Intestinal apolipoprotein A-IV gene expression in the piglet

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Abstract Fetal, newborn, and suckling piglets were used to study the intestinal expression of the apoA-IV gene in the immature mammal. Swine apoA-IV (42 kD) was isolated from fat-fed piglet lipoprotein-deficient plasma by adsorption to IntralipidR followed by preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electroelution. Rabbit anti-swine apoA-IV antibodies were raised, and apoA-IV was immunoprecipitated from small intestinal homogenates after in vivo radiolabeling with [^{3}H]leucine. ApoA-IV synthesis was expressed as a percentage of total protein synthesis from trichloroacetic acid-precipitable counts. Fetal (40 day gestation) whole small intestine synthesis was 2.1%. Postnatally, 2-day-old newborn piglets given high triglyceride and low triglyceride duodenal infusions, as well as bile diversion, were studied. Synthesis rates in jejunal mucosa in all groups were comparable to the fetal whole intestinal value except in the jejunum of the high-triglyceride group, where synthesis was increased sevenfold. In 1- to 2-week-old fasting, cream-fed, and bile-diverted piglets synthesis was again unchanged except in the fat-fed jejunum, where synthesis doubled. Ileal synthesis rates in newborn and suckling animals were lower than jejunal rates and did not increase with lipid absorption or decrease with bile diversion. Northern blot hybridization of intestinal RNA samples from the newborn groups with an authentic cross-hybridizing human apoA-IV cDNA probe revealed a 1.8 kb signal which was strongest in the high-triglyceride jejunal samples. Slot blot hybridization showed eightfold increased apoA-IV mRNA levels in high-triglyceride jejunal samples as compared to low-triglyceride and bile-diverted jejunum with no differences in beta actin mRNA abundance. Fetal, newborn, and suckling piglet small intestine synthesizes a protein similar to human and rat apoA-IV. Jejunal synthesis of apoA-IV is up-regulated in newborn and suckling animals by dietary lipid absorption. In newborn animals this regulation appears to occur at the pre-translational level. Biliary lipid absorption does not play a regulatory role in intestinal apoA-IV synthesis in the immature swine.—**Black, D. D., P. L. Rohwer-Nutter, and N. O. Davidson.** Intestinal apolipoprotein A-IV gene expression in the piglet. *J. Lipid Res.* 1990. **30:** 497–505.

Supplementary key words immunoprecipitation • immunoblot • Northern blot • slot blot • cDNA hybridization

ApoA-IV is an abundant apolipoprotein synthesized by both the liver and small intestine, and both the protein and its gene have been extensively studied in both the adult rat (1–6) and human (6–17). ApoA-IV is a somewhat unusual apolipoprotein in that the major proportion in plasma, especially in humans, exists unassociated with lipoproteins (3, 8, 9, 11, 13, 18, 19). ApoA-IV is a component of nascent intestinal lipoproteins, including chylomicrons and HDL (10, 11, 20, 21), and becomes dissociated from chylomicrons soon after secretion (3, 11, 18, 19, 22, 23). The major metabolic function of apoA-IV is presently unknown. However, recent studies have suggested roles in the activation of lecithin:cholesterol acyltransferase (24, 25), and in reverse cholesterol transport by promoting cellular cholesterol efflux (26) and serving as a ligand for HDL binding to hepatocytes (27).

Studies of the physiologic regulation of intestinal apoA-IV synthesis in the developing mammal have been inconclusive with regard to the factors responsible for the observed increases in intestinal apoA-IV mRNA abundance in the rat pup (28) and in plasma apoA-IV concentrations in the human neonate (29) shortly after parturition. Studies of the effect of luminal lipid absorption, both dietary and biliary, on the developmental expression of the intestinal apoA-IV gene have not been conducted, largely because of the inability to manipulate these variables in the neonatal rat and human. We recently reported the development of the suckling swine as a model for the study of the regulation of intestinal apolipoprotein synthesis in the small intestine of the developing mammal (30). In the present study we have investigated the effects of dietary triglyceride and biliary lipid absorption on intestinal apoA-IV gene expression in the neonatal pig, a model similar in many ways to the human infant.

Abbreviations: EDTA, ethylenediamine tetraacetic acid (Na_2); HDL, high density lipoprotein; LDL, low density lipoprotein; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SSC, sodium chloride/trisodium citrate buffer; TCA, trichloroacetic acid; VLDL, very low density lipoprotein; HTG, high-triglyceride; LTG, low-triglyceride; BiD, bile-diverted.

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MATERIALS AND METHODS

Animals

Two-day-old (newborn) and 1- 2-week-old (suckling) female domestic swine were used in all postnatal studies. Forty-day gestation fetal animals were used in prenatal studies. All animals were obtained from Research Industries Corporation, Monee, IL.

Surgical preparation of animals

Fetal animals. A 40-day gestation pregnant sow was prepared with general anesthesia. A transverse lower abdominal incision was made and the bicornuate uterus was exposed. Starting distally and moving toward the uterine bifurcation, each fetus was isolated and exposed with the umbilical cord intact. Five mCi L-[4,5-^{3}H]leucine (>120 Ci/mmol, Amersham, Arlington Heights, IL) in 0.1 ml of saline was injected into the umbilical vein. Fifteen minutes later the fetus was dissected and the fetal intestine was removed and processed for immunoprecipitation as described below.

Newborn animals. From the time of arrival to the time of surgery the next day, 2-day-old animals were kept in heated isolettes. Animals were fed artificial sow's milk (SPF-LAC^R, Pet-Ag, Inc., Hampshire, IL) by gavage during this period. Anesthesia was induced by intramuscular ketamine (40 mg/kg) and maintained by face mask delivering 1 liter/min O_2 and 0.5–0.8% halothane. A longitudinal midline abdominal incision was made, and the peritoneal cavity was opened. The second portion of the duodenum was cannulated with silicone rubber tubing (i.d. = 0.030 in, o.d. = 0.065 in) through an incision in the gastric antrum. The tubing was then secured in place with a purse-string suture, exteriorized through the right flank, tunneled subcutaneously to the mid-dorsum, and secured through a swivel tether. In bile-diverted animals, the common bile duct was cannulated with silicone rubber tubing (i.d. = 0.030 in, o.d. = 0.065 in) and was then doubly ligated distally. The cystic duct was also ligated, and the gallbladder was completely emptied by aspiration. The bile duct tubing was routed with the duodenal catheter through the flank and dorsum and into the tether. A dual channel tether was used with the bile-diverted animals. Postoperatively the animals were allowed to recover in the heated isolettes. An intraduodenal infusion of 5% glucose in 45 mM NaCl and 20 mM KCl at 100 ml/kg per 24 hr was started during recovery. In bile-diverted animals extra fluid replacement was provided to compensate for bile drainage. After a 24-h recovery period, animals were awake, alert, and mobile. Also, at this time animals were noted to be tolerating the infusion well without vomiting or abdominal distention and to be passing stool. Experimental infusions were started at the end of this recovery period.

After the 24-h recovery period three groups of newborn animals were studied, as illustrated in **Fig. 1**: Group 1: piglets receiving a 24-h intraduodenal infusion of dilute Vivonex^R (Norwich Eaton Pharmaceuticals, Inc., Norwich, NY), a low fat (1.45 g/L of fat in undiluted form) elemental formula, at 50 kcal/kg per 24 h (LTG group, n = 7); Group 2: animals receiving a 24-h intraduodenal infusion of Intralipid^R (Cutter, Berkeley, CA), a triglyceride emulsion, at 50 kcal/kg per 24 h (HTG group, n = 7); and Group 3: bile-diverted animals receiving a 24 h intraduodenal infusion of Vivonex^R at 50 kcal/kg per 24 h (BiD group, n = 4).

All groups received a total fluid volume of 100 ml/kg per 24 h during the experimental infusion using supplemental saline solution. The BiD group also continued to receive bile drainage replacement in addition to the experimental infusion. During the infusions the animals were kept in the heated isolettes and allowed to move freely within the limits of their tethers.

Suckling animals

Proximal and distal small intestinal radiolabeled cytosolic supernatants from 1- to 2-week-old suckling female piglets were prepared as previously described (30). Briefly, these animals were fasted overnight (12 h) before being given cream by gavage through an orogastric tube (fat-fed group) or continued fasting (fasting group). Addi-

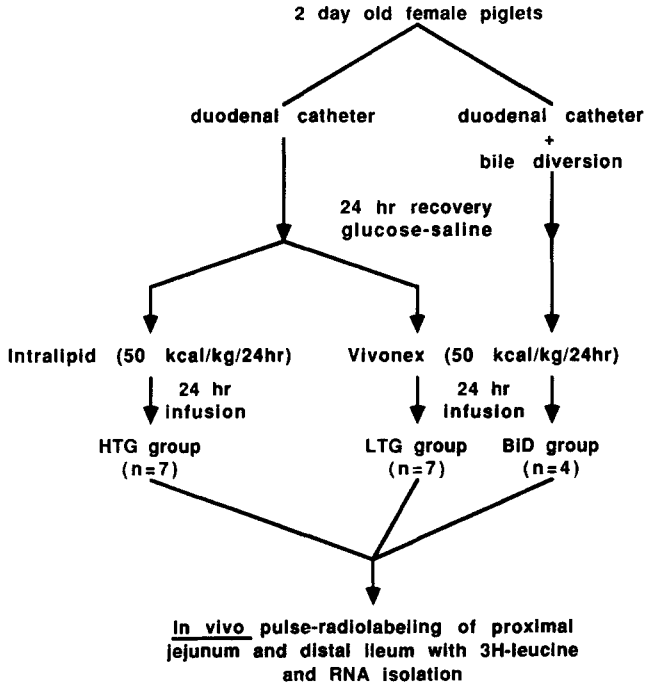

Fig. 1. Experimental protocol for newborn piglets. HTG animals received an intraduodenal infusion of Intralipid^R, LTG animals received an infusion of Vivonex^R, and the bile-diverted (BiD) animals also received the Vivonex^R infusion.

tionally, a bile-diverted group was studied with a matched sham-operated control group. In vivo pulse-radiolabeling of jejunal and ileal segments was carried out 2 h after fat-feeding or 48 h after bile diversion, and cytosolic supernatants were prepared exactly as described in the present study and were stored at −80°C until apoA-IV immunoprecipitation. ApoB and apoA-I synthesis was studied previously in these same animals and reported (30).

Determination of intestinal apoA-IV synthesis

At the end of the experimental manipulations the animals were anesthetized, and a 10-cm segment of proximal jejunum was isolated 10 cm distal to the ligament of Treitz by two ligatures. Likewise, a 10-cm segment of distal ileum was isolated 10 cm from the ileocecal valve. Radiolabeling was performed by instilling 1.5 mCi of L-[4,5-^{3}H]leucine (>120 Ci/mmol) (Amersham, Arlington Heights, IL) into each segment. Nine minutes later the segments were removed and prepared for immunoprecipitation as described below.

Preparation of mucosal cytosolic supernatants for immunoprecipitation

Radiolabeled intestinal segments were flushed with 50 ml of iced PBS (50 mM phosphate, 100 M NaCl, pH 7.4)–20 mM leucine, and the mucosa was scraped and homogenized on ice in 1 ml of PBS–1% Triton X-100-2 mM leucine–1 mM PMSF–1 mM benzamidine, pH 7.4 as previously described (30). Because of the small size, fetal intestine was removed intact and homogenized as a whole. Aliquots of the homogenate were taken for measurement of total protein concentration and TCA-precipitable radioactivity, and the remainder was pelleted at 105,000 *g* for 60 min in a 50.3 Ti rotor (Beckman Instruments, Palo Alto, CA). All procedures were performed at 0–5°C, and the mucosal supernatant samples were stored at −80°C until analysis. In newborn animals segments of jejunum distal to and adjacent to the radiolabeled segments were removed and immediately homogenized in 4 M guanidine thiocyanate–25 mM Na citrate, pH 7.0 as the initial step in RNA extraction as described (31).

ApoA-IV isolation

Preparative isolation of porcine apoA-IV was undertaken using the method of Weinberg and Scanu (13). Serum was obtained from a fat-fed suckling piglet, and the d<1.21 g/ml lipoproteins were removed by ultracentrifugation. The lipoprotein-deficient serum was then incubated with Intralipid as described (13). The Intralipid was then re-isolated and delipidated, and subjected to preparative SDS-PAGE (13). The 42 kD apoA-IV band was identified, sliced out, and the protein was electroeluted. Analytical SDS-PAGE confirmed the purity of the final preparation. Polyclonal antiserum was raised in a New Zealand white rabbit after inoculation with electrophoretically pure porcine apoA-IV in Freund's complete adjuvant.

Apolipoprotein A-IV immunoprecipitation

Intestinal cytosolic supernatant fractions were subjected to specific immunoprecipitation of apoA-IV under conditions of antibody excess as described (32). Aliquots of cytosolic supernatants were mixed with washed IgG-SorbR (The Enzyme Center, Malden, MA) and subsequently reacted with excess anti-apoA-IV antiserum for 18 h at 4°C. After a second addition of IgG-Sorb and extensive washing, the liberated immunocomplex was applied to 5.6% SDS polyacrylamide tube gels. After electrophoresis, gels were sliced into 1-mm slices and incubated in 3% ProtosolR/97% EconofluorR (New England Nuclear, Boston, MA) at 37°C overnight prior to liquid scintillation counting in a Packard Model 2000 liquid scintillation counter (Packard Instruments, Downers Grove, IL). Apolipoprotein species were identified by comparison to stained co-electrophoresed apolipoproteins. ApoA-IV synthesis rates were expressed as the percentage of specific immunoprecipitated apolipoprotein counts as compared to total protein TCA-precipitable counts. Apolipoprotein synthesis was thereby expressed as a percentage of total protein synthesis. All samples were subjected to re-immunoprecipitation to ensure the completeness of the first antigen–antibody reaction.

Electrophoresis methods

During the apoA-IV isolation and purification process, samples were analyzed by SDS-PAGE under reducing conditions on the PhastR System (Pharmacia, Uppsala, Sweden) using pre-cast miniature 8–25% polyacrylamide gradient gels. Delipidated Intralipid samples for preparative SDS-PAGE isolation of apoA-IV and lymph lipoprotein samples for immunoblotting were run under reducing conditions on 10% SDS-PAGE slab gels in a Bio-Rad Protean electrophoresis unit (Bio-Rad, Richmond, CA) according to the method of Laemmli (33). Isoelectric focusing of radiolabeled immunoprecipitates was performed in 6 M urea using pH 4.0–6.0 ampholines (Bio-Rad) (34). After electrophoresis, gels were sliced, solubilized, and counted as described above for SDS-PAGE immunoprecipitate tube gels. For immunoblotting after SDS-PAGE, lipoprotein samples were transferred to nitrocellulose paper and immunostained with pig apoA-IV antiserum as described (35) using a 1:100 and 1:3000 dilution of primary and secondary antisera, respectively.

Analysis of apoA-IV mRNA by Northern blot and slot blot hybridization

Samples of total RNA (25 μg) were subjected to denaturing formaldehyde/agarose electrophoresis and transferred to nitrocellulose as described (36). Intactness of each RNA preparation was verified by methyl mercury gel electrophoresis and visualization of ribosomal subunits. Serial dilutions of RNA were applied to nitrocellulose filters using a slot blot apparatus (Schleicher and Schuell, Inc., Keene, NH). Total RNA in each slot was made constant by the addition of yeast tRNA. Filters were prehybridized and hybridized at 37°C using final concentrations of 50% formamide, 6× SSC, 5 mM Na phosphate, 1 mM EDTA, 1.5 × Denhardt's solution, 100 μg/ml sonicated salmon sperm DNA, and 10% dextran sulfate. The filters were probed with a 300 bp PstI insert from human apoA-IV cDNA (provided by J. Gordon, Washington University, St. Louis, MO) (17) and subsequently with a mouse beta actin cDNA (provided by L. Kedes, Stanford University, CA) (37). Probes were labeled to a specific activity of 2×10^9 cpm/μg by random priming (38). Washes were performed at room temperature in 1 × SSC, 0.1% SDS, and at 42°C in 0.1 × SSC for 1 h. Autoradiograms were developed following exposure to XAR-5 film (Eastman Kodak Co., Rochester, NY) at −70°C for 1–3 days, and scanned using a laser densitometer (LKB Instruments, Inc., Rockville, MD).

Protein measurement

Protein in lipoprotein fractions and cytosolic supernatants was measured by a modified Lowry technique (39).

Statistical analysis

Student's unpaired two-tailed t test was used to compare data between different experimental groups. The null hypothesis was rejected at $P < 0.05$.

RESULTS

ApoA-IV isolation and antibody production

After incubation of the Intralipid emulsion with piglet lipoprotein-deficient serum and ultracentrifugal re-isolation and delipidation, the SDS-PAGE profile (**Fig. 2**, lane B) shows three protein bands as previously described by Weinberg and Scanu during human apoA-IV isolation (13). The middle 42 kD protein band was electroeluted from preparative SDS-PAGE gel slices, and the gel profile of this protein is shown in Fig. 2, lane C. A single 42 kD band is present, and this material was used for antibody production. To test the specificity of the antiserum to the previously described 42 kD protein in piglet mesenteric lymph lipoproteins (30), immunoblot analysis was performed using fatty lymph lipoproteins from a fat-fed lymph-fistulated piglet from a previous study (30). The blot is shown in **Fig. 3**. Reactive 42 kD protein bands are present in lanes containing whole lymph, chylomicrons, VLDL, HDL, and the d > 1.21 g/ml lymph fraction. No immunoreactivity is seen in the LDL fraction.

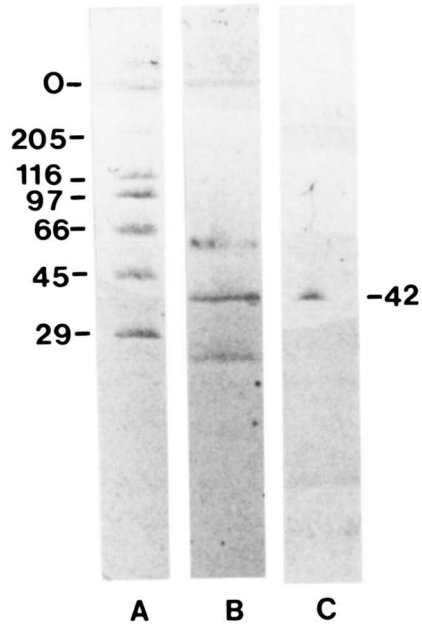

Fig. 2. Phast^R System 8–25% SDS-PAGE electrophoretograms of molecular weight standards marked in kD (lane A), proteins from Intralipid^R incubated with lipoprotein-deficient serum from a fat-fed piglet as described in Methods (lane B), and pure pig apoA-IV (42 kD band) after preparative SDS-PAGE and electroelution from gel slices (lane C).

Intestinal apoA-IV immunoprecipitation

The SDS-PAGE gel profile of the immunoprecipitate obtained from the cytosolic supernatant from the jejunum of a newborn HTG animal is shown **Fig. 4**, top panel. A 42 kD peak is present. Although not shown, profiles of both jejunal and ileal samples from all animal groups studied demonstrated peaks of the same molecular weight. The same jejunal HTG immunoprecipitate was also analyzed by pH 4.0–6.0 isoelectric focusing, and the gel profile is shown in Fig. 4, bottom panel. A pI 5.70 peak containing 33% of the total counts and a pI 5.63 peak with 67% of total counts are noted.

Intestinal apoA-IV synthesis

Jejunal apoA-IV synthesis rates for the various experimental groups studied are shown in **Fig. 5**, top panel. The value for the fetal animals represents synthesis by the entire small intestine, and cytosolic supernatants from three fetuses were pooled for apoA-IV immunoprecipitation and synthesis quantitation. The fasting groups designated in this figure include the 40-day gestation fetal, the

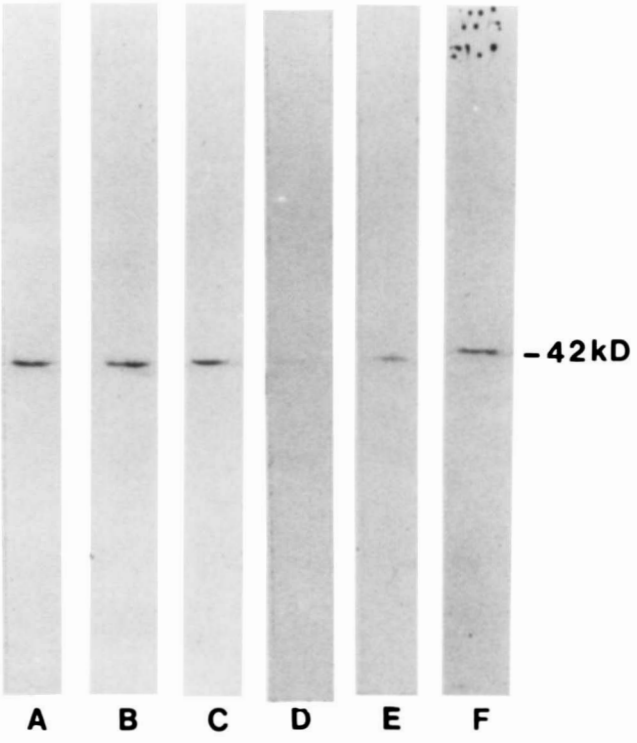

Fig. 3. Immunoblot of delipidated piglet mesenteric lymph and lymph lipoproteins separated by SDS-PAGE using primary antibody prepared from purified porcine apoA-IV. Lanes: A, whole lymph; B, lymph chylomicrons; C, lymph VLDL; D, lymph LDL; E, lymph HDL; and F, lymph d>1.21 g/ml fraction.

2-day-old LTG newborn, and the 7–14-day-old fasted suckling animals. The fat-fed groups include the HTG newborn and the cream-fed suckling animals. For bile-diverted animals, the newborn BiD animals were compared to the LTG newborns, and the suckling bile-diverted piglets were compared to their sham control group values (not shown), which were not different from the fasting animal values. The only significant differences in apoA-IV synthesis were seen in the HTG newborn and fat-fed suckling animals. Triglyceride absorption resulted in a 7-fold increase in jejunal apoA-IV synthesis in the newborn animals, and a 2-fold increase in the suckling animals. Biliary diversion had no significant effect in either group. Ileal apoA-IV synthesis for these animals is shown in Fig. 5, bottom panel. Neither triglyceride absorption nor bile diversion had a significant effect on apoA-IV synthesis in the ileum in either group.

ApoA-IV mRNA analysis

Northern blot analysis of total cellular RNA from newborn LTG, HTG, and BiD jejunal mucosa after hybridization with a human apoA-IV cDNA probe reveals a 1.8 kb signal in all samples, (**Fig. 6**). The strongest signal is noted for the newborn HTG jejunal RNA. Slot blot hybridization using the same apoA-IV cDNA probe and a murine beta actin probe as a control was carried out using jejunal RNA from three animals in each newborn experimental group. Representative blots are shown in **Fig. 7**. Densitometric scanning of the slot blot autoradiograms demonstrated a 763 ± 274% (mean ± SEM) increase in apoA-IV mRNA abundance in the HTG animals compared to the LTG controls. The HTG animals had beta actin mRNA levels that were 93 ± 28% of the LTG controls. The BiD animals had apoA-IV mRNA levels which were 82 ± 25% of the LTG controls and beta actin mRNA levels which were 116 ± 4% of the LTG values.

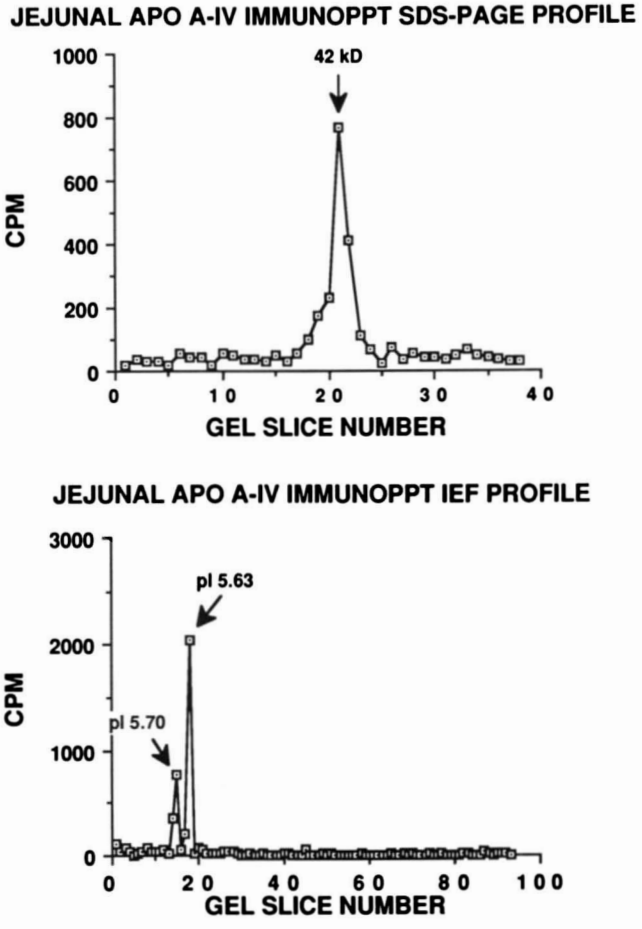

Fig. 4. Electrophoretic profiles of an apoA-IV immunoprecipitate from pulse-radiolabeled newborn piglet jejunal mucosa separated by SDS-PAGE (top) and isoelectric focusing (bottom). For SDS-PAGE the cytosolic supernatant was reacted with excess anti-apoA-IV antiserum, and the immune complex was subjected to electrophoresis on a 5.6% disc gel followed by gel slicing and scintillation counting. Molecular weight was determined by comparison to a stained co-electrophoresed gel containing molecular weight standards. For isoelectric focusing the immune complex was applied to a pH 4.0–6.0 gel for focusing followed by gel slicing and scintillation counting.

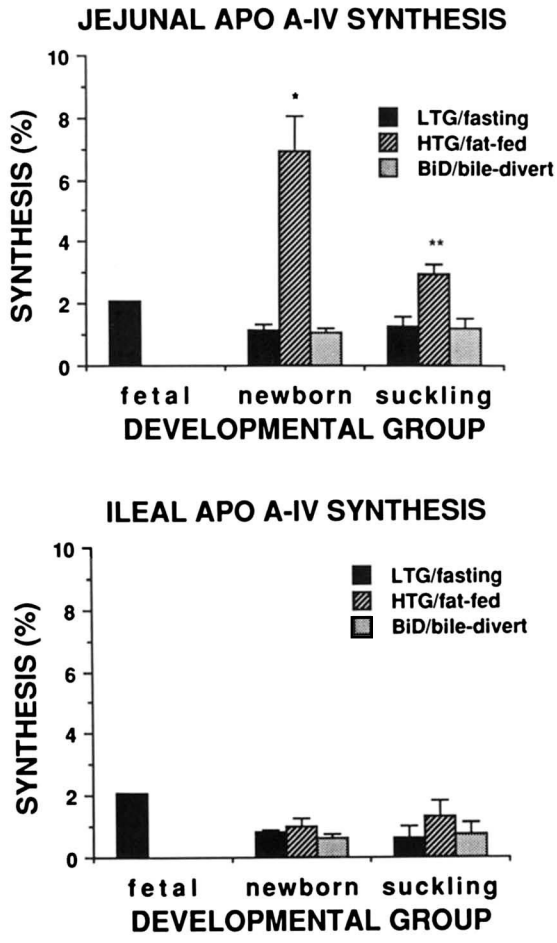

Fig. 5. Jejunal (top panel) and ileal (bottom panel) apoA-IV synthesis rates for the various developmental groups of piglets studied. ApoA-IV synthesis is expressed as a percentage of total protein synthesis per 9-min pulse period. Cytosolic supernatants from the entire small intestine of three radiolabeled fetal animals were pooled and immunoprecipitated to obtain the value shown on the left in each panel, which therefore represents the entire small intestinal, rather than jejunal or ileal, rate. The means ± SEM of synthesis rates from the radiolabeled intestinal mucosa from LTG, HTG, and BiD newborn groups are shown in the center bars, and those from the fasting, fat-fed, and bile-diverted suckling groups are shown on the right of each panel. Significant differences as compared to the corresponding LTG or fasting groups are indicated by • ($P < 0.001$) and •• ($P < 0.022$).

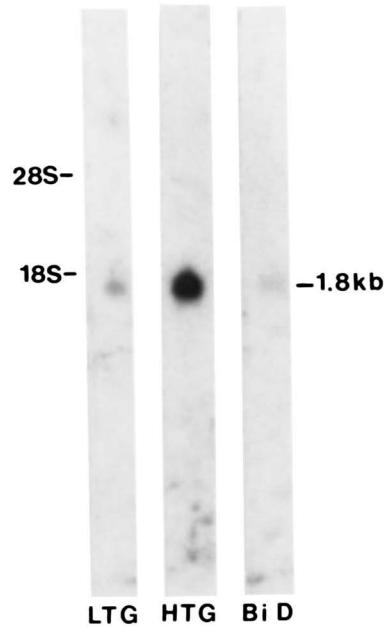

Fig. 6. Northern blots of total cellular RNA from jejunal mucosa from LTG (left), HTG (center), and BiD (right) newborn animals. The filter was hybridized with a ^{32}P-labeled human apoA-IV cDNA probe and autoradiographed as described in Methods. The positions of the 18S and 28S ribosomal subunits are indicated on the left of the figure.

DISCUSSION

Delineation of the factors that regulate apolipoprotein gene expression in the small intestine of the developing mammal has been extremely difficult because of the simultaneous interaction of several potential regulatory factors. These factors include diet, striking changes in the hormonal milieu, and as yet undefined maturational cues. Controlled manipulation of diet followed by study of gene expression in the newborn rat pup or human infant is extremely difficult, if not impossible. Therefore, we have developed a model in the newborn piglet which enables the controlled delivery of lipid to the small intestine in the awake, minimally restrained state followed by in vivo quantitation of intestinal apolipoprotein synthesis and mRNA abundance. In the present report this model was used to study the translational and pre-translational regulation of small intestinal apoA-IV gene expression by luminal lipid absorption in the piglet.

Swine apoA-IV has not been previously isolated or characterized. We previously demonstrated in the suckling pig the intestinal synthesis and incorporation into mesenteric lymph chylomicrons, VLDL, and HDL of a 42 kD protein similar to rat and human apoA-IV (30). However, pig apoA-IV (42 kD) is smaller than both human and rat apoA-IV which have molecular weights of

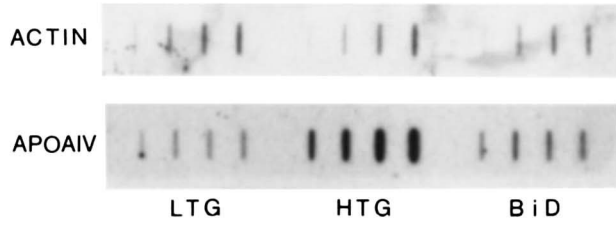

Fig. 7. Representative slot blots of total cellular RNA from jejunal mucosa from LTG (left), HTG (center), and BiD (right) newborn animals. For each animal 25, 10, 5, and 2.5 μg of RNA were applied to the filter (right to left). The filter was first hybridized with a ^{32}P-labeled mouse beta actin cDNA probe (top) and subsequently with a human apoA-IV probe (bottom). Hybridization and autoradiography conditions are described in Methods.

approximately 46,000. Piglet apoA-IV was isolated as previously described by Weinberg and Scanu (13) by adsorption of the apolipoprotein in the lipoprotein-free fraction of serum onto a triglyceride emulsion. This indicates that, as in the human, a large fraction of apoA-IV in the piglet is present in the circulation not bound to lipoprotein particles. Immunoblot analysis of mesenteric lymph lipoproteins with antibodies raised to the purified swine apoA-IV shows that the protein isolated from the lipoprotein-deficient serum is indeed the same 42-kD protein previously noted to be a component of piglet lymph chylomicrons, VLDL, and HDL. Furthermore, a significant amount of lymph apoA-IV appears to be present in the $d > 1.21$ g/ml fraction, although the exact proportion cannot be determined in the present studies because of the probable loss of the apolipoprotein from lipoprotein particles during ultracentrifugal isolation.

Analysis of newly synthesized piglet intestinal apoA-IV by application of a radiolabeled immunoprecipitate to an isoelectric focusing gel revealed a minor isoform (pI 5.70) and a more acidic major isoform (pI 5.63). Human and rat preapoA-IV contain an N-terminal signal peptide, 20 amino acids in length, which is cleaved co-translationally to yield a more acidic mature apoA-IV which is not further altered prior to secretion (14, 16, 40). It is unlikely that either of the isoforms detected in the piglet immunoprecipitate represents the preprotein, since removal of the signal peptide, at least in the human and rat, is a co-translational event and would occur before completion of polypeptide chain elongation to the 42 kD species (14, 40).

With regard to the physiologic regulation of apoA-IV gene expression in the mature mammal, studies in the adult rat have demonstrated an increase in apoA-IV mRNA and synthesis in the small intestine induced by acute triglyceride feeding. Gordon et al. (4) demonstrated a two-fold increase in translatable intestinal apoA-IV mRNA after corn oil feeding in the adult rat. Apfelbaum, Davidson, and Glickman (41) found that acute triglyceride feeding caused an increase in apoA-IV synthesis in both jejunum and ileum in the adult rat. Furthermore, this regulation appeared to occur at the pre-translational level based upon quantitation of translatable apoA-IV mRNA abundance. Studies in adult humans have demonstrated an increase in plasma apoA-IV levels after fat-feeding (11, 19) and an increase in urinary apoA-IV output after lipid ingestion in subjects with chyluria (11), suggesting regulation of intestinal apoA-IV synthesis by dietary lipid absorption. Green et al. (12) studied changes in enterocyte apoA-IV content in normal human adults before and after fat feeding and observed an increase in cellular content by radioimmunoassay accompanied by an increase in apoA-IV immunoperoxidase staining. Therefore, despite early in vivo studies by Windmueller and Wu (42) in the adult rat suggesting no regulation of apoA-IV synthesis by dietary lipid absorption, it now appears that synthesis is indeed modulated by acute dietary fat absorption. This would suggest a role for apoA-IV in the synthesis and secretion of intestinal triglyceride-rich lipoproteins, and there may exist a further role in their peripheral metabolism.

In the present study, we addressed the issue as to whether the dramatic and immediate post-partum increase in intestinal apoA-IV abundance noted previously in the rat pup (28) is regulated by the onset of ingestion of a triglyceride-rich diet, hormonal changes, or some other developmental cue. This issue also has relevance to the newborn human infant, since the plasma apoA-IV concentration has been shown to increase to the adult range during the first week of life (29). Using our piglet model we demonstrated that intestinal apoA-IV synthesis is regulated in the jejunum by acute triglyceride absorption with a sevenfold increase in jejunal synthesis found in the newborn piglet and a twofold increase in the older sucking piglet. Since these two groups were not fat-fed with the same protocol, the difference in magnitude of the increases noted may be due to the amount and timing of the lipid feeding. However, in the absence of dietary triglycride absorption basal jejunal synthesis was very similar in all groups, including the whole fetal intestine. This would suggest that hormonal influences such as the bursts of insulin released immediately pre-partum and of cortisol near weaning do not regulate basal jejunal apoA-IV expression in the piglet. Ileal mucosal apoA-IV basal synthesis was similar in the two post-partum groups, and did not change significantly with either triglyceride absorption or removal of biliary lipid. Lack of effect of fat-feeding on ileal synthesis is different from the previous studies by Apfelbaum et al. (41) in the adult rat, and may be due to species differences or the lack of exposure of the ileal mucosa to the lipid due to more efficient proximal intestinal absorption in the piglet.

Biliary lipid appears to have no regulatory effect on jejunal apoA-IV synthesis in either the newborn or older suckling pig. These observations are in contrast to the effects of biliary exclusion on intestinal apoA-IV gene expression in the rat which produces both a rapid and profound suppression (N. O. Davidson and D. D. Black, unpublished results). Similar species differences in the effects of biliary lipid were previously demonstrated in regard to apoB-48 synthesis which was unresponsive to biliary exclusion in the suckling pig (30), but produced profound, reversible suppression in both jejunal and ileal apoB-48 synthesis in the adult rat (43).

Using the same piglet model as in the present study, intestinal apoB-48 and apoA-I synthesis has been studied in newborn (44) and older suckling animals (30). In contrast to the striking up-regulation of jejunal apoA-IV synthesis by triglyceride absorption in the newborn piglet, apoB-48 synthesis is unaffected and apoA-I synthesis doubles (44). In the newborn piglet ileum apoB-48 and apoA-

IV synthesis is unaffected, but apoA-I synthesis doubles with triglyceride absorption (44). In the older suckling piglet, triglyceride absorption increases only apoA-IV synthesis in the jejunum, and apoB-48 and apoA-I synthesis is not changed in either jejunum or ileum (30). Biliary lipid deprivation has no effect on the synthesis of apoA-IV, apoB-48, or apoA-I in either proximal or distal small intestine in both the newborn and older suckling piglet (30, 44). Therefore, it appears that the synthesis of intestinal apolipoproteins in the developing piglet is regulated differently for each apolipoprotein by dietary lipid absorption, and this regulation seems to also be dependent on developmental cues.

Analysis of intestinal apoA-IV mRNA in the newborn piglet demonstrated a message of a size similar (1.8 kb) to that previously described in both human and rat intestine (4, 15, 16), even though the protein itself is smaller (42 kD vs 46 kD). The significant cross-hybridization of piglet apoA-IV mRNA with the human cDNA probe suggests significant sequence homology. The striking increase in jejunal apoA-IV mRNA abundance with triglyceride absorption in the newborn piglet suggests that the increase in synthesis is mediated at the pre-translational level. Whether the observed increase in apoA-IV mRNA abundance is the result of increased transcription or changes in mRNA stability will require further study. The present study clearly demonstrates that, for apoA-IV, changes in intestinal gene expression early in life are mediated by dietary lipid absorption, but not by biliary lipid flux. Whether or not hormonal and other developmental factors play a permissive role in this regulation will be the focus of future studies.

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