Synthesis and lymphatic transport of intestinal apolipoprotein A-IV in response to graded doses of triglyceride

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Abstract Factors regulating the intestinal synthesis and secretion of apoA-IV are incompletely understood. Although it is known that apoA-IV is stimulated by dietary lipid, it is not known whether graded doses of triglyceride elicit graded responses in apoA-IV synthesis and secretion. We used the chronic intestinal lymph-fistula rat to examine the effect of graded levels of intestinal triglyceride transport on secretion of apoA-IV into lymph and synthesis of apoA-IV in various regions of intestine. Rats were implanted with chronic duodenal and intestinal lymph duct cannulas and infused with lipid emulsions containing 5, 10, 20, 40, 80 μmol triolein (including [^{3}H]triolein, 1.2 μCi), 8.7 μmol phosphatidylcholine, and 57 μmol sodium taurocholate in 3 ml phosphate-buffered saline (pH 6.4) at 3 ml/h for 8 h. Lymph samples were collected for 1 h prior to and at 2, 4, 5, 6, 7, and 8 h after the start of lipid infusion. Lymphatic output of triglyceride, phospholipid, and apoA-IV was measured. Steady-state (8 h) content of radioactive lipid was also measured in the lumen and the wall of the gut. In separate studies rats were given infusions of either 5% glucose in saline ("fasting") or triolein emulsion (10, 20, 40, 80 μmol/h) for 8 h after which incorporation of [^{3}H]leucine into apoA-IV in isolated, in situ loops of duodenum, proximal jejunum, mid-distal jejunum, and terminal ileum was measured. Lipid output increased dose-dependently in response to triolein infusion, with steady-state lipid transport achieved by 5 h after start of lipid infusion. Total recovery of ^{3}H-labeled lipid was similar for all triolein doses. Increase in lymphatic apoA-IV output lagged that of triglyceride by 3–4 h, but by 8 h showed graded increases with triolein dose. ApoA-IV synthesis (apoA-IV radioactivity immunoprecipitated by an apoA-IV monospecific antibody and expressed as % of trichloroacetic acid-precipitable [^{3}H]leucine radioactivity) in the duodenum and proximal jejunum showed a 2-fold increase (compared with fasting) in response to 10 μmol triolein/h, but no further increase at higher doses. However, apoA-IV synthesis in mid to distal jejunum increased dose-dependently. These results demonstrate *1)* graded increases in lymphatic lipid and apoA-IV output, suggesting that lipid-elicited stimulation of apoA-IV output may depend upon the length of intestine recruited for lipid transport; *2)* support for the hypothesis that some factor(s) associated with assembly and transport of chylomicrons stimulates output of apoA-IV; *3)* apoA-IV synthesis in the proximal jejunum is sensitive to low levels of triglyceride; *4)* above that threshold, jejunal apoA-IV synthesis is maximally stimulated, independent of triolein dose; and *5)* graded doses of triolein produce increases in intestinal mucosal synthesis of apoA-IV along a proximal–distal gradient.—**Kalogeris, T. J., K. Fukagawa, and P. Tso.** Synthesis and lymphatic transport of intestinal apolipoprotein A-IV in response to graded doses of triglyceride. *J. Lipid Res.* 1994. **35:** 1141–1151.

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Apolipoprotein A-IV (apoA-IV) is a major protein component of intestinal triglyceride (TG)-rich lipoproteins (1–4). In response to feeding, apoA-IV is secreted into intestinal lymph in chylomicrons (1–4). During plasma passage and metabolism of chylomicrons, apoA-IV dissociates from chylomicrons and is then found circulating in high density lipoproteins (HDL) and the lipoprotein-free fraction (3, 5, 6). Several in vitro actions of apoA-IV have been reported—modulation of activity of lipoprotein lipase (7) and lecithin:cholesterol acyltransferase (8, 9), and binding of HDL to cell membranes (10)—but the physiological significance of these actions remains unclear, as little direct in vivo evidence for these actions has been reported. Moreover, several of these actions are not specific to apoA-IV; most are shared by apoA-I, which is present in higher concentration in plasma (11–14).

Several recent reports provide evidence that apoA-IV may play a physiological role in the short-term control of food intake (15–17), a role not shared by apoA-I. In one study (15), intravenous infusions of chylous lymph (collected from donor rats after duodenal lipid infusions) significantly suppressed 30-min food intake in recipient rats that had been food-deprived for 24 h. Infusion of chylous lymph with apoA-IV removed by immunoprecipitation with an apoA-IV-specific antiserum had no effect

Abbreviations: TG, triglyceride; PL, phospholipid; HDL, high density lipoproteins; EIA, electroimmunoassay; PBS, phosphate-buffered saline.

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on feeding, while chylous lymph with apoA-I removed by specific immunoprecipitation still inhibited food intake. Finally, purified apoA-IV produced a dose-dependent suppression of food intake when infused in amounts considered physiological, while similar doses of apoA-I had no effect.

A second study (16) showed that intravenous infusion of purified apoA-IV (but not apoA-I) decreased meal size in rats feeding ad libitum. Finally, apoA-IV was 50-fold more potent at inhibiting food intake when administered into the third ventricle, as compared with intravenous administration, and third ventricular administration of specific apoA-IV antiserum stimulated feeding in rats (17). The latter findings strongly suggest that apoA-IV acts via central mechanisms to inhibit food intake. Furthermore, as available evidence suggests that de novo synthesis of apoA-IV by brain is unlikely (18), it appears that systemic apoA-IV (or perhaps a smaller fragment of A-IV) can somehow traverse the blood–brain barrier and act at specific receptors in the central nervous system to inhibit food intake. The above studies provide the first strong evidence for a physiological role for apoA-IV, and suggest a link between lipoprotein metabolism and feeding behavior. In view of the above evidence, and as the intestine contributes a significant proportion of apoA-IV to the plasma after a meal (19), it is important to understand the factors regulating the intestinal synthesis and secretion of apoA-IV. At present, these factors remain relatively unexplored.

Among the major intestinal apolipoproteins (A-I, A-IV, and B-48), apoA-IV is the only one whose synthesis and secretion are influenced by acute feeding of dietary fat (20–24). Secretion of apoA-IV into intestinal lymph in rats (20) and into serum in rats (5) and humans (25) markedly increases during acute absorption of lipid. Available evidence suggests that this increased secretion may be explained by increased intestinal synthesis (by a pretranslational mechanism) of apoA-IV that occurs in response to both acute and chronic dietary lipid (24). However, studies of synthesis and secretion of apoA-IV under the same experimental conditions have not been reported. Moreover, it is not known whether graded doses of triglyceride elicit graded responses in apoA-IV synthesis and secretion.

In this study the chronic intestinal lymph-fistula rat model was used to examine the effect of graded levels of intestinal triglyceride transport on intestinal synthesis and lymphatic transport of apoA-IV.

MATERIALS AND METHODS

Animals and surgical preparation

Male Sprague-Dawley rats (300–350 g), adapted to standard diet (Purina Mills 5001 rodent chow) were food-deprived for 24 h, then surgically prepared with intestinal lymph duct and duodenal cannulas. With the rats under halothane anesthesia, the superior mesenteric lymph duct was cannulated with vinyl tubing (0.8 mm O.D.) as described previously (26). A silicone tube (1.6 mm O.D.) was passed through the fundus of the stomach, extended 2 cm into the duodenum, then secured in place with a fundal purse-string suture and a drop of cyanoacrylate glue. After surgery, rats were placed in restraint cages (26) in a temperature-regulated chamber at 30°C and allowed to recover for 24 h. During recovery, they received continuous duodenal infusions of a glucose–saline solution (145 mM NaCl, 4 mM KCl, 0.28 M glucose) at a rate of 3 ml/h.

Lipid infusates

Lipid emulsions were prepared containing 5, 10, 20, 40 or 80 μmol triolein (labeled with [^{3}H]triolein, 1.2 μCi), 8.7 μmol egg phosphatidylcholine, and 57 μmol sodium taurocholate, sonicated in 3 ml phosphate-buffered saline (pH 6.4) (26). For experiments, emulsions were infused at 3 ml/h for 8 h. The doses chosen bracketed the average daily fat intake for rats, assuming they eat about 20 g of standard diet (4% fat by weight) per day (approximately 40 μmol/h). Thus, at 40 μmol/h, the approximate 24-h intake of fat consumed by a rat under ad libitum feeding conditions is delivered in 8 h. Immediately prior to lipid administration, samples were taken from the top, middle, and bottom of every sonicated emulsion for assay of radioactivity, both to confirm emulsion homogeneity and to form the basis for determining hourly and total recovery of radioactive lipid in lumen, gut wall, and lymph.

Lymphatic transport studies

After the rats had recovered from surgery, lymph was collected for 1 h prior to lipid infusion (fasting lymph). From the start of lipid infusion, lymph was collected at 2, 4, 5, 6, 7, and 8 h. All lymph samples were collected in ice-chilled glass tubes. Aliquots of lymph were taken for measurement of radioactivity, for lipid extraction, and subsequent assay of TG and phospholipid (PL), and for assay of apoA-IV. At the conclusion of the infusion, rats were anesthetized using halothane and euthanized by exsanguination. The stomach, small intestine, and cecum + colon were each ligated at both proximal and distal ends, then carefully removed. Lumenal contents of the stomach and cecum/colon were washed into collection tubes using 10 mM sodium taurocholate in 0.15 M NaCl (3 × 10 ml washes). The small intestine was placed on ice, then divided into four equal length segments by ligation: M_1 (duodenum and proximal jejunum), M_2 (proximal to mid-jejunum), M_3 (mid-distal jejunum), and M_4 (ileum). Lumenal contents of each segment were collected as described above (3 × 3 ml washes) for stomach and cecum/colon. All lumenal samples were homogenized, then

aliquots were taken for radioactivity determination. Washed segments of small intestine were cut open longitudinally, placed in stoppered flasks containing 40 ml ice-cold chloroform–methanol 2:1, and minced with scissors. Lipids were then extracted from the intestinal segments by the method of Folch, Lees, and Sloane Stanley (27) and radioactivity in the extracted lipids was measured and reported herein as "wall" lipid, i.e., lipid absorbed but not yet transported into lymph.

Lipids were extracted from lymph using the method of Blankenhorn and Ahrens (28); extracts were assayed for both TG (29) and PL (30). Lymph apoA-IV content was measured using an electroimmunoassay (EIA) previously described (20). Gels for EIA were made of 1.2% Indubiose agarose and 2% dextran in Tris-Veronal buffer containing 1% goat anti-rat apoA-IV antiserum. Samples were subjected to electrophoresis at 3.5 V/cm for 6 h at 20°C. Immunoprecipitates were stained and the areas under the precipitate rockets were measured using a computer-controlled digitizing system (Sigma Scan, Jandel Scientific, Sausalito, CA).

Biosynthesis of apoA-IV in proximal jejunal mucosa

Five groups of rats were used, one infused with glucose–saline for determination of fasting levels of synthesis and one group for each of four doses of triolein: 10, 20, 40, and 80 μmol/h. Lymph-fistula rats were surgically prepared and infused with either glucose–saline or lipid for 8 h as described above except that no radiolabeled triolein was included. Preliminary experiments justified our equating the fasting condition in this experiment (i.e., 8 h infusion of glucose–saline after recovery from surgery) with that in the first experiment (i.e., "fasting" was the first hour of lymph collection immediately after recovery, prior to lipid infusion). Lymph-fistula rats (n = 6) were prepared and allowed to recover for 24 h while being infused intraduodenally with glucose–saline solution. After this period lymphatic output of A-IV was 125.5 ± 17.5 μg/h. During a further 8-h infusion of glucose–saline there was no significant change in lymph apoA-IV output; by the 8th hour, A-IV output was 131.6 ± 15.9 μg/h.

Subsequent methods were those of Davidson and Glickman (21) as adapted by Hayashi et al. (20). After 8 h of infusion of either glucose–saline or lipid, rats were anesthetized with halothane and a 10-cm segment of proximal jejunum (starting at about the ligament of Treitz, corresponding to the distal 6 cm of M_1 and the proximal 4 cm of M_2), was isolated by ligatures. Approximately 0.3 mCi of L-[3,4,5-^{3}H(N)]leucine in 1 ml 0.15 M NaCl was instilled into the loop for 10 min. During the incubation period, the loop was placed back in the abdominal cavity and the rat was kept warm with an incandescent lamp. The intestinal loop was then removed and flushed with 50 ml cold PBS (50 mM sodium phosphate, 100 mM NaCl, pH 7.4) containing 20 mM leucine. The washed segment was placed on a glass plate over ice and was opened longitudinally. The mucosa was gently scraped with a glass slide and homogenized in 1.5 ml PBS + 1% Triton X-100, 2 mM leucine, 1 mM phenylmethylsulfonyl fluoride, 40 μg chymostatin, 160 μg leupeptin, and 6 μg pepstatin. The homogenate was centrifuged at 105,000 *g* for 60 min in a Beckman 50.3 Ti rotor and the cytosolic supernatant was stored at −70°C until analysis.

To determine total protein synthesis, a freshly thawed aliquot of cytosolic supernatant was precipitated with 10% trichloroacetic acid (TCA), solubilized with NCS solubilizer (Amersham, Arlington Heights, IL), mixed with aqueous miscible scintillation fluid, and radioactivity was measured. Protein concentration in the cytosolic homogenate was measured using the Bradford procedure (Bio-Rad, Hercules, CA).

A separate, freshly thawed aliquot of cytosolic supernatant was subjected to specific immunoprecipitation of apoA-IV, using a polyclonal goat anti-rat apoA-IV antiserum described previously (20). All immunoprecipitations were carried out in duplicate. Preliminary experiments using re-incubation of immunoprecipitated samples with additional antiserum established the amount of antiserum necessary to achieve quantitative immunoprecipitation. All subsequent immunoprecipitations were carried out using an excess of antiserum. Immunoprecipitated apoA-IV was resolved using SDS-PAGE followed by autoradiography. The radioactive band corresponding to apoA-IV was removed, solubilized using NCS solubilizer (Amersham, Arlington Heights, IL), and the radioactivity was measured in a scintillation counter. Synthesis of apoA-IV was expressed as the amount of immunoprecipitable radioactivity as a percentage of total TCA-precipitable radioactivity.

Biosynthesis of apoA-IV in different regions of intestine

The lymph-fistula rat is a difficult surgical preparation; examination of the effect of triolein dose on synthesis of apoA-IV in different regions of the intestine would be simpler if absence of the fistula did not influence the results. This issue was directly tested by comparing proximal jejunal apoA-IV synthesis in the presence or absence of lymph fistulas. Rats were surgically prepared with either duodenal cannula and lymph fistula, or with duodenal cannula only, and allowed to recover for 24 h while receiving continuous infusion of glucose–saline solution. Synthesis of apoA-IV in jejunal mucosa was then measured under either fasting conditions (i.e., 8 h glucose–saline infusion) or in response to infusion of lipid emulsion containing triolein at 40 μmol/h. In fasting rats, A-IV synthesis (% of total protein synthesis) was 0.84 ± 0.31 (non-lymph fistula, n = 6) versus 0.71 ± 0.19 (lymph fistula, n = 7); in rats given lipid infusions, A-IV synthesis was 1.53 ± 0.30 (non-lymph fistula, n = 6) versus

1.49 ± 0.30 (lymph fistula, n = 5). There was a significant effect of lipid infusion (P = 0.005), but no effect of surgery (P = 0.942) and no surgery × infusate interaction (P = 0.941, two-way AOV).

For measurement of A-IV synthesis in different regions of the intestine, rats were implanted with duodenal cannulas and allowed to recover for 24 h, while receiving continuous infusion of glucose–saline. For experiments they received 8-h infusions of either glucose–saline or lipid emulsion containing graded doses of triolein (10, 40, or 80 μmol/h). At the conclusion of lipid infusion, rats were anesthetized and 10-cm segments from each of the four intestinal regions (M_{1-4}) used in the transport studies were carefully measured, isolated by ligature, and incubated for 10 min in situ with [^{3}H]leucine as described above. The four segments were: S_1 (duodenum, from pylorus to just distal to ligament of Treitz; proximal portion of M_1); S_2 (proximal jejunum, beginning about 5–10 cm distal to the ligament of Treitz, roughly equivalent to the segment in which synthesis was measured in the previous study; proximal half of M_2); S_3 (mid-distal jejunum; middle of M_3), and S_4 (distal ileum; distal half of M_4). After incubation with [^{3}H]leucine for 10 min, mucosa from each segment was recovered, and cytosolic supernatants were prepared as described above. Total protein synthesis was determined after TCA-precipitation and synthesis of apoA-IV (immunoprecipitable radioactivity expressed as a percentage of TCA-precipitable radioactivity) was determined after immunoprecipitation of apoA-IV as described above. A difference between this and previous synthesis studies was the use of a different tissue solubilizer (Solvable Tissue and Gel Solubilizer, DuPont/NEN Research Products, Boston, MA), because NCS solubilizer was no longer available.

Statistics

Data were analyzed using a commercial statistical software package (31). Time-dependent data on lymph flow and lymphatic output of lipids and apoA-IV in response to triolein dose were analyzed as the change from fasting condition by one-way repeated measures analysis of variance with post-hoc comparisons using a multiple linear general model. The relationship between lymphatic TG or PL output and lymphatic apoA-IV output was evaluated using least squares regression analysis. Data on lumenal and gut wall content of radioactive lipid and apoA-IV synthesis were analyzed by one-way or two-way analysis of variance with multiple comparisons by least significant difference. Presentation of results of F tests in the Results section incorporates the standard practice of subscripting, in order, the degrees of freedom of the numerator and denominator mean squares used to calculate F. Differences were considered significant if $P < 0.05$. All data are presented as mean ± SE.

RESULTS

Lymph flow

Fasting lymph flow rates were similar in all animals tested, ranging from 2.1 ± 0.45 to 3.0 ± 0.1 ml/h. Similar to previous findings (20, 26), lymph flow showed a characteristic increase over time in response to lipid infusion ($F_{5,125}$ = 27.908, $P < 0.001$). In general, all doses produced a similar response pattern (i.e., no significant dose × time interaction, $F_{20,125}$ = 1.368, P = 0.353), but there was a significant dose effect ($F_{4,25}$ = 3.555, P = 0.02) and higher doses tended to produce higher flow rates with the following qualifications: in the early periods after the start of lipid infusion (0–4 h), all doses produced a similar response ($P > 0.1$). At 5 h, flow rate in response to 80 μmol/h was significantly higher than those in response to all other doses ($P < 0.05$), while 20 and 40 μmol/h produced higher flows than did 5 and 10 μmol/h ($P < 0.05$). By 8 h there were no significant differences in lymph flow rates between doses.

Lymphatic lipid output

Fasting output of TG in lymph was similar for all groups, ranging from 4.1 ± 0.25 to 5.9 ± 0.29 μmol/h. Nevertheless, the results depicted in **Fig. 1** are plotted as

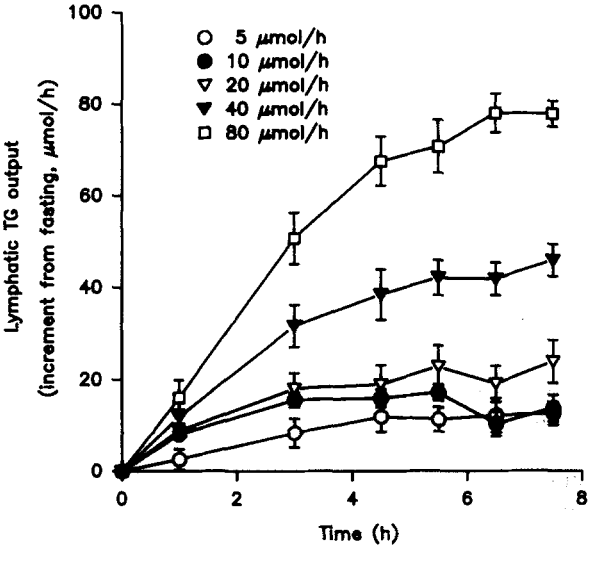

Fig. 1. Triglyceride (TG) output (μmol/h) in intestinal lymph in response to duodenal infusion of lipid emulsions containing graded doses of triolein. Experimental protocol is described in Materials and Methods. TG output was calculated by multiplying lymph TG concentration (determined by TG assay) by average lymph flow rate at each time point. Plotted values are increments (mean ± SE) from fasting (i.e., output during glucose–saline infusion subtracted from output at each collection point). Increments at each time point presented as average over the period of collection so that output at 2 h is plotted versus the midpoint of the 2-h collection period, i.e., 1 h, etc. Plotted data are for n = 5 (5 μmol/h), n = 6 (10 μmol/h), n = 5 (20 μmol/h), n = 8 (40 μmol/h), and n = 7 (80 μmol/h) rats.

the increments in TG output above fasting, obtained by subtracting fasting TG output from values obtained at each collection point after the start of lipid infusion. Overall, lipid infusion produced a dose-dependent increase in TG output over the experimental period ($F_{4,25}$ = 50.358, $P < 0.001$). Lymphatic TG transport was increased compared with fasting output within the first 2 h of lipid infusion; this increase was significant in response to all but the lowest dose (5 μmol/h) at all doses ($P < 0.05$). Subsequent TG transport exhibited a dose-dependent rise to a steady-state by 5 h after the start of lipid infusion (Fig. 1). However, this dose-dependent increase in hourly TG output was significant only from 20 to 80 μmol/h ($P < 0.05$); increments in response to 5, 10, and 20 μmol/h were statistically similar at all time points ($P > 0.06$).

The data on lymphatic TG mass output include contributions from both exogenous and endogenous lipid; transport of exogenous TG was estimated by measuring the lymphatic output of ^{3}H-labeled lipid. There was no significant effect of triolein dose on the proportion of radiolabeled lipid transported into lymph (expressed as a percentage of hourly infused dpm): by 8 h, hourly lymph output of ^{3}H-labeled lipid ranged from 65 to 85% of infused amount) ($F_{5,26}$ = 1.982, P = 0.115). Similarly, cumulative transport of radioactive lipid (expressed as a percentage of total infused radioactivity over 8 h) was similar for all doses (48–66%) (one-way ANOVA, $F_{4,26}$ = 2.228, P = 0.094). Thus, the efficiency of lymphatic TG transport was similar over the range of doses tested.

Fasting output of PL in all groups ranged from 1.2 ± 0.21 to 1.3 ± 0.27 μmol/h. In response to lipid infusion, lymph PL output increased over the study period. Phospholipid output reached a steady-state by 5 h after the start of lipid infusion. There was a significant triolein dose effect on PL output ($F_{4,25}$ = 3.069, P = 0.035). However, this effect was statistically significant only when comparing extremes of the dose range (i.e., 2.91 ± 0.38 μmol/h in response to a triolein dose of 5 μmol/h versus 4.76 ± 0.515 μmol/h in response to triolein at 80 μmol/h).

Lipid in lumen and gut wall

Radioactive lipid in the lumen and gut wall was quantified after 8 h of lipid infusion. As lymphatic transport is the end result of the numerous stages in the digestive/absorptive process, achievement of steady-state TG transport into lymph indicates that lipid flux through the previous compartments is also in a steady-state. In this study we observed steady-state lymphatic transport of TG by 5 h after the start of lipid infusion (Fig. 1). Thus, measuring lumenal and mucosal radioactivity after 8 h ensured that ^{3}H-labeled lipid in these compartments was in a steady-state. There was no effect of triolein dose on lumenal recovery of ^{3}H-labeled lipid ($F_{4,26}$ = 1.323, P = 0.288), which accounted for less than 9% of the total infused dose. Wall lipid (determined by summing the recovered radioactivity in all four small intestinal segments) was similar at the three lowest doses (4.6–7.3% of infused ^{3}H-labeled lipid), but there was a significant increase in the proportion of ^{3}H-labeled lipid in the gut wall in response to 40 and 80 μmol/h (15.9 ± 1.86 and 16.3 ± 0.91% for 40 and 80 μmol/h, respectively, $F_{4,26}$ = 13.392, $P < 0.01$). Triolein dose had no effect on recovery (percent of total infused) of either lymph ($F_{4,26}$ = 2.228, P = 0.094) or total ($F_{4,26}$ = 1.648, P = 0.192) ^{3}H-labeled lipid.

The distribution of ^{3}H-labeled lipid in the gut wall from the duodenum through the ileum was quantified (**Fig. 2**). As these measurements were made at the end of the study under conditions of clearly established steady-state transport of TG (Fig. 1), they probably reflect the length of gut recruited for lipid absorption and transport. In response to triolein doses from 5 to 40 μmol/h, there was a progressive increase in wall lipid in M_1 (duodenum and proximal jejunum) ($F_{4,26}$ = 9.783, P = 0.001), a considerably smaller (albeit significant) proportion of infused radioactivity in M_2, and insignificant spread of counts to M_3 and M_4. In response to the highest triolein dose (80 μmol/h), there was a split of recovered radioactive lipid between M_1 and M_2. Greater spread of labeled lipid into the second quarter of the small bowel at the

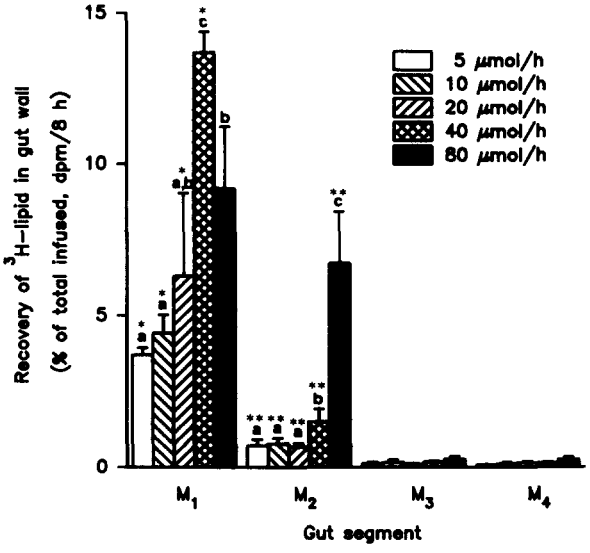

Fig. 2. Effect of triolein dose on recovery of ^{3}H-labeled lipid (% of infused dose) from small intestine. Intestine was divided into quarters: M_1: duodenum to proximal jejunum; M_2: proximal to mid-jejunum; M_3: mid- to distal jejunum; M_4: ileum. Lipid in each segment was extracted after 8 h of duodenal infusion of lipid emulsion; an aliquot was then taken for radioactive counting. Values are means ± SE; number of rats at each dose same as in Fig. 1 except for 40 μmol/h where n = 6. Within each segment, bars with different letter superscripts are significantly different (i.e., dose effect, $P < 0.05$). For a given dose, *indicates M_1 recovery significantly different ($P < 0.001$) from recovery in M_2; **indicates M_2 recovery significantly different ($P < 0.05$) from recovery in M_3.

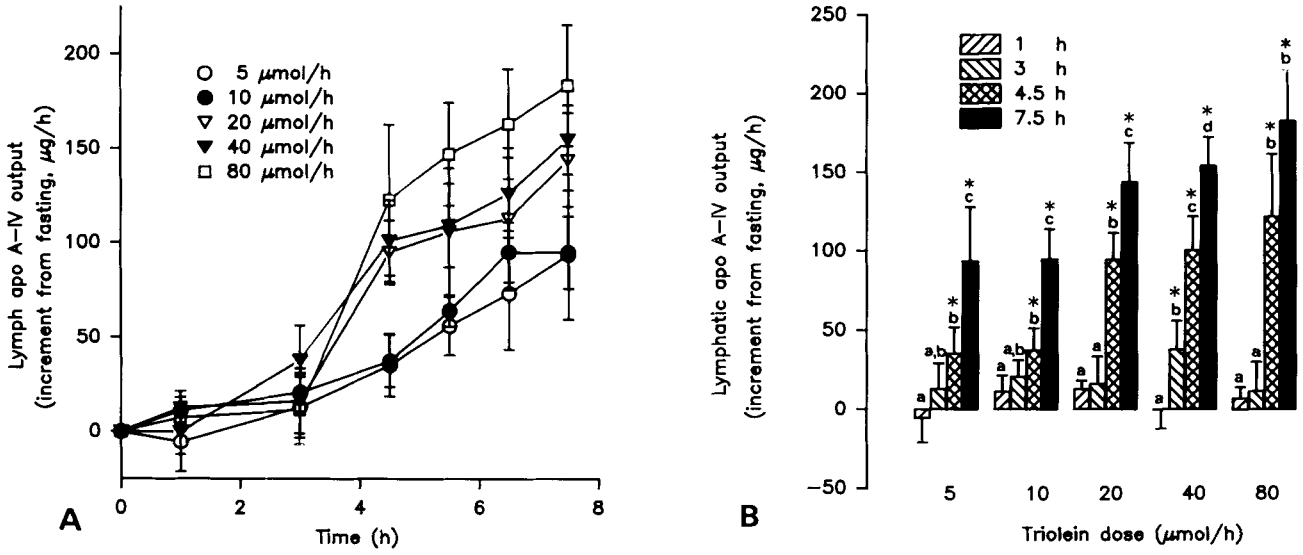

Fig. 3. A: Effect of triolein dose on lymphatic output of apoA-IV (μg/h). Experimental protocol same as in Fig. 1. ApoA-IV output was calculated by multiplying lymph A-IV concentration (determined by electroimmunoassay) by lymph flow rate at each time point. Plotted values are change from fasting values (mean ± SE); number of rats per dose same as in Fig. 1. B: Change in apoA-IV output from fasting (μg/h) at selected time points during 8 h duodenal infusion of graded doses of triolein emulsion. Values are the same as respective time points in Fig. 3A. *indicates significant change from fasting value at each time point ($P < 0.05$); within a given time point, different letter superscripts indicate significantly different outputs ($P < 0.05$).

highest triolein dose suggests recruitment of a longer length of intestine to transport the higher TG load.

Lymphatic apoA-IV output

Fasting lymphatic output of apoA-IV ranged between 90 and 130 μg/h, with a mean for all animals of 115.03 ± 5.08 μg/h. ApoA-IV output in response to lipid infusion for each rat was normalized for varying fasting output by subtracting fasting output from output at each time point after the start of lipid infusion. In contrast to lymphatic TG output (Fig. 1), there was an initial delayed increase in apoA-IV output (**Fig. 3A, B**). This delay was generally independent of triolein dose. At the 2-h collection, outputs were not significantly different from fasting across doses; outputs measured at 4 h were significantly elevated above fasting in response to 40 μmol/h ($F_{1,25} = 6.634$, $P = 0.016$), but not at any other dose. All triolein doses produced significant, and in general, graded increases in apoA-IV output by the 5th hour; this trend continued through the final hour of collection, suggesting a dose–response relationship between triolein dose and apoA-IV secretion during the 7th and 8th h of lipid infusion.

Regression analysis was used to evaluate the relationship between lymph TG and apoA-IV transport. Over the full 8-h period of lipid infusion, the statistical relationship between cumulative lymph TG increment and cumulative lymph A-IV increment was relatively weak (= 0.483; t = 2.866, $P = 0.004$, one-tailed). This was likely due to the initial delay in A-IV output as well as individual variability. This is supported by the results of analyses over the first 4 h of the experiment versus the second half. From the start of lipid infusion to 4 h, there was no relation between TG output and A-IV transport ($r = 0.127$; t = 0.663, $P = 0.256$). However, when cumulative data over the last 4 h of the study were analyzed, there was a highly significant correlation between TG output and A-IV output (**Fig. 4**), indicating that once steady-state lipid transport was achieved, lymph A-IV transport depended upon the level of steady-state TG transport.

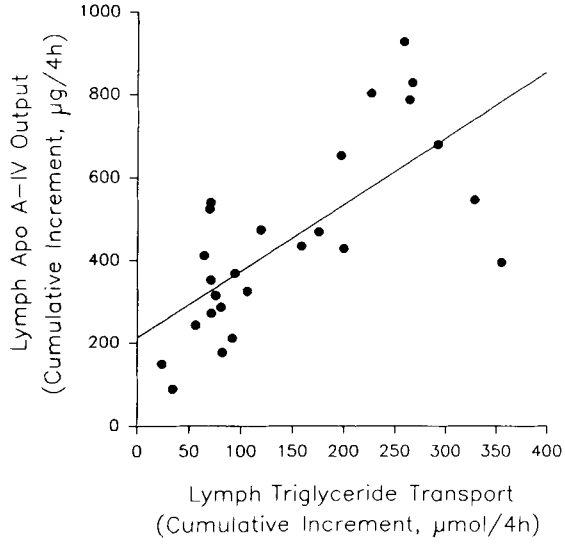

Fig. 4. Lymphatic apoA-IV output versus TG output. This figure depicts the relationship between cumulative increment in lymphatic TG output (μmol/4 h) and cumulative increment in lymphatic apoA-IV output (μg/4 h) during period of steady-state TG transport [i.e., 5–8 h (inclusive) after start of lipid infusion]: y = 1.618X + 213.05; $r = 0.708$; t (one-tailed) = 4.913; $P = 0.00003$; n = 31.

Regression analysis of phospholipid output versus A-IV output did not reveal as strong a relationship. When integrated over the entire experimental period the correlation was not significant (r = 0.291; t = 1.581, P = 0.063). Over the period of steady-state lipid transport (5–8 h), the correlation was still relatively weak (r = 0.526, t = 3.027, P = 0.003).

Proximal jejunal synthesis of apoA-IV

As recovery of radioactive lipid from the gut wall was essentially all from the proximal jejunum (M_{1-2}), a segment of this region was chosen for study of the effects of triolein dose on mucosal synthesis of apoA-IV. Five groups of rats were studied: one group for measurement of fasting synthesis, and the other four for graded doses of triolein. Incorporation of [^{3}H]leucine into apoA-IV was measured in an in situ loop of proximal jejunum, after 8-h infusions of either glucose–saline (fasting) or lipid emulsions (10–80 μmol triolein/h) (**Fig. 5**). Jejunal mucosal synthesis of apoA-IV was measured by quantifying ^{3}H incorporated into apoA-IV by apoA-IV-specific immunoprecipitation, and expressing immunoprecipitated radioactivity as a percentage of ^{3}H incorporated into total protein (determined via TCA precipitation). Fasting synthesis of apoA-IV was 0.71 ± 0.352% of total protein synthesis. In response to triolein infusion at 10 μmol/h, synthesis was 1.51 ± 0.433%, a significant increase

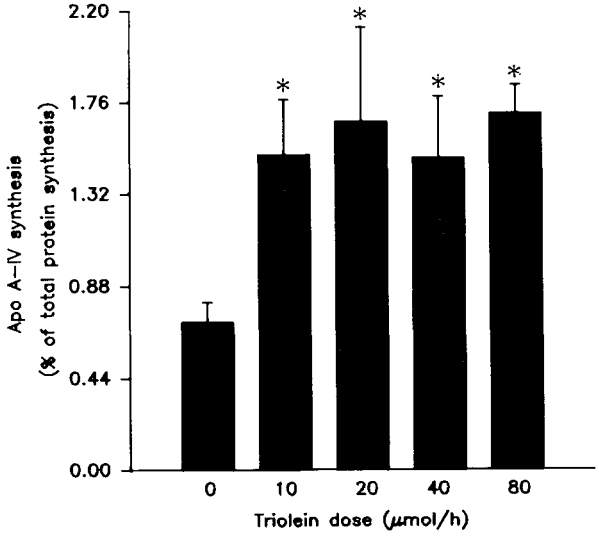

Fig. 5. Effect of triolein dose on apoA-IV synthesis in proximal jejunum. Lymph-fistula rats were given 8-h duodenal infusions of either 5% glucose in saline (fasting, or 0 dose) or lipid emulsion containing graded doses of triolein; then [^{3}H]leucine was instilled into a 10-cm loop of jejunum isolated by ligature. Mucosa was recovered from each segment after 10 min incubation. Synthesis is expressed as % of TCA-precipitable [^{3}H]leucine radioactivity recovered in apoA-IV immunoprecipitated from jejunal mucosal cytosol. Values are means ± SE. Number of rats tested at each dose: n = 7 (fasting), n = 6 (10 μmol/h), n = 5 (20 μmol/h), n = 5 (40 μmol/h), and n = 6 (80 μmol/h). *indicates significantly different from fasting ($P < 0.05$).

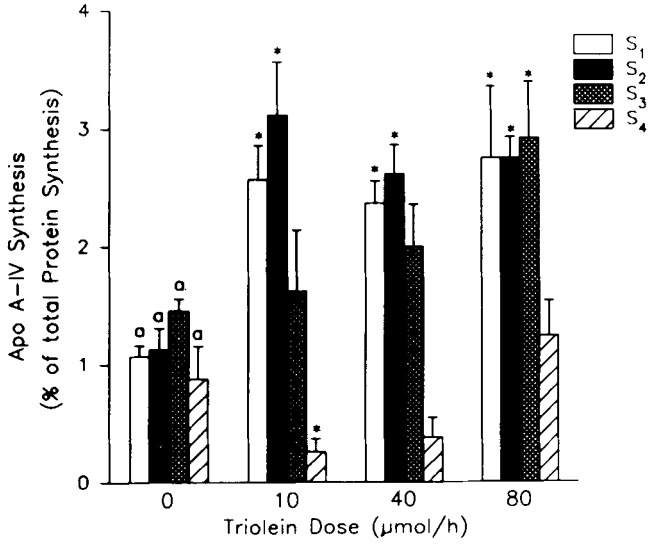

Fig. 6. Effect of triolein dose on synthesis of apoA-IV in different regions of intestine. Rats with duodenal cannulas were given 8-h infusions of either glucose–saline solution or lipid emulsion containing graded doses of triolein (10, 40, 80 μmol/h). Four segments of intestine were analyzed per rat: S_1: duodenum, from pylorus to just distal to ligament of Treitz; S_2: proximal jejunum, starting from about 5–10 cm distal to ligament of Treitz, equivalent to segment tested in experiment depicted in Fig. 5; S_3: mid-distal jejunum; and S_4: distal ileum. Each segment was isolated with ligatures and incubated for 10 min with [^{3}H]leucine. ApoA-IV synthesis was determined as in Fig. 5. Values are means ± SE; n = 7 rats for fasting (0 dose of lipid) and n = 4 rats for each dose of triolein. Similar letter superscripts above values for fasting synthesis indicate no significant difference due to gut segment ($P > 0.05$). *indicates a dose effect significantly different from fasting level of synthesis in a given segment ($P < 0.05$).

($F_{4,24}$ = 2.933, $P < 0.05$). HOwever, higher doses of triolein had no further effect on apoA-IV synthesis. Total protein synthesis (expressed as ^{3}H incorporation into TCA precipitable protein, dpm/mg) was not influenced by triolein dose.

ApoA-IV synthesis in different regions of the intestine

In each rat, A-IV synthesis was measured in four segments from the duodenum to the ileum, in response to infusions of either glucose–saline or graded doses of lipid (**Fig. 6**). In this experiment, the S_2 segment is equivalent to that examined in the previous study. Under fasting conditions (i.e., infusion of glucose–saline), A-IV synthesis was approximately 1% of total, and statistically similar across doses. Consistent with our previous study, synthesis in the S_2 segment was maximally stimulated by triolein at 10 μmol/h, i.e., there was no further increase in A-IV synthesis in S_2 with increasing dose. The same findings held for S_1. However, the A-IV response in S_3 showed a graded increase across doses, although this increase was significant only at 80 μmol/h (repeated measures AOV, $P < 0.001$). With lipid infusion, synthesis in S_4 decreased significantly with 10 and 40 μmol/h; although there was a slight rise in response to 80 μmol/h, this was not significantly different from fasting.

DISCUSSION

An understanding of the role of nutrients (especially lipid) in the regulation of synthesis and secretion of apoA-IV is important in view of recent evidence that apoA-IV may play a physiological role in the control of food intake (15–17). The objectives of this study were to characterize the effect of varying intestinal TG load on *1)* lymphatic transport of both apoA-IV and lipid, and *2)* jejunal mucosal synthesis of apoA-IV. Results of these studies demonstrated *1)* a dose-dependent increase in lymphatic transport of TG and PL; *2)* after an initial delay, a dose-dependent increase in apoA-IV secretion during steady-state TG transport; and *3)* dose-related increases in intestinal synthesis of apoA-IV along a proximal to distal gradient.

We observed that the increase in apoA-IV output in response to lipid infusion lagged that of TG by 3–4 h. Indeed, a dose-dependent effect of triolein on A-IV output was only evident after 5 h of TG infusion, by which time lymphatic TG transport had achieved a steady-state. Previous studies reported that peak apoA-IV output into either human chylous urine (1) or rat intestinal lymph (20) lagged that for TG by several hours. The present observation further supports the hypothesis of Hayashi et al. (20) that secretion of apoA-IV is stimulated by events taking place during TG transport (i.e., assembly and secretion of chylomicrons). The reason for the delay is unknown. Although several studies have shown both increased transcription of the apoA-IV gene and increased synthesis of apoA-IV in response to dietary lipid (20, 24, 32–35), it is not clear whether either or both of these processes could account for the delay in apoA-IV output, or whether some posttranslational event is responsible. In previous studies in rats, Apfelbaum, Davidson, and Glickman (24) observed a 52% increase in jejunal apoA-IV synthesis at 1.5 h and a 34% increase in translatable apoA-IV mRNA by 4 h after a gastric bolus of Intralipid; Gordon et al. (35) observed a twofold increase in translatable intestinal apoA-IV mRNA by 4 h after a gastric bolus of corn oil. However, differences in method (route and method of administration and dose of lipid) make it difficult to directly compare our results with these earlier studies. Furthermore, both groups (24, 35) measured mRNA levels and/or synthesis of apoA-IV; they did not quantify lymphatic output. Whatever the mechanism for the delay in apoA-IV output, it is clear that at least during the early phases of lipid absorption, stimulation of apoA-IV secretion above fasting levels is not required for lipid transport to proceed. Rather, our data support the hypothesis (20) that stimulation of apoA-IV output may be driven by intestinal lipid transport.

Over the dose range of triolein used in this study, 8-h lymphatic output of apoA-IV increased by 80% (at 5 μmol triolein/h) to 171% (at 80 μmol triolein/h). This compares well with a previous report showing approximately a doubling of apoA-IV output in response to 40 μmol triolein/h (20). The range of doses of triolein used in these studies (5–80 μmol/h) was chosen to bracket the approximate daily amount of fat consumed by animals eating standard diet under ad libitum conditions. Thus, at the 40 μmol/h dose, the amount of fat ingested over 24 h was delivered in 8 h. Our infusion rates were lower than gastric emptying rates previously reported in rats (36, 37); recoveries of radioactive lipid were similar over all doses. Moreover, we observed no diarrhea or other indications that the intestine's digestive/absorptive capacity was overwhelmed. It is not clear from the present study what is the maximum output rate for apoA-IV. Krause et al. (38) observed a 400% increase in apoA-IV output in rat mesenteric lymph in response to infusions of Intralipid and olive oil (roughly 1060 μmol/h). Based on previous studies (39) our dose range appears to be on the low end of what has been considered physiological. However, under our conditions, much higher doses did not appear to be warranted; we measured a 245% increase in apoA-IV output in response to a triolein dose of 160 μmol/h, but all animals tested at this dose (n = 3) exhibited diarrhea, and there was a significant amount of lumenal radioactive TG (i.e., undigested) extending into the animals' distal digestive tracts (ileum, cecum/colon) (Kalogeris, T. J., unpublished observations). Because of this observation we did not test higher lipid loads.

Several observations from the lymphatic transport studies suggest that increases in lymphatic A-IV output in response to lipid infusion may be due to recruitment of increasing lengths of intestine as TG dose is increased. First, evidence for increasing recruitment for lipid transport is provided by our measurements of radioactive lipid present in the gut wall under conditions of steady-state transport (Fig. 2). From 5 to 40 μmol/h, there was a progressive increase in the amount of wall lipid in the first quarter of the small intestine (M_1). The second quarter (M_2) did not show significant lipid until 40 μmol/h; at 80 μmol/h there was almost an even split between M_1 and M_2. Second, a reliable statistical relationship between TG transport and A-IV secretion was not observed until steady-state TG transport was achieved. Under these conditions it is likely that the spread of lipid distally will be at a maximum for a given dose. Third, our data on A-IV output in lymph at similar lipid loads (i.e., μmol/h • h) but at presumably different levels of recruitment, support the notion that recruitment, rather than load may be more important. For example, the increment in A-IV output above fasting in response to triolein infusion at 20 μmol/h for 8 h (lymph TG output: 28 ± 4.62 μmol/h) was 143.9 ± 25.1 μg/h, but only 7.3 ± 7.0 μg/h after 80 μmol/h triolein for 2 h (lymph TG output: 21.9 ± 4.33 μmol/h). Finally, results from the synthesis studies (dis-

cussed below) indicate that A-IV synthesis is stimulated in progressively longer lengths of intestine as steady-state TG transport is increased.

Despite the graded response of apoA-IV secretion to triolein dose, measurements of intestinal synthesis of apoA-IV in the proximal jejunum did not show similar dose-dependency; all doses tested produced about a twofold increase in synthesis. The simplest explanation for this result is that lymphatic output of apoA-IV reflects contributions from the entire length of gut involved in apoA-IV secretion, whereas synthesis was measured in a limited segment of jejunum. Rationale for studying apoA-IV synthesis in this segment was twofold: first, a similar segment of proximal jejunum has been used in previous studies of intestinal apoA-IV synthesis and mRNA levels (20, 24). Second, as discussed above, this segment (distal portion of M_1 and proximal portion of M_2) contained most of the radioactive lipid present in the gut wall (Fig. 2), and thus appeared to be the segment most actively involved in lipid transport. The data demonstrate an important point, i.e., apoA-IV synthesis in the proximal jejunum is exquisitely sensitive to low doses of TG. In preliminary studies we have measured the amount of lipid present in 2-cm segments of the intestine from the pylorus to the ileocecal junction after 8-h infusions of triolein at doses from 10 to 80 μmol/h. In the segment of jejunum used in our synthesis studies, these doses produced a 10-fold difference in steady-state wall lipid mass between 10 and 80 μmol/h (Kalogeris, T. J., unpublished observations). Despite this wide range in steady-state mucosal lipid mass (presumably reflecting the rate of lipid transport) in the jejunal segment, the range of triolein doses tested did not produce graded responses in apoA-IV synthesis. Thus, while apoA-IV synthesis in the proximal jejunum is sensitive to low levels of TG, above that threshold, apoA-IV synthesis is maximally stimulated, independent of triolein dose. In order to more rigorously test the question of whether the A-IV lymphatic response to graded doses of triolein reflects recruitment of increasing lengths of intestine, it is important to measure synthesis of A-IV in different regions of the intestine in response to graded doses of infused lipid. This was the rationale for the second set of synthesis studies.

Similar to our previous findings, synthesis of A-IV was approximately doubled in both S_1 and S_2 after triolein infusion at 10 μmol/h; increasing the triolein dose to 40 or 80 μmol/h had no further effect (note that the segment labeled "S_2" is equivalent to the segment of proximal jejunum used for measurement of A-IV synthesis in the previous study). However, A-IV synthesis in a more distal segment (S_3) increased in a graded manner with increasing lipid dose. This result provides strong support for the hypothesis that recruitment of longer lengths of intestine may be sufficient to explain the increases in lymph A-IV transport with increasing levels of steady-state TG transport. However, the present data also suggest that dose-dependent "recruitment" of more distal A-IV-synthesizing mucosa may not be a simple matter of lipid being absorbed and transported by more distal sites. An intriguing finding is that triolein at 80 μmol/h stimulated A-IV synthesis in S_3, despite the fact that the amount of lipid present in that segment was negligible. Figure 4 shows that radioactive lipid in the gut wall under steady-state conditions was recovered essentially all in the first half of the intestine. The third quarter (M_3), containing the S_3 segment from our synthesis studies, contained less than 0.3% of the total infused lipid (roughly 1.8 μmol at a triolein dose of 80 μmol/h). We observed a similar result when we quantified gut wall lipid with 2-cm resolution (Kalogeris, T. J., unpublished observations). In a previous study in rats, Apfelbaum et al. (24) administered 1 g ($>$ 1100 μmol) of TG as an intragastric bolus. Four hours after lipid administration, they measured a 4-fold stimulation of A-IV synthesis in the ileum. The authors did not quantify where in the intestine their lipid load reached. However, assuming gastric emptying was similar to that measured previously (85 μmol/h) (36), and therefore, similar to our highest duodenal dose of 80 μmol/h, it seems unlikely that a significant amount of active lipid transport was occurring in the ileum in the study of Apfelbaum et al. (24). It is possible that the sensitivity of the A-IV response to lipid is even higher than our present data suggest. Alternatively, there may be other factors associated with lipid transport, but independent of the presence of lipid itself, that are capable of stimulating apoA-IV in the rat.

One issue not addressed by the present studies is the route by which apoA-IV enters the lymph. In the rat, not only the intestine but also the liver is a possible source of A-IV (18, 19). As there are well-demonstrated increases in capillary permeability associated with fat feeding (40), it is possible that increases in lymphatic A-IV in response to lipid may be, at least partly, due to leaking of hepatic-synthesized A-IV from blood into lymph. However, since the rats were equipped with lymph fistulas, it is doubtful that significant lipid reached the liver to stimulate A-IV output. Second, studies from this laboratory have shown that under fasting conditions, concentration of A-IV in intestinal lymph is 2- to 3-fold higher than in systemic blood (41). As there is no evidence for active secretion of protein from plasma to lymph, one would not expect significant shunting of liver-derived A-IV into intestinal lymph. Moreover, the presence of a lymph fistula decreases fasting serum A-IV by 60%. Finally, our observations of increased synthesis of A-IV in progressively distal regions of the intestine would appear to be sufficient to explain increases in lymphatic transport of A-IV. Thus, the liver as a significant factor in the present studies appears unlikely.

Although most available data suggest that A-IV is

secreted directly into lymph associated with chylomicrons (1–4), it has been demonstrated by Windmueller and Wu (42) that a considerable amount of intestinal A-IV is also secreted directly into intestinal venous blood. Furthermore, they demonstrated a re-routing of A-IV from intestinal venous blood to lymph in response to fat feeding. It is unknown whether increased direct secretion of A-IV into lymph, or increased re-routing of A-IV from intestinal venous blood to lymph, or both contribute to the increases in lymphatic A-IV transport during lipid absorption. Regardless of the route by which intestinal A-IV gains entry into lymph, our results suggest that increases in intestinal A-IV synthesis may be sufficient to explain increased transport of A-IV in intestinal lymph in response to dietary lipid.

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