Transport of Apolipoproteins A-I and A-II by Human Thoracic Duct Lymph

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ABSTRACT The daily transport of human plasma apolipoproteins A-I and A-II, triglyceride, and total cholesterol from the thoracic duct lymph into plasma was measured in two subjects before and three subjects after renal transplantation. Lymph triglyceride transport was ~83% of the daily ingested fat loads, whereas lymph cholesterol transport was consistently greater than the amount of daily ingested cholesterol. Lymph apolipoprotein transport significantly ($P < 0.05$) exceeded the predicted apolipoprotein synthesis rate by an average of 659±578 mg/d for apolipoprotein A-I and 109±59 mg/d for apolipoprotein A-II among the five subjects. It is estimated that 22–77% (apolipoprotein A-I) and 28–82% (apolipoprotein A-II) of daily total body apolipoprotein synthesis takes place in the intestine.

Lymph high density lipoprotein particles are mostly high density lipoprotein$_{2b}$ and high density lipoprotein$_{2a}$ and have a greater overall relative triglyceride content and a smaller relative cholesteryl ester content when compared with homologous plasma high density lipoproteins. The major quantity of both lymph apolipoprotein A-I (81±8%) and apolipoprotein A-II (90±11%) was found within high density lipoproteins with almost all of the remainder found in chylomicrons and very low density lipoproteins.

The combined results are consistent with a major contribution of the intestine to total body synthesis of apolipoprotein A-I and apolipoprotein A-II. An important role of lymph in returning filtered apolipoprotein to plasma in association with high density lipoproteins is proposed. Accompanying the return of filtered apolipoprotein to the plasma is a probable transformation, both in size and composition, of at least some of the lymph high density lipoprotein$_{2b}$ and high density lipoprotein$_{2a}$ particles into high density lipoprotein$_3$.

INTRODUCTION

The biosynthesis of apolipoproteins (apo)[1] A-I and A-II in man and their subsequent incorporation into plasma high density lipoproteins (HDL) is presently an area of active research. In the rat, both hepatic parenchymal and absorptive epithelial cells of the small intestine have been shown to synthesize apoA-I (1–6). Human apoA-I and apoA-II have been localized by immunochemical techniques in intestinal epithelial cells (7–9). Although some apolipoproteins synthesized in the intestine may enter the blood plasma directly (6), a major portion of the apolipoproteins is thought to be carried by mesenteric lymph lipoproteins into the thoracic duct lymph and subsequently into the venous circulation (2, 4–6, 8).

Human thoracic duct lymph contains lipoproteins of all density classes (10) and a majority of the circulating apolipoproteins (11), including apoA-I and apoA-II. Thoracic duct lymph, which is an admixture of lymph from abdominal organs (e.g., liver and intestine) and the periphery, flows into the blood circulation at a rate of between 50–100% of the plasma volume per day (12). Upon mixing with blood, the apoA-I and apoA-II car-

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[1] *Abbreviations used in this paper*: apo, apolipoprotein; HDL, high density lipoprotein; DTNB, 5,5'-dithionitrobenzoic acid; LCAT, lecithin-cholesterol acyltransferase; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

ried by the lymph can be incorporated into plasma HDL by two mechanisms: (*a*) transfer to plasma HDL from less dense lipoproteins such as chylomicrons (13, 14); and (*b*) as components of lymph HDL that is transformed into plasma HDL. Plasma HDL, in turn, may reach the lymphatic circulation by filtration from peripheral and visceral capillary beds (15). Specifically, an indirect indication of apoA-I and apo-A-II transport between plasma and lymph in man comes from four different metabolic studies (16–19). In each of these studies, compartmental analysis of apolipoprotein catabolism data required the labeled apoA-I or apoA-II in plasma to exchange with an extravascular space. The lymph likely occupies a sizeable portion of that space. Determination of the temporal apoA-I and apoA-II contribution by human thoracic duct lymph to blood circulation is therefore critical in assessing not only the quantitative aspects of lymph apolipoprotein incorporation into plasma HDL, but also the extent of daily intestinal synthesis of these apolipoproteins and the extent of daily lymph apoA-I and apoA-II filtration from plasma.

In the following work, we have undertaken to quantitate the daily transport of apoA-I, apoA-II, triglyceride, and total cholesterol from thoracic duct lymph into plasma, and to determine the distribution of these components among lymph lipoprotein classes. In addition, the composition and physical properties of lymph HDL are compared with those of plasma HDL.

METHODS

Description of subjects. Lymph was obtained by cannulation of the thoracic duct in five subjects undergoing lymph drainage for purposes of immunosuppression either before kidney transplantation (subjects A and B) or after transplantation (subjects C, D, and E). Subjects A and B, both males ages 33 and 45 yr, were dialyzed three times per week. For these subjects, 24-h lymph collection was begun 1 d after dialysis. For subjects C, D, and E, males age 18, 22 and 27 yr, 24-h lymph collection was begun 6 (C), 10 (D), and 11 d (E) after successful kidney transplantation. These three subjects had relatively normal renal function (blood creatinine ≤ 1.5 mg/dl and blood urea nitrogen ≤ 30 mg/dl). The five subjects maintained constant weight on an *ad lib* diet of 2,000–3,700 kcal/d. Dietary cholesterol intake was estimated from standard tables (20) as 0.25–0.75 g/d. Plasma triglyceride and cholesterol concentrations, shown in Table I, were within normal ranges for age and sex-matched controls (21). Furthermore, the concentrations of both lipids did not vary greatly during the 24-h collection period for subjects B, C, D, and E. These data were not available for subject A. The plasma concentrations of apolipoproteims A-I and A-II are also given in Table I. They were also similar before and after the 24-h collection period for subjects B, C, D, and E. The normal range in our laboratory for plasma apoA-I is 131.0±16.0 (SD) mg/dl and for apoA-II, 28.0±2.8 mg/dl (22).

Lymph collection. For each subject lymph collection was begun just before the morning meal and carried out continuously for 24 h in a series of from 8 to 15 sterile collection bags. Noon and evening meals were taken at 12:00 a.m.–1:00 p.m. and 5:00–6:00 p.m., respectively. A sample for analysis (20 ml) was withdrawn from each bag. After lymphocyte removal, the remaining lymph (200–700 ml) was sterilized and returned to the subject by continuous intravenous infusion within 3–4 h of its initial collection. Each subject had been maintained on this schedule for at least 2 wk before the 24-h period, during which samples for analysis were taken. Thus the loss of lymph lipoprotein material to the subject was insignificant. All samples for analysis collected from a given patient were then recombined in proportion to the particular fraction of daily lymph output volume that they represented. In this way, one sample representing the patients' average lymph composition over the 24-h period was obtained. This sample was used for each of the analyses described below.

Electron microscopy. Samples for electron microscopy were prepared according to the negative stain technique described by Forte and Nichols (23).

Lipid and protein analysis. Phospholipid concentrations in lymph and plasma samples were determined as lipid phosphorus by the method of Chalvardjian (24). Total cholesterol and triglyceride concentrations were measured using the Autoanalyzer II (Technicon Instruments, Inc., Tarrytown, N. Y.) (25). Unesterified cholesterol was measured with the Beckman oxygen-enzymic analyzer (26) (Beckman Instruments, Inc., Fullerton, Calif.) and cholesteryl ester calculated as 1.68 × (total cholesterol − unesterified cholesterol). Total protein was determined by the method of Lowry et al. (27).

Analytic ultracentrifugation. Analytic ultracentrifugation of both lymph and plasma samples was performed and the computer-derived schlieren patterns were obtained by pro-

TABLE I

Plasma Lipid and Apolipoprotein Concentrations before and after Lymph Collection

Subject	Plasma apoA-I concentration		Plasma apoA-II concentration		Plasma triglyceride concentration		Plasma cholesterol concentration	
	Before	After	Before	After	Before	After	Before	After
	mg/dl		*mg/dl*		*mg/dl*		*mg/dl*	
A	143±3	NA	50±3	NA	165±5	NA	128±4	NA
B	126±3	130±3	38±3	36±3	203±6	195±3	159±5	152±5
C	157±3	150±3	24±2	19±2	100±3	84±3	142±5	151±5
D	102±2	110±3	21±2	21±2	101±3	79±3	125±4	128±4
E	132±3	135±3	31±3	33±3	103±3	156±5	168±5	182±5

Values are the means and standard errors of three determinations. NA, not available.

cedures (28) that corrected for the concentration dependence of the flotation rate and the Johnston-Ogston effect. HDL_{2b}, HDL_{2a}, and HDL_3 concentrations were determined as previously described (29).

Apolipoprotein quantitation. The concentration of apoA-I and apoA-II in all samples was measured both by electroimmunoassay (30) and radial immunodiffusion (31). All samples were delipidated with methanol/diethyl ether (3:7), and the protein was resolubilized in 0.05 M sodium barbital, 8 mM sodium azide, pH 8.3, for immunochemical measurement. Apolipoprotein standards were obtained as previously described (32, 33), and their concentrations determined by amino acid analysis on a Beckman model 121 Automatic Amino Acid Analyzer (Beckman Instruments, Inc.).

Calculation of apolipoprotein kinetic parameters. Predicted apolipoprotein synthesis rates (PASR) were calculated with the following formula:

$$\text{PASR} = \frac{\text{plasma volume} \times \text{plasma apolipoprotein concentration}}{\text{average apolipoprotein residence time}}.$$

The plasma volume was estimated as 0.045 × body weight. The body weight of each subject is given in Table IV. Average apolipoprotein residence times from a study on 10 normal individuals were 4.69±0.94 (SD) d for apoA-I and 5.07 ±1.06 d for apoA-II (18). Lymph apolipoprotein transport was calculated as the product of the daily volume of lymph flow and the lymph apolipoprotein concentration. The daily volumes of lymph flow are given in Table II.

Column chromatography. Gel permeation chromatography was performed with a 100 × 1.2-cm column (Kontes Co., Vineland, N. J.) of Sepharose 6B-CL (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in NaCl-EDTA buffer (0.85% NaCl, 0.01% EDTA, 1 mM sodium azide, pH 7.4) at room temperature (24°C). Sample volumes applied to the column were 5 ml, and flow rates were ~21 ml/h. Preparations of HDL and low density lipoproteins (LDL) applied to this column were isolated from a normolipemic individual by ultracentrifugation as described below.

Lipoprotein preparations. Lymph and plasma were collected in heparinized containers with a final heparin concentration of 1 U/cm³. To inhibit the action of lecithin:cholesterol acyltransferase (LCAT), 5,5′-dithionitrobenzoic acid (DTNB) in 0.75 M phosphate buffer, pH 7.4, was added during collection of selected aliquots of lymph to a final concentration of 1 mM. Lymph chylomicrons were isolated as the upper 5-ml fraction after 25 ml of whole lymph, layered beneath 5 ml of NaCl-EDTA buffer, was centrifuged for 3 × 10^6 *g*-min. After chylomicron removal, the lymph was subjected to sequential ultracentrifugation at densities 1.006, 1.063, and 1.21 g/ml (Beckman 40.3 rotor, Beckman Instruments, Inc.) for the isolation of very low density lipoproteins (VLDL), LDL, and HDL (34). All isolated density fractions were dialyzed against NaCl-EDTA buffer before immunochemical quantitation. Heparin-Mn^{2+} precipitation of whole lymph was performed according to the method outlined by the Lipid Research Clinics (35).

RESULTS

Electron microscopy. The morphology of lipoproteins from whole thoracic duct lymph collected in the presence or absence of DTNB appears similar. Particles in the size ranges of chylomicrons, VLDL, and LDL are easily identified in negatively stained preparations of whole lymph with DTNB (Fig. 1a). Particles 10 nm in diameter, presumed to be HDL, are also present in Fig. 1a, but are more difficult to discern because of their small number and the proteinaceous background. The HDL isolated from lymph (Fig. 1b) with or without DTNB are spherical, with a mean diameter of 10.2±1.3 (SD) nm. Discoidal HDL particles, such as those recently described in HDL from rat mesenteric lymph isolated in the presence of an LCAT inhibitor (36), were not observed.

Lymph transport of triglyceride and cholesterol. The daily transport of triglyceride and cholesterol by the lymph into plasma was calculated from the average lymph triglyceride and cholesterol concentrations in Table II. As was found by other investigators (37), the ratio of triglyceride to cholesterol varied between 40:1 and 90:1 for the five subjects. Lymph triglyceride transport varied between 65 and 145 g/d, but represented ~83% of ingested fat for each subject. Lymph cholesterol transport varied between 0.75 and 3.10 g/d.

Analytic ultracentrifugal characterization. In Fig. 2 the schlieren patterns of lymph lipoproteins are compared with those of plasma lipoproteins for subjects B, C, D, and E. Over 98% of the lymph total lipoprotein mass was found in the chylomicron fraction. No chylo-

TABLE II
Lymph Triglyceride and Cholesterol Transport

Subject	Average lymph triglyceride concentration	Average lymph cholesterol concentration	Daily volume of lymph flow	Lymph triglyceride transport	Lymph cholesterol transport	Amount fat consumed
	mg/dl	*mg/dl*	*dl/d*	*g/d*	*g/d*	*g/d*
A	3,113±93	75±2	30.2	94.0±2.8	2.26±0.06	105 (2,400)*
B	2,716±81	65±2	30.3	82.0±2.5	1.97±0.06	98 (2,300)
C	690±21	8±2	94.1	64.9±2.0	0.75±0.02	78 (2,064)
D	2,252±68	28±2	60.4	136.0±4.1	1.70±0.05	167.2 (3,150)
E	1,279±38	28±2	112.2	143.5±4.3	3.10±0.09	166.4 (3,744)

Values are means±standard deviations of three determinations.
* Total calories given in parentheses.

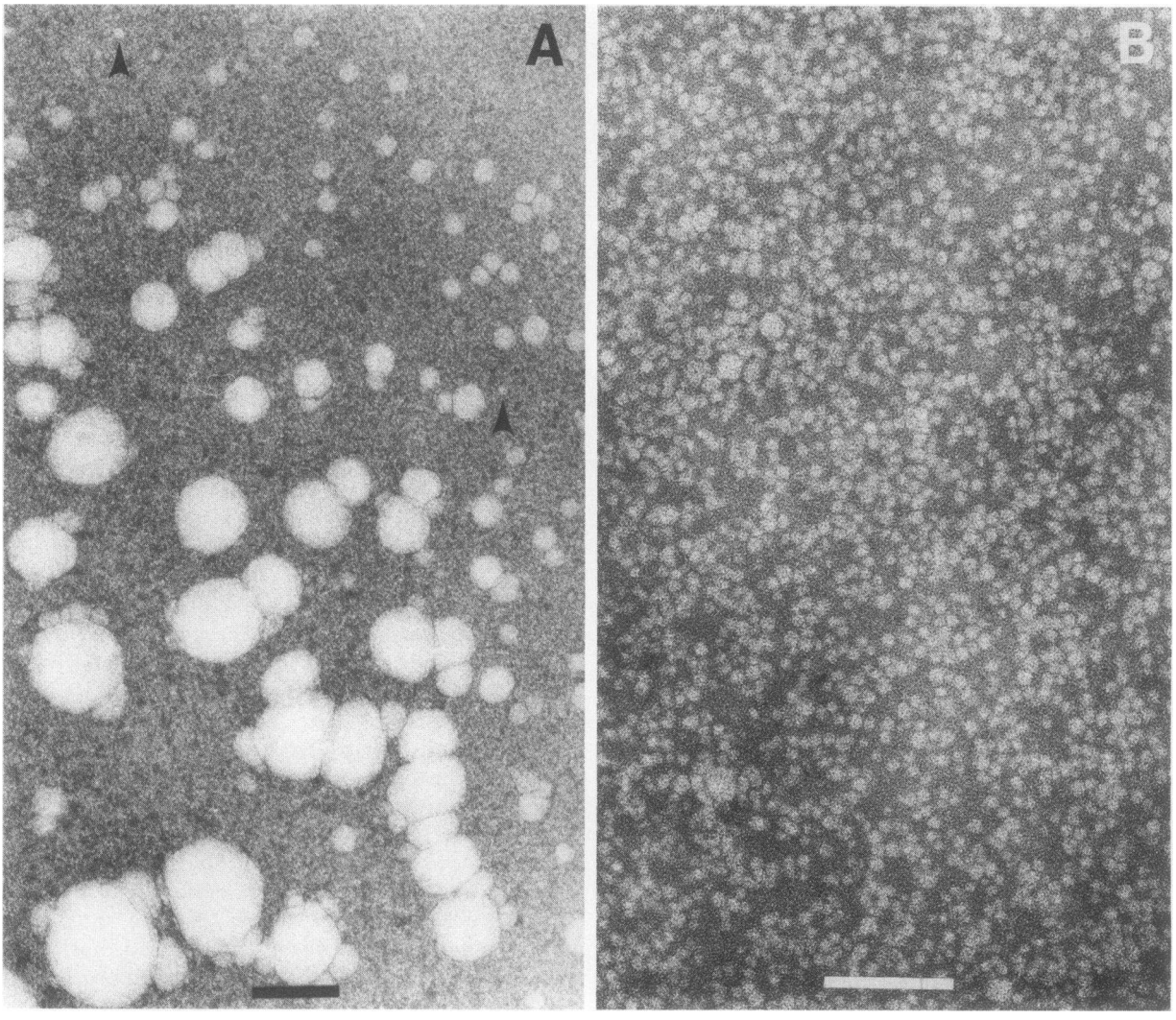

FIGURE 1 Electron microscopy of negatively stained lymph lipoproteins. Micrograph A: whole lymph collected in DTNB. Note the heterogeneity of particle size, which extends from chylomicron-sized particles to almost imperceptible (arrows) HDL-sized particles. Micrograph B: the HDL fraction isolated from lymph collected in DTNB. In both micrographs the bar markers represent 100 nm.

microns were detected in the subjects' fasting plasmas. Similarly, lymph VLDL concentrations were 320–600% higher than plasma VLDL concentrations. Lymph VLDL also displayed a greater proportion of faster floating particles (S_f^0 100–400)[2] than plasma VLDL, which were chiefly slower floating particles (S_f^0 20–100). In contrast, lymph LDL concentrations were only 0.4–9.1% of plasma LDL concentrations. Except for subject C, from whom essentially no schlieren pattern was obtained, the peak flotation rates of lymph LDL were very similar to those of plasma LDL. Lymph HDL concentrations, as with lymph LDL, were only a fraction of plasma HDL concentrations (8–20%). In Fig. 3 the proportion of lymph HDL that exhibits the faster flotation rates of HDL_{2b} and HDL_{2a} (79–100%) is greater than that of plasma HDL (48–67%).

HDL composition. The weight percent composition of lymph and plasma HDL for subjects B, C, D, and E is given in Table III. The consistently lower values for protein content in lymph HDL (38–46%) compared with plasma HDL (50–54%) are consonant with the relatively greater proportion of HDL_{2b} and HDL_{2a} in lymph than plasma. This observation also relates to the mean diameter observed for lymph HDL

[2] S_f^0 is the low density lipoprotein flotation rate, expressed in sverdbergs (10^{-13} centimeters per second per dyne per gram), fully corrected for concentration dependence in a NaCl medium of density 1.063 g/ml at 26°C (1.748 M NaCl).

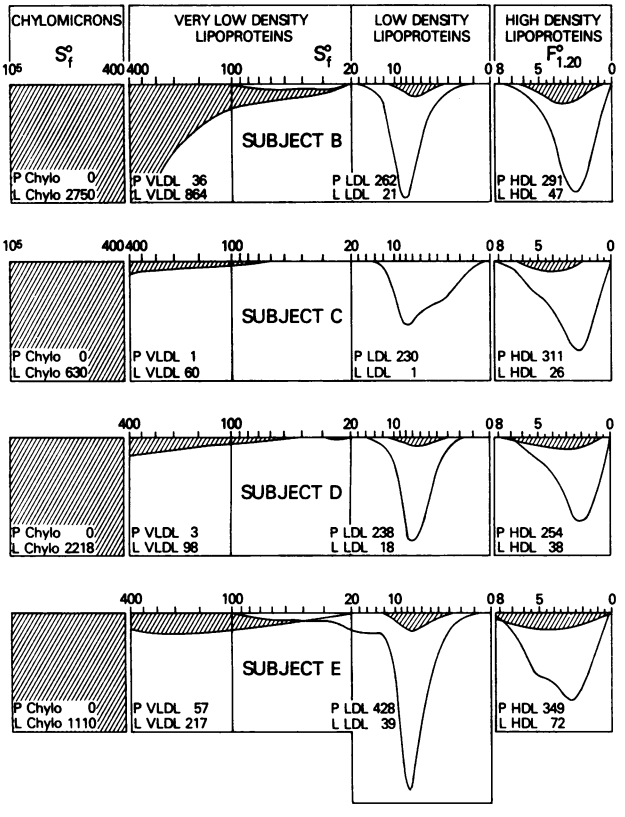

FIGURE 2 Schlieren patterns of homologous lymph and plasma lipoproteins. Crosshatched areas represent lymph schlieren patterns. Concentrations of lipoprotein classes are given as total lipoprotein mass in milligrams per deciliter. Material from subject A did not suffice for this analysis. $F^0_{1.20}$ is the high density lipoprotein flotation rate in a NaCl/NaBr medium of density 1.200 g/ml at 26°C (0.195 M NaCl, 2.762 M NaBr).

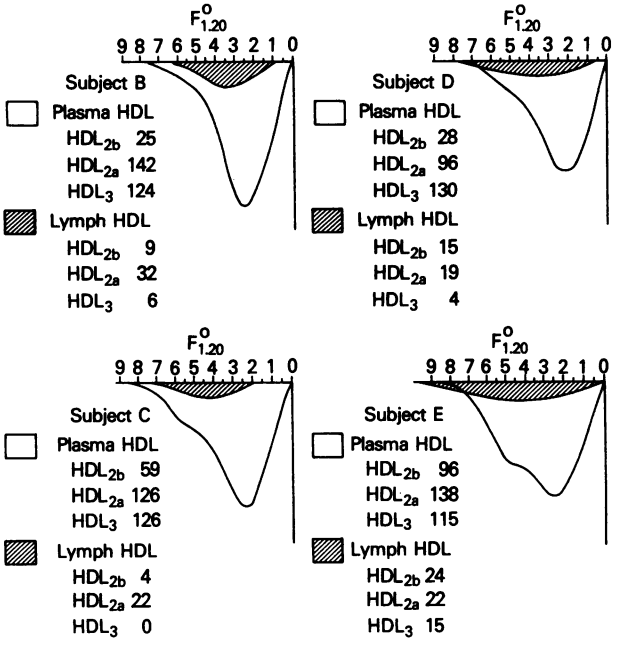

FIGURE 3 Schlieren patterns and subclasses for homologous lymph and plasma high density lipoproteins. Concentrations of HDL subclasses are given as total lipoprotein mass in milligrams per deciliter.

particles (10.2±1.3 nm) by electron microscopy in that the mean diameter value falls in the upper end of the size range reported for HDL_{2a} (9.3–10.5 nm) (38). The greater weight percent values for phospholipid content with diminished values for protein content seen in lymph HDL as compared with plasma HDL reflect requirements for surface constituents of stable lipoproteins (39). There is a strikingly greater weight percent

TABLE III
High Density Lipoprotein Composition

Subject	Protein	Phospholipid	Triglyceride	Unesterified cholesterol	Esterified cholesterol
			%		
B					
Plasma	52±3	23±2	5±1	5±1	15±2
Lymph	46±3	27±3	16±2	1±1	10±2
C					
Plasma	50±3	22±2	7±1	4±1	17±2
Lymph	42±3	28±3	17±2	2±1	11±2
D					
Plasma	54±3	22±3	5±1	4±1	15±2
Lymph	41±3	29±3	18±2	1±1	11±2
E					
Plasma	50±2	22±2	6±1	4±1	18±2
Lymph	38±3	30±3	19±2	1±1	12±2

All values are expressed as percent total lipoprotein weight, and represent the means and standard deviations of three determinations. The material available from subject A did not suffice for this analysis.

TABLE IV
Apolipoprotein Quantitation in Plasma and Thoracic Duct Lymph

Subject	Body weight	Plasma apolipoprotein concentration		Lymph apolipoprotein concentration		Volume of lymph flow	Predicted apolipoprotein synthesis rate		Lymph apolipoprotein transport	
		apoA-I	apoA-II	apoA-I	apoA-II		apoA-I	apoA-II	apoA-I	apoA-II
	kg	*mg/dl*		*mg/dl*		*dl/d*	*mg/d*		*mg/d*	
A	68.2	148±4	50±4	45±3	16±2	30.2	968±196	303±68	1,361±170*	474±59*
B	63.2	128±3	34±3	37±3	11±2	30.3	779±157	191±43	1,122±160*	334±60*
C	55.8	153±4	22±2	10±2	2±1	94.1	857±143	109±37	941±97	188±52*
D	60.9	106±3	21±2	26±2	5±1	60.4	620±68	280±32	1,571±170*	302±70
E	73.7	133±3	32±3	22±2	3±1	112.2	944±185	209±35	2,468±224*	337±101*

Plasma and lymph apolipoprotein concentrations are the means±standard deviation of three determinations.
* Significantly greater than the predicted apolipoprotein synthesis rate at $P < 0.05$ by the Student's t test.

triglyceride in lymph HDL than in plasma HDL. This observation has been previously made by Kostner (40) and is an important distinguishing feature between the two types of HDL. The weight percent esterified cholesterol is lower in lymph HDL, which might be expected in view of the higher value for triglyceride. The ratio of esterified cholesterol to unesterified cholesterol for lymph HDL (5:1–12:1) is significantly higher than for plasma HDL (3:1–4.5:1), $P < 0.05$. The smaller ratio for lymph HDL is of particular interest in view of the reportedly low LCAT activity in thoracic duct lymph (41). Thus, in addition to being of somewhat larger average size and faster flotation rate than plasma HDL, lymph HDL also have significant differences in composition.

Apolipoprotein quantitation. The plasma and lymph concentrations of apoA-I and apoA-II are presented in Table IV for the five subjects studied. The lymph apolipoprotein concentrations represent mean concentrations for the 24-h period. The plasma apolipoprotein concentrations given for subjects C, D, and E are the means of concentrations before and after the 24-h collecting period. Both apoA-I and apoA-II concentrations in the lymph varied between 10–32% of their respective plasma values. Predicted synthesis rates and lymph apoA-I and apoA-II transport are calculated in Table IV. Although apolipoprotein transport rates vary considerably among individual subjects, lymph apolipoprotein transport was equal to and occasionally significantly ($P < 0.05$) greater than the predicted apolipoprotein synthesis rate. This finding is noteworthy in view of the large variation in the volumes of lymph flow shown in Table IV (25.1–112.2 dl/d).

Apolipoprotein distribution among lipoprotein classes. ApoA-I and apoA-II concentrations determined for each lipoprotein density class present in thoracic duct lymph are shown in Table V. The major quantity of both lymph apoA-I (73–95%) and apoA-II (73–100%) was found within HDL, with almost all the remainder found in chylomicrons and VLDL. The distribution of apoA-I and apoA-II was also determined after heparin-Mn^{2+} precipitation for subjects A and B. Lymph apoA-I and apoA-II concentrations in the heparin-Mn^{2+} supernate were 32±3 and 13±2 mg/dl (A) and 27±3 and 8±2 mg/dl (B). Therefore, after precipitation, 71–73% of apoA-I and 73–81% of apoA-II remained in the non-apoB-containing supernate. Whole thoracic duct lymph from subject B was also fractionated by gel permeation chromatography on Sepharose 6B-Cl (Fig. 4). ApoA-I could be detected in the void volume frac-

TABLE V
Apolipoprotein Distribution among Lymph Lipoprotein Classes

Subject	Lymph apolipoprotein concentration	Lymph apolipoprotein concentrations in the lipoprotein classes*			
		Chylomicrons	VLDL	HDL	*d* > 1.21
	mg/dl	*mg/dl*			
A					
apoA-I	45±3	7±1	3±1	35±3	1±1
apoA-II	16±2	3±1	ND	14±2	ND
B					
apoA-I	37±3	5±1	2±1	30±3	1±1
apoA-II	11±2	2±1	ND	8±2	ND
C					
apoA-I	10±2	2±1	ND	8±2	1±1
apoA-II	2±1	1±1	ND	2±1	ND
D					
apoA-I	26±2	4±1	ND	19±2	2±1
apoA-II	5±1	1±1	ND	4±1	ND
E					
apoA-I	22±2	1±1	ND	21±2	1±1
apoA-II	3±1	ND	ND	3±1	ND

* Because there was no apoA-I or apoA-II detected in the LDL fractions from any of the subjects' lymph, this subclass is not included in this table.

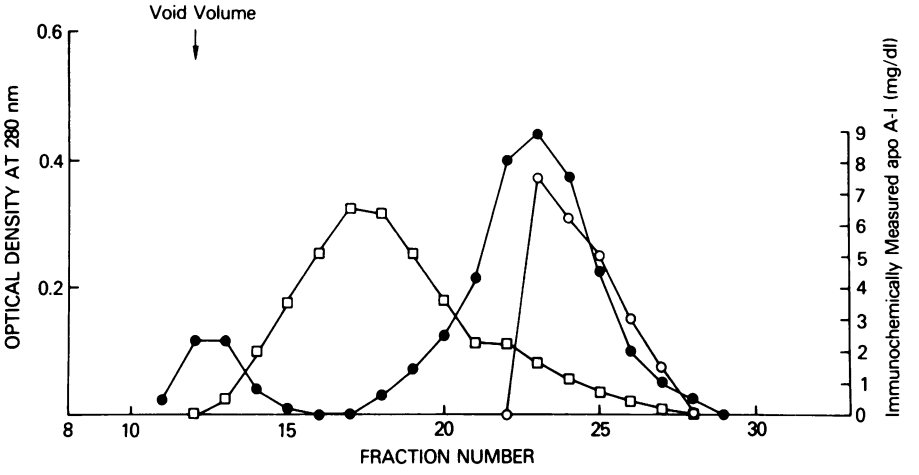

FIGURE 4 Gel permeation chromatography. Chylomicrons and VLDL elute at the void volume. The recovery of immunochemically measured apoA-I from the column was 89%. □, Human LDL, *d* 1.006–1.063; ○, human HDL, *d* 1.063–1.21; ●, immunochemically measured apoA-I in whole thoracic duct lymph.

tions (elution position of VLDL and chylomicrons). However, the major portion (87%) eluted at the position of plasma LDL and HDL fractions. Thus, a similar distribution of apoA-I among lymph lipoproteins was obtained with each of the three fractionation techniques employed.

DISCUSSION

Fat transport and the return of filtered plasma constituents to the blood circulation are functions of human thoracic duct lymph most evident in this study. We found the daily transport of triglycerides by thoracic duct lymph chylomicrons and VLDL to represent ~83% of total ingested fat in each of the five subjects. Although the exact exogenous triglyceride ingestion was not determined, an examination of the subjects' diets revealed that ~95% of the ingested fat was triglyceride or fatty acid.

Absorption coefficients determined for dietary fat in human subjects vary between 90 and 95% (42, 43). The data presented here are thus consistent with the established view of exogenous fat absorption and transport; namely, transport via thoracic duct lymph into the venous circulation is the metabolic fate of virtually all absorbed exogenous fat. There remains the possibility that a small amount of absorbed fat enters the splanchnic circulation through fenestrations in the walls of its capillary endothelium.

Cholesterol transport in the five subjects was generally greater than the 0.25–0.75 g/d dietary intake. Recently, Quintao et al. (43) presented evidence that 64–100% of human mesenteric lymph cholesterol originates from plasma. The thoracic duct lymph is only partially comprised of mesenteric lymph and thus contains a potentially much larger plasma filtration component. However, ~70% of the total lymph cholesterol is transported by chylomicrons and VLDL in the five subjects of this study. Therefore, the 30% of lymph cholesterol in association with HDL and LDL from these subjects may underestimate the plasma filtration component. It seems to us more likely that some of the filtered cholesterol is transferred from HDL and LDL to enrich lymph chylomicrons (43).

In accordance with their function in fat transport, chylomicrons are the predominant lymph lipoproteins, both in mass and number. Schlieren pattern analysis indicated the presence of a considerable amount of VLDL-like particles. A distinction between large and small chylomicrons involves an operational partitioning of what appears on the schlieren pattern to be a continuum of particle sizes and molecular weights. The amount of LDL and HDL detected in the lymph contrasts these two lipoprotein classes from the chylomicrons and VLDL. If there is direct biosynthesis of LDL and/or HDL into the thoracic duct lymph via the mesenteric lymph, it is not sufficient to raise their concentration to plasma levels.

Because of the direct input of lymph HDL into the plasma HDL pool, HDL size and composition are of interest. The greater relative amounts of phospholipid and triglyceride found in lymph HDL result in a particle of larger average size than in plasma. HDL_{2a} predominates among the lymph HDL subfractions, whereas HDL_{2a} and HDL_3 are of approximately equal concentration in plasma. The greater relative proportion of triglyceride in lymph HDL may be a mass action effect of the enormous triglyceride concentration in lymph chylomicrons (44, 45). If the plasma cholesteryl ester transfer protein documented by Chajek and

Fielding (46) is operative in lymph, this may facilitate exchange of triglyceride to HDL from chylomicrons in return for cholesteryl ester.

A process by which cholesteryl esters and unesterified cholesterol were transferred to chylomicrons and VLDL in exchange for triglyceride would provide for the enrichment of the chylomicrons with cholesterol filtered from plasma. Accordingly, the relative cholesteryl ester content of lymph HDL is less than in plasma HDL of the five subjects presented here. Differential exchange with chylomicron triglyceride might account for the higher ratio of cholesteryl esters to unesterified cholesterol in lymph HDL as compared with plasma HDL. This discrepancy may also have to do with the origin of lymph HDL themselves. Green et al. (36) reported the isolation of disc-shaped HDL particles in rat mesenteric lymph. Although we failed to demonstrate any disclike structures in thoracic duct lymph, it is possible that an intestinal HDL particle is synthesized directly.

When compared with the predicted daily synthesis, lymph transport of apoA-I and apoA-II was significantly greater in all five subjects. Green et al. (47), in a study of chyluric subjects, found apoA-I and apoA-II urinary secretion rates to be 104–148% (apoA-I) and 29–66% (apoA-II) of their predicted daily synthesis. Although our results are not directly comparable to those of Green et al., both indicate a major role for lymph in the transport of apoA-I and apoA-II synthesized in the intestine. The results presented in Table IV predict that lymph apoA-I and apoA-II transport could significantly exceed their required synthesis, even if they were only synthesized in the intestine and only entered the plasma via the thoracic duct lymph.

It would therefore appear that as a lower bound, 82±70% (SD) of lymph apoA-I and 69±10% of lymph apoA-II has been derived from plasma and recirculates back into the plasma each day. Assuming that only apoA-I and apoA-II in association with lymph chylomicrons represent the contribution of intestinal synthesis, a lower bound for intestinal contribution can be estimated as 26±10% of total body synthesis for apoA-I and 28±20% for apoA-II. Under this assumption, an upper bound on lymph apolipoprotein recirculation can be estimated as 156±70% for apoA-I and 126±27% for apoA-II. Although not statistically significant, there is a tendency for lymph apoA-I transport to exceed its predicted synthesis by a relatively greater amount than for apoA-II (Table IV).

Further assumptions must be made about the lipoprotein classes that contain apolipoprotein synthesized in the intestine in order to estimate an upper bound for both intestinal apolipoprotein synthesis and extent of recirculation. Such assumptions are complicated by the possibility that the size distribution of lipoprotein particles synthesized in the intestine may be shifted towards chylomicrons or HDL depending upon high or low fat dietary intake and time of day. Indeed, Imaizumi et al. (5), in studies of rat mesenteric lymph, reported the distribution of apoA-I between chylomicrons and HDL to be variable according to the extent of fat and cholesterol feeding. Wu and Windmueller (6) found that ~34% of radioactive amino acids incorporated into apoA-I in perfused preparations of rat intestine could be isolated in chylomicrons and VLDL. The remainder was found to be associated with rat HDL. If these results can be applied to human intestinal synthesis of apoA-I and apoA-II, its upper bound can be estimated as 77±29% of total body synthesis for apoA-I and 82±59% for apoA-II. In another study, Wu and Windmueller estimated that 56% of total rat apoA-I synthesis takes place in the intestine (48).

The combined results presented in this work are consistent with a major contribution of the intestine to total body synthesis of apoA-I and apoA-II. Hence, plasma HDL levels may be affected by factors that relate to mesenteric lymph production and ingested fat load (7). The amount of lymph apoA-I and apoA-II that enters the plasma in association with chylomicrons represents ~27% of predicted daily synthesis. The transfer of chylomicron apolipoprotein to HDL in the plasma (13, 14) thereby constitutes a quantitatively important source of HDL apolipoprotein. In patients with impaired chylomicron catabolism, significantly reduced HDL cholesterol (49) and plasma apoA-I and apoA-II concentrations (50) have been observed.

The results also suggest a vital role for the thoracic duct lymph in returning filtered apolipoprotein to plasma in association with HDL particles. The data in Tables IV and V can be used to calculate that the daily lymph contribution to plasma of apoA-I and apoA-II in association with HDL particles represents 32±14% (SD) (apoA-I) and 32±7% (apoA-II) of the total plasma apolipoprotein pool size. The lymph HDL particles returning to the plasma are primarily HDL_{2b} and HDL_{2a} with altered lipid and protein composition. This fact suggests that upon lymph-plasma mixing, a putative transformation of at least some of the lymph HDL_{2b} and HDL_{2a} particles into HDL_3 particles takes place and is attended by loss of triglyceride and gain of cholesteryl ester. Finally, lymph flow, lymph composition, and capillary permeability are implicated as important physiological factors in the metabolism of human plasma HDL.

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