

Intestinal apolipoprotein synthesis and secretion in the suckling pig

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Abstract The present studies report characterization of intestinal apolipoprotein (apoLp) synthesis and secretion in the suckling pig. Lipoproteins ($d < 1.006$ g/ml) from mesenteric lymph were found to contain both apoB-100 and B-48, in addition to apoA-IV, E, A-I, and Cs. Lymph low density lipoproteins (LDL) and high density lipoproteins (HDL) contained mainly apoB-100 and apoA-I, respectively. Analysis of core cholesteryl ester fatty acid composition suggested filtration from plasma as the major source of lymph LDL and HDL. Dual radioisotope labeling of intestinal and hepatic apoLps in lymph, as well as immunoprecipitation of radiolabeled intestinal mucosa, demonstrated intestinal synthesis of apoB-48, A-IV, and A-I. There was no evidence for apoB-100 synthesis by intestinal mucosa. By contrast, piglet liver synthesized apoB-100, E, A-I, and Cs, but not apoB-48. Newly synthesized intracellular intestinal apoA-I was mainly (basic) isoform 1 (pI 5.58), while lymph and plasma HDL apoA-I were predominantly isoform 3 (pI 5.33), mature apoA-I. Lymph apoB ($P < 0.001$) and apoA-I ($P < 0.04$) mass output increased significantly during lipid absorption. Studies were subsequently conducted in fasting, fat-fed, bile-diverted, and sham-operated animals to determine the role of both dietary and biliary lipid in regulating intestinal apoLp biosynthesis. Proximal and distal small intestinal loops were pulse-radiolabeled with [³H]leucine, and apoB-48 and A-I were immunoprecipitated from cytosolic supernatants. Although a proximal to distal gradient in intestinal synthesis rates for both apoB and A-I was noted in all groups, the acute absorption of dietary lipid did not significantly increase apoB or A-I synthesis in either location. Complete removal of biliary lipid for 48 hr did not alter synthesis rates in jejunum or ileum. ■ These studies suggest that mesenteric lymph apoLps in the suckling pig are derived both by filtration from plasma and by direct secretion from the intestine. Physiologic regulation of intestinal apoA-I and B-48 synthesis rates appears to be independent of luminal lipid availability.—**Black, D. D., and N. O. Davidson.** Intestinal apolipoprotein synthesis and secretion in the suckling pig. *J. Lipid Res.* 1989. 30: 207–218.

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Intestinal lipoprotein and apolipoprotein secretion has been characterized in the adults of several species, including humans. However, information regarding develop-

ment and physiologic regulation is limited by the availability of a suitable animal model for study. Ultrastructural studies have demonstrated lipoprotein particles within the enterocytes of rat pups shortly after birth (1–3). Additionally, studies in the neonatal rat suggest that intestinal secretion accounts for practically all of the plasma triglyceride in 14-day-old animals (4).

The physiologic regulation of apolipoprotein synthesis and secretion in adult rat intestine has been extensively characterized (5–9). However, similar approaches in the neonatal rat have been technically difficult (4, 10, 11). Recently, apolipoprotein synthesis has been demonstrated in explants of rat and human fetal intestine (12–15), but questions of physiologic regulation have been impossible to address. For these reasons, we undertook to characterize aspects of intestinal lipoprotein and apolipoprotein synthesis and secretion in the neonatal pig. Species similarities to humans of lipoprotein metabolism include most of the plasma cholesterol being transported in LDL and HDL, together with extensive homology of certain apolipoproteins (16–20). Differences include absence of plasma cholesteryl ester transfer activity and apoA-II in the pig (20, 21). The present studies report the first characterization of intestinal lymph lipoprotein and apolipoprotein synthesis and secretion following physiologic manipulation of intestinal lipid flux in the suckling pig, a model similar in many ways to the human infant.

Abbreviations: CM, chylomicron; 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; TCA, trichloroacetic acid; VLDL, very low density lipoprotein.

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Animals

Female domestic suckling swine weighing 3.96 ± 0.11 kg (mean \pm SEM), aged 7–14 days, were used in all studies. Animals were obtained from Research Industries Corporation, Monee, IL.

Preparation of animals with lymph fistula

Piglets were fasted overnight prior to surgery. Anesthesia was induced by intramuscular atropine sulfate (0.03 mg/kg) and ketamine (40 mg/kg) and maintained by face mask delivering 1 liter/min oxygen and 0.5–1.0% halothane. The internal jugular vein was cannulated with silicone rubber tubing (Silastic, Dow Corning Corp., Midland, MI, i.d. = 0.058 in, o.d. = 0.077 in) for intravenous access. A longitudinal midline abdominal incision was made, and the peritoneal cavity was opened. The cysterna chyli was visualized following injection of 1% methylene blue solution, and isolated as described by Butterfield, Lumb, and Litwak (22). Heparinized silicone rubber tubing (i.d. = 0.062 in, o.d. = 0.095 in) was threaded into the cisterna, secured to the internal abdominal wall with suture, and exteriorized through the right flank. The second portion of the duodenum was cannulated with silicone rubber tubing (i.d. = 0.078 in, o.d. = 0.125 in) and also exteriorized through the right flank. Post-operatively, animals were placed in a sling suspended in an adjustable slatted wooden restraining cage. As the animals awakened and were able to stand, the sling was removed. Animals could freely stand or lie down and were allowed limited forward-backward mobility.

Intravenous fluids containing 5% glucose and 70 mM NaCl were given for maintenance requirements and to compensate loss from lymph drainage. After a 24-hr recovery period, intraduodenal infusion of 10% glucose in 145 mM NaCl and 4 mM KCl was started to replace one third of fluid requirements. Animals were observed for 12 hr for evidence of intolerance of the infusion whereupon hourly lymph samples were collected into pre-weighed tubes containing a preservative solution containing final concentrations of 0.01% EDTA, 0.02% Na azide, 1 μ g/ml chloramphenicol, and 0.5 μ g/ml gentamicin sulfate. After collection of fasting lymph samples for 4 hr, 10% Intralipid® (Cutter, Berkeley, CA) was added to deliver 1 g/hr of triglyceride, and lymph collection was continued for 8 hr. Samples were immediately refrigerated at 4°C, and isolation of lipoproteins was started 6–48 hr from collection.

In one experiment, the origin of lymph apolipoproteins was studied using the differential double labeling technique described by Wu and Windmueller (6). An animal was prepared with lymph, duodenal, portal vein, and jugular vein catheters. Duodenal lipid infusion and lymph

collection were carried out as described above. After 8 hr of lipid infusion, 250 μ Ci of L-[U-¹⁴C]leucine (> 300 mCi/mmol) (Amersham, Arlington Heights, IL) in 1 ml of saline was injected into the portal vein. Simultaneously, 1.5 mCi of L-[4,5-³H]leucine (> 120 Ci/mmol) in 1.5 ml of saline was injected into the duodenum. The intraduodenal lipid infusion and lymph collection were continued for 2 hr and the animal was killed. Lymph and plasma lipoproteins were isolated as described below, and apolipoproteins were separated by SDS PAGE. Gels were sliced and counted after solubilization in 3% Protosol/97% Econofluor (New England Nuclear, Boston, MA), using dual label techniques in a Packard Model 2000 liquid scintillation counter (Packard Instruments, Downers Grove, IL). Plasma albumin, used as a reference protein of exclusive hepatic origin, was isolated by the method of Iwata, Iwata, and Holland (23), and its purity was confirmed by SDS-PAGE analysis.

Preparation of bile-diverted and sham-operated animals

Animals were anesthetized after an overnight fast and the common bile duct was exposed and ligated through a midline longitudinal abdominal incision. Next, the gallbladder was exposed, and a silicone rubber catheter (i.d. = 0.062 in, o.d. = 0.095 in) was secured in the gallbladder lumen. In sham-operated animals the distal end of the catheter was placed directly into the proximal duodenum. In bile-diverted animals a separate duodenal catheter was placed, and both duodenal and gallbladder catheters were exteriorized through the right flank. The abdominal incision was then closed as described above. Bile-diverted animals were allowed to recover and placed in a restraining cage as described above and were given glucose-saline intraduodenally to fulfill fluid and electrolyte requirements. Sham-operated animals were placed in a regular cage and allowed free access to glucose-saline solution which they readily took perorally. Both groups of animals were maintained as described for 48 hr prior to in vivo pulse-radiolabeling of jejunal and ileal enterocytes as described below.

Fasting and fat-fed animals

Suckling animals were fasted and allowed free access to water overnight. Two groups of fat-fed animals were studied. The first group (2-hr group) was given 60 ml of cream by orogastric tube 2 hr prior to pulse-radiolabeling of enterocytes. The other group (8-hr group) was given 90 ml of cream by the same route, and an additional 90 ml of cream was given again 4 hr after the first dose, with pulse-radiolabeling carried out 4 hr after the second dose of cream. At the time of in vivo pulse-radiolabeling, active lipid absorption was confirmed in both groups by noting the presence of luminal lipid and lymphatics distended with milky chyle. A fasting control group was also studied.

Lipoprotein isolation

Sequential density ultracentrifugation of lymph and plasma samples was accomplished using a Beckman SW 41 Ti rotor (Beckman Instruments, Palo Alto, CA) at 17°C. The density classes separated were CM ($d < 1.006$ g/ml, 1.06×10^6 g-min), VLDL ($d < 1.006$ g/ml, 230×10^6 g-min), LDL (1.006 g/ml $< d < 1.07$ g/ml, 268×10^6 g-min), and HDL (1.07 g/ml $< d < 1.21$ g/ml, 469×10^6 g-min). CM and VLDL samples were washed once through a d 1.006 g/ml overlay. All lipoprotein fractions were dialyzed at 4°C against water containing the above preservative solution.

Density gradient ultracentrifugation using the method of Nilsson et al. was also performed (24). Since this gradient does not resolve triglyceride-rich lipoproteins, some lymph samples were subjected to a preliminary ultracentrifugation step through a d 1.006 g/ml overlay for CM and VLDL removal. Ultracentrifugation was carried out in the Beckman SW 41 Ti rotor at 39,000 RPM for 66 hr at 14°C. Fractions of 0.4 ml were collected with monitoring of the eluate at 280 nm and were immediately frozen at -80°C until analysis.

Electrophoresis methods

Discontinuous 3–20% SDS-PAGE was carried out under reducing conditions according to the method of Laemmli (25). Apolipoproteins were identified by comparison to co-electrophoresed molecular weight standards and scanned by laser densitometry (LKB Instruments, Inc., Rockville, MD). Two-dimensional electrophoresis was performed as described by O'Farrell (26).

For immunoblotting, delipidated apolipoproteins were electrophoresed as described by Gabelli et al. (27). Samples were transferred to nitrocellulose paper and immunostained with swine apoB antiserum as described (28) using a 1:100 and 1:3000 dilution of primary and secondary antisera, respectively.

Determination of intestinal apoA-I and apoB synthesis

Animals were anesthetized, and an 8-cm segment of proximal jejunum was isolated 10 cm distal to the ligament of Treitz by two ligatures. Likewise, an 8-cm segment of distal ileum was isolated 10 cm from the ileocecal valve. L-[4,5- ^3H]Leucine (1.5 mCi) (> 120 Ci/mmol, Amersham, Arlington Heights, IL) was instilled into each segment for 9 min (8, 9). The intestinal segments were then removed, flushed with 50 ml of iced PBS (50 mM phosphate, 100 mM NaCl, pH 7.4)–20 mM leucine, and the mucosa was scraped with a glass microscope slide. Scrapings were homogenized on ice in 1 ml of a solution containing PBS, 1% Triton X-100, 2 mM leucine, 1 mM PMSF, and 1 mM benzamidine, pH 7.4, with a Polytron (Brinkmann Instruments, Westburg, NY) tissue homogenizer for 1 min with the intensity setting at 60%. Aliquots of the homogenate

were taken for measurement of total protein concentration (29) 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 (see below).

The molecular forms of nascent hepatic apoB were investigated by incubating liver slices (taken from multiple sites) in 1 ml Krebs bicarbonate buffer containing 0.5 mCi L-[3,4- ^3H]leucine. Incubations were carried out in a shaking water bath at 37°C with constant gassing with 95% O₂, 5% CO₂. At timed intervals, liver slices were removed, washed in cold PBS containing 20 mM leucine, and homogenized. Cytosolic supernatants were prepared for immunoprecipitation as described above for intestinal mucosa samples.

Intestinal and hepatic cytosolic supernatant fractions were subjected to specific immunoprecipitation of apoB and apoA-I under conditions of antibody excess as previously described (8, 9). Aliquots of cytosolic supernatants were mixed with washed Pansorbin (Calbiochem, La Jolla, CA) and subsequently reacted with excess anti-apoB-100 or anti-apoA-I antiserum for 18 hr at 4°C. Polyclonal antisera were raised in New Zealand White Rabbits following inoculation with electrophoretically pure apoB-100 or apoA-I in Freund's complete adjuvant. Following a second addition of Pansorbin and extensive washing, the liberated immunocomplex was applied to 4% (apoB) or 5.6% (apoA-I) polyacrylamide tube gels. After electrophoresis gels were sliced into 1-mm slices and incubated in 3% Protosol/97% Econofluor at 37°C overnight prior to liquid scintillation counting. Apolipoprotein species were identified by comparison to stained co-electrophoresed apolipoproteins. Synthesis rates of apoB and apoA-I were expressed as the percentage of specific immunoprecipitated apoprotein counts as compared to total protein TCA-precipitable counts. All samples were subjected to re-immunoprecipitation to ensure the completeness of the first antigen-antibody reaction.

Electroimmunoassay quantitation of apoB and apoA-I

Electroimmunoassays for pig apoB and apoA-I were developed according to the technique of Laurell (30) using antibodies to pig plasma LDL apoB and HDL apoA-I. Standards consisted of pooled pig plasma HDL and LDL, and a standard curve was run with each assay.

Analysis of lipoprotein cholesteryl ester fatty acid composition by gas-liquid chromatography

LDL and HDL were isolated from plasma and lymph by sequential ultracentrifugation and extensively dialyzed. Samples were delipidated (31), and lipid classes were separated by thin-layer chromatography on silica gel G using petroleum ether–diethyl ether–acetic acid 80:20:1 (v/v/v). The cholesteryl ester band was identified, scraped off, and transmethylated directly with 14% BF₃-methanol (32), fol-

lowing addition of a C-17:0 internal standard. Samples were analyzed by gas-liquid chromatography (Perkin-Elmer Co., Norwalk, CT). Individual fatty acid peaks were identified by comparison to retention times of authentic fatty acid methyl esters (Nu-Chek Prep, Elysian, MN). Peaks were integrated automatically, and individual fatty acid composition was expressed as a percentage of total fatty acid mass.

Lipid and protein measurement

Triglycerides were determined by the method of Biggs, Erickson, and Moorehead (33). Cholesterol was determined by the method of Zlatkis (34). Phospholipids were determined by the method of Bartlett (35). Protein was measured by a modified Lowry procedure (29).

Statistical analysis

Student's paired and unpaired two-tailed *t* tests were used where appropriate. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Lymph lipoprotein composition

The mass distribution of triglyceride, cholesterol, phospholipid, and protein among fasting and fatty lymph lipoproteins was determined. In fasting lymph, $81.3 \pm 3.1\%$ (mean \pm SEM) of triglyceride was in VLDL, while $74.6 \pm 2.0\%$ of cholesterol and $80.2 \pm 1.6\%$ of phospholipid were in LDL and HDL. Fasting lymph HDL contained $53.6 \pm 3.2\%$ and fasting lymph LDL contained $30.0 \pm 1.6\%$ of lymph lipoprotein-associated protein. During lipid absorption, $99.3 \pm 2.3\%$ of lymph triglyceride, $72.9 \pm 3.5\%$ of the cholesterol, $71.4 \pm 2.1\%$ of phospholipid, and $80.1 \pm 2.9\%$ of lipoprotein-associated protein were found in CM and VLDL.

Fig. 1 shows a 3–20% gradient SDS-PAGE electrophoretogram of fatty lymph CM apolipoproteins. Identifiable protein bands include apoB-100, apoB-48, apoE, apoA-I, and the apoC peptides. A band with a molecular weight of approximately 42,000, which may be homologous to human and rat apoA-IV, is also present. The fatty lymph VLDL pattern (not shown) was similar to lymph CM and contained both apoB-100 and B-48. Fasting lymph VLDL (not shown) also contained both apoB

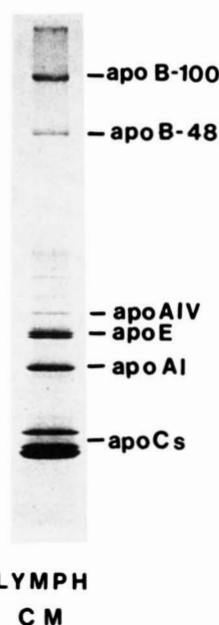


Fig. 1. SDS-PAGE electrophoretogram of piglet lymph CM apolipoproteins. Thirty micrograms of protein was loaded onto a 3–20% polyacrylamide gradient gel. Apolipoproteins were identified by molecular weights derived from comparison to co-electrophoresed standards. Approximate apolipoprotein molecular weights are: apoB-100, 330,000; apoB-48, 220,000; apoA-IV, 42,000; apoE, 33,000; apoA-I, 26,000; and apoCs $< 10,000$.

species, in addition to apoE and the apoC peptides. Results of densitometric scanning of SDS-PAGE gels of lymph CM and VLDL are shown in **Table 1**.

Immunoblotting (not shown) of fatty lymph CM and VLDL with anti-swine apoB antiserum confirmed the immunologic identity of both higher molecular weight proteins as apoB-100 and apoB-48. As will be shown, this apoB-100 does not appear to be synthesized by the intestine.

Lymph and plasma $d > 1.006$ g/ml lipoprotein apolipoproteins

Density gradient ultracentrifugation was performed on samples of plasma and fasting and fatty lymph after a preliminary centrifugation to remove $d < 1.006$ g/ml lymph lipoproteins. The distribution of fasting (**Fig. 2B**) and fatty lymph (**Fig. 2C**) LDL apoB-100 was almost identical to that of plasma (**Fig. 2A**) (fractions 5–14, $d 1.04$ – 1.07 g/ml).

TABLE 1. Apolipoprotein composition of chylomicrons (CM) and VLDL in fatty lymph

Fraction	ApoB-100	ApoB-48	ApoA-IV	ApoE	ApoA-I	ApoCs
CM	4.4 ± 0.8	4.0 ± 1.2	1.3 ± 0.2	8.3 ± 1.4	5.9 ± 1.4	76.1 ± 3.1
VLDL	8.9 ± 0.6	9.6 ± 0.6	1.7 ± 0.1	9.1 ± 0.5	18.4 ± 1.0	52.3 ± 1.6

Results were determined from densitometric scanning of 3–20% gradient SDS-PAGE gels with $30 \mu\text{g}$ protein per lane; mean \pm SEM of percent of total apolipoprotein absorbance; $n = 8$.

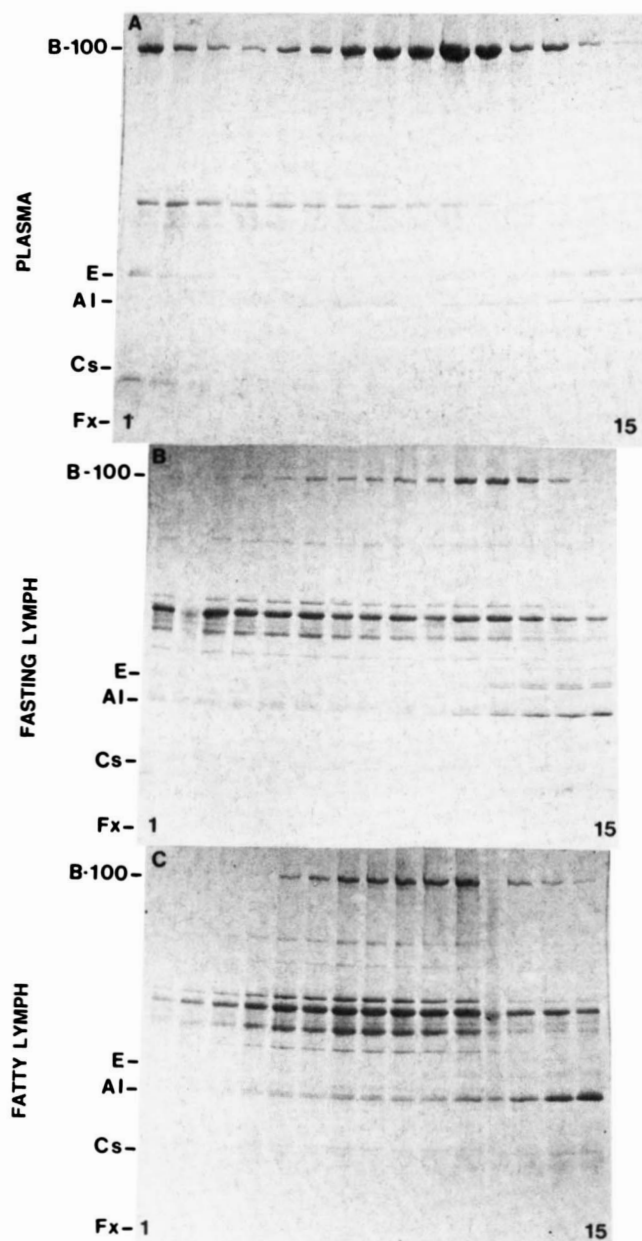


Fig. 2. SDS-PAGE electrophoretograms of ultracentrifugal density gradient fractions of plasma (top, A), fasting lymph (middle, B), and fatty lymph (bottom, C) LDL. The entire protein content of each 0.4-ml gradient fraction was loaded onto each lane of a 3-20% polyacrylamide gradient gel. Density gradient fraction number (Fx) is indicated at the bottom of each gel.

The corresponding electrophoretograms of HDL density gradient fractions 16-24 (d 1.09-1.20 g/ml) of plasma, fasting lymph, and fatty lymph are shown in Fig. 3. In plasma HDL (Fig. 3A), most of the apoA-I and apoC is in the more dense fractions as is a minor band which may be apoA-IV. ApoE is more prominent in the lighter fractions. The very faint apoB-100 band is equally distributed throughout the gradient. In fasting lymph (Fig. 3B), the

apolipoprotein distribution is very similar to that of plasma. However, fatty lymph (Fig. 3C) has a different pattern with most of the apoA-I in the lighter HDL fractions. These results suggest that a major portion of fasting lymph HDL and both fasting and fatty lymph LDL may be derived by filtration from plasma.

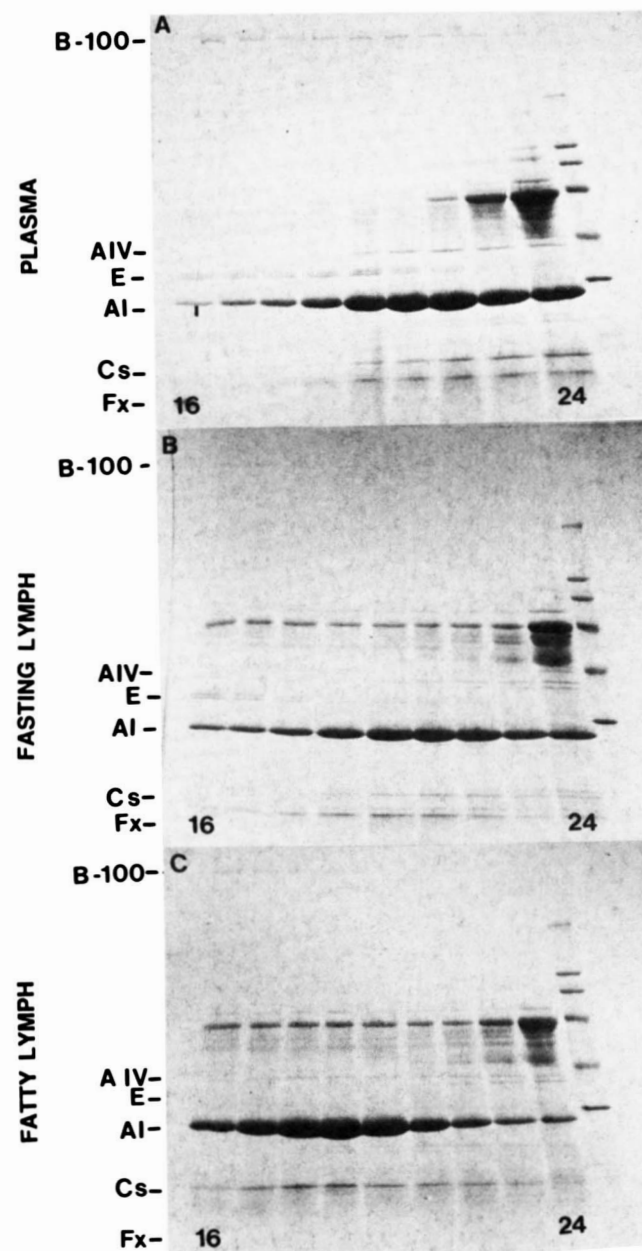


Fig. 3. SDS-PAGE electrophoretograms of ultracentrifugal density gradient fractions of plasma (top, A), fasting lymph (middle, B), and fatty lymph (bottom, C) HDL. The entire protein content of each 0.4-ml gradient fraction was loaded onto each lane of a 3-20% polyacrylamide gradient gel. Density gradient fraction number (Fx) is indicated at the bottom of each gel. The last lane of each gel contains molecular weight standards.

Plasma and lymph LDL and HDL cholesteryl ester fatty acid composition

To determine whether the neutral lipid core of lymph LDL and HDL has an intestinal source, the fatty acid composition of the cholesteryl esters from these lipoproteins was studied. Since the pig does not have plasma cholesteryl ester transfer activity (21), no exchanges of core constituents would be expected between particles, and the pattern of cholesteryl ester fatty acids would therefore be an important clue as to the origin of the lymph particles (i.e., intestinal synthesis vs. filtration from plasma) (36). The data (Table 2) show that the fatty acid composition of plasma and lymph LDL is indistinguishable with the exception of an increase in 14:0 and 14:1 fatty acids in fatty lymph LDL. Fasting lymph HDL cholesteryl ester was found to be enriched in 18:0 species but this difference was not apparent in fatty lymph HDL samples. Taken together, the results suggest that the major source of lymph LDL and HDL core lipid is filtration from plasma.

Differential double-radioisotope labeling of plasma and lymph apolipoproteins

To determine the relative contributions of small intestine and liver to plasma and lymph apolipoproteins, a lymph-fistulated pig was studied (6) during active lipid absorption. Plasma albumin was used as a reference protein made solely by the liver, and its $^3\text{H}/^{14}\text{C}$ dpm ratio (3.01) was divided into the ratios of all apolipoproteins studied, so that a ratio of 1.0 would indicate exclusive hepatic synthesis, and the higher the ratio (> 1.0), the greater the intestinal contribution (6). Table 3 contains the corrected

$^3\text{H}/^{14}\text{C}$ ratios for plasma and lymph lipoprotein apolipoproteins in the animal studied. In lymph CM and VLDL, apoB-48, apoA-IV, and apoA-I appear to have predominantly an intestinal source, whereas apoE and the apoC peptides have a primarily hepatic origin. Although lymph CM and VLDL contained apoB-100 on stained gels, there was no apparent incorporation of either ^3H or ^{14}C into this apolipoprotein as determined by 4% disc gel electrophoresis (Fig. 4). Both lymph and plasma LDL apoB-100 appeared to have a sole hepatic source, suggesting that both the surface and core components of lymph LDL are derived by filtration from plasma. Lymph HDL apoA-IV and apoA-I appeared to have a significant intestinal contribution. Plasma HDL contained apoE and apoA-I of mainly hepatic origin. Plasma VLDL incorporated enough radioisotope only into apoE for analysis, which was liver-derived. Overall, lymph diversion appeared to effectively prevent the entry of intestinally derived apolipoproteins into the plasma lipoprotein pool, since all apolipoproteins isolated from plasma lipoproteins had a $^3\text{H}/^{14}\text{C}$ ratio of near one.

ApoB synthesis by piglet intestinal mucosa and liver slices

In order to determine directly the molecular forms of apoB synthesized in the intestinal mucosa of the suckling pig, immunoprecipitates of enterocyte pulse-radiolabeled apoB from fasting animals were analyzed by SDS-PAGE. Only apoB-48 synthesis was noted in jejunal and ileal mucosa after either 9 (Fig. 5, top) or 30 min (not shown) pulse times. Furthermore, when a 10-fold larger superna-

TABLE 2. Cholesteryl ester fatty acid composition of piglet plasma and lymph LDL and HDL

Fraction	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:2	20:4	22:6
	<i>percent of total</i>											
Plasma LDL	2.26 ± 0.81	0.05 ± 0.03	15.4 ± 1.8	6.28 ± 1.26	6.06 ± 2.37	47.0 ± 3.2	17.0 ± 0.7	0.32 ± 0.23	0.26 ± 0.02	0.68 ± 0.25	4.64 ± 0.61	0.10 ± 0.09
Fasting lymph LDL	2.34 ± 0.52	0.08 ± 0.05	20.8 ± 2.3	6.89 ± 1.15	6.71 ± 1.38	42.2 ± 2.9	16.6 ± 2.5	1.07 ± 0.78	0.46 ± 0.32	1.08 ± 0.73	1.60 ± 1.59	0.10 ± 0.09
Fatty lymph LDL	5.27* ± 0.45	0.13* ± 0.02	20.9 ± 3.3	6.54 ± 1.83	7.91 ± 1.69	38.4 ± 1.9	13.2 ± 3.6	1.36 ± 0.70	0.39 ± 0.24	1.43 ± 1.00	3.53 ± 0.33	0.86 ± 0.72
Plasma HDL	2.60 ± 0.51	0.10 ± 0.03	19.0 ± 1.8	4.85 ± 1.07	3.28 ± 0.91	41.2 ± 4.8	22.4 ± 3.9	0.43 ± 0.23	0.29 ± 0.06	0.34 ± 0.29	5.41 ± 0.76	0.09 ± 0.09
Fasting lymph HDL	3.85 ± 2.26	0.12 ± 0.08	25.2 ± 1.7	1.83 ± 1.83	10.0** ± 2.0	31.7 ± 2.0	14.6 ± 2.3	3.04 ± 0.74	0.19 ± 0.03	2.78 ± 0.85	6.25 ± 0.50	0.43 ± 0.31
Fatty lymph HDL	3.25 ± 1.64	0.10 ± 0.06	15.1 ± 4.0	0.00	8.23 ± 4.18	40.0 ± 10.3	17.4 ± 2.0	3.96 ± 1.70	0.52 ± 0.29	3.63 ± 1.54	7.59 ± 1.41	0.23 ± 0.23

Values given as percent of total, mean ± SEM; n = 3. *Significantly different from the corresponding plasma lipoprotein at $P < 0.02$; **significantly different from the corresponding plasma lipoprotein at $P < 0.05$.

TABLE 3. $^3\text{H}/^{14}\text{C}$ ratios of dual labeled piglet plasma and lymph apolipoproteins

Fraction	B-100	B-48	A-IV	E	A-I	Cs
Lymph CM	--	6.10	21.5	1.13	5.8	1.06
Lymph VLDL	--	11.7	28.6	1.15	4.10	1.11
Lymph LDL	1.01	--	--	--	--	--
Lymph HDL	--	--	6.27	--	2.10	--
Plasma VLDL	--	--	--	1.00	--	--
Plasma LDL	1.00	--	--	--	--	--
Plasma HDL	--	--	--	1.18	1.10	--

Ratio of ^3H dpm to ^{14}C dpm after correction using the serum albumin ratio. An apolipoprotein with a ratio of 1.00 would be exclusively of hepatic origin. The higher the ratio, the greater the intestinal contribution. Dashed lines (--) indicate either complete absence of the apolipoprotein or inadequate isotope incorporation for calculation of an accurate ratio.

tant volume was analyzed, a stoichiometric increase in the apoB-48 peak was noted, but no apoB-100 peak was seen (not shown). Therefore, the apoB-100 found in lymph $d < 1.006$ g/ml lipoproteins does not appear to be synthesized by intestinal mucosa. By contrast, pulse-radiolabeling of liver slices from two fasting animals demonstrated only apoB-100 synthesis at 10, 20, 30, and 40 min incubation times. A representative SDS-PAGE gel profile after a 40-min incubation is illustrated in Fig. 5, bottom.

ApoA-I synthesis by piglet intestinal mucosa

To determine the apoA-I isoform patterns of postsecretory apoA-I in the piglet, whole fatty lymph, lymph HDL, and plasma HDL apoA-I isoforms were studied using two-dimensional SDS-PAGE. Fig. 6 demonstrates five major isoforms of apoA-I. Both plasma and lymph HDL contain predominantly isoform 3 (pI 5.33), presumably mature apoA-I. Whole lymph contains proportionately less isoform 3 as compared to lymph and plasma HDL. Pulse-radiolabeled apoA-I immunoprecipitates from piglet jejunal and ileal mucosa were subjected to isoelectric focusing and gel slicing. As shown in Fig. 7, isoform 1 (pI 5.58) accounted for approximately 90% of total apoA-I counts with isoform 3 containing all remaining counts. Therefore, isoform 1 appears to represent a nascent presecretory form of apoA-I, which is converted to the mature circulating plasma HDL isoform following secretion into lymph.

Mesenteric lymph lipid, apoA-I, and apoB secretion: effects of luminal triglyceride

Five animals were studied in both the fasting and fat-fed state. The fasting lymph flow rate averaged 22.7 ± 3.7 ml/hr (mean \pm SEM) and increased to a peak rate of 43.1 ± 5.3 ml/hr during lipid absorption ($P < 0.01$). Lymph TG output was 14.4 ± 3.1 mg/hr during fasting and increased to 417 ± 108 mg/hr by 6–8 hr of lipid infusion

($P < 0.04$). Cholesterol output was 6.9 ± 0.5 mg/hr during fasting, increasing to 39.9 ± 7.3 mg/hr with lipid infusion ($P < 0.02$). Lymph phospholipid output increased from 15.5 ± 5.8 mg/hr during fasting to 74.0 ± 18.4 mg/hr in fatty lymph ($P < 0.04$). Fasting lymph apoB mass output was 2.92 ± 0.95 mg/hr in fasting lymph and increased to 6.07 ± 0.90 mg/hr during lipid absorption ($P < 0.001$). ApoA-I mass output was 13.1 ± 0.8 mg/hr in fasting lymph and increased to a peak of 33.0 ± 4.5 mg/hr with lipid infusion ($P < 0.04$).

Intestinal apoA-I and apoB-48 synthesis rates: regulation by luminal lipid

Groups of animals were studied as detailed above (Methods) to determine the role of both acute dietary triglyceride and biliary lipid in regulating the in vivo synthesis rates of these two intestinal apolipoproteins. ApoB-48 was the sole apoB species synthesized in all samples analyzed. There was a jejunal to ileal gradient of apolipoprotein in synthesis for both proteins (Table 4). ApoA-I and apoB-48 synthesis rates in both jejunum and ileum were unchanged by the acute administration of triglyceride or complete removal of both dietary and biliary lipid (Table 4). The ileal apoA-I synthetic rate in the 8-hr fat-fed animals was actually significantly lower than in the 2-hr fat-fed group. Total protein synthesis was comparable in all the groups studied (Table 4). Thus, the increase in mesenteric lymph apolipoprotein secretion observed following triglyceride administration was not mediated by an increase in intestinal apolipoprotein synthesis rates, as compared to total cellular protein synthesis.

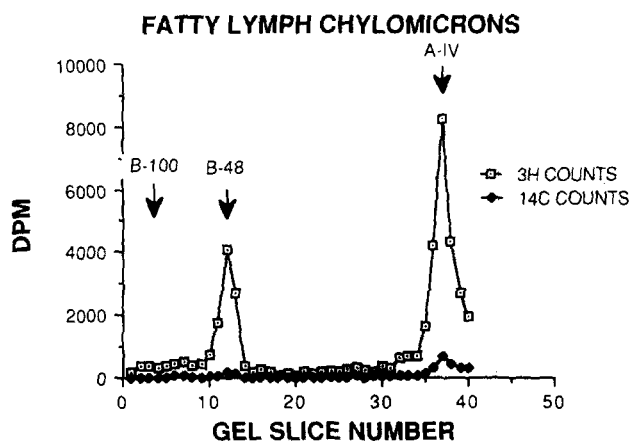


Fig. 4. Radioisotope profile of fatty lymph CM apolipoproteins separated on a 4% SDS-PAGE disc gel. This animal received [^3H]leucine intraduodenally and [^{14}C]leucine intraperitoneally. Even though an apoB-100 band appeared on a stained co-electrophoresed gel, neither isotope appeared in the apoB-100 band. This sample was electrophoresed for 1 hr after complete migration of the tracking dye off the gel to allow optimal resolution of apoB-100 and B-48. This accounts for the absence of detectable apoA-I, which had migrated off the gel.

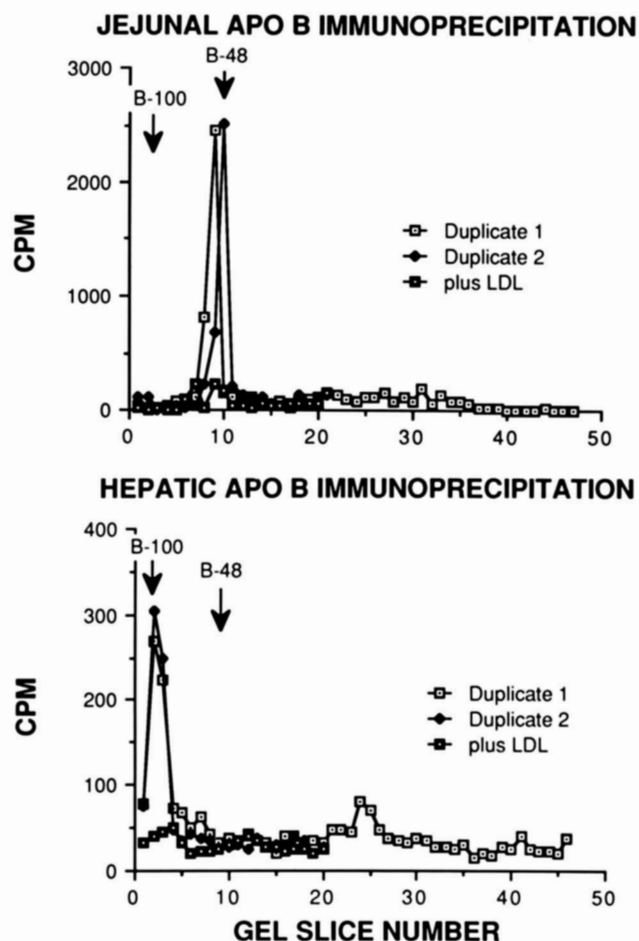


Fig. 5. SDS-PAGE electrophoretic profiles of apoB immunoprecipitates from 9-min *in vivo* pulse-radiolabeled piglet jejunal mucosa (top) and 40-min *in vitro* incubated liver slices (bottom). Duplicate samples of cytosolic supernatant were reacted with excess anti-apoB-100 antiserum, and the immune complex was subjected to SDS-PAGE on a 4% SDS-PAGE disc gel, followed by gel slicing. An aliquot was similarly incubated with 150 μ g of autologous plasma LDL to demonstrate specific complete blocking of the immunoprecipitation. Peaks were identified by comparison to co-electrophoresed standards.

DISCUSSION

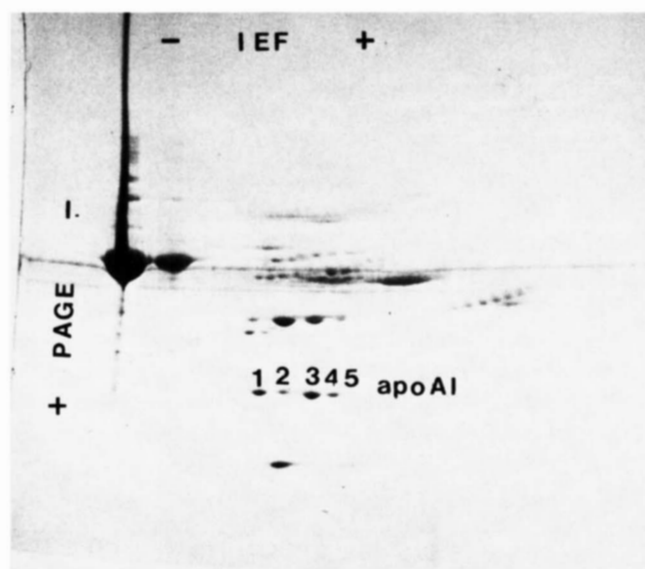
These studies were undertaken to characterize aspects of mesenteric lymph lipoprotein and apolipoprotein secretion in the suckling pig. In particular, the organ sites of origin (intestine and liver) and regulation by luminal lipid were investigated to establish an integrated picture of lipoprotein assembly and secretion in a developing mammal with extensive lipoprotein homology to humans.

Intestinal lipoprotein secretion: origin and composition of mesenteric lymph lipoproteins

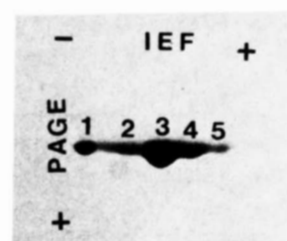
Piglet lymph triglyceride-rich lipoprotein apolipoprotein composition was similar to that of the adult rat and human (37-40). CM and VLDL from fatty lymph contained

the apoC peptides as the major apolipoprotein species. Other apolipoproteins included apoB (see below), apoE, apoA-I, and apoA-IV. Evidence from dual radioisotopic labeling and, in the case of apoA-I and apoB-48, direct immunoprecipitation from intestinal mucosa, suggests that apoA-I, apoA-IV, and apoB-48 are synthesized by the piglet small intestine as the principal apolipoproteins of the triglyceride-rich lipoprotein fraction.

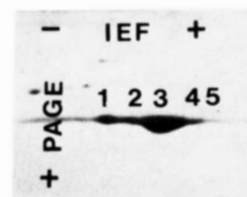
An unexpected observation in the present study was the presence of apoB-100 in mesenteric lymph CM and VLDL.



WHOLE LYMPH



PLASMA HDL APO A I



LYMPH HDL APO A I

Fig. 6. Two-dimensional electrophoretograms of piglet whole fatty lymph (top), plasma HDL (middle), and lymph HDL (bottom) showing apoA-I isoforms. Isoelectric focusing from pH 4.0-6.0 was performed in the first dimension, and SDS-PAGE was performed in the second dimension.

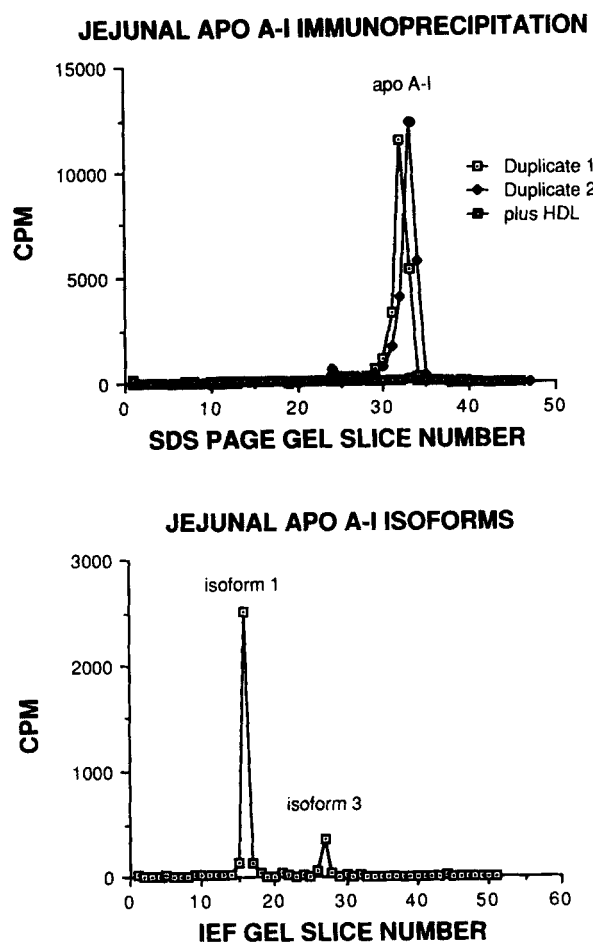


Fig. 7. Electrophoretic profiles of apoA-I immunoprecipitates from pulse-radiolabeled piglet jejunal mucosa separated by SDS-PAGE (top) and isoelectric focusing (bottom). For SDS-PAGE, duplicate samples of cytosolic supernatant were reacted with excess anti-apoA-I antiserum, and the immune complex was subjected to SDS-PAGE on a 5.6% disc gel. An aliquot was similarly incubated with 150 μ g of autologous plasma HDL to demonstrate specific complete blocking of the immunoprecipitation. For isoelectric focusing, the immune complex was applied to a pH 4.0–6.0 gel and peaks were identified by comparison to a co-electrophoresed stained gel of plasma HDL apoA-I isoforms.

Two major lines of evidence suggest that this form of apoB is not synthesized by the small intestine. First, direct immunoprecipitation from small intestinal mucosa pulse-radiolabeled for either 9 or 30 min *in vivo* revealed no evidence of apoB-100 synthesis. By contrast, liver slices incubated *in vitro* with [3 H]leucine and immunoprecipitated in parallel to intestinal samples revealed exclusive synthesis of apoB-100. Secondly, dual radioisotope labeling revealed no incorporation of lumenally administered [3 H]leucine into mesenteric lymph CM or VLDL apoB-100, suggesting that its synthesis may take place in an extraintestinal site. Previous studies of human thoracic duct lymph (41) and chyluric lipoproteins (39) have demonstrated the presence of an apoB-100 band on SDS-PAGE gels, although the source of this protein was not specifically addressed.

Glickman, Rogers, and Glickman (15) have demonstrated the intestinal synthesis of apoB-100 in the human fetus with a gradual switch to apoB-48 synthesis during gestation. The present study demonstrates the synthesis of only apoB-48 in neonatal pig intestine during the second week of life. Recent studies in the rabbit and human have demonstrated that apoB-100 and apoB-48 are translated from the same mRNA sequence with the tissue-specific insertion of a stop codon limiting translation to apoB-48 synthesis in the intestine (42).

Although the source of apoB-100 in piglet lymph CM and VLDL is probably the liver, the mechanism of its entry into lymph triglyceride-rich lipoproteins is unknown at present. Rat hepatic lymph has been shown to contain < 1.006 g/ml lipoproteins containing apoB (43). Therefore, if swine hepatic lymph drains into the cisterna chyli, as in other species, hepatic lymph lipoproteins containing apoB-100 may contaminate lymph CM and VLDL fractions. The lymphatic system of the piglet may be permeable to plasma VLDL, and filtration of this apoB-100-containing particle into lymph and co-isolation with lymph VLDL and CM are possible, especially if permeability increases with lipid absorption. An alternative explanation may be that apoB-100, taken up by enterocytes via plasma LDL, may then be packaged with newly synthesized apoB-48 into CM and VLDL for secretion into lymph. These possibilities and their relationship to the molecular mechanisms for organ-specific apoB production are currently under investigation.

Analysis of piglet apoA-I isoform patterns demonstrated a pattern similar to that of adult humans (44). The predominant isoform in piglet plasma and lymph HDL was isoform 3 (pI 5.33), presumably, mature apoA-I. In pulse-radiolabeled piglet intestinal mucosa, the more basic isoform 1 (pI 5.58), presumably proapoA-I, predominated. Since the lymph and plasma piglet HDL isoform patterns consist of predominantly isoform 3, it appears that the neonatal pig has an active proapoA-I converting enzyme activity, the significance of which is unknown at present (44).

Intestinal apoA-I and apoB-48 synthesis: regulation by luminal lipid

The present studies demonstrate that lymph apoB mass output increases during active lipid absorption in the suckling pig. This is consistent with the observation that apoB output in mesenteric lymph appears to be correlated with output of triglyceride-rich lipoproteins in the adult rat (5). Increased secretion of apoB-48 with lipid absorption must be reconciled with the observation that intestinal synthesis does not concomitantly increase, findings analogous to those in the adult rat (9). Depletion of a preformed intracellular pool of apoB may account for these observations, although this issue was not specifically addressed in the present study. It is also possible that dur-

TABLE 4. Piglet intestinal mucosal total protein specific activities and apoA-I and apoB-48 synthesis rates

Measure	Fasting (n = 8)	Fat-Fed (2 hr) (n = 9)	Fat-Fed (8 hr) (n = 4)	Bile-Diverted (n = 4)	Sham-Operated (n = 4)
Piglet weight (kg)	3.92 ± 0.20	3.93 ± 0.21	4.18 ± 0.23	4.05 ± 0.27	3.65 ± 0.30
Jejunal mucosa					
Sp act (cpm/μg) ^a	981 ± 137	832 ± 89	770 ± 52	1654 ± 241	1299 ± 262
ApoA-I (%) ^b	4.21 ± 0.86	4.63 ± 0.71	3.76 ± 0.91	3.83 ± 0.08	5.11 ± 0.85
ApoB (%)	1.26 ± 0.26	1.11 ± 0.14	0.67 ± 0.15	1.13 ± 0.30	1.16 ± 0.09
Ileal mucosa					
Sp act (cpm/μg)	327 ± 55 ^c	512 ± 74 ^d	310 ± 64 ^c	2033 ± 939	989 ± 408
ApoA-I (%)	1.08 ± 0.20 ^c	2.29 ± 0.48 ^c	0.59 ± 0.15 ^{c,g}	1.18 ± 0.38 ^f	2.38 ± 0.80
ApoB (%)	0.36 ± 0.03 ^c	0.57 ± 0.14 ^c	0.25 ± 0.10 ^c	0.35 ± 0.13	0.54 ± 0.14 ^c

Values given as mean ± SEM.

^aMucosal cytosolic supernatant total protein specific activities (sp act) expressed as cpm/μg protein.

^bSynthesis rates expressed as specific apolipoprotein-immunoprecipitable counts as a percentage of total trichloroacetic acid-precipitable counts from pulse-labeled mucosal supernatants.

^{c,d,e,f}Significantly different from the corresponding jejunal value at ^c*P* < 0.01; ^d*P* < 0.04; ^e*P* < 0.05; ^f*P* < 0.03.

^gSignificantly different from the corresponding 2-hr fat-fed ileal value at *P* < 0.02.

ing fasting newly synthesized intestinal apoB is rapidly degraded intracellularly until it is needed for lipoprotein packaging and secretion. Studies in cultured rat hepatocytes suggest that significant amounts of newly synthesized apoB-48 and apoB-100 are degraded intracellularly (45). A further possibility is that intestinal apoB is shunted into the portal circulation until needed for lipid absorption, but this remains to be proven.

A surprising finding in the piglet was the lack of a significant effect on apoB synthesis by removal of biliary lipid. Previous studies in the adult rat have suggested a major contribution of biliary lipid to the basal expression of intestinal apoB synthesis (9). At this developmental stage in the piglet, apoB synthesis appears to be fixed at a predestined rate and is not acutely regulated by either dietary or biliary lipid absorption.

As noted with apoB, intestinal lymph apoA-I mass output increased with lipid absorption in the piglet with no evidence of increased mucosal synthesis. ApoA-I secretion into intestinal lymph in the adult rat has been shown to increase with triglyceride absorption (46), even though intestinal synthesis rates do not change during acute lipid absorption (8). As in the case of apoB noted above, removal of biliary lipid had no effect on apoA-I synthesis rates in either jejunum or ileum in the piglet. This is in contrast to findings in the adult rat where bile diversion decreases ileal apoA-I synthesis (8). In the suckling pig neither dietary nor biliary lipid appeared to influence apoA-I synthesis in either proximal or distal small intestine, except for a significant decrease in ileal synthesis after 8 hr of lipid absorption. The significance of this decrease is not known at present. As noted for apoB, a proximal to distal gradient for piglet apoA-I synthesis was

noted with jejunal rates higher than ileal rates in all groups except the sham-operated animals.

Although luminal lipid absorption does not acutely regulate intestinal apolipoprotein synthesis in the suckling pig, developmental regulation may be linked to changes in the hormonal milieu as has been noted for other intestinal functions, such as disaccharidase activity (47). Studies in the rat have suggested that developmental regulation of intestinal and hepatic mRNA abundance occurs for apoB, A-I, E, and A-IV (14, 48). The present studies suggest that the suckling pig is a suitable model for the study of developmental aspects of mammalian intestinal apolipoprotein metabolism, sharing many similarities with the human, especially with regard to apoB, apoA-I, and apoA-IV synthesis and secretion. Factors that developmentally regulate intestinal apolipoprotein synthesis and metabolism in this model will be the focus of future studies. ■

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