Apoprotein composition and turnover in rat intestinal lymph during steady-state triglyceride absorption

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Abstract Apoproteins of chylomicrons, very low density lipoprotein (VLDL), and a low density + high density fraction secreted by proximal and distal rat small intestine into mesenteric lymph were examined during triglyceride (TG) absorption. Apoprotein output and composition were determined and the turnover rates of labeled non-apoB (soluble) apoproteins in lipoprotein fractions were measured after an intraluminal [^{3}H]leucine pulse during stable TG transport into lymph. The output of VLDL apoproteins exceeded that of chylomicrons during the absorption of 45 μmol of TG per hour. More [^{3}H]leucine was incorporated into VLDL than into chylomicrons and the decay of newly synthesized VLDL apoproteins was more rapid than that of chylomicrons, in part due to higher concentrations of apoA-I and apoA-IV with a rapid turnover rate. Chylomicrons from proximal intestine contained more apoA-I and less C peptides than chylomicrons from distal intestine. Ninety percent of [^{3}H]leucine incorporated into soluble apoproteins was in apoA-I and apoA-IV, but little apoARP was labeled. The turnover rate of apoA-I and apoA-IV differed significantly in the lymph lipoproteins examined. Although total C peptide labeling was small, evidence for intestinal apoC-II formation and differing patterns of apoC-III subunit labeling was obtained. [^{3}H]Leucine incorporation and apoprotein turnover rates in lipoprotein secreted by proximal and distal intestine were similar. The different turnover rates of apoA-I and apoA-IV in individual lipoproteins suggest that these A apoproteins are synthesized independently in the intestine.—**Holt, P. R., A-L. Wu, and S. Bennett Clark.** Apoprotein composition and turnover in rat intestinal lymph during steady-state triglyceride absorption. *J. Lipid Res.* 1979. **20:** 494–502.

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Increasing experimental evidence indicates that the small intestine is important in the formation of intact lipoproteins and of apolipoproteins (1). Lipoproteins secreted into intestinal lymph are added to the pool of circulating lipoproteins. They contain apoproteins that are rapidly transferred to plasma lipoprotein fractions (2) and may modulate hepatic (3) and extrahepatic cholesterogenesis (4). Studies with isolated perfused intestinal segments have shown that VLDL, LDL, and HDL can be recovered from draining mesenteric lymph (5, 6). Immunofluorescent studies on intestinal tissue (7, 8) and data on lymph lipoprotein labeling after administration of a [^{3}H]amino acid pulse (9) suggest that B apoprotein and apoA-I can be synthesized in the intestine. Since the completion of the present studies, the presence of apoA-IV also has been demonstrated in intestinal tissues and in intestinal lymph (10, 11). In previous experiments, conditions of constant and stable triacylglycerol (TG) absorption and secretion into lymph at high rates were not used and apoprotein turnover studies of individual apoproteins have not been performed.

Previous studies in this laboratory (12–14) have shown that distal rat intestine has a relatively limited capacity to secrete TG into lymph when compared with proximal small intestine. This difference was observed in rats both with (13) and without (14) lymph fistula and appeared to be an intrinsic property of this portion of the intestine (15). Electron microscopic examination of distal intestinal mucosa revealed massive accumulations of TG during trioleoyl glycerol absorption (14), suggesting that distal chylomicron formation and/or secretion might be relatively defective. In the present study, we have examined lipoprotein apoprotein composition and output rates in lymph draining either proximal or distal small intes-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triacylglycerol; PL, phosphoglyceride; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; SDS, sodium dodecyl sulfate; TO, trioleoylglycerol; ApoARP, arginine-rich peptide (apoE).

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tine, using identical and stable perfusion conditions in order to determine whether regional differences might suggest a mechanism for defective secretion of distal chylomicrons. In addition, apoprotein turnover rates were estimated from the rate of ^{3}H-labeled apoprotein decay after a [^{3}H]leucine pulse during stable TG transport into lymph.

MATERIALS AND METHODS

Animals and operative procedure

Male Wistar rats (Charles River Breeding Labs, Inc., Wilmington, MA) weighing 280–320 g were used. The preparation of animals and the experimental procedures have been described previously (12). Briefly, each animal received four cannulas into *1*) the mesenteric lymphatic duct for lymph collection, *2*) the common bile duct for bile and pancreatic juice diversion, *3*) and *4*) two intraintestinal infusion cannulas inserted into either the duodenum (proximal) or the intestinal midpoint (distal) for pancreatic juice return and saline–glucose (basal) or lipid infusion. On the experimental day (48 hr after surgery), trioleoylglycerol (TO) (Sigma Chemical Co., St. Louis, MO; purity >95% as stated by the supplier), emulsified in a solution of glucose (4.5 g/100 ml), vegetable lecithin (Eastman Kodak Co., Rochester, NY; 1.2 g/100 ml), and Pluronic F-68 (Wyandotte Chemical Co., Wyandotte, MI; 0.3 g/100 ml) was infused for 6–7 hr at 45 μmol TO/hr using a constant infusion pump (Harvard Apparatus Co., Mills, MA). At this infusion rate, we have previously determined TO absorption of 95.5–98.7% and lymph recovery of 92–95% (13). Two hours after the beginning of the TO infusion, 1 mCi of L-[4,5-^{3}H]leucine (>60 μCi/mmol; New England Nuclear, Boston, MA; purity >98% as stated by the manufacturer) in 0.5 ml of saline was injected through the intestinal infusion cannula and flushed with 0.2 ml of physiological saline. The TO infusion was continued for 4–5 hr longer. Lymph was collected continuously in hourly aliquots, on ice, into graduated centrifuge tubes containing disodium ethylenediaminetetraacetate (EDTA) (final conc. 0.1 g/100 ml).

Isolation of lymph lipoproteins

Lipoprotein fractions were isolated from hourly lymph collections by sequential ultracentrifugations (16), using a SW 41 rotor in a Beckman L2-65B preparative ultracentrifuge (Beckman Instrument Inc., SPINCO Div., Palo Alto, CA). Lymph, overlayered with a solution of EDTA–NaCl (0.1 g/100 ml–0.85 g/100 ml) of density 1.006 g/ml, was centrifuged at 3×10^6 g-min for isolation of chylomicrons. The top 0.7 ml of chylomicron fraction was sliced off and the subnatant was centrifuged at 1.0×10^8 g-min to float up VLDL. In two pairs of proximally and distally infused rats, lipoprotein particles with densities between 1.006 and 1.21 g/ml (LDL + HDL) were further separated. The VLDL subnatants were adjusted to a density of 1.21 g/ml with solid KBr and the LDL + HDL were floated at 2.8×10^8 g-min. In selected studies, serum lipoproteins were separated by ultracentrifugation into fractions d < 1.006 g/ml (VLDL), d 1.006–1.063 g/ml (LDL), and d 1.063–1.21 g/ml (HDL) (17). All lipoprotein fractions were washed twice by recentrifugation through EDTA–NaCl solution of the appropriate densities. Washed lipoproteins were dialyzed exhaustively against 2 l (three changes) of 0.9 g/100 ml NaCl containing 0.05 g/100 ml EDTA at 4°C. Total protein content of lipoproteins was measured by a modified method of Lowry et al. (18) using bovine serum albumin as standard.

Polyacrylamide gel electrophoresis

Twice-washed lipoproteins were partially delipidated with 10 vol of diethyl ether and heated at 90°C for 3 min in 1% SDS and 5% mercaptoethanol. PAGE was performed in 10% acrylamide (Eastman Kodak Co., Rochester, NY) gels containing 0.1% SDS (BDH Chemical Ltd., Poole, England) using a modification (19) of the method of Shapiro, Vinuela, and Maizel (20). Approximately 20–30 μg of protein containing 10^3–10^6 dpm were applied to each gel. The gels were stained for 1–2 hr with 0.2% Coomassie brilliant blue in ethanol–acetic acid–water 45:10:45 and were destained with several changes of 10% acetic acid. In preliminary experiments, nondelipidated and partially delipidated lipoproteins containing isotopically labeled apoproteins obtained after intraduodenal injection of [^{3}H]leucine were electrophoresed in parallel. The percentage of distributions measured either densitometrically or by ^{3}H incorporation did not differ between nondelipidated and partially delipidated samples. Thus, delipidation caused no specific apoprotein losses. Because the apoprotein bands obtained from nondelipidated lipoproteins were less sharply defined, subsequent PAGE was performed on partially delipidated samples.

All lipoprotein fractions revealed four major apoprotein bands by SDS PAGE (**Fig. 1**). The apparent molecular weights determined by comparison with purified standard proteins of known molecular weight, namely, bovine serum albumin, egg albumin, pepsin, trypsin, chymotrypsin, and cytochrome *c* were 45,000, 34,000, 26,000, and less than 12,000. The apoproteins of molecular weights between 45,000 and

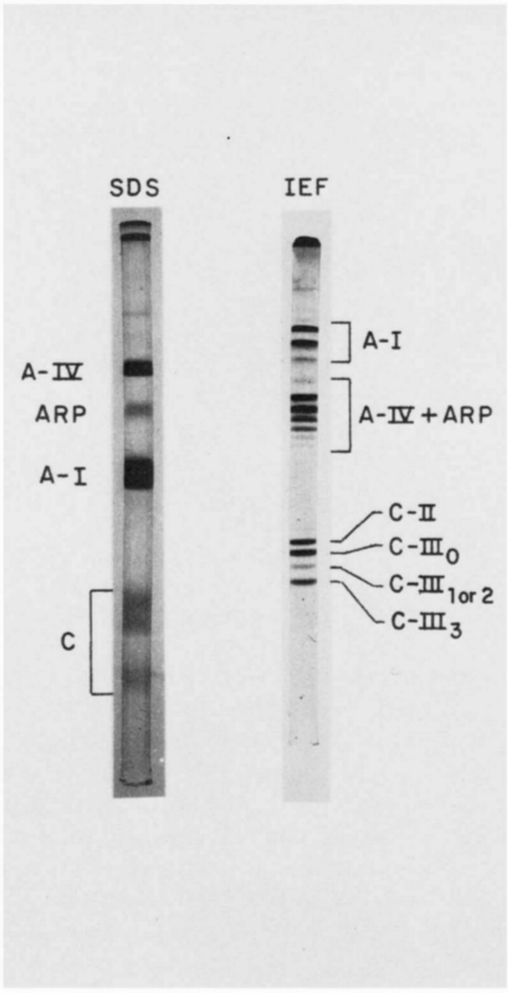

Fig. 1. Typical pattern of VLDL apoproteins separated by SDS PAGE and IEF. On left, SDS polyacrylamide gel electropherogram (approximately 25 μg of protein applied to gel) and on right, isoelectric focusing gel (approximately 80 μg of protein applied to gel) of VLDL obtained from lymph during steady state TO absorption. For details see Methods. ApoB in the SDS electropherogram is represented near the top of the gel.

26,000 were further identified as apoA-IV, ARP, and apoA-I, respectively, by co-electrophoresis against immunologically pure rat apoproteins.[3] The molecular weight range of the apoprotein of less than 12,000 which was partially separated into two bands was consistent with that previously reported for C peptides (21, 22). Variable amounts of a band running proximal to apoA-IV with a molecular weight of 58,000–62,000 was greatly reduced by agarose column chromatography (using Bio-Gel A-50 m 100–200 mesh) and may represent small amounts of contaminating serum protein (23). In preliminary studies, the content of B apoproteins was determined indirectly by the difference between the total protein and the fraction soluble in 50% tetramethyl urea (24).[4]

C apoproteins were further resolved by isoelectric focusing (IEF) in 8% acrylamide and 6 M urea (ultrapure, Schwarz Mann, Orangeburg, NY) deionized before use by passage through Rexyn I-300 column gels according to Gidez, Swaney, and Murnane (21) (Fig. 1). Partially delipidated lipoprotein samples (50–100 μg of protein) were applied to the gels in 6 M urea containing buffer. Gels were focused for 5 hr, fixed with 12% trichloroacetic acid (which also removes the ampholines), rinsed well with water, stained with Coomassie blue, and destained in 30% methanol and 10% acetic acid, followed by 10% acetic acid. Four major C peptide bands were identified according to the classification of Swaney and Gidez (22) as C-II, C-III$_0$, C-III$_1$ or C-III$_2$, and C-III$_3$ with isoelectric points, respectively, of 4.70, 4.61, 4.54, and 4.46.

The stained gels were scanned at 550 nm using a Corning densitometer (Model 750, Corning Scientific Instruments, Medfield, MA). The staining affinity for each individual peptide was not determined in the present study; however, when 20–100 μg of total protein was applied from a single stock of chylomicron and VLDL apoproteins, the chromagenicity of each major band varied linearly with the amount of total protein applied and the percentage distribution of the bands varied by less than 3% over this range. For individual bands the coefficient of variation ranged between 2 and 15% of the mean percentage contributed by that band. Furthermore, apoprotein samples from proximally and distally perfused groups were always electrophoresed in parallel. Comparisons of apoprotein compositions among the samples were therefore made using the densitometer tracings directly.

Determination of apoprotein radioactivity

Bands were sliced from stained gels, digested in 25 μl of 60% perchloric acid, and decolorized with 300 μl of 30% hydrogen peroxide according to Mahin and Lofberg (25). The resultant clear, colorless solutions were counted directly in a liquid scintillation solution (Ready Solv HP, Beckman Instrument Inc., Palo Alto, CA) in a Beckman three-channel liquid scintillation system (LS-250). Aliquots of washed lipoprotein fractions also were counted before partial delipidation in HP scintillation cocktail to determine the total apoprotein ^{3}H content. Count rates obtained were corrected to disintegration rates (dpm) using the external standard ratio method, according to standard

[3] We are indebted to Dr. R. Mahley, N.I.H., for these studies.

[4] We are indebted to Dr. P. Roheim, Albert Einstein School of Medicine, for these determinations.

quench curves prepared with 0.15 M NaCl or perchloric acid and hydrogen peroxide as quenching agents.

Calculations

The total apoprotein contents of the twice-washed chylomicrons, VLDL, and LDL + HDL fractions of the hourly lymph collections were measured and the apoprotein specific activities (^{3}H dpm per μg total apoprotein) were determined. The specific activity of each apoprotein band was expressed as the ^{3}H dpm in that band per μg of total apoprotein applied to the gel. B apoproteins did not consistently enter the gels and were not routinely determined. Since there was no significant trend with time in the distribution of the soluble apoproteins after the ^{3}H pulse in the chylomicron, VLDL, or LDL + HDL fractions and the total apoprotein outputs also remained constant, we assumed there was also no trend with time after the [^{3}H]leucine pulse in the percent of nonsoluble (B) apoproteins. Moreover, in preliminary experiments,[4] no differences were found in proximal and distal B apoprotein content of chylomicrons collected in the third and fourth hours after the [^{3}H]leucine pulse.

Peak ^{3}H incorporation into total apoproteins usually occurred in the first hourly lymph collection. The specific activity of each apoprotein band at the time of peak ^{3}H incorporation was taken as 100% and subsequent hourly collections were calculated as the percentage of the specific activity of that band. The specific activity decay rates were determined from the slopes of the linear regression of the logarithm of the percentage specific activity of each apoprotein band against time. For analysis of individual apoC peptides, after separation by isoelectric focusing, the specific activity was calculated from the percentage of total C peptide ^{3}H per band and the percentage of total C peptide densitometric staining per band. Student's *t* test and paired *t* tests were used to determine statistical significance.

RESULTS

Incorporation into lymph lipoproteins of an intravenous [^{3}H]leucine and an intraluminal [^{14}C]leucine pulse

In preliminary experiments, the relative incorporation into lymph lipoproteins of a simultaneous pulse of [^{3}H]leucine (200–500 μCi) given intravenously and [^{14}C]leucine (50 μCi) given intraduodenally was determined in animals perfused for 6 hr either with or without a TO emulsion. ^{3}H appearance in chylomicron and VLDL apoproteins was relatively delayed compared to that of ^{14}C. The ratio of ^{3}H:^{14}C in lipoproteins increased steadily during the perfusion, but within 5–6 hr it had not reached the ^{3}H:^{14}C ratio of the administered leucine (**Fig. 2**), suggesting preferential incorporation of intraduodenally administered ^{14}C leucine into lymph apolipoprotein. Because of these preliminary observations, the labeled precursor amino acid ([^{3}H]leucine) was pulsed only into the intestinal lumen in subsequent studies.

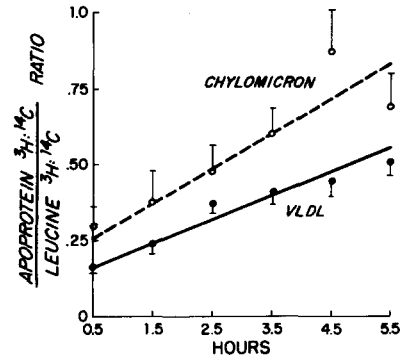

Fig. 2. Ratio of ^{3}H:^{14}C in lymph VLDL and chylomicron apoproteins after simultaneously administered pulse of [^{3}H]leucine given intravenously and [^{14}C]leucine given intraduodenally. Mean ± SEM of chylomicron (– – –) from four rats and VLDL (——) from five rats during perfusion of an 0.85% saline–1% glucose solution or a TO emulsion (45 μmol/hr). Data points are entered at the midpoints of the hourly collection periods.

Apoproteins of mesenteric lymph during proximal or distal TO perfusion

No significant differences in the total apoprotein output of lymph chylomicrons, VLDL, and the LDL + HDL fractions of proximally and distally infused rats were found (**Table 1**). In both groups, VLDL apoprotein output exceeded that of chylomicron apoproteins by over 50%. As much as 58.6 (distal) to 64.8 (proximal) percent of the luminally pulsed ^{3}H appearing in total lymph apoproteins was found in VLDL, whereas only about 20% was present in chylomicrons, and 15–21% in the LDL + HDL fractions. Thus, both the largest amount of protein and the highest specific activity after the [^{3}H]leucine pulse were found in the VLDL fraction under the conditions used (steady TO perfusion at 45 μmol/hr). The rate of decay in VLDL total apoproteins specific activity was faster than that of chylomicron total apoproteins in all animals ($P < 0.005$) (**Fig. 3**), indicating more rapid turnover of VLDL apoproteins. No differences in total apoprotein decay in either lipoprotein fraction were found comparing proximal and distal intestinal perfusion (Fig. 3). The ^{3}H decay rate in LDL + HDL total apoprotein also was slower than that of VLDL; however, the difference did not reach statistical significance ($P < 0.075$) possibly because the d > 1.006 g/ml fraction was analyzed only in two pairs of animals.

TABLE 1. Mesenteric lymph apolipoprotein output from proximal and distal intestine during constant intraduodenal perfusion of a TO emulsion

	Chylomicrons	VLDL	LDL + HDL[a]
		μg per hr	
A. Proximal (4)	201.2[b] (15.9)[c]	358.4 (21.3)	253.5 (51.7)
Distal (4)	244.8 (26.2)	323.9 (34.3)	261.0 (90.3)
		% total lipoprotein apoproteins	
B. Proximal (4)	24.7	44.1	31.2
Distal (4)	29.5	39.1	31.4
		% total ^3H in lipoprotein apoproteins	
Proximal (4)	19.6	64.8	15.6
Distal (4)	20.3	58.6	21.1

A. Protein output determined from mean of 3–5 hourly lymph specimens analyzed in duplicate in each animal. Difference between proximal and distal intestine not significant.

B. Distribution of ^3H incorporation after an intraluminal ^3H pulse measured in duplicate at the time of peak incorporation in the first hourly collection.

[a] Four duplicate specimens from two animals in each group.

[b] $\bar{X}$ (SEM).

[c] Number of animals shown in parentheses.

Chylomicrons from proximal intestine contained significantly more apoA-I and less C peptides than chylomicrons from distal intestine (**Table 2**). VLDL apoproteins from the proximal intestine also contained somewhat more apoA-I and significantly less C peptides than distal intestinal VLDL (**Table 3**). Ninety percent or more of total peak soluble ^3H apoprotein label was incorporated into the apoA-I and apoA-IV fractions of chylomicrons (Table 2), VLDL (Table 3), and the LDL + HDL lipoprotein fractions (**Table 4**). ApoARP and the C peptides were relatively poorly labeled in all the lipoproteins fractions analyzed.

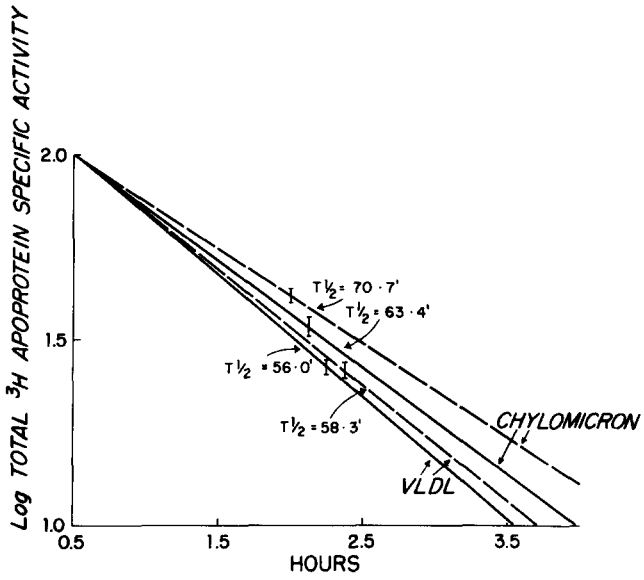

Fig. 3. Decay rates of chylomicron and VLDL total ^3H-labeled apoproteins appearing in mesenteric lymph after an intraintestinal [^3H]leucine pulse during constant TO perfusion. "Specific activity" of ^3H-labeled apoprotein collected hourly for 4 hr after the [^3H]leucine pulse. Mean of four proximally infused (– – –) and four distally infused (——) animals. ±S.E. of the slope.

Turnover rates of individual apoproteins

No differences between proximally and distally infused animals were found in the ^3H decay rates of any of the apoprotein fractions examined by SDS gel electrophoresis; consequently, data from both groups were combined. The mean decay rates for apoA-IV, ARP, apoA-I, and apoC in the individual lipoprotein fractions examined are shown in **Fig. 4.** Overall, the decay rates of labeled A-I and A-IV were much more rapid than those of the other apoproteins. The chylomicron apoA-I decay rate was significantly faster than that of VLDL apoA-I in the same lymph collection ($P < 0.02$). In lymph HDL from both proximally and

TABLE 2. Distribution and peak ^3H incorporation into chylomicron apoprotein after intraluminal [^3H]leucine pulse

Apoprotein Bands (SDS PAGE)	Distribution of Densitometric Areas[a]			[^3H]Leucine Labeling of Apoproteins[b]	
	Proximal (4)[c]	Distal (4)	P vs. D	Proximal (4)	Distal (4)
	%			%	
ApoA-IV	18.4 (2.0)[d]	18.6 (2.4)	NS	29.0 (2.4)	36.6 (3.1)
ApoARP	5.6 (1.7)	7.4 (2.1)	NS	2.7 (0.0)	2.5 (1.3)
ApoA-I	51.0 (3.4)	39.6 (1.7)	$P < 0.05$	62.6 (0.6)	55.0 (2.6)
ApoC	25.0 (2.1)	34.3 (1.9)	$P < 0.025$	5.8 (0.3)	6.0 (2.5)

[a] Distribution of mass determined from densitometric areas in three to five duplicate scans for each animal.

[b] Distribution of ^3H incorporation measured at the time of peak incorporation, usually in the first hour after the pulse.

[c] Number of animals.

[d] $\bar{X}$ (SEM).

TABLE 3. Distribution and peak ^3H incorporation into lymph VLDL apoprotein after intraluminal [^3H]leucine pulse

Apoprotein Bands (SDS PAGE)	Distribution of Densitometric Areas[a]			[^3H]Leucine Labeling of Apoproteins[b]	
	Proximal (4)[c]	Distal (4)	P vs. D	Proximal (4)	Distal (4)
	%			%	
ApoA-IV	27.4 (1.4)[d]	23.3 (2.2)	NS	33.7 (6.3)	37.1 (3.8)
ApoARP	12.7 (4.5)	15.1 (4.7)	NS	2.8 (0.7)	1.9 (0.4)
ApoA-I	46.1 (2.9)	40.5 (3.4)	NS	54.5 (7.3)	54.0 (0.5)
ApoC	13.8 (1.0)	21.1 (0.9)	$P < 0.005$	3.9 (0.3)	1.9 (0.3)

[a] Distribution of mass determined from densitometric areas in three to five duplicate scans for each animal.
[b] Distribution of ^3H incorporation measured at the time of peak incorporation in the first hour after the pulse.
[c] Number of animals.
[d] $\bar{X}$ (SEM).

distally perfused rats, apoA-IV decay rates were found to be faster than that of apoA-I, in contrast to the findings in chylomicrons and VLDL.

In the three lipoprotein fractions, the slopes of total C peptide specific activity decay did not differ significantly from zero. The decay rate of the small amount of labeled apoARP also was very slow. In proximal and distal chylomicrons, apoARP half-times could be calculated as 87.5 and 105 min, respectively, but did not differ from zero in the VLDL and the LDL + HDL fractions.

The specific activities of the individual chylomicron apoC peptides separated by isoelectric focusing did not differ in proximally and distally infused animals. The data from the four hourly collections from both groups were, therefore, also combined. Striking differences in labeling of individual C peptides were noted (**Fig. 5**). There was a rapid decrease in the specific activity of the apoC-II peptide, in contrast to the finding in apoC-III_3 which showed a progressive increase in specific activity. The labeling for the apoC-III_0 and the apoC-III_1 or apoC-III_2 peptides remained quite constant throughout the 4-hr collection after the intraluminal [^3H]leucine pulse. The ^3H labeling patterns of C peptides separated by IEF in the VLDL and LDL + HDL lipoprotein fractions were similar to those found in chylomicrons.

DISCUSSION

The present studies were designed, in part, to explore whether differences in apoprotein synthesis rates might be responsible for the different proximal and distal intestinal secretion of chylomicrons into mesenteric lymph described in this laboratory (14). Apoprotein turnover rates were compared after an intraluminal [^3H]leucine pulse administered 2 hr after beginning a steady TO infusion into either the proximal or distal intestinal lumen. Under these conditions, a relative delay in distal chylomicron transport into lymph previously has been demonstrated (13). No

TABLE 4. Distribution and peak ^3H incorporation into LDL + HDL apoprotein after intraluminal [^3H]leucine pulse

Apoprotein Bands (SDS PAGE)	Distribution of Densitometric Areas[a]			[^3H]Leucine Labeling of Apoproteins[b]	
	Proximal (2)[c]	Distal (2)	P vs. D	Proximal (2)	Distal (2)
	%			%	
ApoA-IV	13.6 (0.8)[d]	7.6 (0.5)	$P < 0.025$	40.1 (3.2)	41.3 (1.1)
ApoARP	14.8 (0.9)	23.0 (0.2)	$P < 0.02$	1.4 (0.1)	3.9 (0.3)
ApoA-I	66.5 (5.3)	63.7 (3.8)	NS	57.6 (4.2)	57.9 (3.0)
ApoC	5.2 (1.2)	5.6 (0.1)	NS	0.8 (0.1)	1.0 (0.1)

[a] Distribution of mass determined from densitometric areas in three to five duplicate scans for each animal.
[b] Distribution of ^3H incorporation measured at the time of peak incorporation in the first hour after the pulse.
[c] Number of animals.
[d] $\bar{X}$ (SEM).

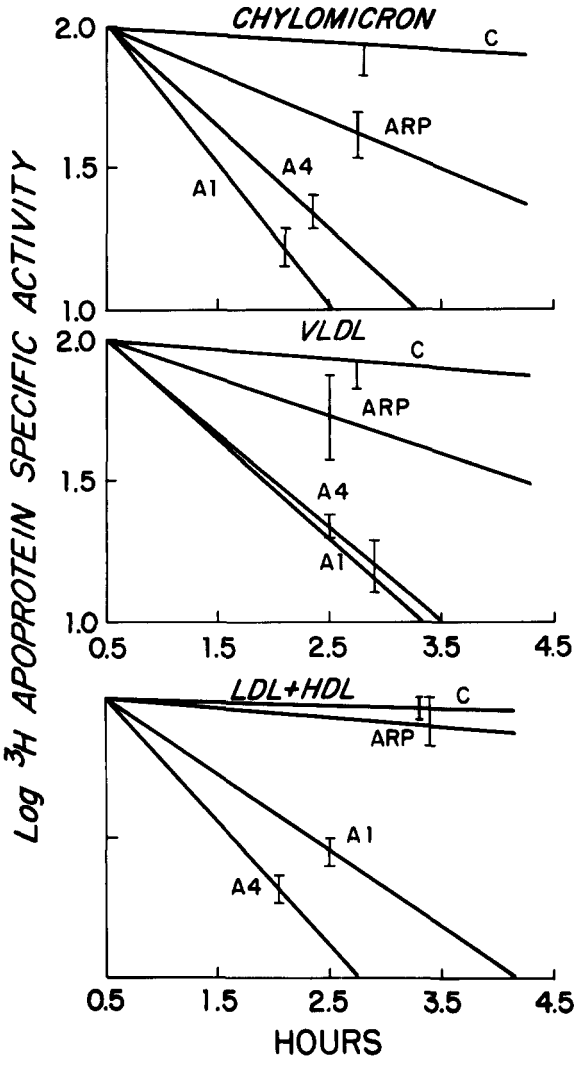

Fig. 4. Decay rates of major lymph chylomicron, VLDL, and d > 1.006 g/ml ^{3}H-labeled soluble apoproteins separated by SDS PAGE after an intraintestinal [^{3}H]leucine pulse during constant TO perfusion. Data from proximally and distally infused animals combined. Eight animals used for chylomicrons, six for VLDL, and four for d > 1.006 g/ml lipoproteins. ^{3}H "specific activity" of each apoprotein fraction determined as described in Methods. ±SEM of the slope.

differences between proximal and distal intestine in chylomicron total apoprotein output rates or [^{3}H]-leucine incorporation were found. The secretion rates into proximal or distal mesenteric lymph of the other apolipoprotein classes also did not differ. These data make it unlikely that the delay in distal intestinal secretion of chylomicrons is due to a relative defect in total apoprotein synthesis.

Recent experiments have shown that the small intestine is an important site of lipoprotein synthesis (6, 9, 11, 26, 27). In the present studies, during absorption of 45 μmol of TO per hr, the output of VLDL apoprotein was greater than that recovered in chylomicrons. Peak specific activity of VLDL apoproteins exceeded that of chylomicron apoproteins by over 100% and the decay rates of [^{3}H]leucine-labeled VLDL apoproteins were significantly faster than those of chylomicrons. These observations could result from independent intestinal chylomicron and VLDL formation or from differences in lipoprotein apoprotein composition with different turnover times. The major apoprotein separated by SDS PAGE in both chylomicrons and VLDL were apoA-I and apoA-IV, and the decay rates of these labeled A apoproteins were much faster than those of the other apoproteins. ApoA-I and apoA-IV represented 61–64% of the total densitometric area in VLDL and only 54–58% in chylomicrons. Thus the more rapid decay of total VLDL than of chylomicron proteins can be explained in part by higher concentrations of rapidly turning over individual labeled apoproteins. When the ^{3}H decay rates of apoproteins in different lipoprotein classes were measured in the same animals, the turnover of apoA-I and apoA-IV differed markedly. For example, chylomicron labeled apoA-I decay was significantly faster than that of VLDL apoA-I. Furthermore in VLDL and chylomicrons, labeled apoA-I and apoA-IV decay rates were similar whereas in d > 1.006 g/ml fraction (LDL + HDL) apoA-IV decay was significantly faster than that of apoA-I. These observations indicate that apoproteins A-I and A-IV

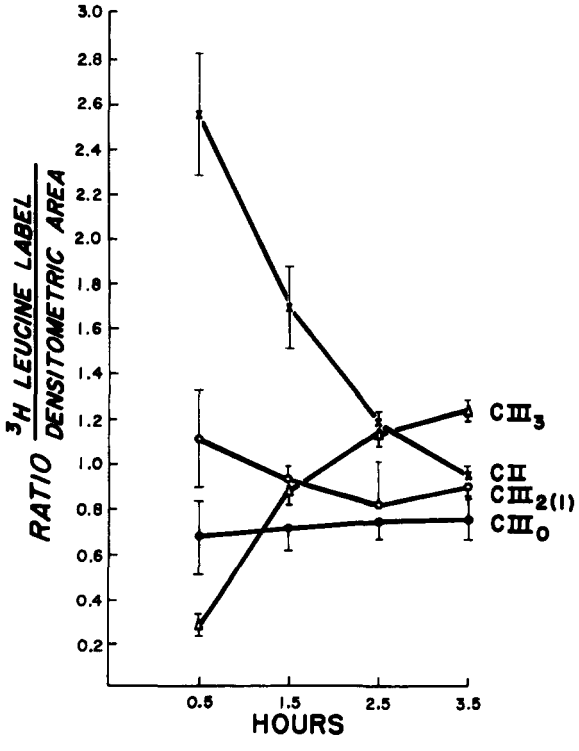

Fig. 5. ^{3}H "specific activity" of chylomicron C peptides separated by isoelectric focusing collected at hourly intervals after a [^{3}H]-leucine pulse during constant TO infusion. Data from two proximally and two distally infused animals combined (±SEM).

destined for incorporation into different lipoproteins may be derived from separate intestinal pools.

The ^{3}H-labeled B apoproteins were not separately isolated in the present studies. In preliminary experiments, the B apoprotein content of chylomicrons from proximal and distal intestine was determined from the difference between tetramethylurea-soluble and insoluble protein content.[4] No differences in B apoprotein concentration between proximal and distal chylomicrons were found. It can be deduced that the decay of ^{3}H-labeled B apoproteins was also rapid in the present studies, in agreement with previous observations (23), since there was little difference between the decay rates of total VLDL apoprotein (t½, approximately 57 min) and that of the major labeled apoA proteins (t½, 49–53 min).

Less than 10% of peak soluble apoprotein labeling was found in ARP and the C peptides. Most experiments suggest that apoARP is not synthesized in the intestine but in the liver (28, 29). Peak specific activity of total C peptides was low in agreement with previous studies which concluded that C peptides were not synthesized by the intestine (5, 23, 26) but were transferred to lymph lipoproteins from circulating lipoproteins (30, 31). However, lymph lipoprotein total C peptide ^{3}H did not fall during the constant TO perfusion. After 4–5 hr of lymph collection, up to 20% of total soluble ^{3}H-labeled apoprotein was present in C peptides. When C peptides were separated by IEF, three turnover patterns were reproducibly detected in all lipoprotein classes. Peak specific activity of the apoC-II band occurred in the initial lymph collection and decreased rapidly, paralleling our observations on apoA decay. On the other hand, apoC-III$_3$ specific activity was minimal initially and rose steadily during the perfusion, but C-III$_0$ and C-III$_1$ or C-III$_2$ labeling was stable throughout. These contrasting observations strongly suggest that apoC-II can be synthesized in the intestine whereas apoC-III$_3$ is probably derived from extra-intestinal sources. The sources for apoC-III$_0$ and apoC-III$_1$ or apoC-III$_2$ found in mesenteric lymph are uncertain. Additional supporting evidence for intestinal chylomicron and VLDL C peptide synthesis comes from studies using the in vivo intestinal segment preparation with high rates of triglyceride perfusion (11). Comparison of lymph and serum lipoprotein composition in patients with chylous ascites also suggests that the human intestine may be a source of C peptide formation (32). It is possible that earlier studies failed to observe labeling of the apoC peptides from precursor amino acids because intestinal synthesis of lipoproteins is not stressed unless TG is perfused continuously at high rates and labeling of fractions with a slow turnover rate otherwise might escape notice. C peptide transport from the intestine directly into capillaries has not been excluded in the present or previous studies. More total C peptides were found in chylomicrons and VLDL recovered from distal intestinal lymph than in proximal lymph. No regional differences were found in the distribution of individual chylomicron and VLDL C peptides separated by IEF.[5] Although our studies indicate that some C peptides are synthesized by the intestine, it is likely that most C peptides found in lymph lipoproteins originate in the circulation (33). The present studies do not explain the mechanism for the difference in C peptide content between proximal and distal lipoprotein. Lymph flow from proximal and distal intestine during TO absorption did not differ, excluding prolonged contact time for apoprotein exchange. On the other hand, distal intestinal chylomicrons do differ in composition, having a lower phospholipid content which might facilitate the transfer of C peptides from other lipoproteins in lymph. Distal intestinal chylomicrons have also been shown to be larger than those derived from proximal intestine.[5] It is of interest that Havel (33) has reviewed data which in general indicate a greater C peptide concentration in large than in small chylomicrons. Whether these compositional differences in distal chylomicrons might alter their subsequent metabolism in the circulation (34) remains to be elucidated.

In summary, the present studies failed to disclose any differences in apolipoprotein turnover in lipoproteins formed by proximal and distal intestine, suggesting that altered apoprotein synthesis is not responsible for a regional change in intestinal fat transport into lymph. Rapid synthesis of several intestinal apolipoproteins during constant fat transport has been demonstrated. The possible quantitative contribution of these intestinally derived apoproteins to systemic apolipoprotein concentrations awaits further study. Our observations that apoproteins destined for incorporation into different intestinal lipoproteins have different turnover rates suggest that they may be derived from separate pools and thus may be susceptible to independent control. Experiments to determine whether dietary manipulation may alter intestinal apoprotein synthesis are presently underway.

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[5] Holt, P. R., A-L. Wu, and S. Bennett Clark. Unpublished observations.

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