Lipid and apolipoprotein concentrations in prenodal leg lymph of fasted humans: associations with plasma concentrations in normal subjects, lipoprotein lipase deficiency, and LCAT deficiency

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Abstract **The extent to which lipid and apolipoprotein (apo) concentrations in tissue fluids are determined by those in plasma in normal humans is not known, as all studies to date have been performed on small numbers of subjects, often with dyslipidemia or lymphedema. Therefore, we quantified lipids, apolipoproteins, high density lipoprotein (HDL) lipids, and non-HDL lipids in prenodal leg lymph from 37 fasted ambulant healthy men. Lymph contained almost no triglycerides, but had higher concentrations of free glycerol than plasma. Unesterified cholesterol (UC), cholesteryl ester (CE), phosphatidylcholine (PC), and sphingomyelin (SPM) concentrations in whole lymph were not significantly correlated with those in plasma. HDL lipids, but not non-HDL lipids, were directly related to those in plasma. Lymph HDLs were enriched in UC. However, as the HDL cholesterol/non-HDL cholesterol ratio in lymph exceeded that in plasma, whole lymph nevertheless had a lower UC/CE ratio than plasma. Lymph also had a significantly higher SPM/PC ratio. The lymph/plasma (L/P) ratios of apolipoproteins were as follows: A-IV > A-I and A-II > C-III and E > B. Comparison with the L/P ratios of seven nonlipoprotein proteins suggested that apoA-IV was predominantly lipid free. Concentrations of apolipoproteins A-II, A-IV, C-III, and E in lymph, but not of apolipoproteins A-I or B, were positively correlated with those in plasma. The L/P ratios of apolipoproteins B, C-III, and E in two subjects with lipoprotein lipase (LPL) deficiency, and of apolipoproteins A-I and A-IV in a subject with lecithin:cholesterol acyltransferase (LCAT) deficiency, were low relative to those in normal subjects. Thus, the concentrations of lipids, apolipoproteins, and lipoproteins in human tissue fluid are determined only in part by their concentrations in plasma. Other factors, including the actions of LPL and LCAT, are at least as important.**—Nanjee, M. N., C. J. Cooke, W. L. Olszewski, and N. E. Miller. **Lipid and apolipoprotein concentrations in prenodal leg lymph of fasted humans: associations with plasma concentrations in normal subjects, lipoprotein lipase deficiency, and LCAT deficiency.** ***J. Lipid Res.*** **2000.** 41: **1317–1327.**

Supplementary key words cholesterol • lipoproteins • tissue fluids • proteins

The extracellular metabolism of lipoproteins occurs in two anatomic compartments: intravascular and extravascular. Our knowledge of lipoprotein metabolism in vivo is derived mostly from studies of venous plasma. Such data reflect the sum of events occurring in the two compartments. Studies in tissue culture have likewise been based almost entirely on plasma lipoproteins, although most cells are exposed only to tissue fluid. What data are available on tissue fluid lipoproteins have shown that they differ from plasma lipoproteins in several ways (1–3). These differences partly reflect the differential transfer of lipoproteins across endothelia, combined with metabolic events in the extravascular space. Such extravascular events cannot be adequately studied by sampling plasma, as lipoproteins entering blood from tissue fluids are immediately mixed with plasma lipoproteins, and are altered by interactions with them and with several enzymes. Furthermore, some products of extravascular lipoprotein metabolism, such as apolipoprotein E (apoE)-containing high density lipoproteins (HDLs) (4) and oxidized low density lipoproteins (LDLs) (5), might be rapidly cleared from plasma by the liver.

The difficulties of sampling tissue fluid under physiologic conditions and in sufficient quantities for studies of

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LPL, lipoprotein lipase; L/P ratio, lymph/plasma ratio; PC, phosphatidylcholine; PL, phospholipid; SPM, sphingomyelin; TC, total cholesterol; TG, triglyceride; TGRL, triglyceride-rich lipoprotein; UC, unesterified cholesterol.

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lipoproteins are considerable. Suction blisters provide insufficient fluid, and may be misleading. The increase in capillary permeability must alter the concentrations of lipoproteins, and the associated tissue damage may release enzymes and free radicals that attack them. The only reliable matrix is prenodal (afferent) lymph, collection of which requires cannulation of a peripheral lymph vessel. Roheim and co-workers (1, 2, 6) have studied lipoproteins in canine peripheral lymph, and have provided valuable information in that species. Almost all the available data on human peripheral lymph lipoproteins were provided by Reichl (3, 7), who cannulated a vessel in the dorsum of the foot. This collection procedure has major problems: a high failure rate (typically 50%), low flow rates (usually 50–100 μL/h), a short cannulation life (typically 1–3 h) and the need to preinject a dye subcutaneously to visualize the vessel. Because of these difficulties, most studies of human lymph lipoproteins have involved small numbers of subjects (usually 4–6), often with lipid disorders, or have been limited to patients with lymphedema. In consequence the extent to which the composition of lymph in healthy humans is determined by that of plasma is poorly defined, and for some components (e.g., apolipoproteins A-IV, C, and E) there is no information.

To enable relatively large volumes of lymph to be reliably collected from healthy subjects under ambulant conditions, we have adapted a procedure (8), originally developed for studies of lymphocytes (9), which uses a larger vessel in the leg. Here we describe the technique, and report our initial findings.

MATERIALS AND METHODS

Subjects

Peripheral lymph was collected from 40 subjects: 37 healthy males, 1 male with familial lecithin:cholesterol acyltransferase (LCAT) deficiency, and 1 male and 1 female with familial lipoprotein lipase (LPL) deficiency (**Table 1**). Subjects were excluded if they had renal, hepatic, endocrine, or cardiovascular disease; if they were taking a special diet (other than for LPL deficiency) or medication; or if they were obese enough to create difficulties during the cannulation procedure. The subject with familial LCAT deficiency had early corneal opacification, abnormal red cells, and a trace of proteinuria. In this subject plasma LCAT activity, assayed with apoA-I/lecithin/[^{14}C]cholesterol proteoliposomes (10), was 1.3 nmol/mL/h (normal range, 39.4–112); plasma cholesterol esterification rate in vitro, assayed according to Stokke and Norum (11), was 6.5 nmol/mL/h (18.0–31.4); plasma HDL cholesterol was 0.02 mM; and the plasma unesterified cholesterol/cholesteryl ester (UC/CE) ratio was 4.47 (0.39–0.55). The two subjects with familial LPL deficiency have been described (12, 13). In each the absence of LPL activity had been demonstrated both in postheparin plasma and in heparin eluates of gluteal adipose tissue. Both subjects had been on a low fat (<10 g/day) diet for several years, and neither had eruptive xanthomas or a recent history of abdominal pain. At the time of study their plasma triglycerides (TGs) were 6.47 and 5.10 mM. The study was approved by the local ethics committee, and all subjects gave informed written consent.

Clinical procedures

Forty-six lymph vessel cannulations were carried out. To test the reproducibility of the measurements, 5 healthy subjects underwent the procedure on two occasions, separated by 119, 181, 274, 282, or 379 days. All cannulations were performed between 8:00 and 11:00 A.M. in a surgical theater under full sterile conditions, with the subject supine throughout. The subjects were fasted during the previous 12–14 h, but were allowed unlimited access to fat-free drinks. The lower part of one leg was shaved to about 20 cm above the ankle, and the skin was sterilized with 0.05% chlorhexidene. An area of skin (~4 cm^2) 6–10 cm above the ankle over the anteromedial aspect of the tibia was anesthetized with subcutaneous 2% lignocaine in 1:100,000 adrenaline. A horizontal incision 15–20 mm wide was made in the center of this area. With the aid of an operating microscope (model M650; Wild Heerbrugg, Glattbrugg, Switzerland), the local subcutaneous lymph vessels were dissected, and one was selected for cannulation. A second small incision (~3 mm wide) was made ~10 mm above the first, through which a cannula (Intramedic® polyethylene tubing PE60; Becton Dickinson & Company, Sparks, MD; cat. no. 427416; i.d. 0.76 mm, o.d. 1.22 mm) was passed into the wound. The cannula had been tapered at one end by drawing out after gentle heating, siliconized with Sigmacote® (Sigma Chemical Co., Poole, UK), and then sterilized in 70% (v/v) ethanol for at least 24 h. It was flushed with sterile NaCl (0.15 mM) immediately before use. The lymph vessel was ligated with silk (Mersilk, 5/0), and then opened distal to the ligature with capsulotomy scissors. The first valve distal to the opening was destroyed by insertion of curved blunt forceps. The cannula was inserted into the vessel for 5–10 mm (toward the foot), and secured with a silk ligature (Mersilk, 5/0). The skin wound was then closed with two or three sutures (Prolene, 3/0). The other (untapered) end of the cannula was passed through a hole in the top of a graduated 2-mL screw-topped polypropylene cryovial (Nunc A/S, Roskilde, Denmark) containing 2 mg of

TABLE 1. Clinical details of the subjects

Category	No. of Subjects	Total No. of Cannulations	Sex	Age	Weight	BMI	Plasma Concentration		
							Chol	TG	HDL Chol
				yr	*kg*	*kg·m^{-2}*		*mM*	
Normal subjects	37	43	M	30.3 ± 1.9 (20–69)	74.8 ± 2.3 (50–105)	23.7 ± 0.6 (18–31)	4.76 ± 0.15 (3.3–7.1)	1.39 ± 0.14 (0.5–3.7)	1.18 ± 0.06 (0.7–1.9)
Familial LPL deficiency	2	2	M	45	74.1	25.6	4.65	6.47	0.59
			F	34	63.0	21.7	3.14	5.10	0.40
Familial LCAT deficiency	1	1	M	28	87.2	27.2	3.34	3.44	0.09

Values are given as means ± SEM (range). BMI, body mass index; Chol, total cholesterol; TG, triglycerides.

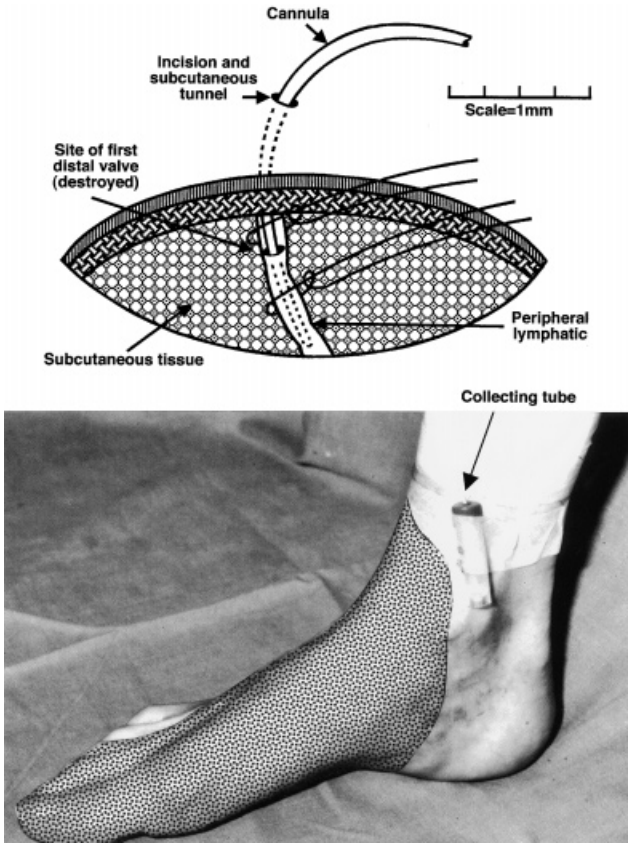

Fig. 1. Diagram of lymph vessel cannulation procedure (top) and photograph of completed cannulation with collection tube in place (bottom). The shaded area is the approximate region of lymph drainage.

Na_2EDTA. A gauze dressing was applied, and the cannula and collection tubes were secured to the leg with surgical tape (**Fig. 1**). The foot was then placed in a loose woollen sock and the subject was transferred to a metabolic ward.

Lymph was collected for 3–6 h, during which the collection vials were changed every 2 or 3 h. Throughout the collections the subjects were ambulant, and were encouraged to walk every 10–15 min to ensure an adequate flow of lymph. Each was given a light fat-free meal and unlimited fruit juice and water. A blood sample was drawn from an antecubital vein into Na_2EDTA (final concentration, 1 mg/mL) during the collection. When the lymph collection was finished, the cannula was withdrawn and a dressing was applied. Sutures were removed 7–10 days later.

In three subjects lymph was collected sequentially (from the same vessel) at both ambient temperature and into vials maintained at 0–4°C. The latter was achieved by keeping the vial immersed in crushed ice in an expanded polystyrene container that had been cut to the shape of the leg and lightly strapped to it with surgical tape. Each collection lasted 3 h. This experiment was done to check that no significant esterification of cholesterol occurred ex vivo during the collections.

Laboratory procedures

Blood and lymph samples were centrifuged at 1,500 *g* for 15 min at 4°C, and the supernatants were transferred to screw-capped polypropylene microcentrifuge tubes. Lymph volumes per collection tube were determined by weighing. In all laboratory analyses plasma–lymph pairs from the same subject were processed together. All assays were done in duplicate.

Lipids, free glycerol, and free choline. Total cholesterol (TC), total TGs, and total choline-containing phospholipids (PLs) were quantified with commercial enzymes (Sigma, Poole, UK) and Trinder-type reporter reagents (Research Organics, Cleveland, OH) in a microtiter plate spectrophotometer (14). Unesterified cholesterol, free glycerol, and free choline were quantified by omitting the cholesterol esterase, lipoprotein lipase, or phospholipase D, respectively. Sphingomyelin (SPM) was assayed according to Blaton et al. (15). The concentration of CEs was calculated as [total cholesterol minus UC], and that of phosphatidycholine (PC) as [PL minus SPM]. Precinorm L® (Boehringer-Mannheim GmBH, Mannheim, Germany) was used as calibrator. Plasma HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with polyethylene glycol 8000 (8%, w/v, final concentration) (16).

HDL and non-HDL lipids. To quantify lipids in HDL and non-HDL lipoproteins, 100 μL of 4-fold diluted plasma and 100 μL of undiluted lymph from 10 subjects were passed through a 10 mm × 300 mm Superose 6 column (HR 10/30; Pharmacia LKB, Uppsala, Sweden) at ambient temperature. A degassed solution of 50 mM Tris-HCl (pH 7.4), 150 mM NaCI, 0.1% (w/v) Na_2EDTA, and 0.1% (w/v) sodium azide was used as eluant, pumped at 0.5 mL/min by a computer-driven HPLC pump (Kontron Instruments, Watford, UK). After excluding the void volume, 200-μL fractions were collected by drop counting into microtiter plates, using a Gilson (Middleton, WI) FC203 fraction collector, and assayed for UC, CE, and PL as already described. Recoveries of total cholesterol were always >90%.

Apolipoproteins. ApoA-I, apoA-II, apoA-IV, apoB, apoC-III, and apoE concentrations were quantified by liquid-phase double-antibody radioimmunoassays, or by rocket immunoelectrophoresis using Tween 20 (0.2%, v/v, final concentration) to expose cryptic epitopes and reduce nonspecific binding and polyethylene glycol 8000 (3%, w/v, final concentration) to enhance reaction kinetics (17). With the exception of anti-apoA-IV, the primary antisera were goat polyclonal IgGs against delipidated human apolipoproteins (International Immunology, Murrieta, CA). Rabbit anti-apoA-IV was a gift from J-C. Fruchart (Pasteur Institute, Lille, France). The precipitating antibodies in the radioimmunoassays were donkey anti-goat IgG or donkey anti-rabbit IgG (Chemicon, Temecula, CA). Radioactivity in antibody-bound pellets (1,500 *g*, 30 min, 4°C) was quantified to ≤0.1% counting error. Radioiodinated delipidated human apolipoproteins were prepared with chloramine-T, and radioiodinated human LDLs (d 1.020–1.055 g/mL) were prepared with iodine monochloride. All apolipoprotein assays were standardized with dilutions of Precinorm L.

Other proteins. Seven other proteins were measured in plasma and lymph to examine the general relationship of molecular size to lymph/plasma (L/P) concentration ratio. α_2-Macroglobulin (750 kDa), complement C3 (180 kDa), total IgG (150 kDa), transferrin (76 kDa), albumin (67 kDa), α_1-antitrypsin (54 kDa), and α_1-acid glycoprotein (38 kDa) were quantified by Laurell rocket immunoelectrophoresis using polyclonal antisera (International Immunology).

Statistical analyses

Data from plasma and lymph samples from the same subjects were analyzed statistically by Student's paired *t*-test. Coefficients of variation for pairs of measurements were calculated as described (18). Associations were examined by the Pearson coefficient of linear correlation. $P < 0.05$ was considered to be significant.

TABLE 2. Concentrations of lipids, free glycerol, and free choline in plasma and lymph

	Lipids					Free Glycerol	Free Choline
	TG	UC	CE	PC	SPM		
	mmol/L					*mmol/L*	
Normal subjects (n = 37)							
Plasma	1.39 ± 0.14	1.38 ± 0.05	3.38 ± 0.11	2.65 ± 0.09	0.73 ± 0.02	0.04 ± 0.01	0.024 ± 0.002
Lymph	0.03 ± 0.01	0.17 ± 0.01	0.50 ± 0.02	0.36 ± 0.02	0.12 ± 0.01	0.08 ± 0.01	0.023 ± 0.002
Familial LCAT deficiency (n = 1)							
Plasma	3.44	2.67	0.67	3.35	0.64	0.04	0.04
Lymph	0.03	0.11	0.02	0.14	0.05	0.06	0.02
Familial LPL deficiency (n = 1)							
Plasma	6.47	1.86	2.79	3.31	0.74	0.03	0.02
Lymph	0.04	0.10	0.24	0.24	0.09	0.16	0.03
Familial LPL deficiency (n = 1)							
Plasma	5.10	1.45	1.69	2.48	0.50	0.03	0.01
Lymph	0.15	0.09	0.10	0.16	0.05	0.07	0.02

TG, triglyceride; UC, unesterified cholesterol; CE, cholesteryl ester; PC, phosphatidylcholine; SPM, sphingomyelin; in the healthy subjects all results are means ± SEM.

RESULTS

Clinical outcome

None of the lymph vessel cannulations failed, and there were no significant complications. The only minor problems were leakage of lymph for 2–3 days after removal of the cannula in one subject, and superficial wound infection after the cannula had been removed in three subjects. The total volume of lymph from each subject varied from 0.80 to 8.18 mL (mean ± SEM, 1.71 ± 1.85), depending on the duration of the collection (3–6 h) and the flow rate (mean, 0.59 ± 0.69 mL/h; range, 0.13–4.09). No lymph sample had visible evidence of contamination with blood.

Normal subjects

Lipids, free glycerol, and free choline. Results for lipid, free glycerol, and free choline concentrations are presented in **Table 2.** With the exception of free glycerol and choline, all concentrations were substantially lower in lymph than plasma ($P < 0.001$). The free glycerol concentration was significantly higher in lymph than in plasma ($P < 0.01$). Among the lipids, the mean L/P ratio was lowest for TG and highest for SPM. The UC/CE ratio was lower (0.348 ± 0.013 vs. 0.411 ± 0.007; $P < 0.0001$) and the SPM/PC ratio was higher (0.589 ± 0.046 vs. 0.387 ± 0.017; $P < 0.0001$) in lymph than in plasma. The concentrations of free glycerol ($r = 0.52$, $P < 0.002$) and free choline ($r = 0.50$, $P < 0.01$) in lymph were positively correlated with those in plasma. However, no significant correlations were found between the concentrations in lymph and plasma of UC, CE, TG, PC, or SPM ($r = 0.14$, 0.05, 0.06, 0.17, and 0.23, respectively).

Apolipoproteins. Results for apolipoproteins in lymph and plasma are presented in **Table 3**. The concentrations of all apolipoproteins were much lower in lymph than in plasma. ApoA-IV had the highest mean L/P ratio and apoB had the lowest. The concentrations of apolipoproteins A-II, CIII, E, and A-IV in lymph were all positively correlated with those in plasma ($P < 0.032$) (**Fig. 2**). However, the lymph–plasma correlations of apoA-I and apoB did not achieve significance. In a subset of three subjects the ratio of apoA-I mass in HDL particles with and without

TABLE 3. Concentrations of apolipoproteins in plasma and lymph

	Apolipoproteins					
	A-I	A-II	B	C-III	E	A-IV
			mg/dL			
Normal subjects (n = 37)						
Plasma	126.7 ± 5.5	32.5 ± 1.1	84.8 ± 4.3	7.8 ± 0.5	2.5 ± 0.1	8.4 ± 0.6
Lymph	26.3 ± 1.4	6.8 ± 0.4	8.0 ± 0.5	0.9 ± 0.1	0.3 ± 0.1	3.3 ± 0.2
Familial LCAT deficiency (n = 1)						
Plasma	19.2	3.5	50.9	8.6	3.6	12.2
Lymph	1.2	0.9	1.6	0.1	0.3	1.7
Familial LPL deficiency (n = 1)						
Plasma	113.2	25.8	72.2	20.5	5.5	5.2
Lymph	17.7	3.5	1.8	0.5	0.2	2.3
Familial LPL deficiency (n = 1)						
Plasma	86.9	18.9	59.1	9.6	3.6	5.8
Lymph	7.7	1.8	1.8	0.2	0.2	1.9

In the healthy subjects all results are means ± SEM.

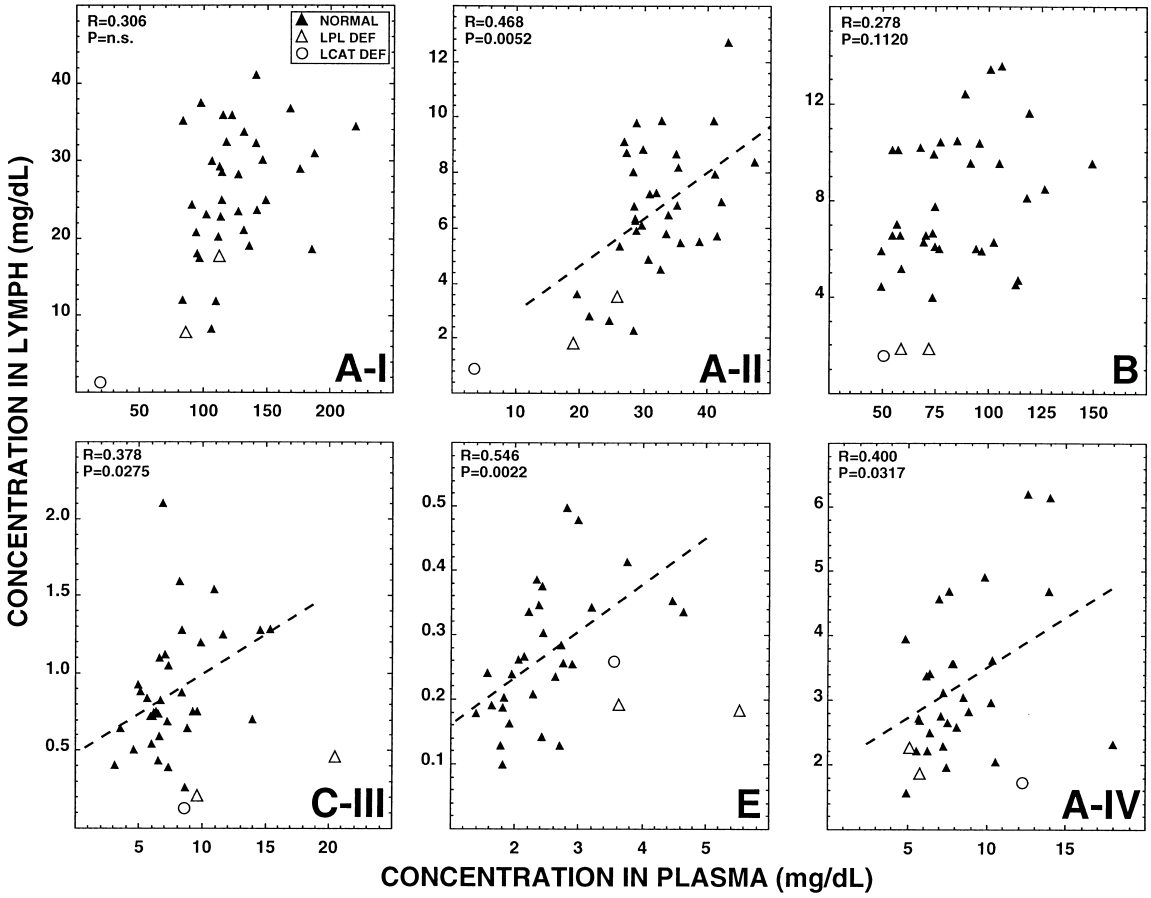

Fig. 2. Relations of apolipoprotein concentrations in lymph to the corresponding concentrations in plasma in normal subjects (solid triangles), one subject with familial LCAT deficiency (open circle), and two subjects with familial LPL deficiency (open triangles). Correlation coefficients are for the normal subjects only.

apoA-II (determined by immunoprecipitation of all apoA-II-containing particles) was similar in lymph and plasma (2.43 ± 0.34 vs. 2.17 ± 0.09).

Other proteins. The L/P ratios of the measured nonlipoprotein proteins were inversely related to the logarithms$_{10}$ of their molecular weights (**Fig. 3**). The mean L/P ratios of apoA-I and apoA-II were close to those predicted from the approximate mean molecular mass of plasma HDLs (200 kDa). The mean L/P ratio of apoA-IV (molecular mass 46 kDa) was close to that expected if it were present as a lipid-free monomer. Assuming that most apoB was in particles of average molecular mass 2,500 kDa, its L/P ratio was always greater than that predicted by the regression line.

HDL and non-HDL lipids. Concentrations of UC, CE, and PL in lymph HDLs were strongly correlated with those in plasma HDLs (**Fig. 4**). In contrast, non-HDL lipids in lymph were unrelated to those in plasma. The L/P ratio of HDL total cholesterol was 2.4-fold greater than that of non-HDL total cholesterol (0.215 ± 0.013 vs. 0.088 ± 0.008, $P < 0.01$). As shown in **Table 4**, lymph HDLs were greatly enriched in UC relative to plasma HDLs.

Day-to-day variation. In 5 normal subjects lymph vessel cannulations were carried out on two occasions 119–379 days apart. Measurements of TC, TG, PL, apoA-I and apoB concentrations in lymph and plasma were made on each occasion. Concentrations in lymph on the first and second occasions were as follows: TC, 0.79 ± 0.10 vs. 0.66 ± 0.11 mM; TG, 0.05 ± 0.01 vs. 0.04 ± 0.01 mM; PL, 0.40 ± 0.06 vs. 0.32 ± 0.02 mM; apoA-I, 28.5 ± 3.2 vs. 28.3 ± 5.3 mg/dL; apoB, 10.4 ± 1.2 vs. 7.1 ± 1.1 mg/dL (mean ± SEM). Coefficients of variation for pairs of results were greater for measurements in lymph than for those in plasma (TC, 27 vs. 7%; PL, 29 vs. 7%; apoB, 29 vs. 25%; apoA-I, 26 vs. 5%).

Ambient versus low temperature collection. In the three subjects in whom collections were made from the same vessel at both ambient temperature and at 0–4°C, the UC/CE ratios in the two samples were essentially identical (0.290 ± 0.001 and 0.289 ± 0.007 at ambient temperature and 0–4°C, respectively).

Familial LPL deficiency

The two subjects with familial LPL deficiency had much lower L/P ratios of apolipoproteins B, C-III, and E than did the normal subjects (Table 3, Fig. 2).

Familial LCAT deficiency

In the lymph from the single subject with familial LCAT deficiency the L/P ratios of all measured lipids and of apolipoproteins A-I and A-IV were low relative to those of the normal subjects (Tables 2 and 3, Fig. 2).

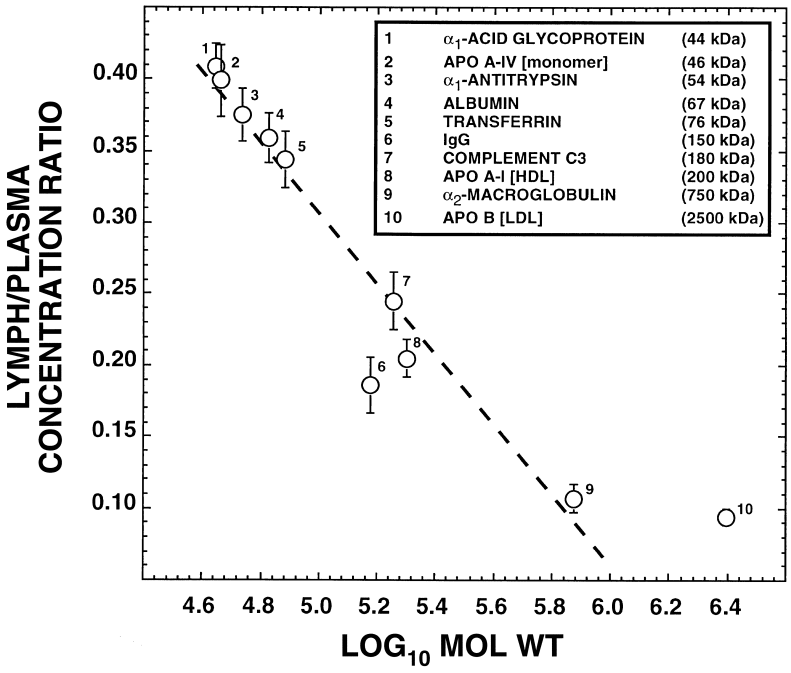

Fig. 3. Relation of the lymph/plasma concentration ratios of different proteins to their $\log_{10}$ molecular masses in normal subjects. Data represent means ± SEM of 37 subjects. Apolipoproteins A-I and B are shown at the approximate mean molecular massess of HDLs and LDLs; apoA-IV is shown at its lipid-free molecular mass. The line was fitted by least-squares regression analysis of the data on non-lipoprotein proteins.

DISCUSSION

This study represents the first attempt to determine the extent to which interindividual differences in the concentrations of the major lipids and apolipoproteins, and those of HDL and non-HDL lipids, in peripheral tissue fluid are determined by their concentrations in plasma in normal humans. Because of the difficulties of cannulating lymph vessels of the dorsum of the foot, previous studies in humans have generally been limited to 4–6 subjects, often including individuals with dyslipidemias of unspecified cause. Other studies were confined to patients with chronic lymphedema, which is known to alter capillary permeability and to prolong the extravascular residence time of

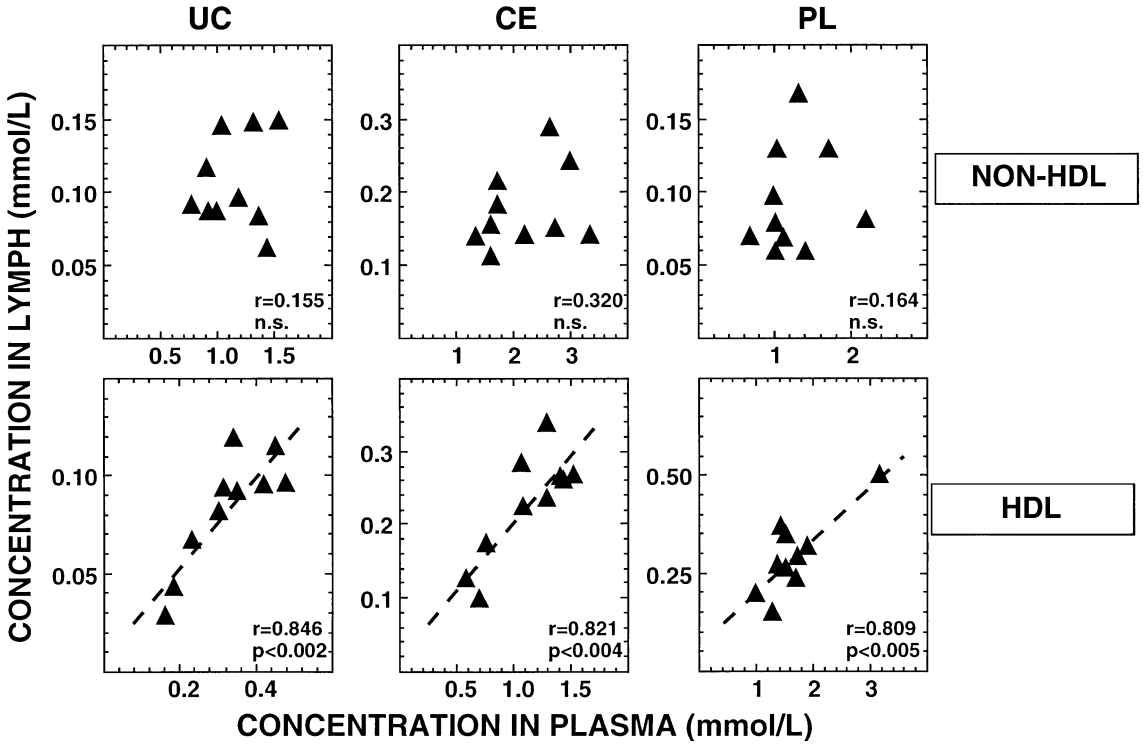

Fig. 4. Relations of lipid concentrations in lymph HDLs and non-HDLs to the corresponding concentrations in plasma. HDLs and non-HDLs were separated by Superose-6 size-exclusion chromatography.

TABLE 4. Unesterified cholesterol and cholesteryl ester concentrations in HDLs and non-HDLs

	Plasma	Lymph	*P*
HDL UC/CE	0.292 ± 0.012	0.362 ± 0.052	0.0026
Non–HDL UC/CE	0.537 ± 0.017	0.610 ± 0.031	0.056
HDL TC/non–HDL TC	0.466 ± 0.059	1.140 ± 0.137	0.0003

Results are means ± SEM of 10 subjects. UC, unesterified cholesterol; CE, cholesteryl ester; TC, total cholesterol. *P* values were obtained by Student's *t*-test.

macromolecules (19). Our study also provides the first data on free glycerol and apolipoproteins A-IV, C-III, and E in normal human lymph, and the first on any lipids or apolipoproteins in lymph from subjects with documented familial LPL or familial LCAT deficiency. It is also the first time that more than two apolipoproteins have been quantified in the same samples of lymph.

Factors influencing lymph composition

The tissue fluids that drain into the lymph vessel that we cannulated come from the skin and subcutaneous tissues in the shaded area shown in Fig. 1. Regional differences in the composition of peripheral lymph have been described in animals. In dogs concentrations of lipids, apolipoproteins, and nonlipoprotein proteins all tended to be higher in skeletal muscle lymph (20) and myocardial lymph (21) than in subcutaneous lymph. However, for most analytes the differences were not large. Other work in animals has provided evidence that the concentrations of proteins in prenodal lymph closely resemble those in local tissue fluid collected by micropuncture (22), insertion of a wick (23), or implantation of a capsule (24). The large size of lipoproteins will favor the lymphatic over the venous capillary route for their return to plasma (3, 19). On these bases our results are likely to be representative of most peripheral tissue fluids in humans.

In the 5 subjects from whom lymph was collected on 2 different days, the day-to-day variations in the concentrations of TC, PL, apoA-I, and apoB were greater than those in plasma. Only some of the variability in lymph could be attributed to changes in plasma concentrations. As all collections were made during the morning after an overnight fast, our results cannot be extrapolated to the nonfasted state. Nor can they be extrapolated to other times of the day. The concentrations of nonlipoprotein proteins in ambulant human peripheral leg lymph have been shown to undergo regular fluctuations during the 24-h cycle (25, 26), as a consequence of postural and exercise-induced variations in capillary filtration, and preliminary observations in our laboratory have indicated that is also true for lipoproteins (27).

The lipids and apolipoproteins of lymph lipoproteins are derived largely from those of plasma lipoproteins, which probably cross capillary endothelia into tissue fluids, mostly by filtration (28–30). The movement of macromolecules across endothelium is strongly influenced by their size, with smaller molecules being favored over larger molecules (19). Our finding that the L/P ratios of seven nonlipoprotein proteins were inversely related to their molecular mass is consistent with previous observations (30–32), and it is likely that the filtration of lipoproteins is influenced in a similar manner. The L/P ratios of all six measured apolipoproteins were positively correlated with the L/P ratio of albumin ($r = 0.49$ to 0.78, $P < 0.003$), suggesting that interindividual differences in apoprotein concentrations in tissue fluid are due at least in part to differences in the transendothelial transport of macromolecules by filtration. However, this does not discount the possibility that some lipoproteins also cross the endothelium by transcytosis (33, 34). Once across the endothelium, the smallest particles will tend to have the largest volumes of distribution, on account of the molecular sieving effect of the extracellular matrix (3, 35).

Lipid concentrations

Triglycerides. As found by others studying human foot lymph and canine peripheral lymph (1–3, 6, 7), the concentrations of all measured lipids and apolipoproteins were much lower in lymph than in plasma. Particularly striking was the near absence of TGs, presumably attributable to the large diameter of TG-rich lipoproteins (TGRLs). Lipolysis of TGRLs by LPL in tissue fluid is unlikely to have contributed to this, as Huang et al. (36) found that canine peripheral lymph contained only extremely low activities of this enzyme. Furthermore, the mean L/P ratio of TGs in our two subjects with familial LPL absence was no greater than that in the normal subjects. Reichl et al. (37) measured TGs in lymph from four subjects with hypertriglyceridemia (plasma TG, 3.1–6.2 mM) and found L/P ratios of 0.02–0.51, but did not report results from subjects with normal plasma triglyceride concentrations.

Free glycerol. The only analyte the concentration of which was higher in lymph than in plasma was free glycerol. This presumably reflected the release of glycerol from subcutaneous adipocytes, the cannulations having been carried out in the fasted state, when hormone-sensitive lipase activity in adipocytes is greatest (38). Our finding that the free glycerol concentration in plasma was positively correlated with that in lymph supports other evidence that adipose tissue lipolysis is a major determinant of plasma free glycerol concentration (38). To our knowledge these are the first data on free glycerol concentration in human peripheral lymph.

Cholesterol and phospholipids. Our failure to find significant correlations between either the TC or PL concentrations in lymph and those in plasma suggests that there are other factors that have major effects on lipid transport in the extravascular compartment. Although Reichl and coworkers studied the lipoproteins of human lymph for several years, their work provided no information on the degree to which lipid concentrations in lymph are determined by those in plasma. Reichl and Sterchi (39) assayed PLs in six subjects, and found a mean L/P ratio of 0.079, approximately half the value observed in the present study. This apparent discrepancy may be related to the fact that all but two of the subjects in the earlier study had hyperlipidemia. Reichl et al. (37) reported a correlation coefficient of 0.62 between plasma and lymph TC concentrations in 7

subjects. However, one of the subjects had severe hypercholesterolemia (20.3 mM). Furthermore, the correlation coefficient was calculated with 13 data pairs: 2 pairs from the hypercholesterolemic subject and from each of 5 of the normal subjects, plus 1 pair from the remaining normal subject. Our results suggest that this led to the strength of the association being overestimated. Other studies in which lymph TC was measured (39, 40) were also too small to provided reliable information of this type.

Cholesterol in HDLs and non-HDLs. Quantification of lipids in particles separated by size-exclusion chromatography showed that the weakness of the association between lymph TC and plasma TC concentrations was owing to the fact that the concentrations of non-HDL lipids in the two matrices were not significantly correlated. In contrast, HDL lipids in lymph were significantly correlated with those in plasma. This is the first time that the correlations between plasma and lymph HDL and non-HDL lipids have been examined. It is of interest that the lymph–plasma correlations of HDL lipids were stronger than those of apoA-I and apoA-II, suggesting that uptake of cholesterol and phospholipids by HDLs in tissue fluids may be one of the determinants of their concentrations in plasma HDLs. Our measurements confirmed earlier evidence (41) that the HDL/non-HDL TC ratio in normal human lymph exceeds that in plasma, presumably reflecting the effect of particle size on the movement of macromolecules across endothelium (32).

Sphingomyelin/phosphatidylcholine ratio. Our finding of a higher SPM/PC ratio in lymph than in plasma is qualitatively consistent with a report by Reichl and Sterchi (39). However, the difference that we observed between the two matrices (means, 0.59 vs. 0.39) was much smaller than that previously recorded (0.90 vs. 0.18). The explanation for this apparent discrepancy is not clear, but it might be related to the small sample size (6 subjects) in the earlier study, and/or the fact that 4 of the subjects were hyperlipidemic. Reichl and Sterchi suggested that the greater SPM/PC ratio in lymph might be a consequence of hydrolysis of PC at the blood-endothelium interface by LPL, as this has no activity against SPM (42). However, our finding that two subjects with familial LPL absence also had higher mean SPM/PC ratios in lymph (0.34) than in plasma (0.21) argues against that mechanism. A novel endothelial lipase that modulates HDL metabolism and has activity against PC has been described (43). However, no data were reported on the relative activities of this enzyme against PC and SPM. Although there are no published data on the lecithinase activity of LCAT in tissue fluids, this seems unlikely to be a contributory factor, as our subject with familial LCAT deficiency also had a greater SPM/PC ratio in lymph than in plasma (0.31 vs. 0.20). Various lysosomal enzyme activities have been demonstrated in peripheral lymph (44, 45), but no studies of specific phospholipases have been reported. Preferential recruitment of SPM from cell membranes by lipid-free apolipoproteins appears unlikely to provide the explanation, as particles formed when lipid-free apoA-I or apoA-II was incubated with cultured cells contained more PC than SPM (46, 47). The PL composition of apoE-containing particles secreted by peripheral cells (48) has not been studied. Whatever its origin, the relatively high SPM/PC ratio in tissue fluids may be important for the extravascular transport of cholesterol from cells, as SPM has a greater affinity for UC (49).

Unesterified cholesterol/esterified cholesterol ratio. The mean UC/CE ratio in lymph from our normal subjects was slightly lower than that in plasma. This result was somewhat unexpected, in view of the reports that human foot lymph (37), human suction blister fluid (50), and canine peripheral lymph (51) all have lower cholesterol esterification rates than plasma. Furthermore, canine lymph (51) and sheep lung lymph (52) have been shown to contain discoidal particles rich in UC, although it is not known whether normal human lymph contains similar particles. The possibility that significant esterification of cholesterol might have occurred ex vivo during the collections in the present study was excluded by the fact that the UC/CE ratios in samples collected at ambient temperature were essentially identical to those in samples collected from the same vessels into vials maintained at 0–4°C. In contrast to the normal subjects, lymph from the patient with LCAT deficiency had a higher UC/CE ratio than plasma (5.5 vs. 4.0).

There are remarkably few data in the literature with which to compare our findings on UC/CE ratios. Reichl et al. (37) reported UC/CE ratios of 0.41–0.48 in lymph from 4 subjects, but did not measure the ratios in plasma for comparison. Similarly, Reichl et al. (40) recorded UC/CE ratios of 0.47–0.67 in lymph from 6 subjects, but again made no measurements in plasma. Rudra et al. (41) found a higher mean UC/CE ratio in lymph than in plasma in 4 subjects, but as no SDs were provided, it is not known whether the difference was significant. Furthermore, when the measurements were repeated in 3 subjects, the UC/CE ratios in lymph and plasma were essentially identical.

UC/EC ratios in HDLs and non-HDLs. When particles were separated by size-exclusion chromatography, we found that HDL-sized particles in lymph were enriched in UC relative to their counterparts in plasma. This is consistent with the results of studies in animals (20, 51, 52). A similar trend was observed for non-HDLs in our study, but this failed to reach a conventional level of statistical significance. There are again surprisingly few published data from normal humans with which to compare our results. Rudra et al. (41) found a greater mean UC/CE ratio in lymph HDLs than in plasma HDLs, in 4 normal subjects, but it is not clear if the difference was significant. Reichl et al. (53) showed that HDLs in human lymphedema fluid were enriched in polar lipids compared with plasma HDLs. However, such data may not be representative of normal physiology, in view of the known effects of lymph stasis on capillary permeability and the extravascular residence time of macromolecules (19). The enrichment of lymph HDLs with UC indicates that the rate of uptake of cell-derived UC by the particles in tissue fluid must exceed its rate of esterification. This suggests that efflux of UC

from cells is not driven by the action of LCAT, and that another factor must be rate limiting. In spite of their enrichment with UC, lymph HDLs retained a much lower mean UC/CE ratio than non-HDL lipoproteins. This fact, combined with the relative preponderance of HDLs over non-HDLs in lymph, explained the paradoxically lower UC/CE ratio in whole lymph compared with whole plasma, as already discussed. These results also explain why the LCAT-deficient patient differed from the normal subjects in having a higher UC/CE ratio in lymph.

Apolipoproteins

ApoA-I and apoA-II. Our study has greatly expanded our knowledge of the relations of apolipoprotein concentrations in human lymph to those in plasma. Reichl and Pflug (54) measured apoA-I in lymph from 8 subjects. The L/P ratios averaged 0.12 (0.09–0.16), compared with the mean value of about 0.2 in the present study. Reichl et al. (55) measured apoA-I and apoA-II in lymph from 6 subjects, but 2 of these had postphlebitic syndrome. In neither of these reports was any information provided on plasma lipids. The L/P ratios of both apoA-I and apoA-II in the present study were approximately those predicted by the general regression of the L/P ratio on $\log_{10}$ molecular mass, determined using a range of nonlipoprotein proteins, if most was associated with particles of average molecular mass 200 kDa. This suggests that HDLs are transferred from plasma to tissue fluid largely or entirely by ultrafiltration. Both apoA-I and apoA-II concentrations were positively associated with those in plasma, although the correlation for apoA-I failed to achieve significance.

ApoB. There is surprisingly little information in the literature on apoB concentrations in normal human lymph. Although Hong et al. (56) studied the physicochemical properties of LDLs and apoB in lymphedema fluid from four subjects, no measurements were made of apoB concentration. Reichl and co-workers measured apoB in lymph in two studies, one with only 2 subjects (40) and another with 10 subjects (57). In both studies the mean L/P ratio was 0.08. In the larger study the lymph–plasma correlation coefficient was 0.52. However, as 4 of the subjects had type III hyperlipidemia and two had type IV, our study is the first to examine the degree to which tissue fluid apoB is determined by plasma apoB concentration in normal humans. A positive trend was observed, but as with non-HDL lipids, this was not significant. Although the mean L/P ratio for apoB was much lower than that of apolipoproteins A-I and A-II, it was always greater than that predicted by regression of the L/P ratio on $\log_{10}$ molecular mass, assuming that essentially all apoB was in particles of approximately 2,500 kDa. This might reflect transcytosis of LDLs through endothelial cells, as proposed by Simionescu and Simionescu (34) and Rutledge (58). However, as it was not possible to assay any proteins of molecular mass greater than that of α_2-macroglobulin, it is not certain that it is valid to extrapolate the linear regression beyond 750 kDa.

ApoC-III and ApoE. Our study provides the first data on apoC-III and apoE concentrations in normal human lymph. The concentration of each of these apolipoproteins was significantly correlated with that in plasma. The L/P ratios of apolipoproteins C-III and E were intermediate between those of apoA-II and apoB, compatible with their being present in both matrices in small TGRL remnants and HDLs. The low L/P ratios of apolipoproteins B, C-III, and E observed in the subjects with LPL deficiency may have reflected the absence of significant lipolysis of TGRLs by LPL at the blood–endothelium interface, leading to reduced transfer of newly generated TGRL remnants into tissue fluids across the endothelium. Some lymph apoE may have been derived from the secretion of newly synthesized apoE by peripheral cells (48, 59, 60). Hata and Nakajima (61) found that the concentrations of apolipoproteins E and C-III in samples of interstitial fluid from the pulmonary arteries of 19 autopsied humans averaged 5 and 14%, respectively, of their concentrations in plasma. Reichl et al. (53) found higher L/P ratios of apoE (mean, 0.38) in chronic lymphedema fluid than we found in lymph. However, as already discussed, the lipoproteins of lymphedema fluid are likely to differ from those of normal peripheral lymph.

ApoA-IV. Our study provides the first data on apoA-IV in human peripheral lymph. Its concentration was positively correlated with that in plasma. Of note is the fact that the L/P ratio was the highest of all the apolipoproteins measured, and was consistent with most or all apoA-IV being present almost as a lipid-free or lipid-poor monomer. The only previous work on apoA-IV in a human peripheral tissue fluid used suction blisters. Duverger et al. (62) studied the composition of apoA-IV-containing particles in blister fluid, but provided no data on total apoA-IV concentration. Furthermore, as already discussed, the composition of suction blister fluid is unlikely to accurately reflect that of normal tissue fluid, as the underlying cause of the blister is an increase in capillary permeability. Previous workers have reported that the concentration of apoA-IV was higher than those of other measured apolipoproteins in rat renal lymph (63), canine subcutaneous lymph (20), and canine skeletal muscle lymph (20). Sloop et al. (20, 64) also found that lymph HDLs became enriched in apoA-IV, when the dogs were fed a high fat, high cholesterol, hypothyroid-inducing diet, suggesting a function in reverse cholesterol transport.

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REFERENCES

1. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* **28:** 225–237.
2. Roheim, P. S., L. Dory, M. Lefevre, and C. H. Sloop. 1990. Lipopro-

teins in interstitial fluid of dogs: implications for a role in reverse cholesterol transport. *Eur. Heart J.* **11 (Suppl E):** 225–229.
3. Reichl, D. 1990. Lipoproteins of human peripheral lymph. *Eur. Heart J.* **11 (Suppl E):** 230–236.
4. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
5. Pieters, M. N., S. Esbach, D. Schouten, A. Brouwer, D. L. Knook, and T. J. C. van Berkel. 1994. Cholesteryl esters from oxidized low-density lipoproteins are *in vivo* rapidly hydrolyzed in rat Kupffer cells and transported to liver parenchymal cells and bile. *Hepatology.* **19:** 1459–1467.
6. Dory, L., C. H. Sloop, and P. S. Roheim. 1986. Interstitial fluid (peripheral lymph) lipoproteins. *Methods Enzymol.* **129:** 660–678.
7. Riechl, D. 1994. Extravascular circulation of lipoproteins: their role in reverse transport of cholesterol. *Atherosclerosis.* **105:** 117–129.
8. Olszewski, W. L. 1981. Physiology and microsurgery of lymphatic vessels in man. *Lymphology.* **14:** 44–60.
9. Engeset, A., B. Hager, A. Nesheim, and A. Kolbenstuedt. 1973. Studies on human peripheral lymph. I. sampling method. *Lymphology.* **6:** 1–5.
10. Chen, C. H., and J. J. Albers. 1992. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
11. Stokke, K. T., and K. R. Norum. 1971. Determination of lecithin:cholesterol acyltransfer in human blood plasma. *Scand. J. Clin. Lab. Invest.* **27:** 21–27.
12. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high-density and very-low-density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* **12:** 113–120.
13. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: *in vivo* and *in vitro* studies in man. *Eur. J. Clin Invest.* **10:** 487–495.
14. Nanjee, M. N., and N. E. Miller. 1996. Sequential microenzymatic assay of cholesterol, triglycerides and phospholipids in a single aliquot. *Clin. Chem.* **42:** 915–926.
15. Blaton, V., M. De Buyzere, J. Spincemaille, and B. Declerq. 1983. Enzymic assay for phosphatidylcholine and sphingomyelin in serum. *Clin. Chem.* **29:** 806–809.
16. Demacker, P. N. M., A. G. M. Hijmans, H. E. Vos-Janssen, A. Van't Laar, and A. P. Jansen. 1980. A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoprotein. *Clin. Chem.* **26:** 1775–1779.
17. Nanjee, M. N., J. E. Doran, P. G. Lerch, and N. E. Miller. 1999. Acute effects of intravenous infusion of apoAI/phosphatidylcholine discs on plasma lipoproteins in humans. *Arterioscler. Thromb. Vasc. Biol.* **19:** 979–989.
18. Mills, G. L., P. A. Lane, and P. K. Weech. 1989. A Guidebook to Lipoprotein Technique. Elsevier, Amsterdam, The Netherlands. 350–351.
19. Olszewski, W. L. 1991. Lymph Stasis: Pathophysiology, Diagnosis and Treatment. CRC Press, London. 243–250.
20. Sloop, C. H., C. K. Castle, M. LeFevre, and L. Wong. 1993. Comparison of the lipid and apolipoprotein composition of skeletal muscle and peripheral lymph in control dogs and dogs fed a high fat, high cholesterol, hypothyroid-inducing diet. *Biochim. Biophys. Acta.* **1169:** 196–201.
21. Julien, P., B. Fong, and A. Angel. 1984. Cardiac and peripheral lymph lipoproteins in dogs fed cholesterol and saturated fat. *Arteriosclerosis.* **4:** 435–442.
22. Rutili, G., and K-E. Arfors. 1977. Protein concentration in interstitial and lymphatic fluids from the subcutaneous tissue. *Acta Physiol. Scand.* **99:** 1–8.
23. Rutili, G., and K-E. Arfors. 1978. Collections of interstitial fluid. A comparison between wick and micropuncture technique. *Microvasc. Res.* **15:** 107–109.
24. Taylor, A., and H. Gibson. 1975. Concentrating ability of lymphatic vessels. *Lymphology.* **8:** 43–49.
25. Engeset, A., W. L. Olszewski, P. M. Jeger, J. Sokolowski, and L. Theodorsen. 1977. Twenty-four hour variation in lymph flow and composition of leg lymph in normal men. *Acta. Physiol. Scand.* **99:** 140–149.
26. Olszewski, W. L., A. Engeset, and J. Sokolowski. 1977. Lymph flow and protein in normal male during lying, getting up and walking. *Lymphology.* **10:** 178–183.
27. Nanjee, M. N., C. J. Cooke, W. L. Olszewski, and N. E. Miller. 1997. Regulation of pre-beta high density lipoproteins in human tissue fluids *in vivo*. *Circulation.* **96:** I-662 (Abstract).
28. Renkin, E. M. 1985. Capillary transport of macromolecules: pores and other endothelial pathways. *J. Appl. Physiol.* **58:** 315–325.
29. Madara, J. L. 1998. Regulation of the movement of solutes across tight junctions. *Annu. Rev. Physiol.* **60:** 143–159.
30. Siflinger-Birnboim, A., P. J. Del Vecchio, J. A. Cooper, F. A. Blumenstock, J. M. Shepard, and A. B. Malik. 1987. Molecular sieving characteristics of the cultured endothelial monolayer. *J. Cell Physiol.* **132:** 111–117.
31. Olszewski, W. L., A. Engeset, and H. Lukasiewicz. 1977. Immunoglobulins, complement and lysozyme in leg lymph of normal men. *Scand. J. Clin. Lab. Invest.* **37:** 669–674.
32. Michel, C. C. 1996. Transport of macromolecules through microvascular walls. *Cardiovasc. Res.* **32:** 644–653.
33. Predescu, D., S. Predescu, T. McQuistan, and G. E. Palade. 1998. Transcytosis of α_2-acid glycoprotein in the continuous microvascular endothelium. *Proc. Natl. Acad. Sci. USA.* **95:** 6175–6180.
34. Simionescu, N., and V. Simionescu. 1985. Interactions of endogenous lipoproteins with capillary endothelium in spontaneously hyperlipoproteinemic rats. *Microvasc. Res.* **30:** 314–332.
35. Bell, D. R., P. D. Watson, and E. M. Renkin. 1980. Exclusion of plasma proteins in the interstitium of tissues from the dog hindpaw. *Am. J. Physiol.* **239:** H532–H538.
36. Huang, J., C. H. Sloop, P. S. Roheim, and L. Wong. 1990. Lipoprotein lipase and hepatic triacylglycerol lipase activities in peripheral and skeletal muscle lymph. *Arteriosclerosis.* **10:** 720–726.
37. Reichl, D., L. A. Simons, N. B. Myant, J. J. Pflug, and G. L. Mills. 1973. The lipids and lipoproteins of human peripheral lymph, with observations on the transport of cholesterol from plasma and tissues into lymph. *Clin. Sci. Mol. Med.* **45:** 313–329.
38. Martin, D. W., P. A. Mayes, and V. W. Rodwell. 1983. Harper's Review of Biochemistry. 19th edition. Lange Medical Publications, Los Altos, CA. 224–229.
39. Reichl, D., and J. M. Sterchi. 1992. Human peripheral lymph lipoproteins are enriched in sphingomyelin. *Biochim. Biophys. Acta.* **1127:** 28–32.
40. Reichl, D., N. B. Myant, M. S. Brown, and J. L. Goldstein. 1978. Biologically active low density lipoprotein in human peripheral lymph. *J. Clin. Invest.* **61:** 64–71.
41. Rudra, D. N., N. B. Myant, J. J. Pflug, and D. Reichl. 1984. The distribution of cholesterol and apoprotein A-I between the lipoproteins in plasma and peripheral lymph from normal human subjects. *Atherosclerosis.* **53:** 297–308.
42. McLean, L. R., R. A. Demal, and L. Socorro. 1986. Mechanism of action of lipoprotein lipase. *Methods Enzymol.* **129:** 738–763.
43. Jaye, M., K. J. Lynch, J. Krawiec, D. Marchadier, C. Mangeais, K. Doon, V. South, D. Amin, M. Perrone, and D. J. Rader. 1999. A novel endothelial-derived lipase that modulates HDL metabolism. *Nature Genet.* **21:** 424–428.
44. Lindena, J., and I. Trautschold. 1983. Enzymes in lymph. *J. Clin. Chem. Clin. Biochem.* **21:** 327–346.
45. Shannon, A. D., J. W. Quin, and F. C. Courtice. 1977. Lysosomal enzyme activities in sheep plasma and lymph. *Res. Vet. Sci.* **22:** 209–215.
46. Hara, H., and S. Yokoyama. 1991. Interaction of free apolipoproteins with macrophages. *J. Biol. Chem.* **266:** 3080–3086.
47. Forte, T. M., J. K. Bielicki, R. Goth-Goldstein, J. Selmek, and M. R. McCall. 1995. Recruitment of cell phospholipids and cholesterol by apolipoproteins A-II and A-I: formation of nascent apolipoprotein-specific HDL that differ in size, phospholipid composition, and reactivity with LCAT. *J. Lipid Res.* **36:** 148–157.
48. Basu, S. K., Y. K. Ho, M. S. Brown, D. W. Bilheimer, G. W. Anderson, and J. L. Goldstein. 1982. Biochemical and genetic studies on the apoE secreted by mouse macrophages and human monocytes. *J. Biol. Chem.* **257:** 9788–9795.
49. Lund-Katz, S., H. M. Laboda, L. R. McLean, and M. C. Phillips. 1988. Influence of molecular packing and phospholipid type on rates of cholesterol exchange. *Biochemistry.* **27:** 3416–3423.
50. Wong, L., L. K. Curtiss, J. Huang, C. J. Mann, B. Maldonado, and P. S. Roheim. 1992. Altered epitope expression of human interstitial fluid apolipoprotein A-I reduces its ability to activate lecithin:cholesterol acyltransferase. *J. Clin. Invest.* **90:** 2370–2375.
51. Dory, L., C. H. Sloop, L. M. Boquet, R. L. Hamilton, and P. S.

Roheim. 1983. Lecithin:cholesterol acyltransferase-mediated modification of discoidal peripheral lymph high density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **80:** 3489–3493.
52. Forte, T. M., C. E. Cross, R. A. Gunther, and G. C. Kramer. 1983. Characterization of sheep lung lymph lipoproteins: chemical and physical properties. *J. Lipid Res.* **24:** 1358–1367.
53. Reichl, D., T. M. Forte, J-L. Hong, D. N. Rudra, and J. Pflug. 1985. Human lymphedema fluid lipoproteins: particle size, cholesterol and apolipoprotein distributions and electron microscopic structure. *J. Lipid Res.* **26:** 1399–1411.
54. Reichl, D., and J. J. Pflug. 1982. The concentration of apolipoprotein A-I in human peripheral lymph. *Biochim. Biophys. Acta.* **710:** 456–463.
55. Reichl, D., D. N. Rudra, and J. J. Pflug. 1989. The concentration of apolipoprotein A-II in human peripheral lymph. *Biochim. Biophys. Acta.* **1006:** 246–249.
56. Hong, J. L., J. Pflug, and D. Reichl. 1984. Comparison of apoB of low density lipoproteins of human interstitial fluid and plasma. *Biochem. J.* **222:** 49–55.
57. Reichl, D., N. B. Myant, and J. J. Pflug. 1977. Concentration of lipoproteins containing apolipoprotein B in human peripheral lymph. *Biochim. Biophys. Acta.* **489:** 98–105.
58. Rutledge, J. C. 1992. Temperature and hydrostatic pressure-dependent pathways of low-density lipoprotein transport across microvascular barrier. *Am. J. Physiol.* **262:** H234–H245.
59. Lin, C-T., Y. Xu, J-Y. Xu, and L. Chan. 1986. Immunoreactive apolipoprotein E is a widely distributed cellular protein. *J. Clin. Invest.* **78:** 947–958.
60. Dory, L., L. M. Boquet, C. R. Tate, and C. H. Sloop. 1986. Peripheral synthesis and isoform distribution of dog apoprotein E. *J. Biol. Chem.* **261:** 811–816.
61. Hata, Y., and K. Nakajima. 1990. Disproportionately high concentrations of apolipoprotein E in the interstitial fluid of normal pulmonary artery in man. *Ann. N.Y. Acad. Sci.* **598:** 49–57.
62. Duverger, N., N. Ghalim, G. Ailhaud, A. Steinmetz, J-C. Fruchart, and G. Castro. 1993. Characterization of apoA-IV containing lipoprotein particles isolated from human plasma and interstitial fluid. *Arterioscler. Thromb.* **13:** 126–132.
63. Roheim, P. S., D. Edelstein, and G. G. Pinter. 1976. Apolipoproteins in rat serum and renal lymph. *Proc. Natl. Acad. Sci. USA.* **73:** 1757–1760.
64. Sloop, C. H., L. Dory, B. R. Krause, C. Castle, and P. S. Roheim. 1983. Lipoproteins and apolipoproteins in peripheral lymph of normal and cholesterol-fed dogs. *Atherosclerosis.* **49:** 9–21.