal functions have been proposed (11, 12, 14, 33, 45), in the enterocyte apo A-IV appears to play a role in the regulation of chylomicron assembly and secretion. Our laboratory (27, 28) has previously demonstrated that increased expression of apo A-IV in a newborn swine intestinal epithelial cell line (IPEC-1) results in the basolateral secretion of larger chylomicrons transporting an increased amount of lipid. In the newborn piglet, acute dietary lipid absorption strongly induces intestinal apo A-IV expression at the transcriptional level (5, 6, 30). However, after weaning this induction is considerably blunted, suggesting a teleological role for apo A-IV in the absorption of a high dietary lipid load, such as that found in breast milk during the suckling period. Therefore, there is considerable interest in delineating the mechanisms of apo A-IV gene regulation by dietary lipid.

The genes for apolipoproteins A-V, A-IV, C-III, and A-I form a cluster on chromosome 11 in the human (23, 38). The apo C-III gene is located downstream from the apo A-I gene and upstream from the apo A-IV gene. The apo A-V gene is located 28 kb downstream of the apo A-IV gene and is transcribed in the same direction as apo A-IV and A-I. The apo C-III gene is transcribed in the opposite direction to that of the other three genes. All four genes are involved in several aspects of lipid metabolism. Tissue-specific and basal regulation of apo A-I, C-III, and A-IV gene transcription has been the focus of extensive study, and relevant cis- and trans-acting factors have been identified (3, 49). Of particular importance in the regulation of the basal and spatial expression of the apo A-IV gene in the small intestine are the proximal apo A-IV promoter (−700/−310) and the distal apo C-III enhancer (−890/−500) regions and associated trans-acting transcription factors (2, 8, 25, 26, 37). However, the regulatory factors involved in lipid-mediated induction of apo A-IV gene expression are poorly understood.

Hepatocyte nuclear factor-4α (HNF-4α) is a nuclear receptor that regulates transcription of numerous genes, including several involved in lipid and glucose metabolism (17, 42). In the small intestine, HNF-4α appears to be a central regulator of gene expression during enterocyte differentiation (43). Although HNF-4α is capable of constitutive transcriptional activation independent of ligand binding, potential endogenous HNF-4α activating ligands have been identified in several studies. There is evidence that these ligands may include fatty acids and fatty acyl CoAs (18, 19), and X-ray crystallography studies have revealed that HNF-4α has a bound fatty acid in a ligand-binding pocket (10). However, this binding pocket appears to be too small to accommodate fatty acyl CoAs (7). Furthermore, this bound fatty acid was not exchangeable with exogenous fatty acids and was required to stabilize the protein for crystallization (46). Collectively, these data suggest that the bound fatty acid may function as a structural cofactor for HNF-4α, rather than a ligand activator. Therefore, a regulatory role for bound fatty acids and fatty acyl CoAs as ligands in the activation of HNF-4α remains controversial (21).

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Recent studies have defined a role for HNF-4α in the transcriptional regulation of apo A-IV in the enterocyte, including the basal spatial expression of apo A-IV in the small intestine with restriction of expression to the villus and a proximal to distal gradient via interaction with the apo A-IV gene proximal promoter and distal apo C-III gene enhancer (2, 37). Peignon et al. (32) demonstrated that E-cadherin-dependent cell adhesion in Caco-2 cells controls the abundance of HNF-4α, which in turn regulates apo A-IV expression. However, data supporting a role for HNF-4α participation in dietary lipid-mediated regulation of intestinal apo A-IV transcription are scarce. Recently, lipid micelles containing fatty acids incubated in the apical medium compartment of Caco-2 cells were shown to enhance apo A-IV transcription and simultaneously increase HNF-4α binding to probes containing the apo A-IV proximal promoter/apo C-III distal enhancer sequences (9). In the present studies, we focused on defining the role of HNF-4α in the regulation of intestinal apo A-IV expression in newborn swine enterocytes in response to lipid absorption using both in vivo and in vitro models.

MATERIALS AND METHODS

Materials. Oleic acid (C18:1n-9; OA) and essentially fatty acid-free bovine serum albumin were purchased from Sigma Chemical (St. Louis, MO). Unless otherwise specified, all other chemicals were molecular biology grade and were purchased from Sigma.

Cloning and sequencing of full-length swine HNF-4α cDNA. Total RNA from swine liver was used for cDNA synthesis by using the SMART RACE CDNA amplification kit according to manufacturer’s instructions (BD Biosciences Clontech, Palo Alto, CA). Gene-specific primers were as follows: 5’ RACE primer, 5’-CTTGTATCTTG-CCCGGTGTCGCTCAAC-3’ (antisense, nt 876 to 901); 3’ RACE primer, 5’-ACGCCTGTCCTAAAGGCATCATCTTC-3’ (sense, nt 830 to 855).

Boils of 5’ and 3’ RACE PCR products were cloned into the TA vector for sequencing. The following primers were then used for full length HNF-4α cloning by high-fidelity PCR: forward, 5’-GGACG-TGGTTGAAACCCAGGAA-3’ (sense, nt 170 to 175); reverse, 5’-AGTTGTCCAAGGCAAGAGG-3’ (antisense, nt 1625 to 1647).

Briefly, 1 µl of cDNA, 5 µl of 10× buffer, 5 µl of PCR enhancer solution, 1.5 µl of 50 mM dNTP mixture, 25 pmol of each primer and 1 µl of Platinum PfX DNA polymerase (Invitrogen, Carlsbad, CA) were used in a 50-µl reaction mixture. PCR was carried out at 94°C for 2 min, followed by 18 cycles of 10 s at 94°C, 15 s at 60°C, and 2 min at 68°C. A 7-min incubation at 68°C was added to the last cycle. The PCR products were cloned for sequencing confirmation.

Animals. Female swine were obtained from Tyson Farms, Plummierville, AR, and used for tissue harvest on the day of arrival. The University of Tennessee Health Science Center Institutional Animal Care and Use Committee approved this protocol. Two-day-old newborn, 3-wk-old weanling, and 2-mo-old weaned animals were fasted for 4–6 h from their last regular breast milk or chow feeding, followed by euthanasia by intravenous pentobarbital overdose. The abdominal cavity was quickly opened for harvest of tissue samples. Samples of jejunum were taken 5 cm distal to the ligament of Treitz for RNA extraction and protein homogenate preparation. A wedge of the anterior right lobe of the liver was similarly sampled. Samples were also taken from kidney, quadriceps femoris skeletal muscle, and intra-abdominal adipose tissue.

To study the short-term (24 h) physiological regulation of HNF-4α and apo A-IV expression by dietary lipid in newborn swine small intestine, 2-day-old female swine were surgically fitted with duodenal catheters exiting through dorsal swivel tethers as previously described (4). Animals were allowed to recover for 24 h while receiving a glucose-saline (5% glucose in 45 mM NaCl and 20 mM KCl) duodenal infusion at 100 ml·kg⁻¹·24 h⁻¹, followed by infusion as previously described of either dilute Vivonex (Norwich Eaton Pharmaceuticals, Norwich, NY), a low-fat elemental formula, or Intralipid (Cutter, Berkeley, CA), a lipid emulsion containing primarily 18-carbon unsaturated fatty acids. Infusions were isocaloric and at a rate of 50 kcal·kg⁻¹·24 h⁻¹ (4). At the end of 4- and 24-h infusions, the animals were euthanized, and jejunal samples were taken as described above.

IPEC-1 cell culture. The derivation of the IPEC-1 cell line has been described previously (15). 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 plastic culture flasks (75 cm², Corning Glassworks, Corning, NY) in growth medium: Dulbecco’s modified Eagle’s medium/F12 medium (GIBCO-BRL, Grand Island, NY) supplemented with 5% FBS (GIBCO-BRL), insulin (5 µg/ml), transferrin (5 µg/ml), selenium (5 ng/ml) (ITS Premix, BD Biosciences, Bedford, MA), epidermal growth factor (5 µg/ml) (BD Biosciences), penticillin (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 75-mm-diameter collagen-coated filters (3.0-µm pore size) in Transwell culture plates (Costar, Corning, Corning, NY). Cells were maintained in serum-containing growth medium for 48 h and then switched to the same medium containing 10⁻⁷ M dexamethasone (Sigma), but without FBS. Medium was then changed every 2 days. We have previously shown that after 10 days, IPEC-1 cells exhibit enterocytic features, including polarization with well-defined microvilli facing the apical membrane (15). Cellular membrane integrity was assessed by measurement of apical medium lactate dehydrogenase activity (Sigma).

Development of IPEC-1 cells with tetracycline-regulatable HNF-4α expression. Undifferentiated IPEC-1 cells were plated and grown to 50% confluency in 60-mm culture dishes. After 24 h, cells were transfected with pTet-On (BD Biosciences) by use of Lipofectamine 2000 (Invitrogen). Transfected cells were allowed to grow another 24 h and then plated in two 10-cm culture dishes, followed by G418 selection for 3 wk. Clones were isolated and transferred to 24-well culture plates to allow an additional 2 wk of growth. Individual clones were screened by luciferase assay with or without doxycycline and further transfected with pTRE2hyg-Luc by use of Lipofectamine 2000 (Invitrogen). Transfected cells were allowed to grow another 24 h and then plated in two 10-cm culture dishes, followed by G418 selection for 3 wk. Clones were isolated and transferred to 24-well culture plates to allow an additional 2 wk of growth. Individual clones were screened by luciferase assay with or without doxycycline and further transfected with pTRE2hyg-Luc by use of Lipofectamine 2000 (Invitrogen). Transfected cells were allowed to grow another 24 h and then plated in two 10-cm culture dishes, followed by G418 selection for 3 wk. Clones were isolated and transferred to 24-well culture plates to allow an additional 2 wk of growth. Individual clones were screened by luciferase assay with or without doxycycline.

Tet-19 cells were transfected with the pTRE-Tight plasmid containing the full-length swine HNF-4α cDNA and linear hygromycin marker by the same protocol that was used to develop the Tet-19 cell line. Both G418 (400 µg/ml) and hygromycin (300 µg/ml) were used for clone selection. After selection and expansion of clones demonstrating the highest induction of mRNA and protein secretion, as determined by real-time PCR and Western blot, the cells were used for experiments. Induction of HNF-4α secretion by differentiated cells was maximal at 6 h of incubation with each dose of doxycycline (data not shown).

Isolation of RNA. Swine tissues and IPEC-1 cells were dissolved in TRI reagent (Molecular Research Center, Cincinnati, OH) at a concentration of 50–70 mg/ml, followed by extraction of total RNA according to the manufacturer’s protocol.

Semi-quantitative RT-PCR. Aliquots of RNA (2–10 µg) were treated with two units of RNase H at 37°C for 30 min in 50 µl of 1× reaction buffer from the DNA-free kit (Ambion, Austin, TX). The DNase was then removed by using DNase inactive reagent from the kit. The supernatant containing 2 µg of total RNA was used in cDNA synthesis with an Omniscript RT kit (Qiagen, Santa Clarita, CA) following manufacturer’s instructions. The single-strand cDNA was amplified using the Taq PCR core kit (Qiagen, Santa Clarita, CA) with 1 µl of cDNA and 50 pmol of each specific primer in a total volume...
of 50 μL. Primers included the following. β-actin: forward, 5'-TGGGATTCGATGACCTG-3' (sense, nt 81 to 100); reverse, 5'-GGGCTCCTCGGAGGATCATG-3' (antisense, nt 471 to 490). Swine apo A-IV: forward, 5'-GAAGCGCGCAGGACGACATG-3' (sense, nt 265 to 284); reverse, 5'-CAGGCTCTCTGACTGCTT-3' (antisense, nt 737 to 756). Swine apo B: forward, 5'-AGGAGTAATGCTGCAGTA-3' (sense, nt 559 to 561); reverse, 5'-CTGCGAATCAGGTCCAGAT-3' (antisense, nt 603 to 610). Swine HNF-4α: forward, 5'-AAGAGAAGGACGCTCGAGAA-3' (sense, nt 349 to 368); reverse, 5'-CTGCGACGCTGATGATGAT-3' (antisense, nt 935 to 954). Swine microsomal triglyceride transfer protein (MTP) large subunit: forward, 5'-CTGGCAGGTCGTTGATGTA-3' (antisense, nt 2046 to 2065); reverse, 5'-CACTCGGGTCTTTGACCTC-3' (antisense, nt 2389 to 2408).

After incubation for 60 s at 94°C, PCR was performed for 23 cycles for β-actin, 25 cycles for HNF-4α, 25 cycles for apo A-IV, 29 cycles for apo B, and 27 cycles for MTP in a thermal cycler (Perkin-Elmer, Boston, MA). For each RNA sample, a negative control was run to check for DNA contamination by using AmpliTaq (Perkin-Elmer), with the sample left on ice during reverse transcription. Additionally, each reaction contained a tube with all the above buffers and enzymes but without RNA to exclude PCR product contamination. The optimal PCR profile was used: 2 min at 50°C, 10 min at 90°C and 40 cycles of 94°C for 10 s, 62°C for 15 s, 68°C for 60 s, and finally 68°C for 7 min. The PCR products were digested with KpnI and MluI and cloned into the corresponding sites of the firefly luciferase reporter plasmid pGL-3-basic (Promega). The integrity of all plasmids was verified by sequencing.

Tet-19 IPEC cells were cultured in normal growth medium with 5% Tet system-approved FBS. On day 1, the cells were seeded on 12-well plates at a density of 3-5 × 10^5 cells per well. On day 2, the cells were cotransfected with LF-2000 reagent (Invitrogen) according to manufacturer’s instructions. Each well of a 12-well plate received 2 μg DNA of pGL-3-intergenic region constructs, pTRE-HNF-4α, and pRL-TK at a ratio of 50:50:1. On day 3, the cells were incubated with either doxycycline 400 ng/ml or OA 0.2 mM plus doxycycline 400 ng/ml. After a 24-h incubation, the plates were washed with cold 1× PBS, then harvested and lysed with 150 μl/well 1× passive lysis buffer (dual luciferase reporter system kit, Promega) by shaking for 20–30 min at room temperature. All transfection experiments were performed in triplicate. Firefly and Renilla luciferase activities were measured using a dual-luciferase reporter assay system (Promega) and LMax microplate luminometer ( Molecular Devices, Sunnyvale, CA). Data are expressed as firefly-to-Renilla luminescence ratios.

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

RESULTS

Swine HNF-4α derived protein sequence. Figure 1 shows the protein sequence of swine HNF-4α derived from the full-length cDNA sequence and aligned with the corresponding human sequence. The nine-amino acid NH2-terminal sequence present in swine, but not human, HNF-4α may represent variation in the translation initiation site in the swine cDNA sequence. There is >95% homology of the swine protein sequence with that of human HNF-4α. The full-length cDNA sequence was submitted to GenBank (accession no. DQ061106).

HNF-4α expression in developing swine. To determine the tissue distribution of HNF-4α mRNA during development, various tissues were harvested from suckling, weaning, and weaned swine and subjected to RNA extraction and conventional RT-PCR analysis. As shown in Fig. 2, the highest levels were found in liver > jejunum > kidney in all three developmental groups.

Regulation of HNF-4α, apo A-IV, apo B, and MTP mRNA expression by dietary lipid in newborn swine jejunum. We next wished to determine the effect of a high-fat intraduodenal
infusion on jejunal expression of HNF-4α and apo A-IV in newborn swine. We have previously described upregulation of apo A-IV at the transcriptional level by such a diet in newborn swine over a 24-h period (30). However, we had not previously measured HNF-4α expression under these conditions. The 24-h high-fat intraduodenal infusion resulted in upregulation of both HNF-4α and apo A-IV mRNA in newborn swine jejunum measured by RT-PCR as shown in Fig. 3. The Western blot at the bottom of the figure documents that HNF-4α protein level parallels the mRNA level.

Another HNF-4α target gene is MTP (39), a heterodimer consisting of a small subunit of protein disulfide isomerase and a large subunit with lipid transfer activity, that functions to lipidate nascent chylomicrons in the endoplasmic reticulum (ER) (40). We have previously shown that MTP expression is regulated by lipid absorption in newborn swine jejunum and by oleic acid in IPEC-1 cells in a fashion similar to that of apo A-IV (27). As shown in Fig. 3, the MTP large subunit mRNA is upregulated by the high-fat infusion in a manner similar to that of apo A-IV.

Apo B-48 is another protein intimately involved in chylomicron production via lipidation by MTP as the first step in chylomicron assembly (40). In small intestine, apo B-48 mRNA is constitutively expressed, and protein levels are regulated by the net balance of proteosome-mediated degradation and rescue by lipidation. Although its gene is considered a target of HNF-4α, this mechanism is not thought to be important in physiological regulation of apo B-48 protein levels. As expected, apo B mRNA levels were not appreciably affected by the high-fat intraduodenal infusion (Fig. 3).

The time course of the upregulation of both HNF-4α and apo A-IV mRNA in newborn swine jejunum by the high-fat intraduodenal infusion, as assessed by both conventional and quantitative real-time PCR, is shown in Fig. 4. The parallel increase in both HNF-4α and apo A-IV mRNA suggests that HNF-4α induction may be involved in apo A-IV upregulation by dietary lipid. This is especially true of the more quantitative and accurate real-time PCR measurements in Fig. 4, B and C, which suggest that HNF-4α mRNA may reach maximal levels by 4 h of high-fat infusion. Also, MTP expression was induced over a time course similar to that of apo A-IV (Fig. 4).
Regulation of HNF-4α, apo A-IV, and apo B expression by fatty acid in IPEC-1 cells. To determine whether the same pattern of regulation of HNF-4α and apo A-IV expression by lipid was also present in IPEC-1 cells, a cell line derived from newborn swine intestinal epithelial cells (15), IPEC-1 cells were incubated with 0.8 mM oleic acid for 24 h. This concentration is in the physiological range and maximally stimulates chylomicron triacylglycerol secretion in this cell line (15). Figure 5 shows that mRNA of both HNF-4α and apo A-IV is upregulated by oleic acid, similar to the observation in newborn swine jejunum. However, as observed in newborn swine jejunum, apo B mRNA levels did not appreciably change with oleic acid incubation.

Regulation of HNF-4α, apo A-IV, and apo B expression by tetracycline-regulatable HNF-4α expression in the absence of fatty acid in IPEC-1 cells. To determine whether the effect of oleic acid incubation on apo A-IV expression was due directly to increased HNF-4α expression, a tetracycline-regulatable expression system was used to allow overexpression of HNF-4α in IPEC-1 cells in the absence of incubation with oleic acid to observe the effect of HNF-4α directly on apo A-IV mRNA levels in the absence of other factors that might be concomitantly induced by fatty acid absorption with potential HNF-4α-independent effects on apo A-IV expression. As shown in Fig. 6, HNF-4α induced by incubation of the cells with doxycycline in the absence of fatty acid resulted in the induction of apo A-IV mRNA. Also, swine MTP large subunit mRNA was strongly induced by HNF-4α overexpression in the absence of fatty acid. Apo B mRNA was induced to a lesser degree than that of either apo A-IV or MTP, even in the face of supraphysiological HNF-4α overexpression (Fig. 6).

Activation of the apo A-IV/C-III intergenic region by HNF-4α in the presence and absence of fatty acid in IPEC-1 cells. Using three intergenic region/reporter gene constructs, cotransfected into IPEC-1 cells with doxycycline-regulatable HNF-4α expression (Fig. 7), we found that HNF-4α overexpression activated all three, with the highest activity in the largest p-5928 construct. Furthermore, treatment with 0.8 mM oleic acid did not further enhance activation of any of the constructs.

DISCUSSION

In the present studies, we describe a previously unreported mechanism for the transcriptional activation of the apo A-IV gene by dietary lipid in the newborn swine proximal small intestine. Data from newborn swine feeding studies and in vitro swine enterocyte culture experiments suggest that absorbed dietary fatty acids result in the upregulation of levels of HNF-4α mRNA and protein, which then activates apo A-IV...
gene transcription without evidence for the direct activation of HNF-4α by ligand binding of fatty acid. This regulatory mechanism was unexpected but may be important in the striking induction of jejunal apo A-IV expression by dietary lipid in the neonatal piglet, a model for the human infant.

Recent studies have defined a role for HNF-4α in the transcriptional regulation of apo A-IV in the enterocyte, including the basal spatial expression of apo A-IV in the small intestine with restriction of expression to the villus and a proximal to distal gradient via interaction with the apo A-IV gene proximal promoter and distal apo C-III gene enhancer located in the apo A-IV/C-III intergenic region (2, 37). Peignon et al. (32) demonstrated that E-cadherin-dependent cell adhesion in Caco-2 cells controls the abundance of HNF-4α, which in turn regulates apo A-IV expression. Although HNF-4α abundance was modulated in their studies by cell adhesion and differentiation, this is not likely to have been a factor in the present experiments, since in both the in vivo and in vitro experiments enterocytes were stably differentiated and appropriate untreated controls were used.

Data from previously reported studies supporting a role for HNF-4α participation in dietary lipid-mediated regulation of intestinal apo A-IV transcription are scarce. Recently, lipid micelles containing fatty acids incubated in the apical medium compartment of Caco-2 cells were shown to enhance apo A-IV transcription and simultaneously activate reporter gene constructs containing the apo A-IV proximal promoter/apo C-III distal enhancer (9). This effect was blocked by transfection with a dominant negative form of HNF-4α. Interestingly, oleic acid-2-monooleoylglycerol-taurocholate-lysophosphatidylcholine-cholesterol micelles were effective in inducing apo A-IV mRNA, whereas the individual micellar components were not. Also in these previous experiments, increased binding of HNF-4α to apo A-IV promoter/C-III enhancer DNA binding

Fig. 6. Regulation of apo A-IV, MTP large subunit, and apo B mRNA by overexpression of HNF-4α in the absence of fatty acid incubation in IPEC-1 cells. A: semiquantitative RT-PCR analysis of HNF-4α, apo A-IV, MTP large subunit, apo B, and β-actin mRNA was performed in the absence of fatty acid incubation in IPEC-1 cells expressing swine HNF-4α driven by the tetracycline response element using the TET-On system, as described in MATERIALS AND METHODS. The concentration of doxycycline added to the culture medium is shown at bottom. Results are representative of those obtained from 3 separate experiments. Quantitative real-time PCR was used for measurement of HNF-4α mRNA (B) and apo A-IV mRNA (C) as ratios to β-actin × 100 and 1,000, respectively, in IPEC-1 cells under the same experimental conditions. Each bar represents mean ± SE of samples measured in duplicate from 3 separate culture wells. Values for treated cells were different from those of untreated cells: *P < 0.001, #P < 0.003.
sites was demonstrated by electrophoretic mobility shift assays after treatment with lipid micelles. Whether this effect was due to increased HNF-4α binding affinity or increased HNF-4α abundance was not addressed. In the present studies, oleic acid/BSA complexes were used to deliver fatty acids to the apical membrane of the IPEC-1 cells and effectively induced HNF-4α and apo A-IV mRNA. However, in the newborn swine studies, mixed micelles would have delivered the infused lipid to the small intestinal mucosa. In the high-fat formula used in the piglet infusion experiments, as well as in the cell culture experiments using oleic acid, 18-carbon unsaturated fatty acids were used. Taken together, these data suggest that this class of fatty acids is effective in the coordinate induction of both HNF-4α and apo A-IV expression in newborn swine enterocytes.

In addition to induction of HNF-4α expression by fatty acid as a mechanism of lipid-mediated regulation of apo A-IV expression, in the present studies we also addressed the issue of whether fatty acid can also directly activate HNF-4α transcriptional activity. By use of the TET-On system, swine HNF-4α was overexpressed in IPEC-1 cells in the absence of oleic acid treatment and resulted in the induction of apo A-IV mRNA. Furthermore, in swine apo A-IV/C-III intergenic region/luciferase reporter gene experiments in the absence of added fatty acid, this overexpression of HNF-4α demonstrated striking transcriptional activation of the apo A-IV gene, presumably mediated by ligand-independent HNF-4α transactivation. As in the human, the swine apo A-IV/C-III intergenic region contains several consensus HNF-4α binding sequences that are probably involved in this regulation. With oleic acid treatment, this activation was not further enhanced, suggesting that oleic acid does not significantly modulate the HNF-4α transcriptional activity by presumed ligand binding.

The mechanism of induction of HNF-4α mRNA by lipid absorption is unknown at present. The HNF-4α gene may be activated by transcription factors that bind fatty acids as ligands or via a signaling mechanism activated by fatty acids. Studies of the transcriptional regulation of HNF-4α have provided insight into non-lipid-mediated mechanisms. Two different promoters appear to drive HNF-4α expression in a tissue-specific manner. The proximal P1 promoter regulates expression in adult liver and kidney (50), and the P1 promoter and a distal P2 enhancer direct expression in embryonic liver and adult pancreatic β-cells (13, 44). These regulatory elements mediate transcriptional regulation via several transcription factors, including HNF-1α, HNF-1β, HNF-3, CCAAT/enhancer binding protein (C/EBP), Sp1, chicken ovalbumin upstream promoter-transcription factor 2 (COUPTFII), retinoic acid receptor-α (RARα), retinoid-X receptor-α (RXRα), HNF-6, and GATA-6. It has also been shown that HNF-4α autoregulates its own expression in liver (31). It is currently unknown whether any of these regulatory factors might be lipid responsive in small intestine. Alternatively, fatty acid-mediated regulation of HNF-4α mRNA levels might occur by regulation of mRNA transcript stability. The basis for regulation of HNF-4α expression in intestine by dietary fatty acids, especially potentially involving P1 and P2, is the focus of ongoing studies in our laboratory.

There are undoubtedly other HNF-4α-dependent and -independent mechanisms involved in the induction of apo A-IV by dietary lipid in small intestine. Modulation of HNF-4α transcriptional activity for various target genes, including apo A-IV in some instances, has been demonstrated to be mediated by phosphorylation (24), as well as interaction with other proteins, including peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (16, 34, 35, 48), sterol regulatory element-binding protein (SREBP) (47), and small heterodimer partner (SHP) (41). However, to date these proteins have not been implicated in dietary lipid-mediated regulation. Recently, estrogen-regulated receptor-α (ERRα) was shown to regulate several genes involved in lipid transport in the small intestine, including apo A-IV, and ERRα−/− mice were found to have significant fat malabsorption (8). However, feeding-induced regulation of apo A-IV expression was not observed, even though ERRα mediated basal apo A-IV expression in cooperation with PGC-1α. Finally, we recently identified a novel putative repressor protein that binds to the proximal promoter of the swine apo A-IV gene that may regulate apo A-IV expression in jejunum during lipid absorption in newborn piglets fed a high-fat diet (30). Identification of other dietary lipid-specific factors that regulate apo A-IV in the intestine awaits further study.

Finally, in addition to apo A-IV, there are other genes involved in intestinal lipid transport that are HNF-4α targets that may also be activated by lipid-mediated HNF-4α induction and thereby enhance intestinal lipid absorption in concert with apo A-IV. These include MTP that serves to lipidate apo B-48 as the first step in chylomicron assembly, as well as in the subsequent addition of bulk lipid to the nascent chylomicron in the ER (39, 40). We have previously shown that MTP large subunit expression is regulated by lipid absorption in newborn swine jejunum and by oleic acid in IPEC-1 cells in a fashion similar to that of apo A-IV (27). In the present studies we documented that the MTP large subunit gene is indeed a specific target of overexpressed HNF-4α in the absence of fatty acid, similar to apo A-IV.

We also examined the effect of HNF-4α overexpression of apo B mRNA levels. Although supraphysiological HNF-4α overexpression increased apo B mRNA levels, the magnitude was less than that of apo A-IV and MTP large subunit. Also, the intraduodenal infusion of a high-fat formula in newborn swine oleic acid treatment of IPEC-1 cells did not appear to significantly alter apo B mRNA levels, despite striking increases in HNF-4α, apo A-IV, and MTP large subunit mRNA levels. These observations, coupled with the fact that apo B-48 protein levels are generally regulated at the posttranslational level, make it unlikely that HNF-4α is a physiological regulator of apo B, despite the presence of HNF-4α binding sites in apo B gene regulatory elements (49). Although not addressed in the present report, liver fatty acid binding protein has been demonstrated to be regulated by HNF-4α in liver and is also expressed in the enterocyte, where it may function in fatty acid trafficking (20, 36).

In conclusion, the present studies demonstrated coordinate upregulation of both HNF-4α and apo A-IV expression by lipid absorption in newborn swine proximal small intestine. Furthermore, the same pattern of upregulation was present in IPEC-1 cells derived from newborn swine small intestinal epithelial cells. Fatty acid-independent overexpression of HNF-4α in IPEC-1 cells induced apo A-IV expression at the transcriptional level, and fatty acid treatment did not further enhance this transcriptional activation. These data suggest that the acute
induction of the apo A-IV gene by dietary lipid in newborn intestine may occur, at least in part, via ligand-independent transactivation by HNF-4α that is itself induced by a lipid-mediated mechanism. Absorbed fatty acids may induce enterocyte apo A-IV expression via induction of HNF-4α in newborn small intestine as a novel mechanism to enhance small intestinal lipid absorption. This enhancement would occur via the assembly and secretion of larger chylomicrons mediated by increased availability of apo A-IV, as we have demonstrated previously (28, 29). This enhancement may be augmented by upregulation of other HNF-4α target genes involved in lipid absorption, such as MTP. Thus HNF-4α may serve as a “master switch” for coordinating chylomicron assembly in the newborn.

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