Transport of lipid and apolipoproteins A-I and A-IV in intestinal lymph of the rat

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Abstract Intestinal lipid absorption is associated with marked increases in the synthesis and secretion of apolipoprotein A-IV (apoA-IV) by the small intestine. Whether the increased intestinal apoA-IV synthesis and secretion results from increased fat uptake, increased cellular triglyceride (TG) content, or increased secretion of TG-rich lipoproteins by the enterocytes is unknown. Previous work from this laboratory has shown that a hydrophobic surfactant, Pluronic L-81 (L-81), is a potent inhibitor of intestinal formation of chylomicrons (CM), without reducing fat uptake or re-synthesis to TG. Furthermore, this inhibition can be reversed quickly by the cessation of L-81 infusion. Thus L-81 offers a unique opportunity to study the relationship between lymphatic TG, apoA-I and A-IV secretion. In this study, we studied the lymphatic transport of TG, apoA-I, and apoA-IV during both the inhibitory phase (L-81 infused together with lipid) and the subsequent unblocking phase (saline infusion). Two groups of lymph fistula rats were used, the control and the experimental rats. In the experimental rats, a phosphate-buffered taurocholate-stabilized emulsion containing 40 μmol [^{3}H]triolein, 7.8 μmol of phosphatidylcholine, and 1 mg L-81 per 3 ml was infused at 3 ml/h for 8 h. This was then replaced by glucose-saline infusion for an additional 12 h. The control rats received the same lipid emulsion as the experimental rats, but without L-81 added, for 8 h. Lymph lipid was determined both by radioactivity and by glyceride-glycerol determination, and the apoA-I and apoA-IV concentrations were determined by rocket electroimmunophoresis assay. L-81 inhibited the rise in lymphatic lipid and apoA-IV output in the experimental rats after the beginning of lipid + L-81 infusion. Upon cessation of L-81 infusion, the mucosal lipid accumulated as a result of L-81 treatment was rapidly cleared into lymph as CM. This was associated with a marked increase in apoA-IV output; the maximal output was about 3 times that of the fasting level. There was a time lag of 4–5 h between the peak lymph lipid output and the peak lymph apoA-IV output during the unblocking phase in the experimental rats. There was also a comparable time lag between the maximal lipid and apoA-IV outputs in the control animals. Incorporation studies using [^{3}H]leucine showed that apoA-IV synthesis was not stimulated during lipid + L-81 infusion, perhaps explaining the lack of increase in lymphatic A-IV secretion. Upon relief from L-81 inhibition (glucose-saline infusion), apoA-IV synthesis was markedly stimulated, which may account for the marked increase in lymph apoA-IV secretion. In both the experimental and the control rats, there was a small, but not significant, rise in the lymph apoA-I output 5–8 h after the beginning of lipid infusion. We conclude from this study that the stimulation of apoA-IV synthesis and secretion by lipid infusion is not mediated by lipid uptake into the enterocytes or cellular TG content. Rather, it is probably the events involved in the packaging and secretion of CM that are responsible for increasing apoA-IV synthesis and secretion. This study also supports findings by other investigators that acute lipid feeding has relatively little effect on lymph apoA-I synthesis and secretion.—**Hayashi, H., D. F. Nutting, K. Fujimoto, J. A. Cardelli, D. Black, and P. Tso.** Transport of lipid and apolipoproteins A-I and A-IV in intestinal lymph of the rat. *J. Lipid Res.* 1990. **31:** 1613–1625.

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Numerous studies have been published about apolipoprotein A-IV (apoA-IV) since it was first identified in rat plasma in 1977 (1). Human apoA-IV is a glycoprotein produced by the small intestine and has a molecular weight of 46,000 (2–4). ApoA-IV has a high percentage of α-helical structures (5), which result from the presence of multiple amino acid repeating sequences (6). These amino acid repeats are amphipathic in nature, and the lipid binding capacity of apoA-IV is dependent on the integrity of the secondary structure of apoA-IV (7). Unlike apolipoprotein B, apoA-IV can readily be displaced from lipoproteins by other apolipoproteins (8, 9). ApoA-IV binds to lipoproteins weakly, and as much as 80% of human apoA-IV circulates as a free protein (10). Early work revealed that rat apoA-IV is major component of high density lipoprotein (HDL) (2, 3, 11). In intestinal lymph,

Abbreviations: apoA-I, apolipoprotein A-I; apoA-IV, apolipoprotein A-IV; CM, chylomicron; EIA, electroimmunoassay; FA, fatty acid; HDL, high density lipoprotein; L-81, Pluronic L-81; MG, monoglyceride; PAGE, polyacrylamide gel electrophoresis; PC, phosphatidylcholine; PL, phospholipid; SDS, sodium dodecyl sulfate; TG, triglyceride; VLDL, very low density lipoprotein.

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triglyceride (TG)-rich lipoproteins such as chylomicrons (CM) and very low density lipoproteins (VLDL) contain one half of the total amount of apoA-IV (4). When the TG-rich lipoproteins enter the circulation, apoA-IV is displaced from CM to HDL and the lipoprotein-free fraction in both rats (12) and humans (13). Although the amino acid sequence and the gene loci of apoA-IV have already been determined (6, 14, 15), the physiological role of apoA-IV is unclear. The redistribution of human apoA-IV from lipoprotein-free fraction to HDL suggests a possible role for apoA-IV in the action of lecithin:cholesterol acyltransferase (16, 17). On the other hand, the fact that apoA-IV is a major component of rat HDL led investigators to propose that apoA-IV is a ligand for the binding of rat HDL to liver (10, 18) or aortic endothelial cells (19).

Apolipoprotein A-I (apoA-I) is a major component of the proteins associated with HDL (20). As revealed by circular dichroism, apoA-I has considerable α-helix present in its structure (21). Both the intestine and the liver are capable of making apoA-I (22–24), but only the intestine can make apoA-IV (25). The data of Wu and Windmueller (24) from the rat seem to indicate that the gut and liver contribute equally to the circulating apoA-I. ApoA-I is an activator of lecithin:cholesterol acyltransferase (26). Numerous reports have indicated that intestinal apoA-I secretion into lymph is increased after fat feeding (11, 23). However, whether the mucosal synthesis of apoA-I is increased after a fat feeding is less certain (24, 27, 28). Gordon et al. (29) have studied the effect of acute fat feeding on the translatable mRNA levels for apoA-I in the rat small intestine and demonstrated no change in the mRNA level up to 8 h after the intragastric feeding of corn oil. Studies by Davidson and Glickman (30) have clearly demonstrated a regional difference in the regulation of apoA-I synthesis in the intestine. In the jejunum, apoA-I synthesis is not acutely regulated by the flux of TG from lumen to lymph. However, after prolonged dietary TG withdrawal, introduction of fat induces a small, but significant increase in jejunal apoA-I synthesis.

ApoA-IV synthesis in intestinal cells and secretion to mesenteric lymph are known to be enhanced by lipid feeding (4, 31). However, whether the increased intestinal apoA-IV synthesis and secretion results from either increased fat uptake or increased secretion of TG-rich lipoproteins by the enterocytes is unknown. Work from this laboratory has clearly demonstrated that Pluronic L-81 (L-81), a non-ionic hydrophobic detergent, is a potent inhibitor of formation of CM (32, 33). A small dose of L-81 (0.5 mg/h) infused together with the lipid test meal is sufficient to exert this inhibitory effect. L-81 did not inhibit the uptake of lipolytic products or the re-esterification of monoglyceride (MG) and fatty acid (FA) to form TG. The inhibitory action of L-81 is rapid ($T_{1/2}$ of 69 min for 0.5 mg/h dose and 35 min for 1 mg/h), and the inhibition of CM secretion can be reversed quickly by stopping L-81 infusion. During the unblocking period, CM are rapidly formed from the accumulated mucosal lipid and then secreted into the lymph (34). Thus L-81 offers a unique opportunity to study the relationship between lymphatic TG secretion and the secretion of apoA-I and apoA-IV. In this study, we investigated the lymphatic transport of TG, apoA-I, and apoA-IV during both the inhibitory phase (L-81 infusion) and the unblocking phase (after termination of L-81 infusion). These outputs of lipid and apolipoproteins were compared with the lymphatic outputs of these components in control animals during the absorption of a comparable lipid test meal but without the presence of L-81.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 350–400 g, were fasted overnight, and the main mesenteric lymph duct was cannulated with a clear vinyl tubing (OD 0.8 mm) according to the method of Bollman, Cain, and Grindlay (35) as described previously (33). Silicone tubing (OD 2.2 mm) was introduced about 2 cm down the duodenum through the fundus of the stomach. The fundal incision was closed by a purse-string suture and sealed with a small drop of cyanoacrylate glue. Postoperatively, the animals were infused via the intraduodenal tube at a rate of 3 ml/h with a glucose–saline solution (145 mM NaCl, 4 mM KCl, 0.28 M glucose) and allowed to recover in restraint cages maintained at 30°C for at least 24 h before the lipid infusion.

Experimental protocol

Two groups of intestinal lymph fistula rats were used in the study. On the day of the experiment, a lipid emulsion containing 40 μmol triolein (labeled with tri[9, 10 (n)-^{3}H]oleoyl glycerol, 2.5 nCi/μmol), 7.8 μmol egg phosphatidylcholine (PC), 57 μmol sodium taurocholate, and 1.0 mg L-81 in 3 ml of phosphate-buffered saline (pH 6.4) was infused intraduodenally at a rate of 3 ml per h for 8 h in the experimental rats. The preparation of the infusate has been described in detail previously (33). The lipid plus L-81 infusion was followed by the infusion of glucose–saline for an additional 12 h. Since L-81 inhibits intestinal lipid transport, the first 8 h period with L-81 infusion was designated as the blocking phase and the period with glucose–saline infusion was designated as the unblocking phase. Lymph samples were collected before the lipid infusion (fasting lymph), 0–2 h, 2–4 h, 4–6 h, 6–7 h, and 7–8 h in the blocking phase and hourly in the unblocking phase. The control animals were given the same lipid infusate for 8 h, but without L-81 added, and the experiment was terminated at the end of the lipid infusion.

Determination of lipid content

An aliquot of lymph was taken for measurement of radioactivity. Another aliquot was extracted by the method of Folch, Lees, and Sloane Stanley (36), and the TG (37) and phospholipid (PL) (38) contents were determined.

Development of apoA-I and A-IV electroimmunoassays

Isolation of apoA-I and A-IV. Rat plasma was obtained from the abdominal aorta blood of 40 fed rats. Potassium bromide was added to adjust the density to 1.21 g/ml. Lipoproteins of $d < 1.21$ g/ml was isolated by flotation ultracentrifugation using a Beckman SW 41 rotor and centrifuging at 40,000 rpm for 48 h. The harvested lipoproteins were washed once by ultracentrifugation. The sample was then lyophilized and delipidated with ethanol–ether 3:1 (v/v) at 4°C for 24 h. The protein pellet was precipitated by centrifugation and the ethanol–ether was aspirated. The protein precipitate was delipidated with ethanol–ether 3:1 (v/v) twice more, then with ethanol–ether 1:1 (v/v) once, and finally with ether alone. Residual ether was removed under nitrogen and the protein pellet was dissolved in 0.1 M Tris, pH 8.2, containing 1% sodium decyl sulfate. The protein concentration was determined by the modified Lowry procedure (39).

The rat apolipoproteins were isolated by preparative 8% polyacrylamide gel electrophoresis (PAGE) containing 0.2% sodium dodecyl sulfate (SDS). Twelve disc gels (1.0 × 10 cm) were loaded with ~800 μg of apoproteins per tube. After electrophoresis for 6 h at 10 mA per tube, the individual apolipoproteins were located by staining half of one gel. The areas of gels corresponding to apoA-I and apoA-IV were removed into separate Erlenmeyer flasks, and 150 ml of 0.1% SDS in 0.05 M NH_4HCO_3, 0.1 M sodium azide, 0.1 mM phenylmethylsulfonyl fluoride (PMSF, protease inhibitor), pH 8.0 was added. The gel was macerated with a Polytron tissue homogenizer (Brinkmann Instruments). After an overnight incubation at 37°C in a shaking water bath, the gel fragments were centrifuged and eluted again with 100 ml of the NH_4HCO_3 buffer. The combined eluates were lyophilized and redissolved in 10 ml of distilled water. The apoA-I and apoA-IV solutions were then dialyzed against two consecutive changes of 4 l each of a buffer containing 10 mM Tris-HCl, 2 mM sodium decyl sulfate, 1 mM EDTA, and 0.01% sodium azide, pH 8.5, at 4°C. We performed SDS-PAGE on the isolated apoA-I and apoA-IV and found them to be homogenous (one band). The migration patterns of the apoA-I and apoA-IV agreed with the reported molecular weights of these two proteins identified on the basis of molecular weights relative to known protein standards. Furthermore, the migration patterns also agreed with published SDS gel electrophoretograms of rat apolipoproteins (31, 40–42).

Antisera to apoA-I and A-IV. Antiserum was prepared in rabbits (New Zealand White). Solutions of apoA-I or apoA-IV (~250 μg) were emulsified with Freund's complete adjuvant and injected at multiple sites subcutaneously. This was then followed by additional two or three injections of the apoprotein plus incomplete Freund's adjuvant in the 6–10 weeks after the primary injection. Antibody titer was measured by the conventional Ouchterlony technique.

Specificity of the antiserum as determined by Western blot analysis. Apolipoproteins were first separated using discontinuous SDS polyacrylamide slab gel electrophoresis according to the method of Laemmli (43). The stacking gel was 3% polyacrylamide, and the running gel was a linear gradient from 3.5 to 20% polyacrylamide. Electrophoresis was performed immediately after formation of the stacking gel in a Hoeffer vertical slab gel apparatus using a constant current of 25 mA per slab. Immediately after gel electrophoresis, the apolipoproteins were electrophoretically transferred to nitrocellulose paper in an LKB electroblotting apparatus. The blotting buffer contained 25 mM Tris, 192 mM glycine in 20% (v/v) methanol, pH 8.3. The electroblotting was performed at constant voltage of 100 v for 4 h at 4°C, and the transfer was found to be complete by staining the gel with Coomassie blue after the transfer. The nitrocellulose membrane was then incubated in a blocking solution of 0.05% Tween 20 in saline (0.9% NaCl, 10 mM Tris-HCl, pH 7.4). To test the specificity of antiserum to apoA-I, the membrane was incubated for 10 min with a 1:3000 dilution of the rabbit anti-rat apoA-I serum in a solution of 50 mM NaCl, 10 mM Na_2HPO_4, 10 mM dithiothreitol, 0.5% Zwittergent detergent, 0.15% SDS, 0.5% bovine serum albumin, and 20 μM leupeptin (pH 7.4). Afterwards, the membrane was washed four times by incubation in saline containing 0.05% Tween 20. Next, the membrane was incubated for 5 min with a 1:2000 dilution of a second antibody of goat anti-rabbit IgG conjugated with horseradish peroxidase in the same solution used in the incubation with the first antibody. The antigen-antibody complex was then identified by the color development after incubating the membrane with a solution containing 1 mg/ml 3'3'-diaminobenzidine, 1 mg/ml imidazole, and 1 μl/ml 30% H_2O_2. The specificity of antiserum to apoA-IV was confirmed by the same method as apoA-I. The antibodies for both apoA-I and apoA-IV were monospecific as shown in **Fig. 1**.

Electroimmunoassay (EIA) procedure

Electroimmunoassay was performed according to the procedure described by Laurell (44). Gels for EIA were made of 1.6% Induboise agarose and 1% dextran in Tris-Veronal buffer containing 1% rabbit antisera for apoA-I or 1.2% Induboise agarose and 2% dextran containing

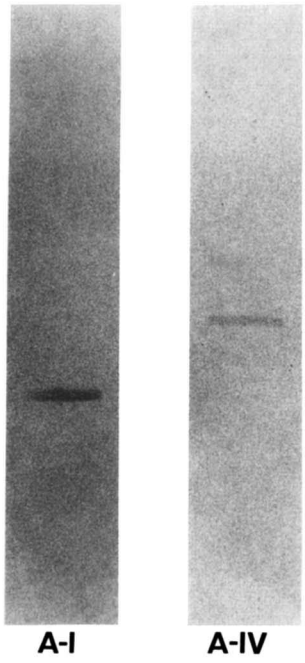

Fig. 1. Specificity of anti-apoA-I and apoA-IV serum. The anti-rat apoA-I and A-IV serum were raised in the rabbit. To test specificity of the serum, a lipoprotein fraction of density <1.21 g/ml was electrophoresed on 3.5–20% gradient SDS polyacrylamide gel under reducing conditions. Proteins were electrophoretically transferred to nitrocellulose membrane as described in Materials and Methods. The nitrocellulose membrane was incubated with either apoA-I or A-IV antiserum. After washing, the membrane was then incubated with a second antibody (against rabbit IgG) conjugated with horseradish peroxidase. Positive antigen–antibody complex was visualized by color development after introduction of substrate.

1% rabbit antisera for apoA-IV. A series of standards diluted with Tris-Veronal buffer was applied to each gel. All aliquots were applied to the gels in duplicates. Electrophoresis was complete in 6 h at 20°C with 3.5 V/cm. Immunoprecipitates were stained and each area was calculated by the height and width. Purified rat apoA-I was used as the primary standard for EIA of apoA-I. When fitted with a linear regression, it yielded a correlation coefficient of 0.995 and an equation of a = 3.32 + 33.31 b (a = area in mm^2; b = conc. of apoA-I in mg/dl). A similar curve was established for apoA-IV and a correlation coefficient of 0.998 was obtained, with an equation of a = 1.70 + 29.97 b. To conserve the supply of apolipoprotein standards, a pool of rat serum was subsequently used as the secondary standard. To further validate our assay, we sent our rat serum to Dr. Paul Roheim's laboratory at the Department of Physiology, Louisiana State University Medical School at New Orleans. The values we obtained from our laboratory agreed within ± 2% for both apoA-I and apoA-IV with those obtained in Dr. Roheim's laboratory. The amount of apolipoprotein in the sample was calculated from the standard curve generated by the serial dilutions of the pooled serum. In the apoA-I assay, the inter-assay variation was 8.6 ± 0.5% (mean ± SE) and intra-assay variation was 5.9 ± 1.0%; in the apoA-IV assay, these were 7.6 ± 0.6% and 5.2 ± 0.8%, respectively.

Hourly apolipoprotein output in lymph was calculated by multiplying the lymphatic apolipoprotein by the lymph flow.

Determination of apoA-I and apoA-IV biosynthesis

The methods used in the determination of apoA-I and apoA-IV synthesis were similar to those described by Black and Davidson (45) with some modifications. Four groups of lymph fistula rats were prepared as described above. These rats were then fed glucose–saline (fasting), triolein emulsion for 8 h (normal lipid transport), triolein + L-81 for 8 h (CM transport blocked), or triolein + L-81 for 8 h followed by 3.5 h of glucose–saline (unblocking). After the experiment, the animal was anesthetized. An 8-cm segment of proximal jejunum was isolated by ligatures starting 10 cm distal to the ligament of Treitz. Approximately 0.3 mCi of L-[3,4,5-^{3}H(N)]leucine (150.0 Ci/mmol, NEN Products, Boston) in 1 ml physiological saline was instilled in the loop for 10 min. The intestinal loop was then removed and the contents were flushed with 60 ml of cold PBS (50 mM sodium phosphate, 100 mM NaCl, pH 7.4) containing 20 mM leucine. The mucosa was scraped with a glass microscope slide, and scrapings were placed in a centrifuge tube with 1.5 ml homogenization buffer containing PBS, 1% Triton X-100, 2 mM leucine, 1 mM PMSF, 40 μg chymostatin, 160 μg leupeptin, and 6 μg pepstatin. Scrapings were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westburg, NY) for 1 min with the intensity setting at 60%. Aliquots of the homogenate were pelleted at 105,000 *g* for 60 min in a 50.3 Ti rotor (Beckmann Instruments, Palo Alto), and the cytosolic supernatant was collected. All procedures were performed at 4°C, and the mucosal supernatant samples were stored at −70°C until immuno- and trichloroacetic acid (TCA) precipitation.

Cytosolic supernatant samples were subjected to specific immunoprecipitation of apoA-I and A-IV. Aliquots of cytosolic supernatants were mixed with washed IgG sorb (IGSL 100, The Enzyme Center) at 4°C and centrifuged at 10,000 *g* for 2 min. The supernatant was mixed with an excess of apoA-I or apoA-IV antiserum and incubated at 4°C for 24 h. Washed IgG sorb (containing protease inhibitors) was added to the sample followed by centrifugation at 10,000 *g* for 2 min. The pellet was washed 3 times, resuspended in reducing buffer, and applied to a gradient 3.5–20% polyacrylamide slab gel. After electrophoresis, the gel was sliced into 1-mm slices and dissolved in NCS™ (Amersham, Canada) solubilizer for liquid scintillation counting. **Fig. 2** shows the SDS-PAGE electrophoretic profiles of apoA-IV immunoprecipitates from a 10 min in vivo [^{3}H]leucine pulse-labeled jejunal mucosa. Only one major labeled protein was immunoprecipitated from the rat jejunal mucosa. Addition of purified

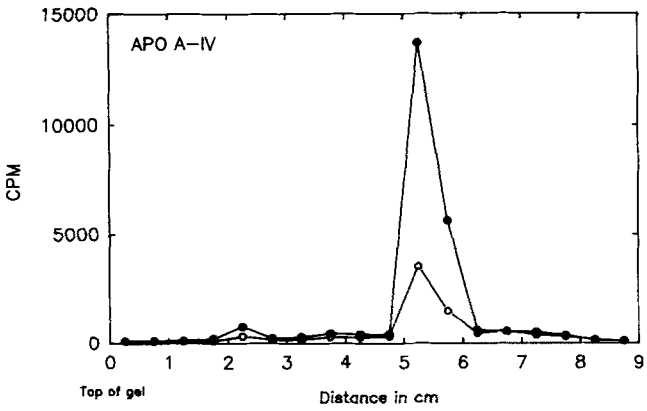

Fig. 2. Electrophoretic profile of the apoA-IV immunoprecipitates from the pulse-radiolabeled jejunal mucosa separated by SDS-PAGE. A sample of the cytosolic mucosal sample was incubated in the presence of excess apoA-IV antiserum and the immuno-complex formed was separated by gradient gel SDS-PAGE (●-●). To test for specificity of the immunoprecipitation for apoA-IV, another sample was processed in similar manner but with purified apoA-IV added during the immunoprecipitation (O-O). As seen, the addition of purified apoA-IV markedly reduced the amount of radiolabeled apoA-IV precipitated.

apoA-IV markedly reduced the amount of radioactive A-IV precipitated from the jejunal mucosa (Fig. 2).

ApoA-I and A-IV synthesis is expressed as the amount of immunoprecipitable radioactivity as a percentage of the TCA precipitable radioactivity.

Statistical analysis

Differences within groups were compared by analysis of variance using repeated measures for time-dependent comparisons. Group differences were compared by the independent two-tailed *t*-test. Differences were considered significant when the probability of the difference occurring by chance was less than 5 in 100 ($P < 0.05$) for both the *t*-tests and analysis of variance.

RESULTS

Lymph flow

The fasting lymph flow was 2.65 ± 0.14 ml/h (mean ± SE) in the experimental rats (n = 6) and 2.29 ± 0.19 ml/h in the control rats (n = 6). These figures agree with those reported in previous studies (32, 33). In the blocking phase of the experimental rats (**Fig. 3B**), lymph flow did not change significantly after the beginning of lipid plus L-81 except for a statistically significant decrease at 0–2 h as compared to the fasting state. This decrease was not specific to L-81 infusion because we observed a similar fall in lymph flow during the same period in the control rats (Fig. 3A). Soon after the beginning of glucose-saline infusion during the unblocking phase in the experimental rats, lymph flow increased slightly. With the exception of a small decrease in flow at 0–2 h, lymph flow increased

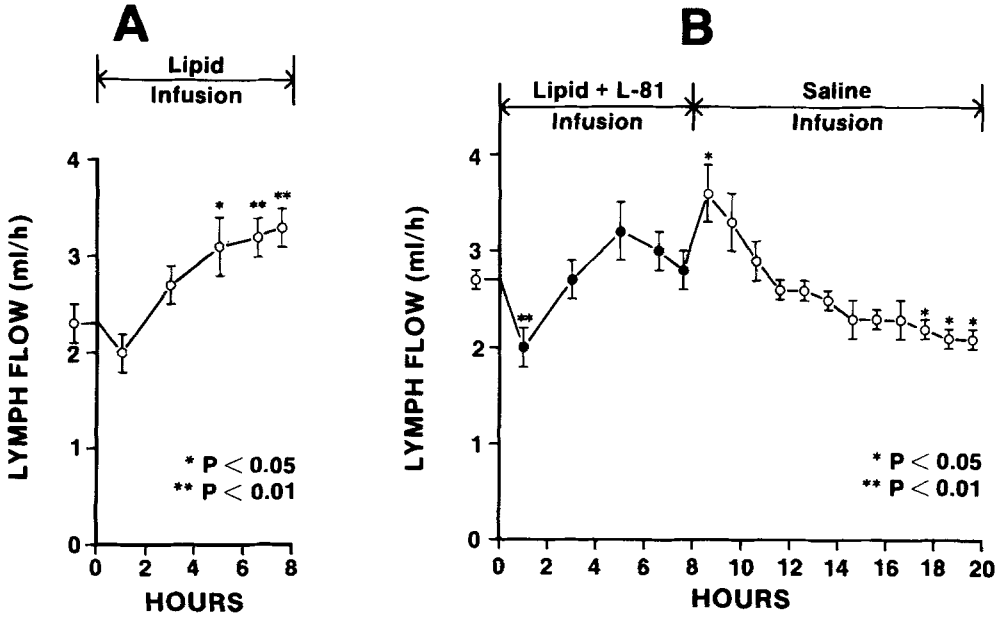

Fig. 3. Lymph flow rate expressed as ml/h. In the control animals (Fig. 3A), lymph flow was measured before and during 0–2, 2–4, 5, 6, 7, and 8 h of lipid infusion. In the experimental rats (Fig 3B), a lipid emulsion with L-81 was infused for 8 h (●) and then replaced with glucose-saline infusion during the unblocking phase (O). Lymph flow was measured at the same intervals as the controls animals during the blocking phase and hourly during the unblocking phase. Six rats were used for each group and values are given as mean ± SE. Each measurement of lymph flow was compared with the fasting lymph flow. Statistically significant differences are labeled with *($P < 0.05$) and **($P < 0.01$).

continuously during the remaining period of lipid infusion in the control animals (Fig. 3A). The lymph flow during the 4–8 h period was significantly higher than the fasting flow.

Lipid output in lymph

During the infusion of lipid plus L-81 in the experimental group, the lymphatic radioactive lipid output increased slightly (**Fig. 4B**), but was markedly suppressed throughout the entire blocking period (4–9% of hourly infused lipid). During the unblocking phase (glucose–saline infusion), the lymphatic radioactive lipid output increased rapidly and peaked at 12–13 h. Then the lymph radioactive lipid output declined gradually, reflecting the depletion of radioactive lipid in the intestinal mucosa. In the control animals (Fig. 4A), the lymphatic radioactive lipid output increased rapidly after the beginning of lipid infusion. The 6–8 period h of radioactive output into lymph was 59–63% of the hourly infused radioactivity. During the entire 8 h of lipid infusion, the output of lymphatic radioactive lipid was significantly higher in the control rats than in the experimental rats.

Although the majority of the lymph (radioactive) lipid is TG, there are also other classes of lipid that can carry (radioactive) FA. Thus we also performed chemical determination of the TG transported in lymph (μmol/h) (**Fig. 5**). In the control rats, the fasting TG output was 4.33 μmol/h. After the infusion of lipid intraduodenally, the lymphatic lipid greatly increased and the lymphatic TG output was 31–36 μmol per h during the 6–8 h period. Since the fasting lymph TG output was about 4.3 μmol per h, the net increase (minus fasting output) in lymph TG output as a result of lipid infusion during the 6–8 h period was 27–32 μmol/h. This is higher than the TG output of 23.5–25.0 μmol per h measured by the radioactive lipid output in lymph. Most of this difference between the two estimates can be explained by the FA contributed by the 7.8 μmol/h of egg PC added to stablize the lipid emulsion and from endogenous lipids. It is well established that biliary lipid output increases with fat feeding (45). In the case of experimental rat (Fig. 5B), the lymphatic TG output as determined chemically was a mirror image of the lymphatic radioactive lipid output described in Fig. 4B. Again, the chemical estimates were consistently higher than the TG as measured by radioactivity for reasons similar to those described above for the control rats.

The PL output in lymph followed that of lymph TG ouptput (**Fig. 6**). The maximum lymphatic PL output in the experimental group was again reached during the 12–14 h period but the lymphatic PL output declined more slowly than the TG output. One other difference between lymphatic TG and PL outputs is that the maximum TG output was about 13 times (the experimental group) or 8 times (the control group) that of the fasting output, but the maximum PL output was only 3 times (both groups) that of the fasting output. These differences likely reflect the much smaller surface-to-volume ratio of CM as compared to fasting VLDL.

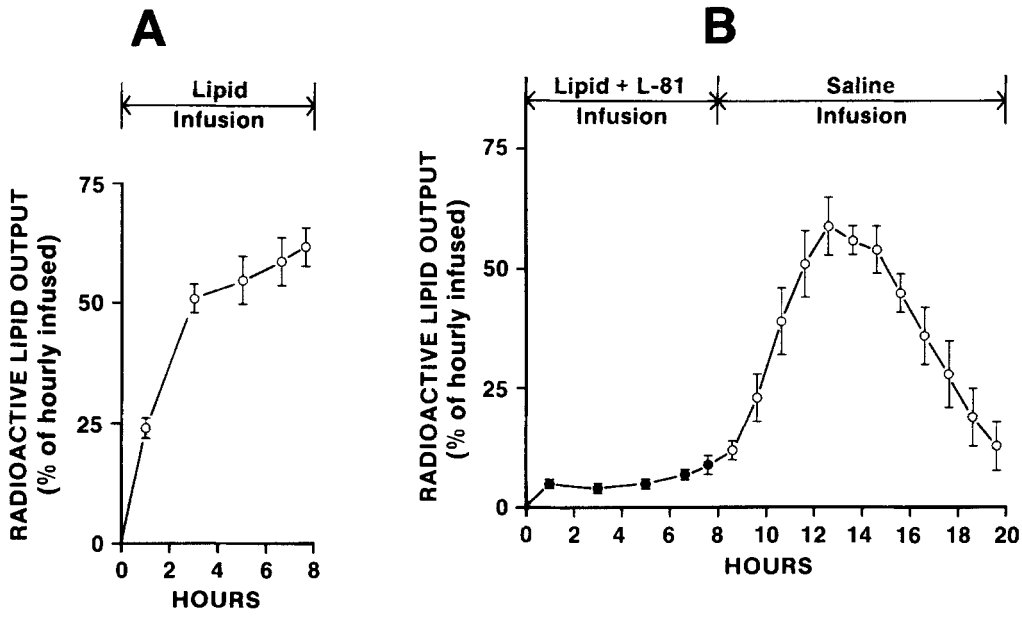

Fig. 4. Radioactive lipid output in lymph. Output in lymph is expressed as a percentage of the dose of radioactive lipid infused per h. Six rats were used for each study and the values are expressed as mean ± SE. The data from control rats are depicted on the left (Fig. 4A) and those from the experimental rats are on the right (Fig. 4B).

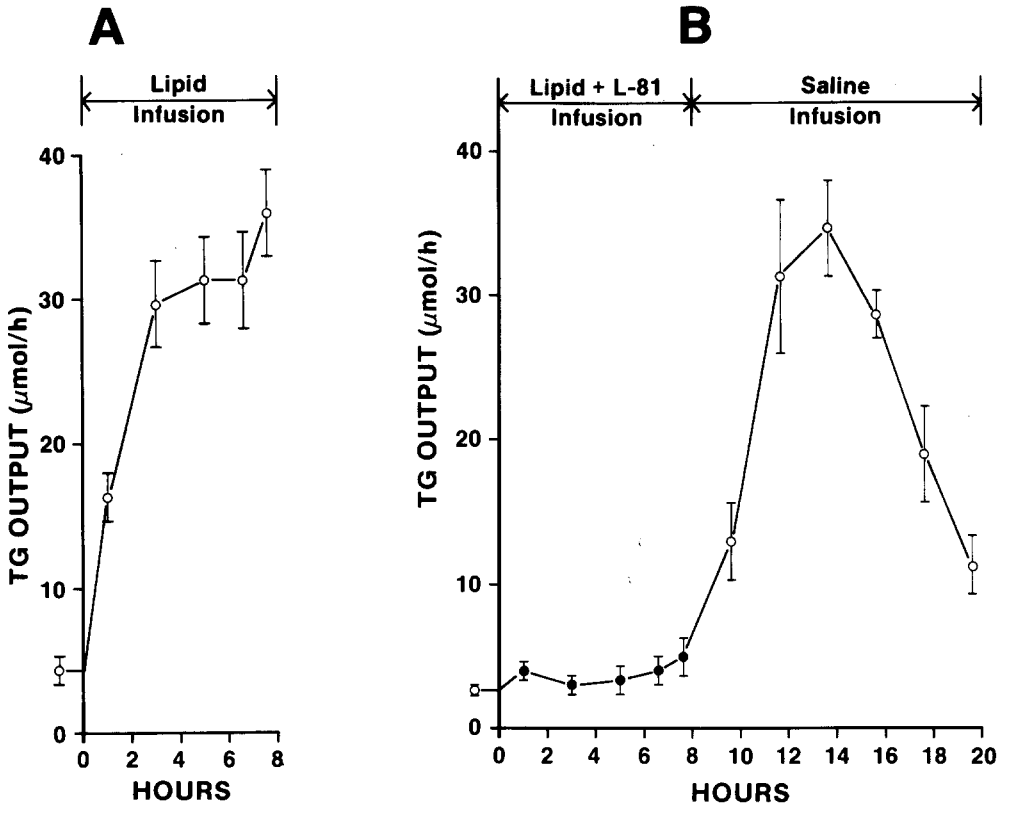

Fig. 5. Triglyceride output in lymph. Lymph TG was determined chemically and the output is expressed as μmol/h in the same six rats per group as in Fig. 4. The data from control rats are depicted on the left (Fig. 5A) and those from the experimental rats are on the right (Fig. 5B).

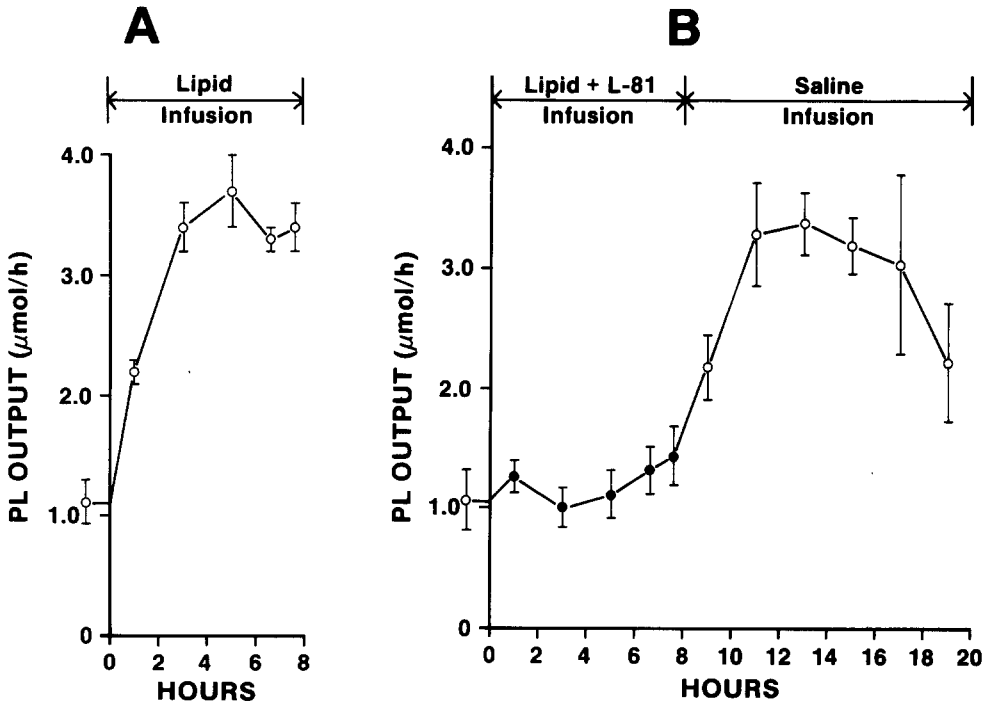

Fig. 6. Phospholipid output in lymph. Lymph PL output was measured by determining the phosphorus content of the PL present in lymph and the output is expressed as μmol/h. Six rats were used in each study and the values are expressed as mean ± SE. The data from controls are depicted on the left (Fig. 6A) and those of the experimental rats are on the right (Fig. 6B).

Apolipoprotein output

The fasting lymph apoA-IV output in the experimental group was 68.2 ± 5.6 μg/h and did not change significantly during the blocking phase (**Fig. 7B**). After the cessation of the infusion of lipid plus L-81, the lymphatic apoA-IV output increased and reached the maximum output of 230.8 ± 15.1 μg/h (3.4 times more than the fasting value) during the 17–18 h period. It is important to note that there was time lag of 4–5 h between the maximum output of TG and apoA-IV. While the TG output in lymph was decreasing during unblocking (14th h onwards), the apoA-IV output continued to increase. Thus these data would seem to indicate that apoA-IV secretion is undoubtedly linked to CM transport and the normal increase in lymphatic apoA-IV output associated with lipid feeding was prevented by the presence of L-81. In the control rats (Fig. 7A) the fasting apoA-IV output in lymph was 86.4 ± 7.6 μg/h, comparable to the fasting output in the experimental rats. After lipid infusion, apoA-IV output in lymph increased and reached an output of 201.4 ± 5.5 μg/h during the 8th hour of lipid infusion. It is interesting that the lymphatic apoA-IV output increased linearly during the entire period of lipid infusion in the control rats while TG output seems to reach a steady output during the 4–6 h period.

Unlike that of apoA-IV, lymphatic apoA-I output did not change much in either the control or the experimental rats (**Fig. 8A and B**). The fasting lymphatic apoA-I output was 109.4 ± 17.9 μg/h in the control rats and 81.4 ± 8.1 μg/h in the experimental rats. In the control animals, the lymphatic apoA-I output increased after lipid infusion and an output of 124 ± 28.2 μg/h was observed during the 7–9 h period of lipid infusion. Due to the large variation among animals, none of the differences in lymphatic apoA-I output was statistically significant in the control rats. In the experimental rats, the lymphatic apoA-I output increased after the beginning of infusion of lipid plus L-81. Although not statistically significant, the lymphatic apoA-I output during the 7–8 h period was 1.3 times that of the fasting output. Interstingly, the apoA-I output actually declined after the beginning of glucose–saline infusion, when the lymphatic TG output was increasing markedly as a result of clearance of mucosal lipid. Then from the 14–15 h period onwards, the lymphatic apoA-I output increased while the lymphatic lipid output was decreasing. However, none of these differences

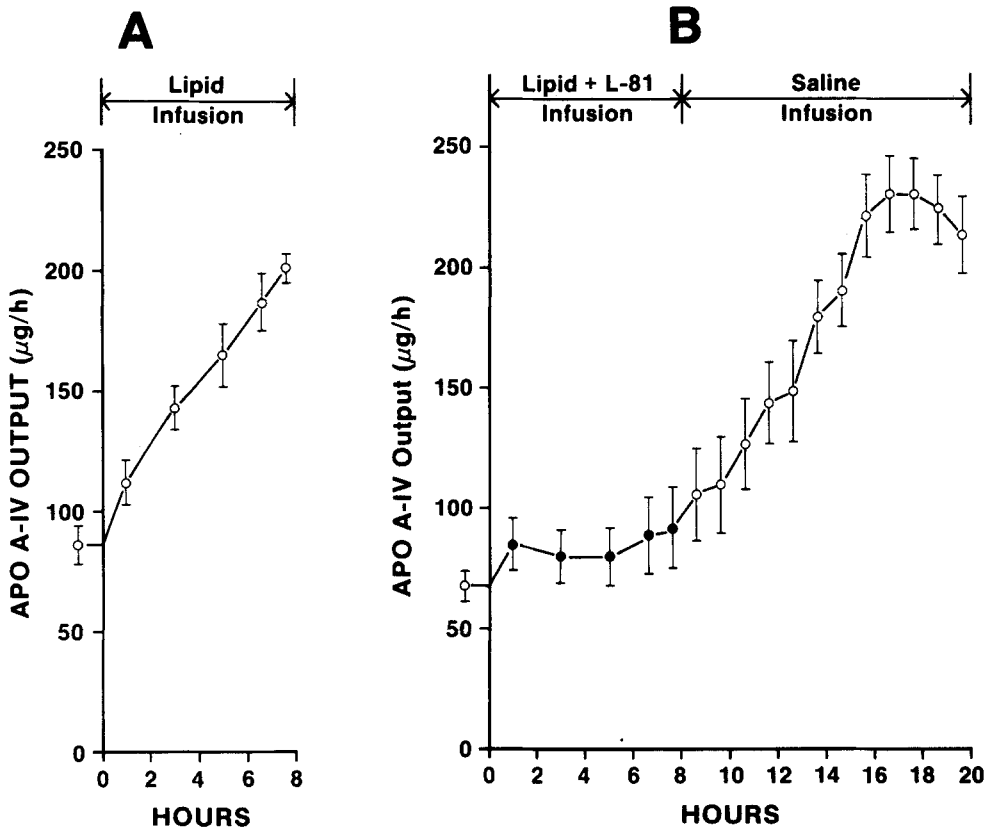

Fig. 7. Lymph apoA-IV output. Lymph apoA-IV concentration was measured by rocket electroimmunophoresis procedures as described by Laurell (44). A pooled rat serum was used as the standard. By multiplying the concentration and lymph flow, we obtained the lymph apoA-IV output in μg/h. Six rats were used in each group and the values are given as mean ± SE. The data from the control rats are depicted in Fig. 7A and those from the experimental rats are in Fig. 7B.

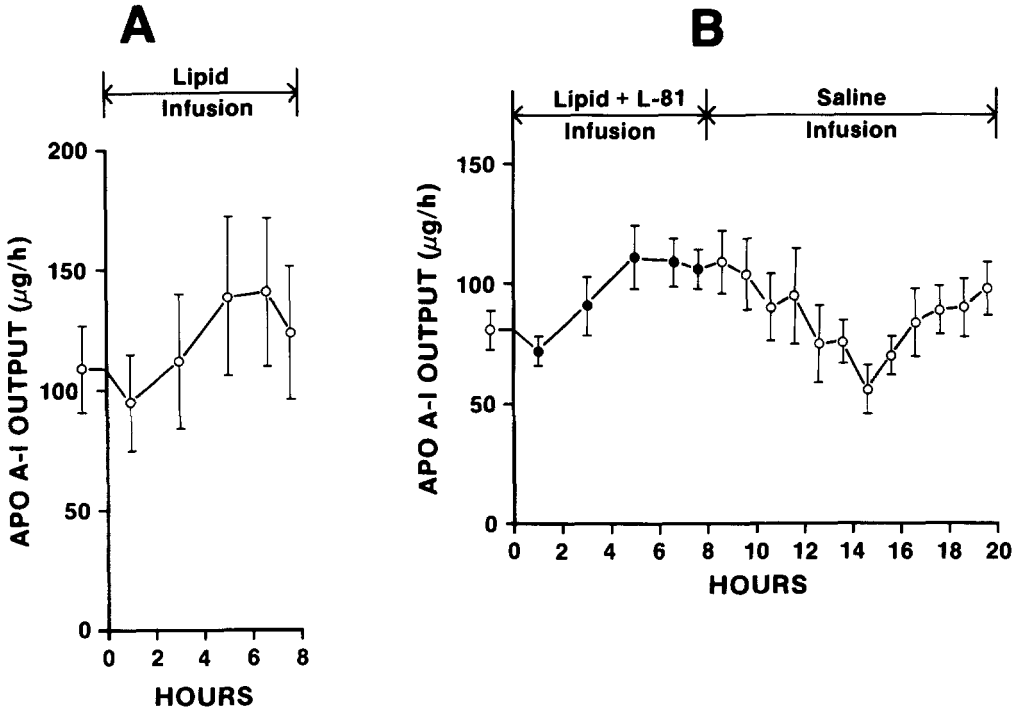

Fig. 8. Lymph apoA-I output. Lymph apoA-I concentration was determined by rocket electroimmunophoresis assay as described by Laurell (44). A pooled rat serum was used as the standard. By multiplying the concentration and lymph flow, we obtained the lymph apoA-I output in μg/h. Six rats were used in each group and the values are expressed as mean ± SE. The data from the control rats are depicted in Fig. 8A and those from experimental rats are in Fig. 8B.

was statistically significant as a result of the large among-animal variation.

Mucosal synthesis of apoA-IV and apoA-I

Four groups of lymph fistula rats were studied. The four groups were: rat fed glucose-saline (fasting); rats fed triolein emulsion for 8 h (normal lipid transport); rats fed triolein + L-81 for 8 h (CM transport blocked); or rats fed triolein + L-81 for 8 h followed by 3.5 h of glucose saline (unblocking). As shown in **Table 1**, apoA-IV synthesis as determined by the incorporation of [^{3}H]leucine increased approximately twofold as a result of the feeding of the triolein lipid emulsion (group 2 vs group 1, $P < 0.01$). Interestingly, the mucosal incorporation of radioactive leucine into apoA-IV did not increase as result of feeding of lipid + L-81, although much lipid had accumulated as TG in the intestinal mucosa because of the inhibition of CM formation by L-81 (32, 33). With the cessation of L-81 infusion, mucosal lipid was rapidly cleared as CM. This was also associated with a marked increase in the synthesis of mucosal apoA-IV measured at 3.5 h during the unblocking phase (group 4 vs 3, $P < 0.01$). Contrary to apoA-IV, apoA-I synthesis was

TABLE 1. Effect of dietary lipid and Pluronic-81 on intestinal apolipoprotein A-I and A-IV synthesis

Group	Apolipoprotein A-I	Apolipoprotein A-IV
	% of total protein synthesis	
1 Fasting	0.52 ± 0.09[a]	1.50 ± 0.19
2 Lipid only	0.64 ± 0.09	2.50 ± 0.25
3 Lipid + L-81	0.45 ± 0.07	1.28 ± 0.10
4 3.5 h after end of lipid + L-81 infusion	0.51 ± 0.06	2.17 ± 0.11
P values		
1 versus 2	NS	<0.01
2 versus 3	NS	<0.01
3 versus 4	NS	<0.01

[a]Values are expressed as mean ± SE; n = 4/group.

not significantly affected by active lipid absorption, the inhibition of CM formation by L-81 (blocking phase), or the unblocking from L-81.

DISCUSSION

This study was conducted to examine the relationship between intestinal lipid transport and apoA-I and A-IV secretion by the small intestine, under three experimental conditions. Under the first condition, we had normal lipid uptake and transport in the control animals (high intracellular TG content and large TG efflux from the enterocytes). Under the second condition, we studied the lymphatic outputs of lipid, apoA-I and apoA-IV when a lipid test meal was fed together with L-81 (very high intracellular TG content with little TG efflux, mainly as VLDL). Lastly, we studied lymphatic transport of lipid, apoA-I and apoA-IV during the reversal of L-81 inhibition, i.e., during the unblocking phase (high, but decreasing, intracellular TG content with large TG efflux). It should be noted that there is a difference in the first and third experimental conditions. Under the first condition, the TG-rich lipoproteins are presumably formed from growing small lipid droplets with the addition of surface coat of PL and protein. In the latter case, the TG-rich lipoproteins are formed from huge existing intracellular droplets. Consequently, it was interesting to compare the lymphatic outputs of lipid and apolipoproteins under these two different experimental conditions.

When the lymphatic lipid output was studied by measuring the radioactivity and chemical glyceride-glycerol outputs, it was obvious that the lymphatic lipid output was depressed by the presence of L-81. The lymphatic TG output during the 7–8 h of lipid infusion in the experimental rats was 4.86 ± 0.33 μmol/h, much lower than the output of 35.83 ± 3.13 μmol/h in the control rats. When the infusion of lipid plus L-81 was replaced by saline infusion, the mucosal lipid accumulated as a result of L-81 treatment was rapidly cleared as lipoproteins transported in the lymph (46, 47). Previous morphologic and biochemical studies have demonstrated that, during unblocking phase, CM are the major lipoproteins carrying the lipid cleared from the intestinal mucosa. The maximum lipid output as measured by radioactivity in lymph was 23.5 ± 2.5 μmol/h of TG, which was achieved between 12 and 13 h of lipid infusion. This agreed extremely well with data obtained by Halpern, Tso, and Mansback (34). In their study, they found that the clearance of mucosal lipid as CM is a rapid and efficient process. Unlike liver TG, the mucosal TG does not have to be hydrolyzed and then re-esterified to form TG before it can be packaged to form TG-rich lipoproteins. The process of conversion of these large lipid droplets into the much smaller CM particles is unknown.

In the control animals, the lymph TG output increased significantly as a result of lipid infusion. The lymphatic lipid output measured by radioactivity ranged between 23.5 and 25.0 μmol/h during the 6–8 h of lipid infusion. This is slightly lower than the values measured by glyceride-glycerol determination. This difference between the two methods probably reflects the slight increase in endogenous lipid output in lymph during the lipid infusion. The lymphatic lipid output during the 6–8 h period of lipid infusion observed in the control animals is comparable to the maximum output observed during the 12–13 h period in the experimental animals. The lymph PL output did not parallel TG output. The presence of L-81 in the lipid infusate prevented the rise in lymphatic PL output observed in the control animals. The lymphatic PL output increased significantly during the unblocking phase. However, the ratio of PL/TG decreased during the unblocking phase, indicating the clearance of mucosal TG as large CM transported in lymph. This ratio decreased to a minimum at around 14 h and started to increase afterwards, indicating smaller CM or VLDL being transported with the diminishing mucosal TG.

The apoA-IV output in lymph was between 50 and 110 μg per h during fasting in both groups of animals. In the control animals, the infusion of a triolein test meal induced a significant increase (2.3-fold) in lymphatic apoA-IV output and reached an output of 201.4 μg/h in the 8th hour of lipid infusion. This agrees with the increased intestinal apoA-IV synthesis and secretion observed by other investigators (4, 31, 48) and is also supported by the apoA-IV synthesis as measured by [^{3}H]leucine incorporation in this study. In contrast to the control animals, the lymphatic apoA-IV did not increase during the infusion of lipid plus L-81 (blocking phase) of the experimental rats. The incorporation study also failed to demonstrate an increase in apoA-IV synthesis during the infusion of lipid + L-81. These data suggest that intestinal apoA-IV secretion may be linked either directly or indirectly to the intestinal CM transport. This notion is further supported by the fact that after cessation of L-81 infusion, lymphatic apoA-IV increased markedly and reached a maximum output of 230.8 μg/h. This is about a threefold increase over the fasting output of 68.2 μg/h. From the [^{3}H]leucine incorporation study, the reversal of L-81 inhibition (Table 1, group 4) also resulted in a significant increase in the synthesis of apoA-IV. Thus the lymph apoA-IV output and also the mucosal synthesis of apoA-IV seemed to agree with each other.

However, it should be emphasized that there is a marked difference in the pattern of lymphatic apoA-IV transport and lymphatic TG output during the unblocking phase. Lymph lipid output peaked at 12–13 h whereas that of apoA-IV peaked at 17–18 h. It seems that the maximal apoA-IV output lags behind the maximal lymphatic

TG output by 4–5 h. Interestingly, a similar lag was observed in the control rats. In the control animals, the TG output in lymph reached a steady output during the 4–6 h period of lipid infusion. In contrast, the apoA-IV output continued to rise during the entire period of lipid infusion.

Thus the data on lymph apoA-IV output and also mucosal apoA-IV synthesis seemed to indicate that lipid uptake in the enterocyte is probably not a sufficient signal for induction of increased apoA-IV synthesis and secretion into lymph during fat feeding. The reason is because L-81 has no effect on the digestion and uptake of TG by the small intestine (32, 33). Neither is the re-esterification of absorbed MG and FA to form TG a sufficient signal for increased apoA-IV synthesis and secretion, since lipid accumulated as TG in the mucosa with L-81 inhibition. Rather, it may be the actual formation and/or secretion of CM that is responsible for the increased apoA-IV synthesis and release by the enterocytes.

As yet, we have little information regarding the mechanism of how L-81 inhibits intestinal CM formation and secretion. To explain why L-81 prevented the normal increase in lymphatic apoA-IV output after beginning lipid infusion, there are possible explanations. First, it is possible that L-81 inhibited apoA-IV synthesis. However, this is unlikely because, although apoA-IV synthesis failed to respond to feeding of lipid + L-81, neither synthesis nor secretion of apoA-IV decreased. Second, it is possible that apoA-IV needs to be associated with CM before it can be secreted by the enterocytes. This again unlikely because apoA-IV should accumulate in the enterocytes during the blocking phase and should then be released in association with CM during the unblocking phase, i.e., the lymph apoA-IV output should follow closely the lymph TG output. The last and most likely reason is that during L-81 treatment, the stimulus for increased apoA-IV production normally associated with lipid feeling is missing. It is tempting to speculate that it may be the trafficking of CM inside the enterocyte, e.g., from the endoplasmic reticulum to the Golgi apparatus, that acts as the stimulus for apoA-IV synthesis and release by the enterocyte. In contrast, VLDL trafficking may be important in maintaining the basal apoA-IV production during fasting and the blocking phase of the experimental group. Thus, the apoA-IV data suggest that there are differences in the packaging and secretion of CM and VLDL particles by the enterocytes. We have proposed in a previous study that there may be two separate pathways for the packaging and secretion of intestinal CM and VLDL particles (49). This proposal has been suggested by other investigators. The first evidence came from study of Ockner, Hughes, and Isselbacher (50) showing that the infusion of palmitate resulted in a marked increase in VLDL transport, whereas the infusion of oleate and linoleate left VLDL output in lymph unchanged. Then Mahley and co-workers (51) showed that Golgi vesicles contained either CM particles or VLDL particles, with little mixing of particle sizes. Studies by Feldman et al. (52) demonstrated that cholesterol and TG differentially affect the particle size of intestinal lymph lipoproteins. More lipid was carried by VLDL with increasing cholesterol absorption, in contrast to the preferential increase in CM when TG was absorbed. Lastly, the study of Vahouny et al. (53) provided evidence that the secretion of protein in CM and VLDL by the female rat small intestine was inhibited to a different degree by puromycin, suggesting that CM and VLDL are formed under different regulation.

Lymph apoA-I output increased slightly as a result of lipid infusion in the control animals. The magnitude of increase in lymph A-I output as a result of lipid infusion was significantly less than that of apoA-IV. The data on the mucosal synthesis of apoA-I also supported the data on lymph apoA-I output. Thus, our data support the findings reported by Gordon et al. (29) and Davidson and Glickman (30). Davidson and Glickman (30) found that, in the jejunum, apoA-I synthesis is not acutely regulated by the flux of TG from the lumen to lymph (30). However, after prolonged dietary TG withdrawal, the introduction of fat induces a small but significant increase in jejunal apoA-I synthesis. In our L-81-treated rats, there was a small increase in lymph apoA-I output after infusion of lipid plus L-81. Similar to the control rats, this difference in apoA-I output was not statistically significant. Thus L-81 did not seem to prevent the small increase in apoA-I output normally observed during lipid absorption. During the unblocking phase, it seems that lymph apoA-I output was actually inversely proportional to lymph TG output. We do not have an explanation for this at the moment. Thus this study fully supports previous observation that acute feeding of lipid does not significantly affect the mucosal translatable mRNA levels for apoA-I nor the synthesis and secretion of apoA-I.

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