Characterization of sheep lung lymph lipoproteins: chemical and physical properties

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Abstract We have determined the composition and distribution of plasma and lung lymph lipoproteins from unanesthetized ewes. Cholesterol, triglyceride, and phospholipid levels in lung lymph were 45%, 50%, and 50%, respectively, of those in plasma. Lipoproteins from both lymph and plasma were separated into two major fractions: d < 1.063 g/ml or "LDL", and d 1.063–1.21 g/ml or HDL. HDL was the major lipoprotein species in the plasma and lymph. Gradient gel electrophoresis of HDL on 4–30% gels showed that, in lymph, HDL particles were shifted to larger sizes; in addition to a peak at 8.5 nm, which was similar to plasma HDL, there were two additional components of larger size, one at 9.2 nm and the other at 12 nm. Electron microscopy revealed that lymph HDL contained two new particles not seen in plasma: large, round particles, 13.6 nm diameter, and discoidal particles, 18.7 by 4.9 nm, long and short axis, respectively. Compositional analysis of lymph HDL revealed a relative enrichment in free cholesterol as well as an enrichment in apolipoprotein E. Lymph "LDL" on gradient gel electrophoresis was extremely heterogeneous. Several peaks were evident in the 23–30 nm size range (similar to plasma "LDL"), but a supplementary component at approximately 15–16 nm was also present. Whereas plasma "LDL" on electron microscopy contained only round particles 26 nm in diameter, lymph contained an additional, unusual particle which was close-packed, with square geometry, and was 15 nm in diameter. Lymph apolipoprotein composition differed from that of plasma by the appearance of apoE and A-I as well as apoB. Particles containing apoE and A-I were separated from apoB-containing particles in a fraction of d 1.047–1.063 g/ml by density gradient centrifugation. On electron microscopy, this fraction revealed square-packing particles; the density and apolipoprotein composition suggest that these unusual particles are a continuum of HDL. Changes in the physical and chemical properties of lung lymph lipoproteins suggest that these particles are metabolically modified.—**Forte, T. M., C. E. Cross, R. A. Gunther, and G. C. Kramer.** Characterization of sheep lung lymph lipoproteins: chemical and physical properties. *J. Lipid Res.* 1983. **24:** 1358–1367.

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Lung lymph is believed to be representative of lung interstitial fluid; hence chronic lymph fistula preparations have been frequently utilized to study changes in lung permeability (1–4). These investigations have focused on the transmicrovascular movement of proteins in the lung but have devoted scant attention to the presence of lipoproteins in lung interstitial fluid. However, since the lung represents a tissue active in the synthesis of surfactant lipids, it is probable that interstitial lipoproteins play a significant role in lipid metabolism of the lung. Studies on rat lung lipoprotein lipase activity suggested that triglyceride-rich lipoproteins served as substrate lipids for surfactant synthesis (5, 6). This observation is strengthened by recent studies which demonstrated that rat plasma cholesterol levels controlled phosphatidylcholine synthesis by the lung (7).

We have recently shown, by analytic ultracentrifugal techniques, that both low density lipoproteins (LDL) and high density lipoproteins (HDL) are present in sheep lung lymph at approximately 50% of plasma levels (LDL, plasma 55 mg/dl, lymph 28 mg/dl; HDL, plasma 100 mg/dl, lymph 45 mg/dl) (8). Furthermore, analytic ultracentrifugal studies on the distribution of lung lipoproteins indicated that there were changes in the flotation rate of lymph HDL. This observation suggested that lipoproteins in the lymph had undergone modification of their physical and chemical properties during transit through the lung interstitial compartment. In the present study, the two major density classes of sheep lung lymph lipoproteins have been isolated and further characterized. The investigations reveal that lung interstitial lipoproteins, as reflected by lung lymph, possess several new components not found in the plasma.

METHODS

Lymph and plasma collection

Adult Columbia-Suffolk ewes (43–55 kg) were prepared with chronic lung lymph fistulas by cannulation

Abbreviations: HDL, high density lipoproteins isolated at d 1.063–1.21 g/ml; "LDL", low density lipoproteins isolated at d < 1.063 g/ml; apo, apolipoprotein; GGE, gradient gel electrophoresis; PAGE, polyacrylamide gel electrophoresis; LCAT, lecithin:cholesterol acyltransferase.

of the efferent lymphatic of the caudal mediastinal lymph node. The lymphatic cannulation was performed through a right thoracotomy of the sixth intercostal space. Abdominal contamination was minimized by a double ligation and resection of the tail of the node below the inferior pulmonary ligaments through a lower second thoracotomy. An additional maneuver was then performed to further remove any diaphragmatic lymphatics as reported by Drake et al. (9) in order to enter the node cephalad to the inferior pulmonary ligament. This consisted of cauterizing the mediastinal pleural and the superficial fibers of the esophagus along the right border of the node for a distance of 5–8 cm. Animals used in this study ranged from 3 to 35 days postsurgery (mean 15.8 ± 11.7 days for 11 animals) and were allowed free access to food and water. Only sheep in good health with steady state lymph flows and protein concentration and no evidence of acute or chronic inflammation were used. Lymph was drained into chilled tubes containing sodium EDTA (1 mg/ml) to prevent clotting. Corresponding blood samples (EDTA, 1 mg/ml) were obtained from the superior vena cava. Lymph and blood samples were centrifuged at 1000 *g* at 4°C for 20 min to sediment cells.

Lipoprotein isolation

Lymph and plasma lipoproteins were isolated by standard sequential preparative ultracentrifugal techniques at 15°C (10). In initial studies, the d < 1.006 g/ml fraction was isolated after 18-hr centrifugation at 40,000 rpm in a Beckman 40.3 rotor. After removal of the d < 1.006 g/ml fraction, the density was adjusted to d 1.063 g/ml and the solution was centrifuged for 24 hr at 40,000 rpm; low density lipoproteins (LDL), d 1.006–1.063 g/ml, were removed with a pipette. After adjusting the density to d 1.21 g/ml, high density lipoproteins, d 1.063–1.21 g/ml, were obtained by centrifugation at 40,000 rpm for 24 hr. Lipoprotein fractions were concentrated eightfold to plasma or lymph concentration as previously described (11). Examination of these fractions by analytic ultracentrifugation, gradient gel electrophoresis, and electron microscopy revealed a lack of lipoproteins in the d < 1.006 g/ml fraction. Hence, subsequent lipoprotein isolations were carried out by initially adjusting plasma or lymph to d 1.063 g/ml and removing the d < 1.063 g/ml fraction (termed "LDL" in this report); in a second centrifugation step the d 1.063–1.21 g/ml fraction was removed.

Subfractionation of lung lymph "LDL" was carried out in an equilibrium density gradient according to the procedure described by Shen et al. (12).

Electrophoretic procedures

Lipoprotein fractions and subfractions were analyzed by gradient gel electrophoresis in order to determine size and heterogeneity of lipoproteins. Precast Pharmacia 4–30% slab gels (Pharmacia, Piscataway, NJ) were used to analyze HDL fractions according to the procedure of Blanche et al. (13); reference proteins used to determine diameter consisted of thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin. To analyze "LDL" fractions, 2–16% slab gels were employed according to the method of Krauss and Burke (14); carboxylated latex beads (Dow Chemical), thyroglobulin, and apoferritin were used as standards.

Polyacrylamide gel (10%) electrophoresis in the presence of 0.1% SDS was used to determine the molecular weights of lymph and plasma apolipoproteins; procedures used were essentially those of Weber and Osborn (15). Standards consisting of bovine serum albumin (66,000 mol wt), bovine apoA-I (28,000 mol wt), human apoE (37,000 mol wt, gift from Dr. Karl Weisgraber), and egg lysozyme (14,300 mol wt) were co-electrophoresed in order to determine molecular weights. "LDL" apolipoprotein B was electrophoresed on 3% polyacrylamide gels according to the method of Kane, Hardman, and Paulus (16) to determine the presence of apoB subspecies. Densitometric scans on polyacrylamide gels and gradient gels were obtained with a Transidyne RFT densitometer.

Chemical composition

Protein was determined by the method of Markwell et al. (17) using bovine serum albumin as a standard. Phospholipid was measured according to the method of Bartlett (18). Cholesterol and triglyceride were determined with the enzymatic reagent kit from Gilford Diagnostics. Free cholesterol and cholesteryl ester were determined on selected samples using the gas–liquid chromatography method of Hindricks, Wolthers, and Groen (19).

Electron microscopy

The procedures used for visualizing and sizing lipoprotein particles were those previously described (20). A minimum of 200 free-standing particles per sample was measured.

RESULTS

Lung lymph and plasma lipid and lipoprotein concentrations

The mean concentrations of lipids and lipoproteins in sheep lung lymph and plasma are shown in **Table 1**.

TABLE 1. Lipid and lipoprotein concentrations in sheep lung lymph and plasma[a]

	Cholesterol	Triglyceride	Phospholipid	HDL[b]	"LDL"[b]
			mg/dl		
Plasma (P)	42.1 ± 10.5	12.0 ± 3.0	43.2 ± 7.6	84 ± 16	52 ± 17
Lymph (L)	18.8 ± 5.8	6.1 ± 1.3	21.8 ± 4.9	37 ± 8	26 ± 8
L/P Ratio	0.45	0.50	0.50	0.44	0.50

[a] Values represent the mean and standard deviation of seven sheep.
[b] HDL and "LDL" concentrations were calculated from protein and lipid mass.

Lymph cholesterol, triglyceride, and phospholipid levels are approximately 50% that of plasma, which is consistent with the observed reduction of both HDL and "LDL" in lung lymph. Triglyceride levels are extremely low in both plasma and lymph, which is compatible with the reported absence of VLDL in these fluids in the sheep (8, 21).

As indicated in Table 1, HDL are the major lipoprotein constituents in both plasma and lymph. Both HDL and "LDL" levels in lymph, however, are approximately 50% of those in plasma as indicated by the lymph to plasma ratios. In keeping with our previous observations from analytic ultracentrifugation (8), the ratios of HDL to "LDL" in plasma and lymph are similar, 1.6 and 1.4, respectively.

Gradient gel electrophoresis

Isolated "LDL" (d < 1.063 g/ml) and HDL (d 1.063–1.21 g/ml) fractions were examined by gradient gel electrophoresis (GGE) in order to determine whether major changes in lipoprotein peak positions occurred in lymph as compared with plasma. Plasma HDL electrophoresed on 4–30% slab gels (see representative gel scan in Fig. 1) revealed the presence of a major peak at approximately 8.6–8.9 nm; occasionally a slight shoulder was present at 8.0–8.2 nm. In contrast, lymph HDL consistently showed new components with larger diameters. These new peaks had diameters of approximately 9.0 and 12.0 nm. The largest particle species can probably account for the faster floating lipoprotein species reported previously in analyic ultracentrifugal patterns (8).

Plasma "LDL" demonstrated greater particle size heterogeneity than HDL. Three to five peaks between 23–30 nm were typically in evidence as demonstrated in the representative scan of plasma "LDL" on 2–16% slab gels in **Fig. 1**. It is noteworthy that no peaks with particles of diameters less than 23.0 nm were encountered in the plasma d < 1.063 g/ml fraction. Lymph "LDL", on the other hand, contained an additional new

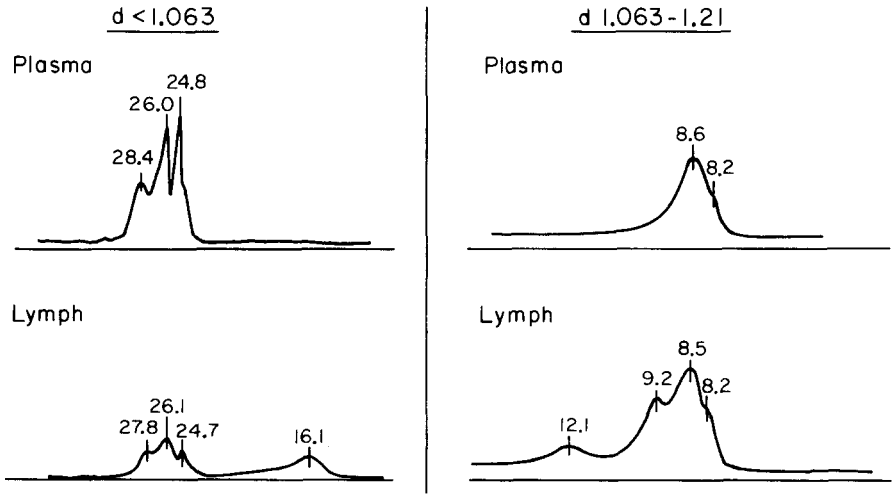

Fig. 1. Gradient gel electrophoresis scan from a representative sheep plasma and lymph HDL (d 1.063–1.21 g/ml) and "LDL" (d < 1.063 g/ml) fraction (nine different samples were examined and were similar to the illustration). Numbers above peaks and shoulders denote particle diameter in nm. HDL was electrophoresed on precast 4–30% gels. Plasma HDL typically has a sharp, single peak which may possess a shoulder of smaller-size particles. Lymph HDL is more complex and showed several new, larger components. "LDL" was electrophoresed on 2–16% gels. Plasma "LDL" possesses great particle heterogeneity; even greater heterogeneity is seen in lymph "LDL" where a broad peak is apparent at 16 nm.

component of smaller size with a peak diameter between 15–16 nm (Fig. 1).

Electron microscopic analysis of lipoprotein fractions

Electron microscopy was carried out on HDL and "LDL" from both plasma and lymph, and the data are summarized in **Table 2** and **Fig. 2**. Plasma HDL (Fig. 2a) are fairly uniform in size and consist of spherical particles with a mean diameter of 9.4 nm, which agrees well with the gradient gel data. Lymph HDL show heterogeneity (Fig. 2b) of particle size and morphology; both small and large spherical particles are evident with diameters of 9.7 and 13.6 nm, respectively (Table 2). Some discoidal particles with long and short axes of 18.7 and 4.9 nm, respectively, are also evident, however these particles constitute a variable but minor component.

Plasma "LDL" are heterogeneous in size as indicated by the micrograph in Fig. 2c and have a mean diameter of 26.2 nm (Table 2). Most particles are round in profile, with some particles forming short linear "chains" when packed. Lymph "LDL", however, clearly have two types of particles—one which is round in profile and another which forms square-packing arrays (Fig. 2d). This latter particle is considerably smaller than the round species of particles and has a diameter of 14.9 nm (Table 2). The size of the square-packing species is very similar to the peak diameter of the new "LDL" component seen by gradient gel electrophoresis. Square-packing particles were isolated from the larger, round structures by density gradient centrifugation and a representative electron micrograph is seen in Fig. 2e. The square-packing particles were recovered at a density of d 1.047–1.063 g/ml and formed extensive packing arrays.

Protein and lipid composition of lipoprotein fractions

Table 3 summarizes the lipid and protein composition of lipoproteins isolated from both lymph and plasma. Lymph HDL differs from plasma HDL in that there is a slight decrease in protein and a slight increase in total cholesterol. Free and esterified cholesterol concentrations in plasma and lymph HDL were determined on three animals; the mean plasma and lymph HDL concentrations for unesterified cholesterol were 3.59 ± 0.42 mg/dl and 3.13 ± 0.05 mg/dl, respectively. The esterified cholesterol values for plasma and lymph were 25.13 ± 3.90 mg/dl and 9.17 ± 2.0 mg/dl, respectively. The free to esterified cholesterol ratio for lymph HDL was 0.36 as compared to 0.15 for plasma; this twofold increase in the lymph free to esterified cholesterol ratio indicates that free cholesterol is relatively enriched in lymph HDL.

TABLE 2. Size of plasma and lymph HDL and "LDL" determined by electron microscopy

Fraction	N[a]	Morphology	Size
			nm ± S.D.
Plasma HDL	3	small round	9.4 ± 1.4
Plasma LDL	2	round	26.2 ± 3.7
Lymph HDL	3	small round	9.7 ± 1.7
		large round	13.6 ± 2.0
		discs: long axis	18.7 ± 3.1
		discs: short axis	4.9 ± 0.5
Lymph "LDL"	3	square-packing	14.9 ± 2.1
		round	26.8 ± 3.8

[a] N represents the number of individual animals examined.

Lymph "LDL" clearly shows significant compositional differences from plasma "LDL". Phospholipid content of lymph "LDL" is considerably greater (34% increase) than that of plasma, while triglyceride is considerably less (41%). Although the percent total cholesterol content of lymph "LDL" is slightly less than plasma (8% decrease), the relative distribution of free versus esterified cholesterol changed in lymph. Plasma "LDL" free cholesterol concentration in three animals was 9.25 ± 1.9 mg/dl while esterified cholesterol was 31.0 ± 9.6 mg/dl. In lymph "LDL", free cholesterol concentration was 5.49 ± 1.5 mg/dl and esterified cholesterol concentration was 10.21 ± 3.3 mg/dl. The free to esterified cholesterol ratio of 0.54 for lymph "LDL" versus 0.31 for plasma indicates that free cholesterol is preferentially increased in lymph "LDL".

The apolipoprotein distribution of lymph and plasma HDL and "LDL" on SDS-PAGE (10%) is shown in **Fig. 3**. HDL from both lymph and plasma contain proteins with molecular weights similar to apoA-I (28,000 daltons) and apoC's (12,000 and 8,000 dalton peaks). Our methods did not permit us to determine whether one of the small molecular weight proteins was monomeric apoA-II. ApoA-I constitutes the major component in both plasma and lymph HDL; assuming equal chromogenicity, apoA-I accounts for 84% of plasma HDL protein and 85% of lymph HDL protein. The small proteins (apoC's) account for 16% and 12% of plasma and lymph HDL Coomassie staining material, respectively. In addition to apoA-I and apoC's, lymph HDL also possess approximately 3% apoE (37,000 daltons). Plasma "LDL" contains predominantly apoB (95% of Coomassie staining material) and a slight band (5%) in the apoE position. Lymph "LDL", however, contains a pronounced apoA-I band which accounts for approximately 65% of the total "LDL" staining material while

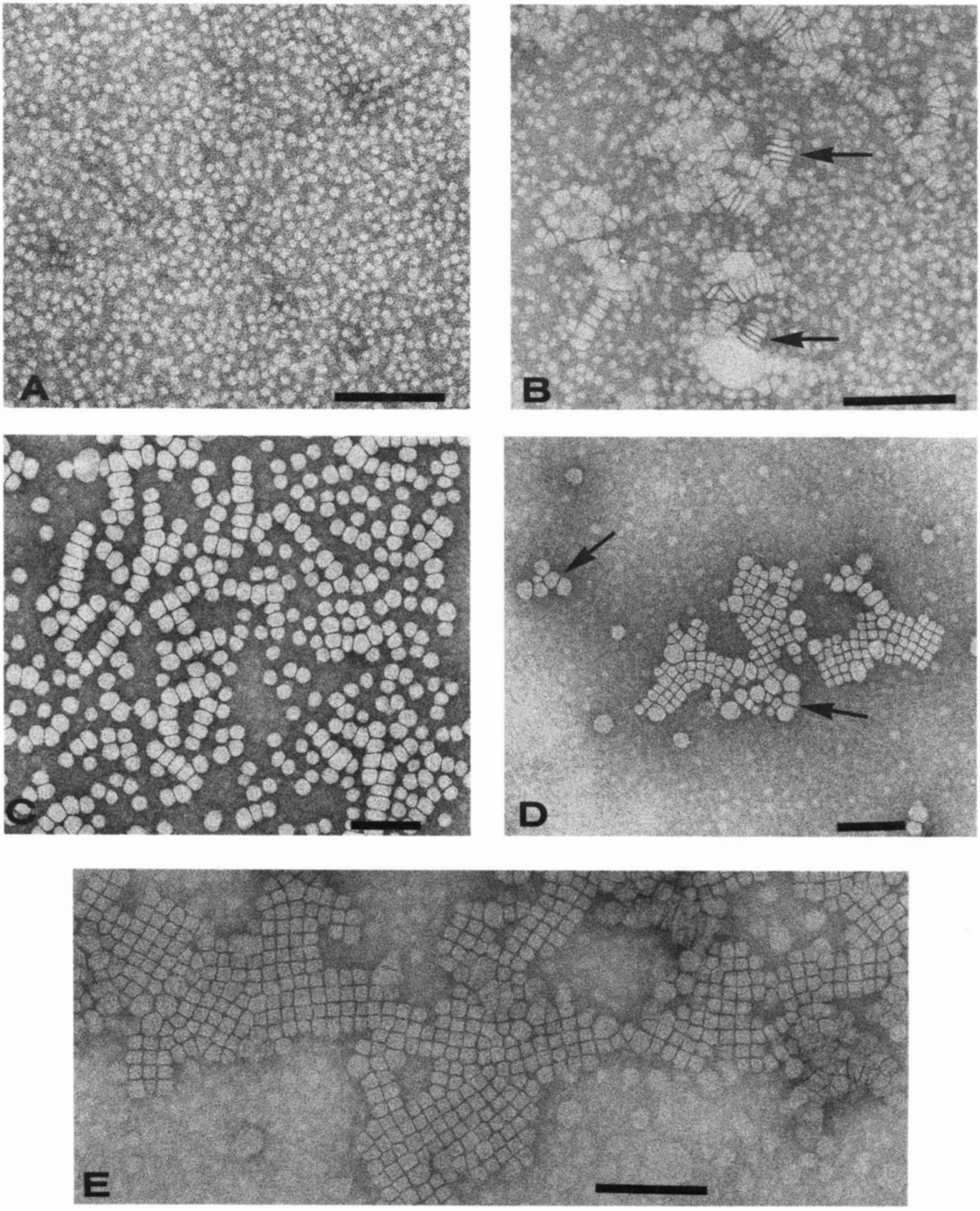

TABLE 3. Lipoprotein composition: sheep lung lymph versus plasma

Fraction		% Total Weight[a]			
		Protein	Phospholipid	Total Cholesterol	Triglyceride
HDL	Plasma	49.5 ± 5.0	29.8 ± 4.2	17.3 ± 1.2	2.2 ± 0.6
	Lymph	46.5 ± 4.1	30.3 ± 5.3	20.7 ± 1.9	2.6 ± 1.1
"LDL"	Plasma	23.9 ± 2.3	31.2 ± 3.5	36.1 ± 4.6	8.6 ± 3.3
	Lymph	19.6 ± 1.1	41.9 ± 7.1	33.3 ± 5.3	5.1 ± 2.5

[a] Total weight is the summation of protein, phospholipid, total cholesterol, and triglyceride. Data represent the mean and standard deviation of samples from five sheep.

apoE accounts for approximately 14% of the total. In lymph "LDL", apoB is approximately 21% of the staining protein. Analysis of apoB variants on 3% PAGE revealed that both lymph and plasma "LDL" contained a single, large molecular weight component similar to human B-100 (data not shown).

In order to ascertain whether apoE and apoA-I in lymph LDL are associated with the smaller, more dense particles (15–16 nm) seen in gradient gel electrophoresis, LDL was subfractionated by density gradient centrifugation and the subfractions were analyzed in 10% PAGE. The distribution of apolipoproteins is seen in **Fig. 4**. Lymph LDL subfractions contain at least two types of particles, one with predominantly apoB (d 1.025–1.029 g/ml) and the other with apoA-I and E (d 1.057–1.065 g/ml). The latter correspond to the square-packing particles seen in electron microscopy (Fig. 2e). Intermediate fractions of d 1.035–1.057 g/ml possess apoB, A-I, and E, and probably represent a mixture of particles.

DISCUSSION

The chronic unanesthetized sheep lung lymph fistula model has frequently been used to study the permeabilities of the pulmonary microvasculature. Such studies were predicated upon the assumption that fluid from the efferent duct of the mediastinal lymph node represented relatively uncontaminated and unmodified lung interstitial fluid. This concept is somewhat controversial since recent acute studies on anesthetized sheep indicate that diaphragmatic lymph vessels may on occasion contaminate lung lymph (9, 22). Additionally, there is some evidence from studies with the popliteal lymph node that passage of fluid through the node can alter protein concentration in the lymph (23); however, the degree of alteration appears to vary with nodes from different regions of the body (24). Experiments by Staub et al. (2) in sheep suggest that lymph collected from the efferent lymphatic of the caudal mediastinal lymph node is over 90% pulmonary lymph and is not significantly modified during its nodal passage. The present studies demonstrate that lipoproteins in the lung extravascular compartment undergo physical and chemical modification; additionally these investigations establish baseline values for future studies in lipid metabolism with the unanesthetized sheep lung lymph model.

The lung lymph to plasma ratio for cholesterol (0.45) in the sheep is not unlike that found for cardiac lymph in the dog (25, 26). This ratio, however, is considerably higher than the ratio of 0.10 reported for human lymph from the dorsum of the foot (27, 28) and the ratio of 0.10 from peripheral lymph of the dog (29). These differences in cholesterol ratios may in part be due to regional differences in endothelial permeability or to differences in lipoprotein metabolism by the surrounding tissue.

Several investigators have previously shown that HDL is the major plasma lipoprotein species in sheep (21, 30–32), and our present study is consistent with these earlier reports. We and others have shown that sheep plasma (8, 21) and lung lymph (8) are deficient in VLDL. Deficiency of triglyceride-rich particles in plasma and lymph may in great part be due to dilution by extracellular fluids and rapid turnover. In the present investigation, HDL accounts for 60% of the total lipoprotein mass in lung lymph as well as in plasma. Moreover, the HDL to "LDL" ratios for plasma and lymph are quite similar (1.6 and 1.4, respectively), which suggests that although total lipoprotein concentration

Fig. 2. Electron micrographs of negatively stained lipoprotein fractions. (A) Plasma HDL: fairly homogeneous small round particles. (B) Lymph HDL: particle size and morphology is heterogeneous. Variable sized round particles predominate, but discoidal particles are also evident (arrows). (C) Plasma "LDL": heterogeneous round particles; some have a tendency to form short chains. (D) Lymph "LDL": two types of particles are evident—one which is similar to plasma "LDL" (arrows) and the other which forms square-packing arrays. (E) Lymph "LDL" fraction of mean density of 1.057 g/ml. This fraction contains mainly square-packing structures. Bar markers indicate 100 nm.

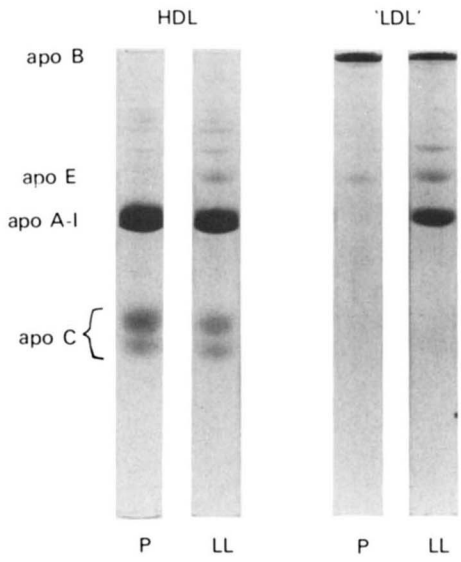

Fig. 3. Polyacrylamide gel (10%) electrophoresis of plasma (P) and lung lymph (LL) lipoproteins from a representative sheep. Samples are unreduced, and 30 μg of protein was loaded onto each gel. Major apolipoproteins are identified based on estimated molecular weights.

is lower in lymph, HDL and "LDL" are present in the same relative proportions as in plasma. A similar phenomenon has been noted for dog and pig cardiac lymph (26).

Gradient gel electrophoresis indicates that lymph HDL was physically modified as compared with the plasma HDL. The latter particles according to GGE had a major peak at 8.6 nm; this small size is consistent with the reported analytic ultracentrifugal pattern which indicated that sheep plasma HDL is similar to human HDL_3 (8). Lymph HDL consistently showed the presence of two additional larger components; one (9.2 nm) which is equivalent to human $(HDL_{2a})_{gge}$ and the other (12.0 nm) to human $(HDL_{2b})_{gge}$ (13). The presence of larger species corroborated our previous findings that an additional fast floating component can be seen on analytic ultracentrifugation (8). Our electron microscopic data indicate that at least three species of particles (small spheres, large spheres, and discs) are present in the HDL density region of lung lymph, but their relationship to a specific GGE subpopulation has yet to be established. The appearance of a large HDL subspecies has also been recently reported for human lymph from the dorsum of the foot (33) and in peripheral lymph of control and cholesterol-fed dogs (34). In vitro incubation studies with human plasma by two laboratories have also shown that HDL_3 can shift to HDL_2-like particles (35, 36). This interconversion may be produced by the synergistic action of enzymes such as lecithin:cholesterol acyltransferase (LCAT) and lipoprotein lipase together with lipid transfer proteins. At present it is not known whether all these systems operate in the lymph; however, there is some evidence that LCAT, although low, is present in human dorsum of the foot lymph (27) and in dog cardiac lymph (25). The observation by Julien and Angel (37) that VLDL of dog cardiac lymph is depleted in triglyceride suggests that lipolytic enzymes

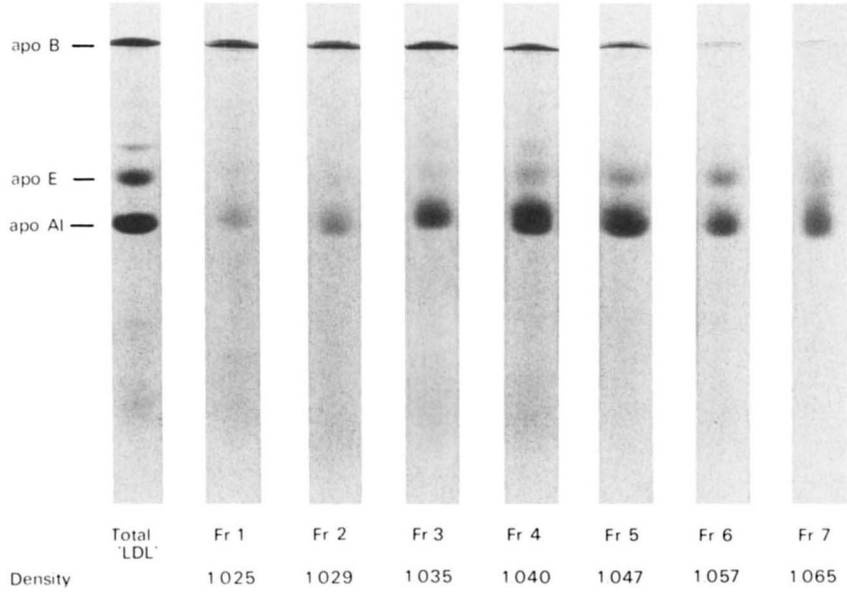

Fig. 4. Polyacrylamide gel (10%) electrophoresis on lung lymph "LDL" subfractions obtained by density gradient ultracentrifugation. The first tube shows total "LDL", d < 1.063 g/ml fraction. The remaining tubes are numbered fractions 1 through 7, and the mean density of each fraction is indicated.

may be present in this lymph. Furthermore, a preliminary report by these authors (38) indicates that both hepatic lipase and lipoprotein lipase are detectable in cardiac lymph.

Sheep plasma HDL have a protein and lipid composition not unlike that previously reported by others (29, 30); however, no data are available on the apolipoprotein composition of this ruminant. According to our data, the apolipoproteins in sheep HDL are similar to those reported for the bovine (39–41) where apoA-I is the major apolipoprotein with small quantities of apoC. No apoA-II was identified under our conditions. In contrast to plasma HDL, lymph HDL showed the presence of apoE; based on Coomassie staining of apolipoproteins, lymph HDL contained approximtely 0.5 mg/dl apoE, whereas plasma HDL appeared to be deficient in apoE. However, it is not known whether the apoE is associated with a particular morphological entity of lung lymph HDL. Recent studies by Dory et al. (42, 43) indicate that, in peripheral prenodal lymph of the hypercholesterolemic dog, apoE is associated with a phospholipid and unesterified cholesterol-rich discoidal particle. Basu et al. (44) have shown that mouse peritoneal macrophages and human monocyte-derived macrophages can also secrete apoE-rich discoidal particles. Further studies are clearly required to establish whether lung cells are capable of a similar secretory mechanism.

In addition to the presence of apoE, lung lymph HDL exhibits a pronounced increase in unesterified cholesterol relative to esterified cholesterol (ratio 0.36 for lymph versus 0.15 for plasma). Although speculative, the increase in unesterified cholesterol may arise by "reverse cholesterol transport" during interaction of HDL with cells and tissue. Such a mechanism for the removal of cellular cholesterol has been proposed by others (44–47). Maintenance of elevated unesterified cholesterol levels in the lymph may be the result of decreased LCAT activity which would normally esterify circulating cholesterol. This suggestion is supported by the studies of Marcel et al. (48) who showed that HDL rich in apoE are poor substrates for LCAT.

The composition of sheep plasma "LDL" in the present study is similar to that reported by others (21, 30). Like its human counterpart, plasma "LDL" possesses only apoB; however, there are no apoB variants in sheep "LDL", where only the equivalent to B-100 is apparent.

Sheep plasma "LDL" reveals particle heterogeneity with typically three to five peaks apparent on GGE. The degree of heterogeneity is not unlike that seen in humans (14). In contrast to plasma, lymph "LDL" possesses an additional component of smaller diameter when examined by GGE. The presence of particles with large and small diameters in GGE was corroborated by electron microscopy which indicated that two major populations of particles exist in this density region: one that is morphologically similar to plasma and the other that is unique in its square-packing tendency.

Protein and lipid analysis showed that lymph "LDL", compared with plasma, contained large increases in the percentage of phospholipid and unesterified cholesterol, and possessed apoE and A-I in addition to apoB. Our density gradient fractions, examined by SDS-polyacrylamide gel electrophoresis, revealed a particle with a density between 1.047–1.063 g/ml that possessed apoE and A-I and no apoB and that formed square-packing arrays on electron microscopy. Square-packing of these particles may be related to the transport of excess surface lipids, such as phospholipid, since in vitro studies with bovine HDL loaded with excess phospholipid also form similar particles.[1] The fact that the square-packing particles contain apoE and A-I suggests that they are a lower density continuum of the HDL density region. By virtue of their increased lipid content, these apoE and A-I particles float into the d < 1.063 g/ml region. In their high apoE and A-I content, these particles are similar to HDL_1 found in control dogs and to apoE-rich HDL (HDL_c) described in cholesterol-fed swine (49).

The present studies suggest that lipoproteins undergo modification during transit in the lung. Modification may, in part, be related to the metabolic function of lipoproteins in the lung since it appears that there is a possible relationship between lipoproteins and surfactant synthesis. Evidence that lipoproteins play a role in lipid metabolism in the lung was provided by Hass and Longmore (50), who showed that both LDL and HDL can modulate cholesterol metabolism in rat lung cells and that LDL catabolism is greater than that of HDL. Suzuki and Tabata (7) found that treatment of rats with 4-aminopyrazolo(3,4d)pyrimidine, which decreases plasma cholesterol levels, concomitantly decreased synthesis of phosphatidylglycerol and phosphatidylcholine moieties of lung surfactant. Interactions between cells in the lung (macrophages, epithelial and/or endothelial cells) and lipoproteins in concert with lipid-hydrolyzing enzymes and lipid-transfer proteins may well account for the modified or processed lipoprotein species found in the lung lymph. Clearly, further studies are required to identify specific lipoprotein species with specific metabolic functions occurring in lung tissue.

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[1] Forte, T. M. Unpublished observation.

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