HNF-4-dependent Induction of Apolipoprotein A-IV Gene Transcription by an Apical Supply of Lipid Micelles in Intestinal Cells*

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Apolipoprotein (apo) A-IV, a component of triglyceride-rich lipoproteins secreted by the small intestine, has been shown to play an important role in the control of lipid homeostasis. Numerous studies have described the induction of apoA-IV gene expression by lipids, but the molecular mechanisms involved in this process remain unknown. In this study, we have demonstrated that a lipid bolus-induced transcription of the apoA-IV gene in transgenic mice and that the regulatory region of the apoA-IV gene, composed of the apoC-III enhancer and the apoA-IV promoter (c3-A4), was responsible for this induction. In enterocyte Caco-2/TC7 cells, a permanent supply of lipids at the basal pole induced expression of the apoA-IV gene both at the transcriptional level and through mRNA stabilization.apoA-IV gene transcription and protein secretion were further induced by an apical supply of complex lipid micelles mimicking the composition of duodenal micelles, and this effect was not reproduced by apical delivery of different combinations of micelle components. Only induction of the apoA-IV gene by lipid micelles involved the participation of hepatic nuclear factor (HNF)-4, as demonstrated using a dominant negative form of this transcription factor. Accordingly, lipid micelles increased the DNA binding activity of HNF-4 on the eC3-A4 region. These results emphasize the importance of physiological delivery of dietary lipids on apoA-IV gene expression and the implication of HNF-4 in this regulation.

Apoptiprotein (apo) A-IV is an abundant apolipoprotein that is synthesized and secreted by the small intestine in humans and by both the liver and small intestine in rodents. The small intestine is the major organ responsible for circulating apoA-IV, the plasma concentration of which essentially depends on the nutritional status (see Ref. 1 for review). Apolipoprotein A-IV appears to have myriad functions. This protein has been shown to modulate plasma lipoprotein metabolism, suggesting a major role for apoA-IV in the prevention of atherosclerosis. ApoA-IV may also have an impact on gastrointestinal functions such as gastric motility. Furthermore, a series of studies showed that apoA-IV, secreted by the intestine or specifically expressed in the hypothalamus, may act as a signal for satiation (see Refs. 1 and 2 for reviews).

Numerous studies have reported that the intestinal synthesis and secretion of apoA-IV were increased following lipid supply, but the molecular mechanisms involved in the induction of apoA-IV gene expression remain unknown (see Ref. 1 for review). Hayashi et al. (3) have reported that stimulation of apoA-IV synthesis and secretion were associated with chylomicron assembly after lipid supply. Another study showed that induction of apoA-IV gene transcription by lipids in newborn swine small intestine involves the DNA binding of a repressor protein on the swine apoA-IV promoter (4). However, because the induction of apoA-IV gene expression was not markedly observed near weaning in the swine intestine (5), Black et al. suggested that this regulatory mechanism was specific to the newborn swine.

The apoA-IV gene is comprised within a cluster with apoA-I and apoC-III genes. We have demonstrated previously that apoA-IV gene is expressed in the intestine according to cephalo-caudal and crypt-to-villus axes and that the apoA-IV promoter, when fused with apoC-III enhancer (6), was necessary and sufficient to restrict apoA-IV expression to villus enterocytes. We have also shown that this specific pattern of apoA-IV expression requires the presence of a functional hormone-responsive element, located within the distal promoter of the apoA-IV gene (7). Mutations or deletions of DNA-binding sites for hepatic nuclear factor (HNF)-4 located on the apoC-III enhancer (6) or on the apoA-IV promoter markedly decreased the expression of apoA-IV (7, 8), suggesting a major role for HNF-4 in the control of apoA-IV expression in the small intestine.

Other transcription factors, such as peroxisome proliferator-activated receptors, retinoid X receptor, or sterol regulatory element-binding protein, have also been involved in the control of lipid metabolism, and several studies indicate that such factors are affected by dietary lipids (see Ref. 9 for review). However, the liver-specific invalidation of the HNF-4α gene in mice (10) clearly demonstrated that HNF-4 is an important factor in the control of expression of genes involved in lipid metabolism. Accordingly, in humans, mutations in the HNF-4α gene cause maturity-onset diabetes of the young (MODY-1), which displays hypolipidemia resulting from a reduced expression of HNF-4α-responsive genes, e.g. apolipoproteins and microsomal triglyceride transfer protein (MTP) (11, 12). A direct or indirect role of fatty acids on regulation of HNF-4 activity is still widely discussed. Hertz et al. (13) showed that defective
transactivation by HNF-4 mutants was rescued by exogenous fatty acid agonist ligands of HNF-4, whereas Petrescu et al. (14) reported a physical and functional interaction of HNF-4 with acyl-CoA-binding protein that exhibits a high and specific affinity for long chain fatty acyl-CoAs.

The purpose of our study was to analyze the molecular mechanisms involved in lipid-dependent regulation of the apo-AIV gene in intestinal cells and the potential role of HNF-4 in this regulation. We have considered the effects of a physiological supply of lipids to intestinal cells, i.e. a permanent supply of plasma lipids at the basal pole of the enterocytes and an acute apical delivery of lipids micelles, which occurs after a fat-rich meal. Indeed, in vivo, dietary lipids reach the duodenum as complex micelles resulting from their solubilization by bile components (biliary acids, cholesterol, and phospholipids) and their concomitant digestion by pancreatic hydrolases into fatty acids, 2-monoacylglycerol, and lysophospholipids. We have reported recently that such lipid micelles were efficient inducers of the secretion of apoB-containing triglyceride-rich lipoproteins (15) and specifically control apoB traffic in the enterocytic Caco-2 cell line (16). In the present study, we demonstrate that the supply of lipids at the basal pole of the enterocytes induces expression of the apo-AIV gene both at the transcriptional level and through mRNA stabilization, whereas an apical supply of lipid micelles further induces apo-AIV transcription through an HNF-4-dependent mechanism.

**EXPERIMENTAL PROCEDURES**

**Caco-2/TC7 Cell Culture—**Caco-2/TC7 cells (17) were seeded at 6 × 10^5 cells/cm² on 6-well semi-permeable filters (1-µm pore size; BD Biosciences). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen). After confluence, only the medium of the day of culture.

Concentration of lipids was obtained by addition of the rest of the solution in serum-free medium using an ultrasound bath. The final concentration of lipids in chloroform-methanol (2:1, v/v) was stored at -20 °C. The final concentration of fatty acids to albumin (ITS/L medium). To maintain a 4:1 molar ratio of fatty acids to albumin (ITS/L medium). To maintain a 4:1 molar ratio when lipids were added separately, the ITS medium supplemented with palmitic acid, oleic acid, or cholesterol contained 0.33%, 0.66%, and 0.1% of FAP-BSA, respectively. An appropriate stock solution of lipids in chloroform-methanol (2:1, v/v) was stored before use. Lipids were added with an aliquot of the appropriate FAP-BSA solution in serum-free medium using an ultrasound bath. The final concentration of lipids was obtained by addition of the rest of the FAP-BSA solution. The Caco-2/TC7 cells were cultured in the presence of ITS medium supplemented with lipids from confluence until the last day of culture.

For analysis of lipid uptake, [1-14C]oleic acid (51 µCi/mmol; 2 µCi/ml final medium) was added to lipids. Results are expressed as nmol/mg protein. Protein concentrations were determined with the Bio-Rad DC protein assay (Bio-Rad).

**Plasmids, Transfection Experiments, and Luciferase Assay—**The pc3-A4-LUC plasmid was generated by subcloning the eC3-A4 fragment containing the -520/-890 human apoIII enhancer fused with the -700/+10 apo-AIV promoter region, obtained from the eC3-A4-CAT plasmid (6), into the pGL2 basic vector. Caco-2/TC7 cells were stably co-transfected using the calcium phosphate precipitation method with pc3-A4-LUC and pPUR vector (Clontech), which contains the puromycin resistance gene. Stable transfectants were selected by puromycin (10 µg/ml; Sigma). The total puromycin-resistant resulting Caco-2/TC7 eC3-A4-LUC cell population was analyzed. After selection, all experiments were performed on filters, using the appropriate culture medium without puromycin.

The recombinant adenovirus Ad-DN-HNF-4, which expressed a deletion mutant of HNF-4 that lacked residues 381–465, was constructed by digression of pcDNA1-HNF-4-CD1b (kindly provided by Margarita Hadzopoulou-Cladaras; Ref. 20) with HindIII and NotI. The HNF-4/CD1b fragment obtained was then subcloned by blunt ligation into pTG-6600 vector (kindly provided by Luis Garcia from the Gene Vector Production Network of Genethon, Ervy, France) using the EcoRI restriction site. The recombinant adenovirus was then generated by homologous recombination with the pKp1.3 vector (kindly provided by Luis Garcia). The production of infectious particles was performed by Gene Vector Production Network. The recombinant adenovirus Ad-GFP, which expressed green fluorescent protein under the control of the cytomegalovirus promoter, was produced and provided by Gene Vector Production Network. For adenovirus infection experiments, Caco-2/TC7 eC3-A4-LUC cells were seeded at 6 × 10^4 cells/cm² on 6-well semi-permeable filters and cultured in the appropriate culture medium. After 10 days of culture, post-confluent cells were infected with recombinant adenovirus Ad-DN-HNF-4 or Ad-GFP at a multiplicity of infection of 50 plaque-forming units/cell. Lipid micelles were added 3 days after infection, and the cells were harvested 24 h later.

Luciferase activities were assayed as described previously (21).

**Nuclear Extracts and Electrophoretic Mobility Shift Assay—**Caco-2/TC7 nuclear extracts were prepared as reported previously (22) with the addition of 1% sodium deoxycholate (Sigma) to the nuclear extraction buffer (Sigma and phosphatase inhibitors (2 mM Na3VO4, 50 µM NaF, and 40 µM β-glycerocephosphate). Electrophoretic mobility shift assays were performed as described previously (23). The oligonucleotides used were as follows: 5'-GGGAGATTTGAATCC GACCC-3' for AIE, 5'-TGATAGTTCTCAGGTC AAAAAGTCGACAAGGACCC-3' for ANV, and 5'-CTCCATCTTGTGCTGACAGCACTGACA-CTGA-3' for CIII. Supershift experiments were performed with antibody directed against HNF-4a (C-19X), according to the manufacturer's instructions (Santa Cruz Biotechnology, Tebu-bio, Le Perray en Yvelines, France).

**Animals and Chloramphenicol Acetyltransferase (CAT) Assay—**Transgenic eC3-A4-CAT mice (8) were fasted throughout the experiments. Groups of three mice (5-8 g) were given either (24 h after the first administration) oral doses of olive oil (400 µl/mouse) by gavage. The control group received an equivalent volume of water by the same route. Animals were sacrificed 4 h after their last gavage. The proximal part of the jejenum was excised quickly for measurement of CAT activity (as described previously; Ref. 6) and RNA extraction.

**Reverse Transcription and Real-time PCR Analysis—**Total RNA was isolated using RNA plus reagent (QiGen, Illkirch, France) according to the manufacturer's protocol. The real-time reverse transcription experiments were performed as described previously (18) with 2 µg of total RNA in a total volume of 40 µl. Messenger RNA was quantified using the Light-Cycler System according to the manufacturer's procedures (Roche Applied Science). PCRs were performed with a 1.1000 final dilution of the reverse transcription product. The apo-AIV mRNA level was quantified using hybridization probes designed by Gentest (Proligo, Paris, France). PCR conditions were one step of denaturation (8 min at 95 °C) followed by 40 cycles (each cycle consisted of 15 s at 95 °C, 10 s at 60 °C, and 15 s at 72 °C). The mRNA levels of the other genes were quantified using SYBR Green, and the PCR conditions were one step of denaturation (8 min at 95 °C) followed by 40 cycles (each cycle consisted of 10 s at 95 °C and 30 s at 60 °C). All steps of the primers used in this study were as follows: (a) for ApoA IV: 5'-AGGCAAGACAAGACTCTCTC-3' (forward primer), 5'-TCTCACCTCCCACTGGAC-3' (reverse primer), 5'-CCCCCTTGAGGACTCTTGCTC-3' (anchor probe), and 5'-LCr6C400-CCCCCTGAGGACTCTTGCC-P-3' (deletion probe); (b) for ApoB: 5'-CCCAAGCAAAGTAATG-3' (forward primer) and 5'-GTCTGAGTGTGAAACGTAAG-3' (reverse primer); (c) for acyl-CoA:cholesterol O-acyltransferase 2, 5'-CTGCTGCAGGAGACA-
GAAG-3′ (forward primer) and 5′-GGTTCGGTCCATTGTACC-3′ (reverse primer); and (d) for MTP, 5′-TTCAGACCTCTAGGACTGC-3′ forward primer and 5′-GTCGAGGCTCTGACAGAG-3′ reverse primer. The quantification of 18S RNA (using TaqMan probes from Applied Biosystems, Courtaboeuf, France) was used as control for the RNA extraction and reverse transcription experiments. Results were expressed as the ratio between the levels of mRNA of interest and 18S RNA.

mRNA Stability Studies—Caco-2/TC7 cells were cultured on semipermeable filters in the presence of ITS or ITS-L medium. On the last day of culture, 0.2 mM 5′,6-dichloro-1-deoxyuridine (DRB; Invitrogen) was added to the culture medium in the presence or absence of an apical supply of lipid micelles and maintained throughout the experiment. Cells were harvested at the indicated time of culture after the addition of DRB. Total RNA was extracted, and the mRNA level of ApoA-IV was determined.

Western Blot Analysis—Cells were cultured in ITS condition with or without lipid micelles in the apical compartment during the last 24 h of culture. The basal media were collected, supplemented with protease inhibitor mixture, and concentrated 6-fold using Centricon YM-10 (Millipore, Saint-Quentin en Yvelines, France). An equal volume of each concentrated culture medium was separated by 9% SDS-PAGE under reducing conditions and transferred onto nitrocellulose membrane. The blots were incubated in 1% polyvinylpyrrolidone (PVP-400000) in PBS-T (phosphate-buffered saline containing 0.1% Tween 20). They were then probed with a rabbit antibody against rat apoA-IV (1:2000; kindly provided by Dr. A. Mazur, INRA, Theix, France) or with a rabbit antibody against human α-fetoprotein (1:1500; Behring, Marburg, Germany) in 1% polyvinylpyrrolidone, followed by a peroxidase-conjugated goat anti-rabbit antibody (1:10,000; Vector Laboratories, Burlingame, CA) in PBS-T. Blots were developed with ECL reagents according to the manufacturer’s instructions (Amersham Biosciences).

Statistical Analyses—Statistical analyses were performed using Student’s t test.

RESULTS

ApoA-IV Gene Transcription Is Induced in Vivo by Dietary Lipids—The effect of lipids on transcription of the apoA-IV gene in intestinal cells was first analyzed in vivo using transgenic mice expressing the CAT reporter gene under the control of the regulatory sequence eC3-A4. This sequence contains the human −700/+10 apoA-IV promoter fused to the human −500/−890 apoC-III enhancer. It is responsible for the specific pattern of expression of the apoA-IV gene along the cephalo-caudal and crypt-to-villus axes of the intestine (6, 7). Transgenic mice were given one or two bolus of olive oil, and CAT activity and endogenous ApoA-IV mRNA were analyzed in jejunum samples. The administration of olive oil increased CAT activity up to 3-fold after the second bolus (Fig. 1A), indicating that dietary lipids induce human apoA-IV gene transcription in vivo through the eC3-A4 regulatory region. The physiological relevance of this result was demonstrated by the observation, in the same animals, of a parallel lipid-dependent induction of the endogenous mouse apoA-IV mRNA level (Fig. 1B).

ApoA-IV mRNA Expression Requires the Presence of a Permanent Supply of Lipids at the Basal Pole of Caco-2/TC7 Cells—In vivo studies cannot determine whether transcriptional induction of apoA-IV expression was due to a direct effect of the apical supply of dietary lipids on enterocytes or whether it resulted from an increase in plasma lipids. We thus further characterized the lipid-dependent expression of human apoA-IV in Caco-2/TC7 cells, which display morphological and functional characteristics of enterocyte differentiation after confluence (17). The culture of Caco-2/TC7 cells on semi-permeable filters allows mimicking of the asymmetry of physiological conditions of lipid supply in intestinal cells, with plasma and luminal lipids supplied at the basal and apical poles of the cells, respectively (16).

In order to evaluate the respective influence of an apical supply and a basal supply of lipids on expression of the apoA-IV gene in Caco-2/TC7 cells, we first modulated the presence of lipids in the basal compartment (Fig. 2). We compared cells incubated under standard conditions (i.e. in the presence of serum) with cells incubated with a serum-free, lipid-free ITS defined medium, which allows Caco-2/TC7 cells to grow and differentiate as well as under standard culture conditions (16, 24). In the presence of ITS medium, the level of apoA-IV mRNA was dramatically lower (10-fold) than it was in the presence of serum (Fig. 2). Because serum provides both lipids and growth factors, we further analyzed the effect of a permanent supply of lipids (oleic acid, palmitic acid, and cholesterol) in ITS medium in the basal compartment (ITS/L). Such a condition was previously shown to restore the physiological effect of serum on apoB traffic in Caco-2 cells (16). In the presence of ITS/L, the expression of apoA-IV rose up to the level observed in the presence of serum (Fig. 2), indicating that the expression of apoA-IV mRNA requires a lipid supply at the basal pole of enterocytes. In order to characterize which lipids played a role in apoA-IV expression, we cultured cells with either oleic acid, palmitic acid, or cholesterol complexed to BSA in the basal compartment. As reported in Fig. 2, the effect on apoA-IV expression of lipid supplementation in ITS medium was mainly due to the presence of oleic acid (Fig. 2, compare ITS/L condition with ITS-OA-BSA) and, to a lesser extent, the presence of palmitic acid (Fig. 2, compare ITS/L condition with ITS-PA-BSA). The presence of cholesterol combined with BSA in the basal compartment had no significant effect on apoA-IV expression.

Under the same culture conditions, we have also analyzed the expression of apoB and MTP, both of which are involved in the assembly and secretion of chylomicrons, and acyl-CoA cholesterol Oacyltransferase 2. The different culture conditions analyzed had no effect on the mRNA levels of apoB and acyl-CoA-cholesterol Oacyltransferase 2, but we observed that the mRNA level of MTP was markedly reduced (10-fold) in ITS.
medium as compared with the serum condition. Moreover, MTP expression was only partially restored after lipid supplementation in ITS medium, indicating that it also requires serum factors. Taken together, these results showed the specificity of the regulation of apoA-IV gene expression by a permanent supply of lipids in the basal compartment.

**ApoA-IV Gene Expression and Protein Secretion Were Induced by an Apical Supply of Lipid Micelles—**Physiologically, after a meal, enterocytes face complex micelles made of fatty acids, 2-monoacylglycerol, lysophospholipids, cholesterol, and biliary acids, resulting from the solubilization of dietary lipids by bile and their digestion by pancreatic hydrolases. We therefore compared the effect of an apical delivery of complex micelles to Caco-2/TC7 cells with that of oleic acid combined with BSA (OA-BSA), which represents a current manner of fatty acid administration in cell culture experiments. At the concentration used, micelles displayed neither toxicity nor modification in cell morphology (16). In the presence of serum in the basal compartment, the addition of micelles induced apoA-IV expression by 3-fold, whereas the apoA-IV mRNA level was increased only 1.7-fold by OA-BSA (Fig. 3A). This result cannot be explained by a more efficient absorption of oleic acid when presented as micelles because its uptake was similar in both conditions (675 ± 60 versus 625 ± 18 nmol/mg protein under micelles and OA-BSA supply, respectively). No modification in the mRNA levels of apoB, acyl-CoA:cholesterol O-acyltransferase 2, and MTP was observed after lipid micelle supply (Fig. 3A). These results indicate that complex lipid micelles are more efficient inducers of apoA-IV gene expression than oleic acid combined with BSA. This specific effect of micelles on apoA-IV mRNA level was also observed when cells were cultured in the presence of ITS medium in the basal compartment (Fig. 3B). The 12-fold increase observed under this condition resulted in a level of apoA-IV mRNA in the range of that observed with Caco-2/TC7 cells incubated with apical micelles in the presence of serum in the basal compartment. No variation in apoB and acyl-CoA:cholesterol O-acyltransferase 2 mRNA levels was observed under these conditions, but an increase of MTP mRNA (3-fold) was obtained only when lipid micelles were supplied. In accordance with the variation of apoA-IV mRNA level, lipid micelles provoked an important increase of apoA-IV protein secretion in the basal compartment (Fig. 3C). This demonstrates that the apical supply of lipid micelles was able to induce apoA-IV gene expression and secretion in the absence of a supply of lipids at the basal pole.

Because lipid micelle components could act individually as apoA-IV mRNA inducers, we performed incubation with different combinations of lipids in the apical compartment (Fig. 3B). Although these combinations resulted in a slight increase in apoA-IV mRNA level (1.5–1.8-fold), no combination was able to reproduce the induction observed with complete micelles, indicating that the specific effect of the apical supply of complete micelles on apoA-IV gene expression could not be explained by an additive effect of their components.

**Apical Lipid Micelles and Basal Lipid Supply Modulated ApoA-IV Gene Expression through Different Mechanisms—**To determine whether the lipid-dependent expression of the apoA-IV gene in Caco-2/TC7 cells was due to a transcriptional effect, as in vivo (Fig. 1), these cells were stably transfected with a construct containing the luciferase gene reporter under the control of the eC3-A4 regulatory region of the human apoA-IV gene and cultured in FBS, ITS, or ITS/L (Fig. 4). In the presence of serum in the basal compartment, we observed a 2.5-fold increase in luciferase activity after apical administration of micelles (Fig. 4A). Consistent with the variations observed in apoA-IV mRNA level (Figs. 2 and 3), the effect of apical micelles on apoA-IV transcription was more important (7-fold) when cells were cultured in the absence of lipids in the basal compartment (ITS condition) (Fig. 4B). Addition of lipid to the basal ITS medium resulted in a 5-fold increase in luciferase activity, as compared with the ITS condition. These results indicated that both apical and basal lipid supply control apoA-IV gene transcription through the eC3-A4 regulatory region.

The effects of lipid supply on apoA-IV mRNA stability were also analyzed. As described in Fig. 4C, the apical supply of micelles did not modify the half-life of apoA-IV mRNA as compared with the ITS condition (t1/2 = 5.5 h). Conversely, a 2-fold increase of apoA-IV mRNA half-life was measured when ITS medium was supplemented with lipids in the basal compartment (ITS/L condition). These results indicate that apoA-IV was regulated at the transcriptional and post-transcriptional levels by lipids present in the basal compartment, whereas apical micelles only induced apoA-IV gene transcription.

**A Dominant Negative Form of HNF-4 Prevents the Induction of ApoA-IV Transcription by Apical Lipid Micelles—**Because HNF-4 plays a peculiar role in the enterocyte-specific expres-
HNF-4 and Lipid-dependent ApoA-IV Gene Transcription

**Fig. 3. Effects of apical lipid supply on apoA-IV, apoB, acyl-CoA:cholesterol O-acyltransferase 2, and MTP mRNA levels and on apoA-IV protein secretion in Caco-2/TC7 cells.** A, cells were cultured on filter supports in the presence of serum (FBS) in the basal compartment and in the presence of oleic acid combined with bovine serum albumin (OA-BSA) or micelles in the apical compartment of the filter. *, *p < 0.05 as compared with FBS condition; **, *p < 0.01 as compared with FBS condition; ***, *p < 0.001 as compared with ITS condition. TC, taurocholic acid; Chol, cholesterol; LPC, lyso-phosphatidylcholine; MO, 2-monoooleoylglycerol; ACAT2, acyl-CoA:cholesterol O-acyltransferase 2. C, Western blot analysis of apoA-IV secretion. Post-confluent Caco-2/TC7 cells were cultured on filter supports in the presence of ITS in the basal compartment and in the presence or absence of lipid micelles in the apical compartment. The concentrated basal media were separated by SDS-PAGE, immunostained using a polyclonal anti-apoA-IV antibody, and then re-probed with a polyclonal anti-a-fetoprotein (AFP) antibody as loading control.

section of apoA-IV and in lipid-dependent gene regulation, we wondered whether HNF-4 was involved in the induction of apoA-IV transcription by lipids. We therefore analyzed the effects of a dominant negative form of HNF-4 (DN-HNF-4) on the lipid-dependent induction of apoA-IV transcription. This mutant form (HNF-4-D1b), resulting from the deletion of residues 361–465, has lost its transactivation potential but is still able to bind to its cognate DNA binding sites and has been demonstrated to inhibit transcription induced by endogenous HNF-4 in HepG2 cells (20).

Caco-2/TC7 eC3-A4-LUC cells were cultured under different conditions of lipid supply in the basal compartment, with or without apical micelles, and in the presence of an adenovirus expressing dominant negative HNF-4 (Ad-DN-HNF-4) (Fig. 5). The amount of Ad-DN-HNF-4 used in this study corresponds to the maximal amount of adenovirus that does not provoke cellular deleterious effects (data not shown). In the absence of apical micelles, apoA-IV transcription was marginally affected by Ad-DN-HNF-4 (<10% inhibition), as compared with Ad-GFP-treated cells, regardless of the presence (FBS and ITS/L conditions) or absence (ITS medium) of lipids in the basal compartment. Conversely, Ad-DN-HNF-4 markedly reduced luciferase activity measured under supply of apical micelles, regardless of the presence or absence of basal lipids. Indeed, a 66% and 50% inhibition of micelle-induced apoA-IV transcriptional activity was provoked by Ad-DN-HNF-4 under the serum and ITS conditions, respectively. These results clearly indicate that the induction of apoA-IV transcription by basal lipids and by apical micelles occurs via distinct molecular mechanisms and that only the apical delivery of lipid micelles recruits the participation of HNF-4.

**Apical Micelles Increase the DNA Binding Activity of HNF-4a on the ApoA-IV Promoter**—We then wanted to determine whether the HNF-4-dependent induction of apoA-IV transcription after an apical supply of lipid micelles was due to a direct effect of HNF-4 on the eC3-A4 regulatory sequence of the human apoA-IV gene (Fig. 6). Three HNF-4 binding domains have been characterized previously in this region: AIVC and AIVE elements, located within the apoA-IV promoter; and CIII-I element, present within the apoC-III enhancer (25). The AIVC element is necessary for maximal promoter activity (8), and the AIVE site is a hormone-responsive element responsible for the restriction of apoA-IV expression to villus enterocytes (7). The hormone-responsive element CIII-I drives the intestinal expression of the apoA-IV gene (6–8). Electrophoretic mobility shift assays were performed using these HNF-4-DNA binding sites and nuclear extracts from Caco-2/TC7 cells cultured with serum in the basal compartment and in the presence or absence of an apical supply of lipid micelles. We observed that the addition of lipid micelles results in an increase of the HNF-4-DNA binding activity on these three sites (Fig. 6). The presence of HNF-4a was confirmed by supershift experiments using an anti-HNF-4a antibody that completely supershifted the DNA-protein complexes formed with AIVE and CIII-I probes (Fig. 6, A and B). Although the major complex formed with the AIVC probe was not totally supershifted with increasing amounts of anti-HNF-4a antibody (Fig. 6C), the intensity of the remaining complex was not changed in nuclear
extracts from lipid micelle-treated cells as compared with control conditions. Taken together, these results suggested that the induction of apoA-IV gene transcription by lipid micelles occurs via a direct effect of HNF-4 on the eC3-A4 regulatory region of the apoA-IV gene.

**DISCUSSION**

The present study demonstrates that transcription of apoA-IV gene requires the presence of lipids at the basal pole of enterocytes and that the apical delivery of lipid micelles specifically induces apoA-IV gene transcription through a pathway involving the transcription factor HNF-4. The biological significance of this effect of apical micelles on apoA-IV gene transcription was highlighted by the subsequent increase in the secretion of apoA-IV, a protein proposed as a signal for satiety and control of gastrointestinal functions.

The importance of HNF-4 for basal apoA-IV gene expression has already been assessed by liver-targeted invalidation of this transcription factor in mice (10). We have reported previously that the HNF-4/DNA binding sites of the eC3-A4 regulatory region of the human apoA-IV gene were necessary to restrict its expression to villus cells of the intestine (6, 7). Here, in addition

**FIG. 5**. Effect of dominant negative HNF-4 on lipid-dependent transcription of the apoA-IV gene. Caco-2/TC7 eC3-A4-LUC cells were cultured on filter supports in the presence of serum (FBS), ITS, or ITS/L in the basal compartment and in the presence or absence of micelles in the apical compartment. Post-confluent cells were infected with adenovirus expressing dominant negative HNF-4 (Ad-DN-HNF-4) or the green fluorescent protein (Ad-GFP, as control for adenovirus transfection) as described under "Experimental Procedures." For each lipid condition, the luciferase activity obtained in the presence of Ad-GFP was set at 100%. The results reported in the graph represent, for each lipid condition, the percentage of inhibition of luciferase activity obtained in the presence of Ad-DN-HNF-4 as compared with Ad-GFP-treated cells. Results represent the means ± S.E. from three independent experiments.

**FIG. 6**. Effect of apical lipid micelles on HNF-4-DNA binding activity. Electrophoretic mobility shift assays were performed with the HNF-4-DNA-binding sites AIV-I (A), CHI-I (B), and AIVC (C) of the human apoA-IV gene and with nuclear extracts from Caco-2/TC7 cells cultured in the presence of serum in the basal compartment and in the presence or absence of micelles in the apical compartment. Supershift experiments were performed with anti-HNF-4α antibody. The arrow on the left indicates the major HNF-4-DNA complex that is supershifted by anti-HNF-4 antibodies.

extracts from lipid micelle-treated cells as compared with control conditions. Taken together, these results suggested that the induction of apoA-IV gene transcription by lipid micelles occurs via a direct effect of HNF-4 on the eC3-A4 regulatory region of the apoA-IV gene.
HNF-4 and Lipid-dependent ApoA-IV Gene Transcription

to its role in basal and cell-specific transcription of apoA-IV, we point out another function for HNF-4 in the specific induction of apoA-IV gene transcription by lipid micelles, the form in which dietary lipids reach the intestine. Indeed, we showed that the eC3-A4 regulatory region was involved in this regulation, which was accompanied by an increase of the binding of HNF-4 on its binding domains within the eC3-A4 regulatory region. Different components of micelles used in this study, such as cholesterol, oleic acid, and taurocholic acid, are known to directly modulate the activity of transcription factors such as sterol regulatory element-binding protein, peroxisome proliferator-activated receptor, and farnesoid X receptor, suggesting that these transcription factors could also be involved in this regulation. However, the apical delivery of these micelle components provoked a much lower induction of apoA-IV mRNA than complex lipid micelles. Furthermore, several studies have shown that the binding of these factors on hormone-responsive elements precluded that of HNF-4 (26, 27). Moreover, an increase of apoA-IV expression has been observed in the liver and in the small intestine of farnesoid X receptor-null mice, suggesting a negative role of farnesoid X receptor on the regulation of apoA-IV expression (28). Thus, the activation of this transcription factor by individual components of lipid micelles could not account for the induction of apoA-IV transcription by complex micelles.

Previous studies have reported that fatty acids or acyl-CoAs were able to modulate HNF-4 DNA binding activity in hepatocytes (29, 30). However, a direct involvement of fatty acids in HNF-4-dependent apoA-IV gene transcription cannot account for our results. Indeed, the apical delivery of oleic acid, brought by albumin, provoked a much lower induction of apoA-IV expression than complex lipid micelles, although the uptake of oleic acid was similar under these different conditions. This clearly demonstrates that the regulation of apoA-IV expression could not be explained simply by a differential lipid entry due to different physical forms of the substrates being presented at the apical cell surface. Moreover, fatty acid supply in the basal compartment increased apoA-IV gene transcription through a mechanism independent of HNF-4 because the dominant negative form of HNF-4 did not abolish this effect. In addition, we observed that the permanent presence of lipids combined with BSA at the basal pole of enterocytes increased apoA-IV mRNA stability, a process that was not observed with apical micelles. Taken together, our results indicate that distinct molecular mechanisms are involved in the lipid-dependent induction of apoA-IV gene expression by basal lipids and apical micelles and that fatty acid supply alone could not account for the HNF-4-dependent activation of apoA-IV transcription.

The discrepancies we observed in the effects of fatty acids on apoA-IV transcription according to their mode of delivery led us to favor the hypothesis that complex lipid micelles may induce a specific cellular signaling pathway leading to the increase of HNF-4 activity and resulting in the induction of apoA-IV transcription. Our hypothesis is reinforced by the observation that MTP gene expression, which was reported to be regulated by lipids (31) and HNF-4 (32), varied in a manner similar to that of apoA-IV under the different conditions of lipid supply. Such a specific micelle-dependent signaling was already suggested by recent results of our group, underlining the importance of the mode of lipid delivery on the bi-directional traffic of apoB. Indeed, the permanent presence of basal lipids led to the formation of an apical pool of apoB, whereas the addition of lipid micelles specifically signaled the chase of this apical pool of apoB toward the secretory pathway (16). In our present report, we show that micelles did not induce variations of apoB mRNA levels, a result that is in accordance with previous studies from other groups. Indeed, whereas HNF-4 has been shown to be essential for apoB expression, numerous studies performed in the liver or in intestinal cells showed that lipids regulate apoB expression at the translational level rather than at the transcriptional level (33, 34). The modulation of apoB expression by lipids involves specific events such as the regulation of mRNA editing, protein synthesis and degradation, as well as protein secretion (see Ref. 35 for review).

The activity of HNF-4 has been shown to be modulated by phosphorylation/dephosphorylation (36–38) and, more recently, by its interaction with other proteins such as peroxisome proliferator-activated receptor γ coactivator 1 (39, 40), sterol regulatory element-binding protein (41), or the small heterodimer partner (42). Such modulations of HNF-4 activity have been demonstrated to be responsible for regulation of the expression of several metabolic genes. Whatever the signaling pathway involved in HNF-4 activity, it could be triggered by lipid micelles by different means. A receptor/transporter located in the brush border membrane of the enterocyte could specifically detect the lipid micelles. Such a mechanism has been suggested for the sensing of dietary sugars by intestinal cells, in which dietary glucose would be sensed by the glucose co-transporter SGLT1 and would generate an intracellular signal involving the activation of G-protein and a cAMP/protein kinase A signaling cascade (43). Dietary lipids are also known to modify the composition of the cell membrane and its fluidity (44, 45), with major consequences on the functions of the membrane and membrane-embedded proteins such as enzymes or receptors (46, 47).

In conclusion, our results point out the importance of the polarity and mode of lipid delivery on apoA-IV transcription in enterocytes. Plasma lipids supplied at the basal pole of enterocytes may regulate gene expression by similar mechanisms in the intestine and liver. By contrast, we report a particular HNF-4-dependent regulation of apoA-IV transcription in enterocytes by an apical supply of lipid micelles, an intestine-specific mode of lipid delivery. This effect is not reproduced by individual components of the micelles, supporting the hypothesis of the presence of a sensor for dietary lipids in enterocytes, which may control enterocyte function in lipid metabolism.

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HNF-4-dependent Induction of Apolipoprotein A-IV Gene Transcription by an Apical Supply of Lipid Micelles in Intestinal Cells
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