Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats

Katsumi Imaizumi, Richard J. Havel, Menahem Fainaru, and Jean-Louis Vigne

Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143

Abstract Transport of apolipoprotein A-I and arginine-rich apolipoprotein in mesenteric lymph was examined in rats given constant intraduodenal infusions of saline, glucose in saline, or emulsified fat. Lymph flow in all groups was constant from 5 to 50 hr after beginning the infusions. Lymphatic transport of triglycerides was about 20-fold greater and transport of apoprotein A-I was about twofold greater in fat-infused rats than in the other two groups. In each group transport of apoprotein A-I bore a significant positive relationship to transport of triglycerides. Lymphatic transport of the arginine-rich apoprotein was only 6–12% of that of apoprotein A-I and was more closely related to lymphatic transport of total protein than to that of triglycerides. In fat-infused rats given [^{3}H]lysine intraduodenally, about two-thirds of the ^{3}H in the chylomicron proteins was in apoprotein A-I and only about 1% was in the arginine-rich apoprotein. Estimated specific activity of chylomicron proteins was highest for apoprotein A-I and apoprotein A-IV, and lowest for the arginine-rich apoprotein and proteins of low molecular weight (mainly C apoproteins). In fat-infused rats given constant intravenous infusions of radioiodinated high density lipoproteins from blood plasma, the specific activity of apoprotein A-I in lymph chylomicrons was only about 5% of that of apoprotein A-I in blood high density lipoproteins, indicating that more than 90% of the apoprotein A-I in chylomicrons was synthesized in the intestine. From these and other data it is concluded that both the intestine and liver are significant sources of apoprotein A-I whereas only the liver synthesizes significant amounts of the arginine-rich apoprotein.

Supplementary key words intestine · chylomicrons · high density lipoproteins · apolipoprotein A-IV · apolipoprotein C · apolipoprotein synthesis

Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoproteins (HDL) in mammalian blood plasma (1). In nascent HDL isolated from perfusates of rat liver, however, the major protein component is the arginine-rich apolipoprotein (ARP) (2–4). The rate of secretion of apoA-I from perfused rat livers is about one-eighth that of ARP (4). For several years, it has been known that a protein resembling apoA-I is present in rat lymph chylomicrons (5, 6) and we (7) and others (8) have recently shown that apoA-I is the major protein component of chylomicrons in rat mesenteric lymph. The distribution of the major soluble protein components of chylomicrons from fat-fed or glucose-fed rats and those of HDL in blood plasma is similar (apoA-I, apoA-IV, ARP, and the proteins of low molecular weight—C apoproteins and apoA-II) (9). Estimates based upon the determined protein composition of large chylomicrons obtained from fat-fed rats (7, 9, 10) and small chylomicrons obtained from glucose-fed rats (9) indicate that transport of apoA-I in lymph is greater than the apparent rate of secretion of apoA-I from the perfused liver (4), whereas lymphatic transport of ARP in triglyceride-rich lymph lipoproteins must be much smaller than the rate of hepatic secretion of this apoprotein. Our measurements of transport of apoA-I and ARP in whole lymph, reported in preliminary form elsewhere (11), have confirmed these calculations.

In this report, we summarize our measurements of transport of apoA-I and ARP in mesenteric lymph of fat-fed, glucose-fed, and fasted rats. We also present the results of radioisotopic experiments which indicate that more than 90% of the apoA-I in lymph is synthesized in the intestine and which support our previous suggestion (9) that lymphatic ARP is derived largely, if not entirely, from lipoproteins in blood plasma.

METHODS

Collection of lymph and separation of lymph lipoproteins

Male Sprague-Dawley rats, weighing 300–350 g and maintained up to the time of lymph duct cannulation on standard Purina rat chow (Ralston Purina

Abbreviations: Apo, apoprotein; ARP, arginine-rich protein; VLDL, very low-density lipoproteins ($d < 1.006$ g/ml); HDL, high density lipoproteins ($1.063 < d < 1.21$ g/ml or $1.09 < d < 1.21$ g/ml); SDS, sodium dodecyl sulfate.

Co., St. Louis, MO), were used in all experiments. The main intestinal lymph duct and duodenum were cannulated in the morning as described previously (9) and the rats were placed in restraining cages. One of three liquid diets was infused through the duodenal cannula at a rate of 2.8 ml hr^{-1} from an infusion pump (Harvard Apparatus Co. Inc., Millis, MA). Diet A contained 0.9% NaCl; diet B contained 0.9% NaCl and 10% glucose; and diet C contained an emulsion of 2% triolein (Grade II, Sigma, St. Louis, MO), 0.04% cholesterol (C.P. ash-free, Pfanstiehl Laboratories, Inc., Waukegan, IL) and 0.4% plant lecithin ("practical", Eastman Kodak Corp., Rochester, NY) together with 2% Intralipid triglycerides (Vitrum, Stockholm, Sweden) in 0.72% NaCl (9). Lymph was collected at intervals of 6–12 hr for 1–3 days in a container placed in ice. Samples of lymph were allowed to clot at room temperature and the fibrin was removed by filtration through glass wool. Lymph lipoprotein fractions were separated by sequential ultracentrifugation in the 40.3 rotor of a Beckman ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA (12). In some experiments large chylomicrons were separated from lymph by brief ultracentrifugation and chromatography on 2% agarose gel (9). In other experiments the total complement of triglyceride-rich lipoproteins from 1 ml of whole lymph was collected directly in the void volume from a 1.2 × 50-cm column of 4% agarose gel (Biogel A-15, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA). The columns were equilibrated and eluted at 18–20°C at a rate of 7 ml/hr with 0.2 M NaCl, 0.002 M EDTA (disodium salt), 0.02% sodium azide, pH 7.0 Fractions of 1.5 ml were collected and elution of triglyceride-rich lipoproteins was monitored by measurement of light scattering (Micronephelometer, Particle Data Inc., Elmhurst, IL).

Preparation of [^{3}H]lysine-labeled chylomicrons

L-[4, 5-^{3}H]Lysine, 83 mCi/mmol, (Amersham/Searle Corp. Arlington Heights, IL) was added to solution C (0.025 mCi ml^{-1}) and the mixture was infused through the duodenal cannula of rats with intestinal lymph fistulae as described above. In preliminary experiments, ^{3}H in lymph chylomicrons increased during the first 2 hr and then remained approximately constant. Therefore, lymph collected 2–5 hr after beginning the infusion was pooled and large chylomicrons were separated as above.

Transfer of proteins of blood HDL to lymph lipoproteins

Blood, obtained by aortic puncture of rats fed a diet rich in saturated fat for 3 days (13), was mixed with 0.1% disodium EDTA. HDL were separated from plasma at 40,000 rpm for 24 hr in the density range of 1.09–1.21 g/ml in the 40.3 rotor of a Beckman ultracentrifuge and recentrifuged at a density of 1.21 g/ml for 48 hr. After dialysis against 0.15 M NaCl, 0.04% disodium EDTA, pH 7.4, the HDL were iodinated with ^{131}I or ^{125}I (carrier-free, Amersham/Searle Corp.) by the iodine monochloride method of MacFarlane (14) as modified for iodination of lipoproteins by Langer, Strober, and Levy (15). Unbound iodine was separated from the iodinated HDL on a column of Sephadex G 10 (Pharmacia Fine Chemicals, Piscataway, NJ). The HDL were then dialyzed against 0.15 M NaCl, 0.04% disodium EDTA, pH 7.4. After dialysis the radioiodinated preparations contained less than 2% unbound iodine, as determined by precipitation with 15% trichloroacetic acid in the presence of carrier albumin, and less than 1.5% of the radioiodine in the lipid moiety, as determined by extraction of lipids into chloroform–methanol 2:1 (v/v).

The solution of radiolabeled HDL was infused at a constant rate of 0.6 ml hr^{-1} (0.2–0.4 μCi ^{131}I or ^{125}I, 0.04–0.08 mg of apoprotein) through a cannula (Silastic tubing, i.d. 0.020 in, o.d. 0.037 in, Dow Corning Corp. Medical Products, Midland, MI) placed in the jugular vein at the time of lymph duct cannulation. The infusion was preceded by a priming dose equivalent to 3–7 times the hourly rate of infusion. The rats were placed in a restraining cage, after lymph duct cannulation as above, and received solution C through the duodenal cannula.

After administering the priming injection of radiolabeled HDL, blood samples of 0.1 ml were withdrawn at intervals of 1–2 hr from an indwelling cannula of Silastic tubing placed in a carotid artery, or from a tail vein, and samples of lymph were collected at intervals of 15–60 min. After 10–12 hr the rats were anesthetized with diethyl ether and exsanguinated from the aorta.

Lymph samples and serum from the final blood sample were subjected to sequential ultracentrifugation as described above and the triglyceride-rich lipoproteins and HDL were purified by recentrifugation under the original conditions. Large chylomicrons were also separated from the lymph as above.

Analyses

Triglycerides and total protein were measured by standard techniques (16, 17)) and apoA-I and ARP were determined by radioimmunoassay (7, 10). For analysis of ^{3}H and radioiodine, blood and lymph lipoproteins were delipidated with ethanol–ether 3:1 (v/v) and ether (18). Apolipoproteins were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel

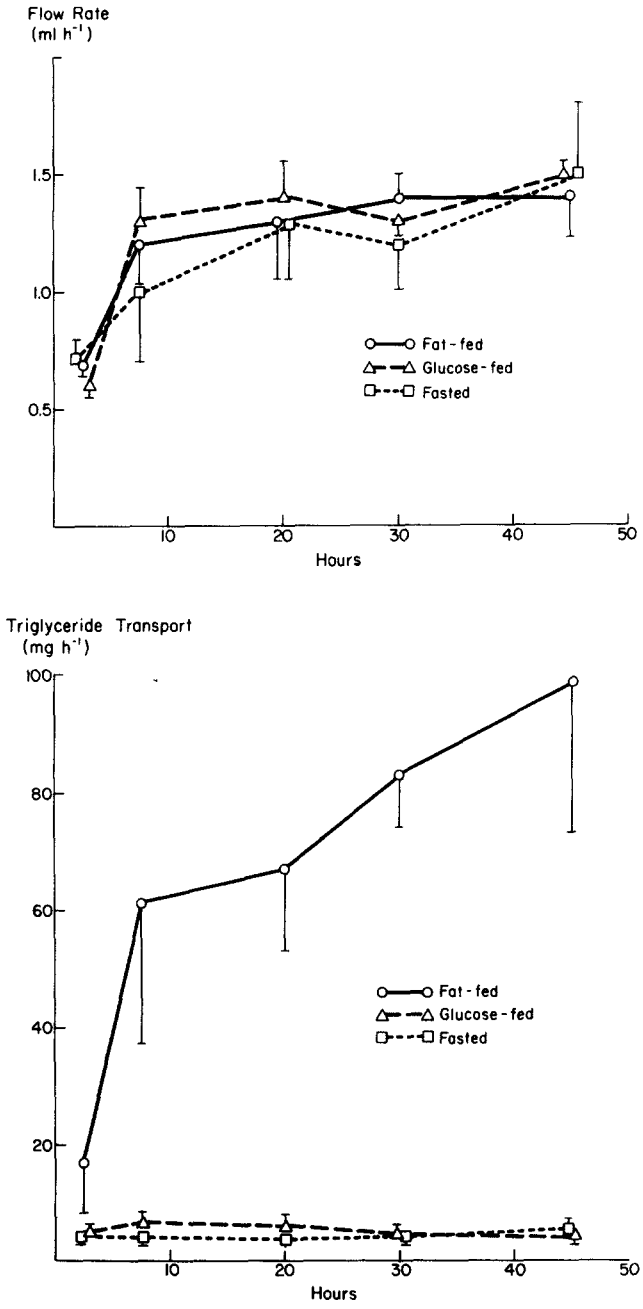

Fig. 1. Rates of lymph flow and triglyceride transport in rats infused intraduodenally with physiological saline (diet A, "fasted"), 10% glucose in saline (diet B), and emulsified fat (diet C). Values are mean for 4–5 animals at each time. Bars indicate SEM.

electrophoresis (19) as described elsewhere (9). Lymph fractions of $1.063 < d < 1.21$ g/ml were mixed with carrier apo HDL from blood serum before electrophoresis. For experiments with [^{3}H]lysine, duplicate gel samples were run and one of each pair was stained with Coomassie brilliant blue (9). The other gel was frozen with dry ice and then sliced with a razor blade into segments 1–2 mm thick. Each segment was dissolved by incubation in a mixture of 0.9 ml of NCS solution (Amersham/Searle Corp.) and 0.1 ml of water at 50°C for 2 hr. After addition of 10 ml of toluene containing 4.8 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)] benzene per 1, ^{3}H was assayed by liquid scintillation spectrometry. The extent of quenching was constant and unaffected by the amount of polyacrylamide present. For experiments with ^{131}I and ^{125}I, segments cut from stained gels were assayed in a gamma scintillation spectrometer.

RESULTS

Transport of apoA-I and ARP in mesenteric lymph

Rates of lymph flow rose during the first few hours after cannulation but remained almost constant during the next 40 hr (**Fig. 1**). Flow rates were similar in fasted rats (infused with physiological saline), glucose-fed rats, and fat-fed rats but rates of triglyceride transport were about 20-fold greater in fat-fed rats (Fig. 1 and **Table 1**). Lymphatic transport of total protein was similar in fasted and glucose-fed rats but was higher in fat-fed rats, particularly during the latter part of the first day of collection (**Fig. 2** and Table 1). Transport of apoA-I was consistently higher in fat-fed rats than in the other two groups and increased progressively with duration of fat-feeding. Rate of transport of ARP was similar in fat-fed and glucose-fed rats, and higher in both than in fasted rats. Within each of the three groups of rats, the rate of transport of apoA-I correlated directly with the rate of triglyceride transport, but not with the rate of transport of total protein (except for a low-order correlation in fat-fed rats) (**Table 2**). Except in fasted rats, the rate of transport of ARP correlated more closely with transport of total protein than with transport of triglycerides. Transport of total protein was positively correlated with triglyceride transport in fasted rats but not in fat-fed rats or those fed glucose.

Distribution of apolipoproteins in lymph lipoproteins (Table 3)

In fat-fed rats more than 80% of apoA-I was in ultracentrifugally separated triglyceride-rich lipoproteins ($d < 1.006$ g/ml). Most of the remainder was in the HDL fraction. In two samples of lymph from fat-fed animals, the ratio of apoA-I to triglycerides in the triglyceride-rich lipoproteins eluted in the void volume from a 4% column of agarose gel was 92 and 82% of the ratio in whole lymph, respectively, in agreement with the results obtained by ultracentrifugation. Recovery of triglycerides in the void volume fraction was 80–90% of that placed on the column. In rats

TABLE 1. Lymphatic transport rates

		Flow Rate	Transport Rates			
			Triglycerides	Total Protein	ApoA-I	ARP
		ml hr^{-1}	*mg hr*$^{-1}$	*mg hr*$^{-1}$	*μg hr*$^{-1}$	*μg hr*$^{-1}$
Fasted	(n = 4)	1.2 ± 0.10	4.0 ± 0.5	7.2 ± 1.4	129 ± 27.1	8.6 ± 3.2
Glucose-fed	(n = 7)	1.3 ± 0.12	4.9 ± 0.8	10.7 ± 1.6	140 ± 19.2	26.0 ± 4.7
	P^a	N.S.[c]	N.S.	N.S.	N.S.	<0.05
Fat-fed	(n = 8)	1.3 ± 0.08	89.9 ± 7.2	17.1 ± 2.5	255 ± 26.6	30.9 ± 4.4
	P^a	N.S.	<0.001	<0.05	<0.05	<0.01
	P^b	N.S.	<0.001	0.06	<0.01	N.S.

Values are mean ± SEM for the period of 5–50 hr after beginning infusion of diet into duodenal catheter.

[a] Significance of difference from fasted rats by Student's t test.

[b] Significance of difference from glucose-fed rats by Student's t test.

[c] Not significant.

fed glucose, about two-thirds of apoA-I was in ultracentrifugally separated triglyceride-rich lipoproteins and the remainder was predominantly in HDL. The distribution of ARP in fat and glucose-fed rats was similar to that of apoA-I.

Incorporation of [^{3}H]lysine into large chylomicrons

As shown in **Fig. 3,** discrete peaks of [^{3}H]lysine were associated with all major apoproteins of large chylomicrons except ARP. When related to the known composition of chylomicron proteins (apoB, 8%; apoA-IV, 8%; ARP, 4%; ApoA-I, 31%; apoC, 38% (9), estimated specific activity was highest for apoA-I and lowest for ARP and proteins of low molecular weight (predominantly apoC).

Transfer of apoproteins from blood to lymph

When radioiodinated HDL were infused intravenously into fat-fed rats bearing mesenteric lymph fistulae, the isotopically labeled apolipoproteins rapidly appeared in lymph (**Fig. 4**). Stable levels of radioiodine were achieved in plasma by a priming injection followed by a constant infusion except in one experiment in which the priming injection exceeded the hourly rate of infusion by a factor of 7. In the other two experiments, this ratio was 3.3. In lymph, levels of radioiodine were generally stable or declined slowly after the third hour of infusion. In blood serum, 86–92% of the radioiodine remained in HDL (1.063 < d < 1.21 g/ml), but in lymph obtained 3–9 hr after beginning the infusion more than 60% was in chylomicrons (d < 1.006 g/ml). About two-thirds of the radioiodine in the blood HDL was in apoA-I and most of the remainder was in apoproteins of low molecular weight (apoC and apoA-II) (**Table 4**). Lymph HDL had an even greater preponderance of radioactivity in apoA-I (85%), but 80% or more of the radioactivity in large chylomicrons and the total d < 1.006 g/ml lipoproteins was in apoproteins of low molecular weight (Table 4 and **Fig. 5**). The specific activity of apoA-I in blood HDL and lymph chylomicrons, determined in each experiment in samples taken 8–10 hr after starting the infusion, is shown in **Table 5.** The specific activity of apoA-I in chylomicrons was 3–6% of that in blood HDL.

DISCUSSION

The incorporation of [^{3}H]lysine into apoA-I of lymph chylomicrons is consistent with other evidence that this protein is synthesized in the intestine. Windmueller, Herbert, and Levy (6) found that this amino acid is incorporated into a protein of intermediate mobility in alkaline urea polyacrylamide gels in chylomicrons secreted from the isolated, perfused intestine and which can now be taken to represent apoA-I. In addition, Rooke and Skinner have shown that [^{35}S]methionine is incorporated into immunoprecipitable A-I by intestinal mucosal scrapings from rats (20) and Glickman and Green have confirmed the presence of apoA-I by immunofluorescence in isolated rat intestinal epithelial cells (8). Glickman and Green also demonstrated the incorporation of [^{3}H]leucine into apoA-I of mesenteric lymph chylomicrons and HDL. The results of our experiments, in which radioiodinated HDL was infused intravenously into rats bearing lymphatic fistulae, indicate further that only about 5% of the apoA-I in lymph chylomicrons has its origin in blood plasma. It is therefore reasonable to conclude that more than 90% of this protein in lymph chylomicrons is synthesized in the intestine.

Our results are consistent with previous evidence (6,21,22) that apoB is synthesized in the intestine and also suggest that apoA-IV is synthesized there. We found some [^{3}H]lysine to be incorporated into the

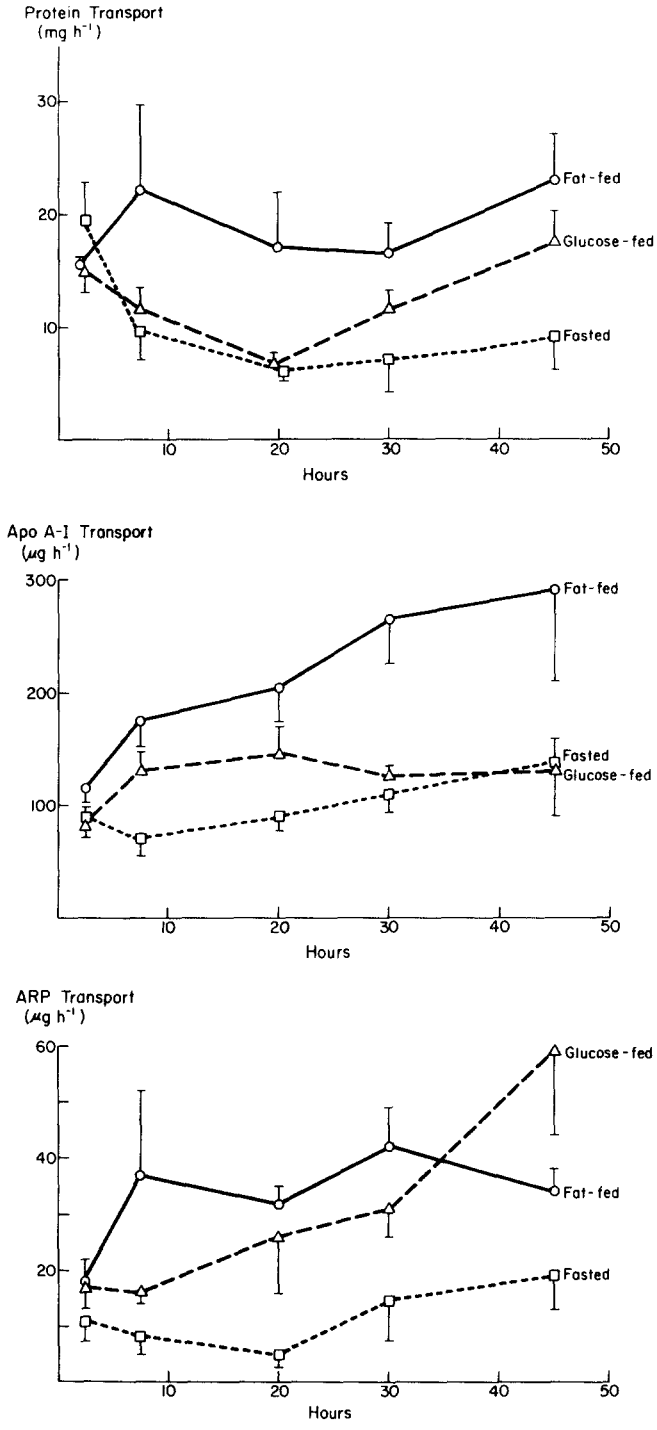

Fig. 2. Rates of transport of total protein, apoA-I, and ARP in lymph of fasted, glucose-fed, and fat-fed rats. Values are mean for 4–5 animals at each time. Bars indicate SEM.

proteins of small molecular weight (mainly C apoproteins), but their estimated specific activity was much lower than that of apoB, apoA-IV or apoA-I. The experiments in which radioiodinated HDL was infused intravenously into rats bearing lymphatic fistulae show that at least some of these proteins are extensively transferred from the blood into intestinal lymph. Our results are therefore not in conflict with the conclusion of Windmueller et al. (6) and of Mahley et al. (22) that these proteins are not synthesized to any extent by the intestine in rats.

We found little or no incorporation of [^{3}H]lysine into ARP of lymph chylomicrons. As with the other apoproteins, some ARP was transferred from blood to lymph. We have previously shown that ARP is transferred to lymph chylomicrons from HDL when chylomicrons are incubated with blood serum (9). The present results therefore suggest that, as with the proteins of low molecular weight which also transfer readily to chylomicrons upon exposure to blood serum lipoproteins, most if not all ARP in lymph is derived from the blood rather than from intestinal synthesis. Estimates based upon the measured radioiodine in proteins of low molecular weight and on their mass indicate that their specific activity in lymph chylomicrons relative to their specific activity in blood HDL was about 10-fold greater than that of apoA-I. Precise calculations from such data would require separate determinations of the specific activity of each species.

The rate of intestinal secretion of apoA-I was fairly steady for 2 days in fasted and glucose-fed rats. During the last day, the rates were closely similar under these two nutritional conditions. This result cannot be taken to indicate that secretion of apoA-I is unaffected by the rate of tissue catabolism, because only about 26 kilocalories of glucose were given daily and the estimated caloric requirement of rats of this weight is about 73 kilocalories per day (23). All rats lost about 30 g during the period of lymph collection, but there was no tendency for the rate of secretion of apoA-I to fall during this time. The fat-fed rats also lost weight, as expected, because most of the fed fat appeared in the drained lymph (fed fatty acids = 109 mg hr^{-1}; average rate of transport of lymphatic fatty acids = 86 mg hr^{-1}). However, rates of lymphatic transport of triglycerides and apoA-I increased progressively during the 2-day period of lymph collection (Figs. 1 and 2). The increased rate of fat absorption may reflect gradual postoperative recovery of absorptive capacity, although lymph flow rates stabilized during the first day of collection.

Secretion of apoA-I was higher in fat-fed rats than in those fed glucose or fasted. This observation agrees with those reported by Glickman and Green (8), but the absolute rates obtained by them with a "rocket" immunoelectrophoretic technique were several fold higher than ours. They found much higher concentrations of apoA-I in lymph lipoproteins of $d > 1.006$ g/ml in both fasted and fat-fed rats. The

TABLE 2. Relationship between transport rates of triglycerides, total protein and apolipoproteins in mesenteric lymph of fasted, glucose-fed and fat-fed rats

x	Total Protein			Triglycerides	
y	Triglycerides	ApoA-I	ARP	ApoA-I	ARP
Fasted	(n = 16)	(n = 14)	(n = 14)	(n = 15)	(n = 15)
a	2.24	0.096	0.001	0.055	−0.004
b	0.21	0.0007	0.0007	0.011	0.004
r	0.63	0.11	0.63	0.58	0.68
P	<0.01	N.S.	<0.05	<0.05	<0.01
Glucose-fed	(n = 20)	(n = 20)	(n = 20)	(n = 22)	(n = 22)
a	5.25	0.12	0.001	0.024	0.028
b	0.003	0.0009	0.005	0.020	−0.001
r	0.006	0.062	0.59	0.76	−0.16
P	N.S.	N.S.	<0.01	<0.01	N.S.
Fat-fed	(n = 19)	(n = 31)	(n = 28)	(n = 19)	(n = 19)
a	39.5	0.13	0.0009	0.037	0.015
b	1.74	0.005	0.001	0.002	0.0002
r	0.44	0.36	0.63	0.80	0.46
P	N.S.	<0.05	<0.01	<0.01	<0.05

Correlation coefficients (r) were obtained by least squares fitting of transport rates (mg hr^{-1}) to the linear regression equation; $y = a + bx$. n = number of paired observations. NS = not significant.

reasons for these apparent differences are obscure. The increased secretion of apoA-I in fat-fed rats suggests that intestinal synthesis of the apoprotein is in part driven by the rate of intestinal triglyceride synthesis or secretion. This interpretation is supported by the observation that, within each group of rats, the two rates were significantly correlated (Table 2). However, a 20-fold increase in triglyceride secretion in fat-fed rats was accompanied by less than a twofold increase in secretion of apoA-I. The mass ratio of triglyceride to apoA-I in large chylomicrons produced during active fat absorption is about 400:1 whereas the ratio in small chylomicrons produced in the absence of dietary fat is about 45:1 (9). The ratio of the rates of lymphatic transport of triglycerides and apoA-I in fat-fed rats was 353:1, and in fasted and glucose-fed rats the ratios were 31:1 and 35:1, respectively (Table 1). Clearly the increased size of chylomicrons during active fat absorption permits greatly increased rates of fat transport with only a small increase in synthesis of the major protein component of the particle. A 20-fold increase in triglyceride transport could occur with no increase in number of chylomicron particles if the volume-average diameter of chylomicrons increased 2.7-fold. The calculated diameter (24) of small chylomicrons produced in glucose-fed rats (9) is ~600 Å and that of fat-fed rats is in the range of 1000–2000 Å (25). It therefore seems possible that the mass of apoA-I per secreted particle varies little under various conditions, i.e., the rate of

TABLE 3. Percentage distribution of apoA-I and ARP in lipoprotein fractions from mesenteric lymph

	Exp. No.	d < 1.006	1.006 < d < 1.063	1.063 < d < 1.21	d > 1.21
ApoA-I:					
Glucose-fed	1	58.1	1.5	38.5	1.9
	2	73.8	4.6	14.1	7.5
Fat-fed	1	96.4	0.7	2.8	0.2
	2	82.4	2.7	14.1	1.3
ARP:					
Glucose-fed	1	50.3	15.3	30.5	4.5
	2	53.0	7.7	29.9	9.4
Fat-fed	1	88.9	4.2	5.7	1.3
	2	98.2	1.2	0.5	0.2

The period (hr) of lymph collection after beginning infusion of diet into duodenal catheter was 10–20 (Exp. 1) and 5–23 (Exp. 2) in glucose-fed rats and 5–20 (Exp. 1) and 5–16 (Exp. 2) in fat-fed rats. Lipoprotein fractions were separated by sequential single ultracentrifugations (12).

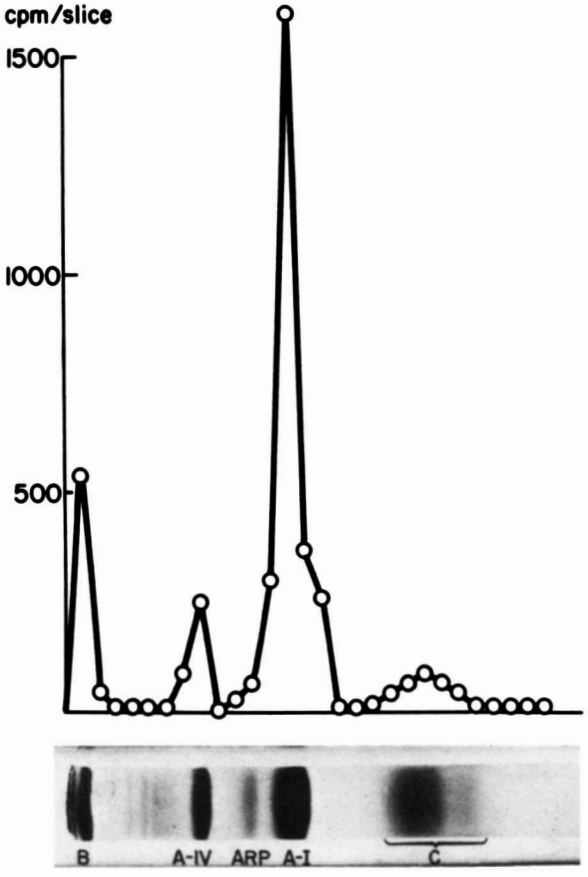

Fig. 3. Distribution of ^{3}H in apoproteins of large chylomicrons separated by brief ultracentrifugation and gel chromatography on 2% agarose from lymph obtained between 2 and 4 hr after beginning intraduodenal infusion of [^{3}H]lysine mixed with diet C. Each point represents cpm in one segment cut from polyacrylamide gel after electrophoresis of chylomicron proteins in SDS. A duplicate gel, stained with Coomassie brilliant blue, was used to locate individual proteins (photograph below graph). 98.5% of the ^{3}H applied to the gel was recovered in the segments and was distributed as follows: apoB, 14.9%; apoA-IV, 9.1%; ARP, 1.4%; apoA-I, 65.8%; apoC, 7.7%.

secretion of apoA-I may be determined mainly by the rate at which triglyceride-rich particles are formed.

In fat-fed rats, more than 80% of the apoA-I in lymph was associated with triglyceride-rich lipoproteins whereas only about two-thirds was so associated in glucose-fed animals. We have previously shown that apoA-I dissociates from large chylomicrons during repeated brief centrifugations (9). The extent to which the recovery of apoA-I in other lipoprotein fractions, particularly HDL, reflects an ultracentrifugal artifact is therefore uncertain. Our observation that not all apoA-I is associated with triglyceride-rich lipoproteins separated from lymph by gel chromatography suggests that some HDL exist in native lymph. Like others (6, 26), we have recovered lipid and protein in the HDL density fraction from mesenteric lymph of fasted or fat-fed rats,[1] but these HDL have not been shown to be secreted as such into lymph from the intestine. At least some components of HDL in blood plasma evidently transverse the intestinal capillary bed. All of the apoproteins of blood HDL appeared in mesenteric lymph after intravenous injection of radioiodinated HDL. Most of these labeled apoproteins were associated with lymph chylomicrons, but the extent of association varied. The labeled C apoproteins were associated with chylomicrons to a greater extent than apoA-I (Fig. 5 and Table 4).

In contrast to apoA-I, lymphatic transport of ARP was related more closely to that of total protein rather than triglycerides. The coefficient of correlation between ARP and triglyceride transport in each of the three groups of rats paralleled that of total protein

[1] Vigne, J. L., and R. J. Havel. Unpublished observations.

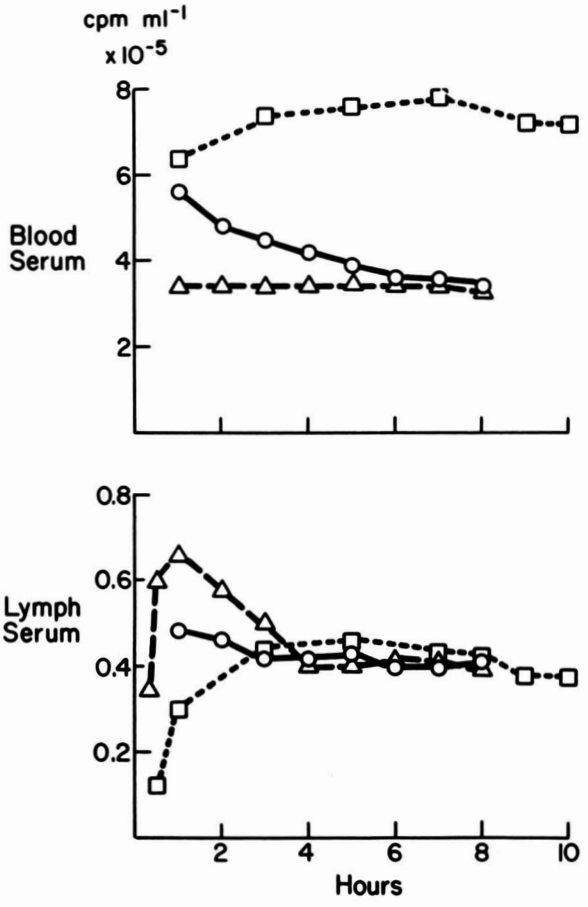

Fig. 4. Concentration of radioiodine in blood and lymph serum of three rats given a priming injection of radioiodinated HDL intravenously followed by a constant infusion for 8–10 hr. Diet was infused through a duodenal cannula throughout. In experiment 1 (○ —— ○) the priming injection of radioiodinated HDL was equivalent to 7 times and in experiments 2 (△ —— △) and 3 (□ —— □) to 3.3 times the hourly rate of infusion.

and triglycerides (Table 2). These correlations are consistent with the isotopic evidence that lymphatic ARP is derived from the blood.

The intestinal contribution to total body synthesis of apoA-I can be estimated by comparing the rates of lymphatic transport of apoA-I with transport of this apoprotein in blood plasma. Sigurdsson, Noel, and Havel (27) estimated transport of the total protein of HDL, in the same strain of rats used by us, from analysis of the removal of radioiodinated, screened HDL ($1.09 < d < 1.21$ g/ml). The data fitted a two-compartmental model and yielded a mean fractional catabolic rate in rats fed ad libitum of 10.9% hr^{-1} and an absolute catabolic rate of 986 $\mu g\ hr^{-1}$. Removal of apoA-I paralleled that of total apo HDL. Hence the rate of synthesis of apoA-I can be estimated from the content of apoA-I in HDL (62% of total protein) to be about 600 $\mu g\ hr^{-1}$. Mean lymphatic transport rates of apoA-I in fasted and glucose-fed rats were 22% and 23% of this value, respectively, suggesting that at least one other major source of this apoprotein exists. Rates of secretion of apoA-I from isolated perfused livers of Long-Evans rats fed a standard chow diet ad libitum are on the order of 75 $\mu g\ hr^{-1}$ (4). It therefore appears that both intestine and liver contribute apoA-I to blood plasma, but the observed rates in the two tissues together account for only about one-half of the rate estimated by Sigurdsson et al. (27). In the experiments with perfused rat livers, no amino acids were supplied in the perfusate nor were hormones added that might stimulate protein synthesis. A recirculating system was used so that the actual rate of secretion may also have been underestimated owing to hepatic uptake of the secreted apoprotein. Experiments in which rat livers were perfused with radioiodinated, screened HDL from blood plasma indicate that the rate of uptake of apo HDL is very low (27), but the possibility remains that nascent HDL secreted from perfused livers are metabolized more efficiently than HDL in blood plasma. Additional research is required to quantify the contribution of intestine and liver under different conditions, but it is evident that a fat-rich diet is likely to increase the intestinal contribution. It should be pointed out that the possibility that apoA-I synthesized in the intestine is transported into the blood via the portal vein or that it is synthesized in tissues other than intestinal mucosa and liver has not been excluded.

The rate of secretion of ARP from perfused rat

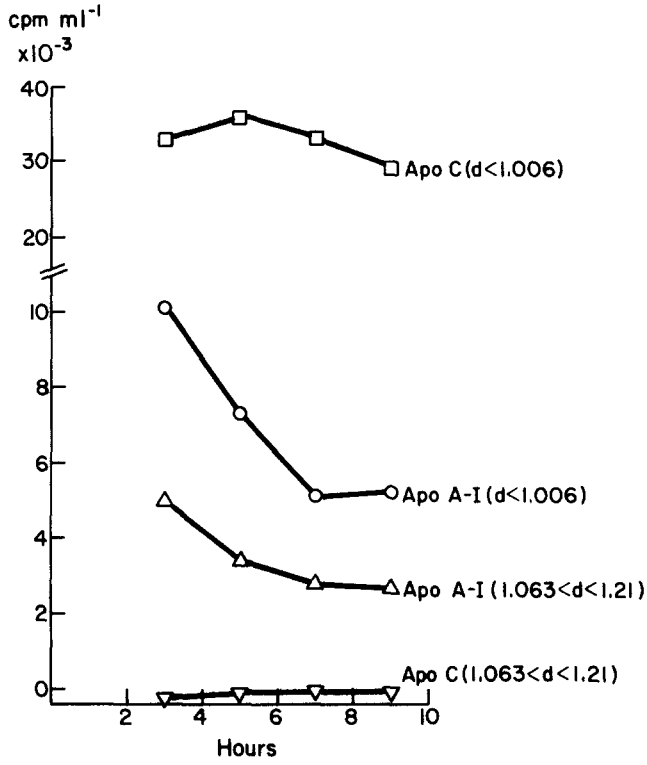

Fig. 5. Concentration of radioiodine (as cpm/ml of unfractionated lymph) in apoA-I and proteins of low molecular weight (apoC + apoA-II) of triglyceride-rich lipoproteins ($d < 1.006$ g/ml) and HDL fraction ($1.063 < d < 1.21$ g/ml) from lymph during intravenous infusion of radioiodinated HDL in a rat receiving diet C intraduodenally.

TABLE 4. Percentage distribution of radioactivity in apoproteins of blood HDL and lymph lipoprotein fractions of fat-fed rats infused intravenously with radioiodinated HDL

	ApoA-I	ApoC + ApoA-II
Infused HDL	54.5 ± 5.6	26.3 ± 1.4
Blood HDL[a] ($1.063 < d < 1.21$ g/ml)	67.8 ± 2.3	21.8 ± 0.7
Large chylomicrons[b]	13.3 ± 3.5	83.5 ± 5.6
Lymph $d < 1.006$ g/ml lipoproteins[b]	19.0 ± 1.1	79.1 ± 1.1
Lymph HDL[b] ($1.063 < d < 1.21$ g/ml)	84.9 ± 2.3	4.2 ± 0.8

Values represent percent of radioiodine added to gels and are mean ± SEM of three experiments. Radioiodine in other apoproteins was insufficient for analysis.

[a] HDL were separated from blood serum obtained at the end of infusions lasting 8–10 hr.

[b] Lymph lipoproteins were obtained from 3 to 8 or 10 hr after beginning the infusions.

TABLE 5. Specific activity of apoA-I in blood HDL and lymph chylomicrons of fat-fed rats infused intravenously with radioiodinated HDL

Exp. No.	Specific Activity		$\frac{a}{b} \times 100$
	Chylomicrons (a)	HDL (b)	
	$cpm\ mg^{-1} \times 10^{-4}$		
1	2.52	43.8	5.8
2	0.78	28.1	2.8
3	3.08	74.8	4.1

livers is about 500 μg hr^{-1} (4). This rate greatly exceeds that of lymphatic transport of ARP. Therefore, even if a fraction of the ARP transported in mesenteric lymph is derived from intestinal synthesis, the intestinal contribution to the synthesis of ARP in blood must be negligible.

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