review

Interstitial fluid lipoproteins

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Peripheral cell cholesterol metabolism is regulated primarily by interaction with lipoproteins in the interstitial space. Peripheral cells acquire cholesterol by receptor-mediated uptake mechanisms that presumably require direct contact between interstitial lipoproteins and the cell membrane (1). In a similar manner, the initial events of cholesterol efflux (reverse cholesterol transport) also occur through direct or indirect interaction between interstitial lipoproteins and the cell membrane (2, 3). Thus, events occurring in the immediate environment of the peripheral cell are important to the regulation of both cellular cholesterol influx and efflux and may affect an organism's susceptibility to atherosclerosis.

This review examines the various methods of interstitial fluid collection and summarizes the current knowledge of interstitial fluid lipoprotein composition and metabolism.

THE INTERSTITIAL SPACE

The interstitium has been defined as "...the connective tissue space outside the vascular and lymphatic system and the cells" (4). It contains a heterogeneous meshwork of insoluble collagen and elastin fibers embedded in a matrix of hydrophilic hyaluronate and proteoglycans (5). There is general agreement that the interstitial space comprises two compartments (6). The colloid-rich gel phase is dominated by interaction between water and hyaluronate-proteoglycan aggregates, whereas the colloid-poor sol phase of interstitial fluid consists of free fluid channels and prelymphatics about 100 nm in diameter (7). Larger plasma proteins are apparently confined to the sol phase, whereas smaller plasma proteins also occupy a fraction of the gel phase (4). It is not clear whether the sol phase is in direct contact with the plasma membranes of peripheral cells.

Factors that affect the composition of interstitial fluid include: *1)* the plasma composition; *2)* the balance of Starling forces across the capillary wall (principally capillary hydraulic pressure and plasma oncotic pressure); *3)* the permeability-surface area of the capillary endothelium; *4)* lymph transport; *5)* the physicochemical properties of the matrix within the interstitial space; and *6)* modification, addition, or removal of components by peripheral cell metabolism.

EXPERIMENTAL COLLECTION OF INTERSTITIAL FLUID

Although the total volume of the interstitial space is three or four times that of plasma, the interstitial fluid compartments surrounding individual cells are microscopic. The small size of these spaces makes it difficult to obtain significant volumes of interstitial fluid. This difficulty has given rise to a variety of "models" of interstitial fluid. All of these techniques have both experimental and theoretical disadvantages. As a result, no method of collecting interstitial fluid is universally accepted, and its detailed composition remains controversial.

We have included a wide range of data from techniques that have not yet been used for lipoprotein studies. These data give general information on the composition of interstitial fluid collected by various means from vascular beds with different capillary permeabilities and allow comparison with the available lipoprotein data (**Table 1**).

Direct sampling of interstitial fluid

Conceptually, the simplest method of sampling interstitial fluid is to collect it directly with micropipettes inserted into the interstitial space. Creese, D'Silva, and Shaw (8) collected 100–200 nl of "interfibre" fluid by means of capillary tubes inserted between muscle fibers. Others (9, 10)

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; L/P ratio, lymph/plasma concentration ratio; CTP, cholesterol transfer protein; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ISS, interstitial space.

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TABLE 1. Interstitial fluid/plasma protein concentration ratios

Method of Collection	Species	Tissue	R_{TP}[a]	R_{Alb}[b]	Reference
Micropipette	rabbit	subcut.	0.37	0.42	(10)
Paper absorption	pig	aortic intima		0.54	(12)
Capillary tube	guinea pig	muscle		0.48	(8)
Paraffin cavity	rabbit	subcut.	0.32		(9)
Wick	rat	subcut.	0.56	0.61	(15)
Wick	rat	muscle	0.52	0.37	(16)
Capsule	rat	subcut.	0.42		(84)
Capsule	rat	subcut.	0.52	0.64	(30)
Capsule	rabbit	subcut.	0.42		(9)
Lymphedema	human	subcut.		0.42	(22)
Inflammatory fluid	rabbit	subcut.	0.54		(18)
Peripheral lymph	dog	paw	0.29		(32)
Peripheral lymph	rabbit	paw	0.42	0.47	(81)
Peripheral lymph	dog	paw	0.36	0.38	(82, 83)
Peripheral lymph	dog	paw	0.13		(53)
Skeletal muscle lymph	rabbit	leg	0.56	0.76	(85)
Skeletal muscle lymph	dog	leg	0.34	0.58	unpublished[c]

[a] R_{TP} Interstitial/plasma concentration ratio for total plasma proteins.
[b] R_{Alb} Interstitial fluid/plasma concentration ratio for albumin.
[c] Unpublished observations, Sloop, C. H. et al.

have used micropipettes to collect 2–50-nl samples of interstitial fluid from subcutaneous tissue. The extremely small amount of fluid collected greatly limits the utility of these techniques. Micropipette collection of fluid may also damage surrounding tissue and affect the composition of the fluid that is collected.

Collection of aortic wall interstitial fluid by absorption

Filter paper squares have been used to sample interstitial fluid from the subintimal space of human and animal aortic walls collected after death (11–13). A flap of intima and subintima is dissected free of the aorta on three sides. Weighed squares of filter paper are then placed under the flap for 3 hr and the volume of their absorbed interstitial fluid is determined by weight gain. The concentrations of various apoproteins and proteins are measured by rocket immunoelectrophoresis of the filter paper square. The amount of interstitial fluid collected per filter paper square varies from 1–5 mg. From various studies, the interstitial fluid/plasma concentration ratio for albumin was 0.54 (Table 1 and **Table 2**). Almost all of the aortic wall LDL was in the subintimal space. Its measured concentration was twice that of plasma.

Wick technique

Scholander, Hargens, and Miller (14) devised a "wick" technique for measuring interstitial fluid pressure in vivo. This technique has been modified to permit collection of interstitial fluid from muscle, skin, and connective tissue of both humans and laboratory animals (15–17). Saline-soaked nylon thread is sewn into the tissue and left for several hours to absorb interstitial fluid. The thread is then removed, weighed, and placed into a known volume of saline for 24 hr. Interstitial fluid volume is determined by weight difference. Protein concentrations are determined colorimetrically or by rocket immunoelectrophoresis. The typical wick contains about 10 μl of fluid. Calculated interstitial fluid/plasma concentration ratios for total protein and albumin were 0.56 and 0.61, respectively (15). This technique permits sampling from skin, skeletal muscle, and connective tissue (15, 16) and has been used in unanesthetized humans (17). The technique's major dis-

TABLE 2. Interstitial fluid/plasma concentration ratios for lipoproteins

Method of Collection	Species	Tissue	R_{TP}	R_{Alb}	R_{VLDL}	R_{LDL}	R_{HDL}	Reference
Peripheral lymph	rabbit(cf)[a]	paw		0.35	0.08(< 1.119)	0.14	0.31	(40)
Peripheral lymph	dog	paw	0.13		0.03	0.07	0.13	(53)
Paper absorption	human	aortic intima		0.55		2.00	0.36	(12)
Blister fluid	human	skin		0.3		0.16	0.24	(21)
Cardiac lymph	dog	myocard.	0.58		0.67	0.30	0.35	(63)
Cardiac lymph	dog	myocard.		0.69			0.25	(86, 64)

[a] cf, Cholesterol-fed.

advantages are the small interstitial fluid volume obtained and the possibility that local trauma or inflammation may increase capillary permeability and alter the composition of the collected interstitial fluid.

Sponge technique

Raymond and Reynolds (18, 19) studied the lipoprotein composition of inflammatory fluid collected from control and cholesterol-fed rabbits. Polyvinyl sponges are implanted aseptically into the subcutaneous tissue on the animals' backs. After 8–72 hr the sponges are removed and the fluid is squeezed from them. Inflammatory fluid LDL has a larger diameter, increased electrophoretic mobility, heterogenous size, more triglyceride, and less cholesterol and cholesteryl ester than plasma LDL. Inflammation greatly increases capillary permeability, and the large number of inflammatory cells present in the fluid may alter the interstitial lipoproteins.

Suction blister fluid

Another technique for collection of interstitial fluid involves separation of epidermis and dermis by suction (20). A small plastic chamber containing a septum with several holes is applied directly to the skin of a human volunteer. The chamber is then sealed, and a pressure of about 200 mm Hg is applied for 100 min. Several blisters (about 0.7 ml total volume) can be raised with one application of suction. The blister fluid/plasma ratio was 0.3 for albumin. The concentration ratios for HDL and LDL were 0.24 and 0.16, respectively (21). For the various classes of lipid, the blister fluid/plasma concentration ratios varied from 0.20–0.27. This technique allows collection of useful amounts of fluid and can be performed on unanesthetized human volunteers. The technique is relatively simple; however, it may traumatize underlying tissue, which would increase capillary permeability and alter the composition of interstitial fluid collected by this method.

Lymphedema

Primary lymphedema fluid has been used as a model of interstitial fluid (22). Clear lymphedema fluid is aspirated directly from incisions made during surgical resection of intractable lymphedematous tissue. Virtually all of the apoB-containing lipoproteins of lymphedema fluid float at LDL density (1.019–1.063 g/ml) but have pre-beta mobility. The lymphedema fluid/plasma concentration ratio for albumin is 0.42 (Table 1). Isoelectric focusing experiments have shown that LDL from lymphedema fluid has a higher net charge than plasma LDL (23). Chronic lymphedema is characterized by extensive subcutaneous fibrosis (24), chronic inflammation (25), and "cellulitis" (26). Under these conditions, the microvascular bed of subcutaneous connective tissue probably does not have normal permeability properties. The long-term stasis of peripheral lymph and accompanying inflammation also exposes the interstitial lipoproteins to unphysiologic conditions. The extensive fibrosis present probably alters the exclusion properties of the interstitial space. These abnormalities may limit the usefulness of this model.

Capsular fluid

Implanted subcutaneous capsules were first designed to allow measurement of interstitial fluid pressure under in vivo conditions (27). This technique has been applied to the collection of interstitial fluid (28–30). Coetzee, Hattingh, and Mitchell (30) implanted simple, open-ended plastic tubes into the subcutaneous connective tissue of rats. Six weeks were allowed for healing and resolution of local inflammation. Approximately 0.1 ml of fluid could be withdrawn from each capsule. The interstitial fluid/plasma concentration ratios for total protein and albumin were 0.52 and 0.64, respectively (30). The physiologic properties of implanted capsules have been well studied because of the controversy involving subatmospheric interstitial fluid pressure measurements (31). This technique allows collection of useful quantities of interstitial fluid without accompanying inflammation. One possible drawback to the capsule technique is the reported presence within the capsule of granulation tissue with "notoriously leaky capillaries" (7). The reported interstitial fluid/plasma concentration ratios for capsules are similar to those reported by other methods (See Table 1).

Lymph

Although the composition of lymph has traditionally been assumed to be similar to that of interstitial fluid (32–34), there have been few direct comparisons of lymph and interstitial fluid collected simultaneously from the same organ. Rutili and Arfors (10) compared peripheral lymph and interstitial fluid collected from rabbit subcutaneous tissue by means of micropipettes (**Table 3**). These data support the concept that the composition of peripheral lymph within the initial lymphatic segment is similar to that of the interstitial fluid of subcutaneous connective tissue. Although this view is not universally accepted (35, 36), most scientists believe that prenodal peripheral lymph represents an adequate sample of the interstitial fluid of skin and connective tissue (4). Studies using postnodal lymph are complicated by the fact that macromolecules within lymph may be either concentrated or diluted by water movement during passage across lymph nodes or lymphatic walls (37–39). The intimate contact between plasma and lymph within the node may also allow some exchange between them (4).

Literature concerning intestinal and hepatic lymph lipoproteins will not be discussed in this review. Intestinal lymph contains exogenous components absorbed from the diet, whereas hepatic lymph is derived from an extremely permeable microvascular bed and has a composition which is similar to that of plasma.

TABLE 3. Subcutaneous tissue interstitial fluid/plasma and lymph/plasma concentration ratios

	Total Protein	Albumin	Globulin	Transferrin
Interstitial fluid (SD)	0.37 (0.06)	0.42 (0.08)	0.32 (0.07)	0.42 (0.09)
Lymph (SD)	0.38 (0.06)	0.46 (0.08)	0.30 (0.09)	0.41 (0.09)

[a] Reproduced with permission from Acta. Physiol. Scand. (10).
[b] SD, standard deviation.

STUDIES OF PERIPHERAL LYMPH LIPOPROTEIN COMPOSITION

F. C. Courtice (40, 41) was one of the first scientists to study the lipoprotein composition of lymph collected from experimental animals. He used ultracentrifugation and electrophoresis in the study of lipoproteins in lymph collected from leg, cervical, hepatic, and thoracic ducts of cats, rabbits, and dogs. He was mainly interested in lipoproteins as molecular size markers for the study of capillary permeability changes during injury to peripheral vascular beds. Courtice noted that all plasma lipoprotein classes, including chylomicrons, appeared in the peripheral lymph of uninjured animals. He found that the concentration of lipoproteins in lymph is inversely proportional to their molecular size. He reported lymph/plasma concentration ratios of 0.37 for albumin, 0.31 for HDL (1.063–1.20 g/ml), 0.13 for LDL (1.019–1.063 g/ml), and 0.07 for VLDL (< 1.019 g/ml) in rabbits (40). Cholesterol feeding increased the lipid concentrations of both plasma and lymph.

Reichl and co-workers (23) pioneered studies of lipoprotein metabolism in human peripheral lymph collected from normal and hyperlipidemic subjects. Peripheral lymph is collected under local anesthesia by cannulating lymphatics on the dorsum of the foot. Total lymph collection from each subject is usually less than 1 ml (23). Total lymph/plasma cholesterol concentration ratios average 0.1. Low levels of LCAT activity have also been reported (42). Radiolabeled VLDL and LDL, injected intravenously, appeared in lymph (43). The apoprotein B lymph/plasma ratio was 0.05–0.1 in both normal and hyperlipemic subjects (44) whereas apoA-I ratios varied from 0.09 to 0.16 (45) (**Table 4**). The average size of lymph HDL was larger than that of corresponding plasma HDL and contained more cholesterol relative to A-I (45, 46). The free cholesterol content of HDL was not increased; however, Reichl et al. postulated that free cholesterol was transferred to lymph HDL from peripheral tissues (47) and esterified by LCAT in lymph. One of the difficulties with human studies is the relatively small amount of peripheral lymph collected.

Considerable volumes (30–50 ml) of peripheral lymph can be collected in acute experiments in dogs, and this model has been extensively used to study the permeability characteristics of the microvascular bed of skin and connective tissue (48–50). We have also developed a chronic lymph cannulation procedure that yields over 200 ml of lymph collected over 5 days. These relatively large amounts of lymph are advantageous for characterization of interstitial fluid lipoprotein subfractions. Although control dog plasma lipoproteins are almost entirely HDL, atherogenic diets substantially increase the plasma content of VLDL, IDL, and LDL and cause the appearance of HDLc and β-VLDL in plasma (51, 52). Such diet-induced changes in the plasma lipoprotein content enable one to study the entire size spectrum of interstitial fluid lipoproteins under conditions of increased cholesterol flux.

All lipoprotein fractions found in the plasma of normal and hypercholesterolemic animals are present in peripheral lymph. There was no apparent change in the electrophoretic mobility when lymph lipoproteins were compared with those of plasma. The lymph/plasma concentration ratios of lipoproteins, as measured by their predominant apoprotein content, followed the expected inverse relation to their molecular size in control, and cholesterol-fed/hypothyroid dogs (53, 54) (Table 4).

Considerable differences between plasma and lymph lipoproteins in the HDL density fraction were observed. These differences were present in the lymph HDL collected from control, hypercholesterolemic euthyroid, and hypercholesterolemic hypothyroid dogs. They were more accentuated, however, in the hypothyroid animals. Compared with plasma HDL, lymph HDL was larger, had an increased cholesterol/protein ratio, and contained proportionately more phospholipid and free cholesterol (**Table 5**) (55). Consistent with these compositional changes, which also occurred in control dogs, lymph HDL was found to contain discoidal particles (**Fig. 1**). Total plasma HDL fractions collected from the same animal contained no demonstrable discoidal particles. Major differences in apoprotein composition were also observed when lymph HDL was compared with plasma HDL. Lymph HDL contained disproportionately large quantities of apoE and apoA-IV (**Table 6**), indicating that peripheral modification of HDL occurs either by redistribution of apoproteins or de novo peripheral synthesis. The marked size-and-shape heterogeneity of lymph HDL suggests that it consists of a mixture of modified and unmodified HDL that could be subfractionated on the basis of size. Lymph and plasma HDL were, therefore, chromatographed on 10% agarose columns (56). **Fig. 2** shows the protein elution profiles. The larger

TABLE 4. Interstitial fluid/plasma concentration ratios of apoproteins

Subject (Ref.)	ApoB	ApoE	ApoA-I	ApoA-IV
Human peripheral lymph (45)	0.16		0.12	
Human lymphedema fluid (22)	0.06	0.38	0.12	
Human aortic wall (12)	2.0			
Human blister fluid (20)	0.16		0.24	
Control dog peripheral lymph (53)	0.07	0.11	0.10	0.15
Cholesterol-fed dog peripheral lymph (53)	0.02	0.04	0.09	0.12
Rat renal lymph (69)	< 0.02	0.14	< 0.07	0.50

HDL particles (Fraction I) were predominant in peripheral lymph from cholesterol-fed animals. These particles contained over 80% unesterified cholesterol, represented more than 60% of the total lymph HDL cholesterol present, and were almost exclusively of discoidal shape. In plasma, HDL particles in the same fraction contained about 50% unesterified cholesterol and accounted for only 20% of the total plasma HDL cholesterol. Few of these particles were of discoidal shape. The smaller HDL particles in both lymph and plasma (Fraction II) were uniformly spherical. Similar results were obtained in control dogs (**Table 7**).

The apoprotein composition of the large HDL was different from that of small HDL particles in both plasma and lymph (**Fig. 3**). Compared with apoA-I, the larger lymph HDL was enriched in both apoE and A-IV. Using heparin-Sepharose affinity chromatography, we were able to isolate two types of particles present in the larger HDL of plasma and lymph. One fraction contained apoE, A-IV, and A-I, whereas the other fraction contained only A-I and A-IV. The increased apoE content of lymph HDL is, at least partially, due to peripheral synthesis (57). The newly synthesized and secreted apoE is associated with the large, discoidal fraction (Fraction I) of lymph HDL (**Fig. 4**). Although no peripheral synthesis of apoA-I and apoA-IV could be demonstrated, the increased apoA-IV content of lymph HDL may be the result of the higher rate of capillary filtration of the small "free" plasma apoA-IV and its subsequent association with lymph HDL. The vastly different composition of these HDL particles may result in differing metabolic fates.

Although we have shown that small amounts of discoidal HDL particles occur in peripheral lymph of control dogs, the most startling increase in discoidal HDL occurred in lymph collected from hypercholesterolemic hypothyroid animals (55). To eliminate the effects of hypothyroidism, we studied lymph from hypercholesterolemic euthyroid dogs treated as described by Mahley and co-workers (51, 52, 58). Lymph from these animals also showed increased formation of apoE-, apoA-IV-enriched discoidal particles. About 5% of the lymph HDL particles of euthyroid hypercholesterolemic dogs were discoidal (**Fig. 5**). In contrast, the lymph HDL of control dogs contained occasional discs, whereas the lymph HDL of hypothyroid hypercholesterolemic animals was mostly discoidal. The difference in disc content between hypothyroid and euthyroid hypercholesterolemic animals could be explained by the sharp reduction in plasma spherical HDL particles that occurs in hypothyroid but not euthyroid animals (**Table 8**). The 80% decrease in plasma spherical HDL particles reduces filtered spherical HDL content of lymph in the hypothyroid hypercholesterolemic animals. Thus, hypercholesterolemia is clearly associated with an increased number of discoidal HDL particles, regardless of the thyroid status of the animals.

TABLE 5. Chemical composition of plasma and lymph HDL (d 1.063–1.21 g/ml) obtained from control and cholesterol-fed dogs[a]

	Control (n = 3)		Cholesterol-Fed (n = 3)	
	Plasma	Lymph	Plasma	Lymph
	% (by weight)			
Protein	43.2 ± 0.4[b]	37.1 ± 0.3	40.7 ± 0.9	35.7 ± 1.2
Cholesteryl ester	12.8 ± 1.5	11.0 ± 0.5	19.1 ± 0.8	9.0 ± 0.3
Free cholesterol	4.4 ± 0.4	8.1 ± 0.4	4.2 ± 0.4	15.3 ± 0.4
Phospholipid	39.1 ± 1.1	41.9 ± 1.8	35.2 ± 1.1	40.0 ± 0.8
Triglyceride	0.5 ± 0.1	1.9 ± 0.3	0.8 ± 0.3	ND[c]
Free cholesterol/ester cholesterol	0.36 ± 0.06	0.74 ± 0.07	0.23 ± 0.04	1.70 ± 0.02

[a] Reproduced with permission from J. Lipid Res. (55).
[b] Values (% by weight) ± SEM.
[c] Not detectable.

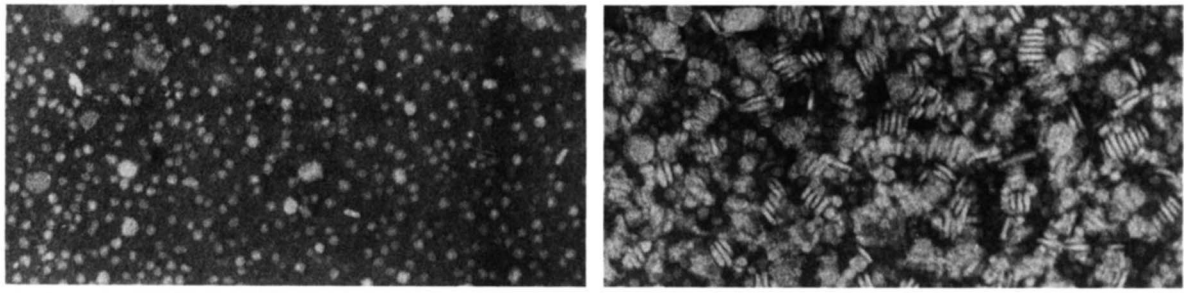

Fig. 1. Electron micrographs of plasma (left) and interstitial high-density lipoprotein (right). Reproduced with permission from J. Lipid Res. (55).

Muscle lymph

The interstitial space (ISS) of skin and connective tissue constitutes 20–40% of the total ISS. Another 20–40% of the ISS is associated with skeletal muscle. For this reason, it is important to determine whether the unique HDL particles observed in peripheral lymph occur in lymph derived from skeletal muscle. Preliminary experiments with skeletal muscle lymph collected from femoral lymphatics indicate that apoE- and apoA-IV-enriched discoidal HDL particles are also formed in the interstitial space of skeletal muscle.

Cardiac lymph

Collection of cardiac lymph has been used to study the lipoprotein composition of interstitial fluid derived from the myocardial microvascular bed. Cardiac lymph is collected by cannulation of one of several cardiac lymphatics caudal to the cardiac lymph node (59–61). Although cardiac lymph is collected before it traverses the cardiac lymph node, it may be contaminated by postnodal lymph from scattered pretracheal nodes (62). Myocardial capillaries are apparently quite permeable to macromolecules. Lymph/plasma concentration ratios for total plasma protein vary from 0.60 to 0.70 (59, 63). Cardiac lymph contains considerable quantities of lipoproteins of all density classes. Stokke et al. (63) measured lymph/plasma concentration ratios of 0.67, 0.30, and 0.35 for VLDL, LDL, and HDL, respectively (Table 2). Cardiac lymph VLDL is smaller (39 nm vs. 66 nm diameter) and has less triglyceride than corresponding plasma VLDL (64). This finding and the measurement of low specific activities of cardiac lymph LDL after intravenous injection of labeled VLDL have led to the hypothesis that LDL is continuously formed from VLDL within the cardiac interstitium (64).

TABLE 6. Lymph/plasma apoprotein concentration ratios of HDL in control and cholesterol-fed dogs[a]

	ApoA-I	ApoE	ApoA-IV
Control	0.07	0.70	0.23
Cholesterol-fed	0.10	1.05	2.33

[a] Based on data in Tables 3 and 4 of reference 55.

Lung lymph

Lung lymph lipoproteins have been characterized in chronically instrumented awake sheep (65, 66). The lung microvascular bed is highly permeable (67), as is reflected in lymph/plasma concentration ratios of HDL and LDL of about 0.5. Despite this relatively high permeability, lymph lipoproteins differed considerably from their plasma counterparts in size, shape, and chemical composition

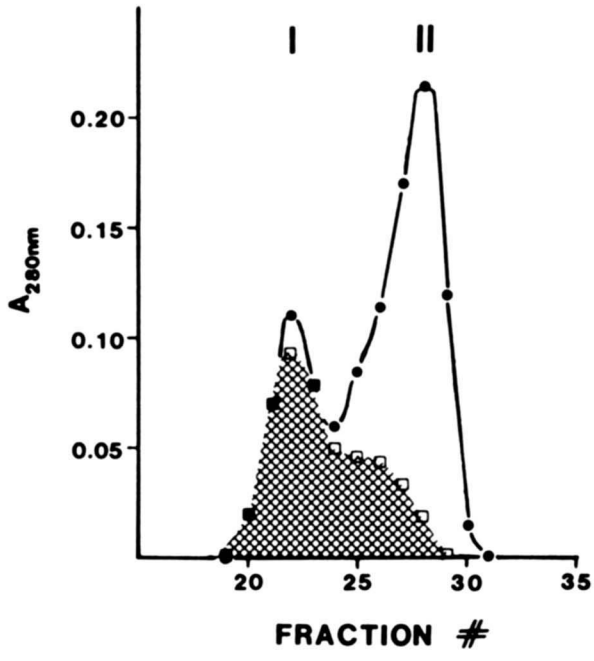

Fig. 2. Ten percent agarose (A-0.5m) column chromatography of interstitial fluid (shaded area □ — □) or plasma (clear area ● — ●). The HDL mixture was obtained from a cholesterol-fed dog. Fractions I and II were pooled separately as indicated. Reproduced with permission from J. Lipid Res. (56).

TABLE 7. Chemical composition (% weight) of interstitial fluid and plasma HDL subfractions I and II from control and cholesterol-fed dogs[a]

	Control				Cholesterol-Fed			
	Plasma		Interstitial Fluid		Plasma		Interstitial Fluid	
	I	II	I	II	I	II	I	II
Protein	24 ± 2	42 ± 1	26 ± 3	43 ± 2	20 ± 2	41 ± 1	21 ± 1	36 ± 3
Unesterified cholesterol	14 ± 1	5 ± 0.2	15 ± 1	6 ± 1	16 ± 2	5 ± 0.5	28 ± 1	15 ± 3
Cholesteryl ester	20 ± 1	14 ± 1	15 ± 1	13 ± 1	17 ± 6	14 ± 2	6 ± 2	12 ± 2
Phospholipid	43 ± 5	39 ± 1	44 ± 1	38 ± 2	47 ± 3	40 ± 2	45 ± 1	37 ± 1
Unesterified cholesterol/protein	0.58	0.12	0.58	0.14	0.14	0.80	1.33	0.42
% Unesterified cholesterol	42	25	50	32	48	26	82	56

[a]Reproduced with permission from J. Lipid Res. (56).

(lipid and apoprotein). Lung lymph HDL particles are larger and more heterogeneous than those of plasma, as measured by gradient gel electrophoresis. Electron microscopy shows fairly uniform spherical particles in the plasma HDL. Lymph HDL contains both large and small spherical particles as well as a variable amount of discoidal particles (65, 66). Lymph LDL also contains a distinct subgroup of smaller particles that are not found in plasma. Electron microscopy of these particles shows that they have a tendency to form square packing arrays. Their principal apoproteins are apoE and apoA-I. The authors suggested that these particles may be related to transport of surface components such as phospholipids. The phospholipid contents of lymph whole LDL was 41.9% compared with 31.2% for plasma LDL (66). The free-to-esterified cholesterol ratios were dramatically increased in both lymph HDL and LDL (**Table 9**). These findings show that several density classes of filtered plasma lipoproteins increase their free cholesterol content within the interstitial space and may be important in reverse cholesterol transport. Distinct differences in plasma and lymph lipoprotein composition exist, even in tissues that exhibit high microvascular permeability. Lung metabolism apparently has dramatic effects on the composition of filtered plasma lipoproteins.

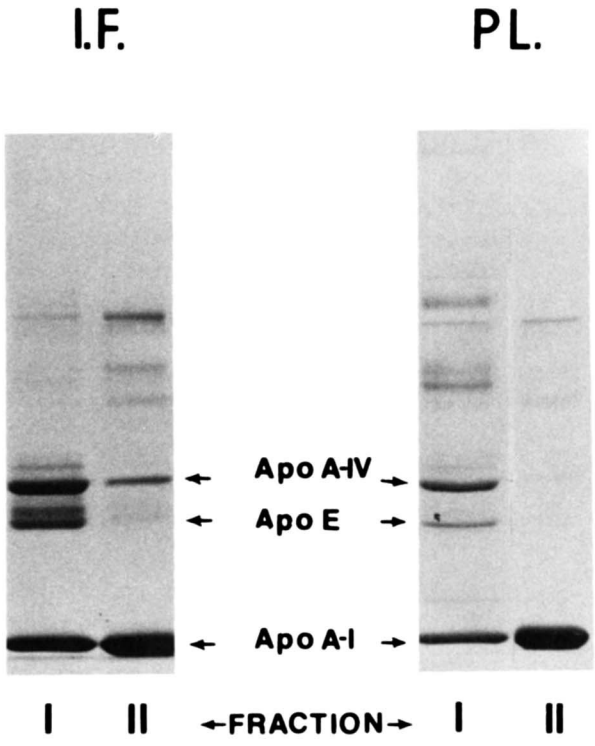

Fig. 3. SDS-PAGE of interstitial fluid and plasma HDL subfractions obtained from a cholesterol-fed dog. Reproduced with permission from J. Lipid Res. (56).

Renal lymph

Renal lymph is derived mainly from fenestrated capillaries from the cortical peritubular capillary plexus. The renal medullary interstitial space is not thought to contribute to the formation of renal lymph (68). Measured lymph/plasma concentration ratios for albumin are 0.25 (69). Lymph/plasma concentration ratios (L/P) for apoA-IV were 0.50, whereas apoE had an L/P ratio 0.18. The L/P ratio of apoA-I was less than 0.07. The high L/P ratio of apoA-IV reflects the high plasma content of freeA-IV. The L/P ratio of apoE is probably a function of its peripheral synthesis by the kidney, as well as filtration of apoE-containing lipoproteins. The low L/P ratio for apoA-I may be related to its catabolism by the kidney (70, 71).

ENZYMES OF LIPID METABOLISM WITHIN THE INTERSTITIAL SPACE

In view of the volume of the interstitial space (three to four times that of plasma) and its lipoproteins content, a considerable fraction of the body's total lipoproteins could be exposed to a variety of enzymes that are present in the interstitial fluid (72). This review emphasizes the experi-

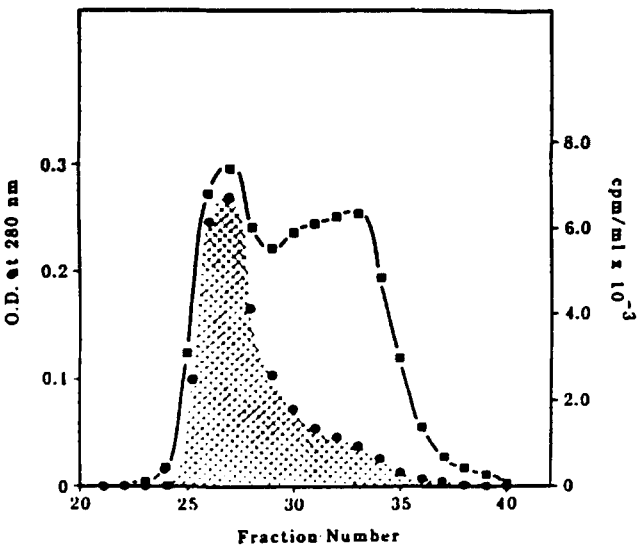

Fig. 4. Column chromatography of the d 1.05–1.21 g/ml fraction of a cholesterol-fed dog from pooled interstitial fluid after an injection of 2 mCi of [^{35}S]methionine into the skin of the toes of the hindlimb. Interstitial fluid gathered in a 3-hr period was collected and the d 1.05–1.21 g/ml lipoproteins were isolated. Concanavalin A-Sepharose affinity chromatography was used to remove the apoB-containing lipoproteins, and the resulting HDL mixture was subfractionated using a 1 × 120-cm column of 10% agarose. The first peak corresponds to HDL_I and the second peak to HDL_{II}. Shaded area (● — ●) represents the elution of [^{35}S]methionine, while the clear area (■ — ■) represents absorbance at 280 nm. Reproduced with permission from J. Biol. Chem. (57).

mental data concerning lecithin:cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL) and their action on lipoproteins within the interstitial fluid.

Lecithin:cholesterol acyltransferase has been postulated to play an important role in reverse cholesterol transport (2). The enzyme forms cholesteryl esters from free cholesterol and is thought to control the relative flux of free cholesterol between peripheral cells and the lipoproteins of interstitial fluid. LCAT activities of 1–10% that of plasma have been reported in lymph derived from low-permeability peripheral sources (42, 54, 73). Consistent with the low LCAT activities of peripheral lymph are the very high free-to-esterified cholesterol ratios reported for peripheral lymph lipoproteins (46, 55). In contrast to low-permeability peripheral sites, cardiac lymph contains 10–30% that of plasma LCAT activity (63, 74).

Lipoprotein lipase activities are not detectable (or extremely low) in peripheral lymph of humans and dogs (42, 75). This is also true when plasma lipoprotein lipase concentrations are increased by heparin injection. Under these conditions, non-steady-state L/P ratios of LPL were about 0.003 compared with measured steady-state L/P ratios of 0.3 for albumin. Normal steady-state LPL activity in peripheral lymph is likely less than 1% of the activity measured in plasma (75). In contrast, the post-heparin lipoprotein lipase activity of cardiac lymph reached 10–30% that of plasma (74). This relatively high activity of LPL within the myocardial interstitial space may be responsible for the low triglyceride content of cardiac lymph VLDL particles and increased LDL formation (64).

All of these reports were based on measurement of enzyme activities and not enzyme mass; however, no inhibitors have been reported in peripheral lymph (42, 54, 75).

Interstitial fluid lipoprotein metabolism

Few studies of the metabolism of interstitial fluid lipoproteins are available, due in part to the difficulty of obtaining adequate quantities of material for the study. The low LCAT activity found in peripheral lymph is not due to the fact that interstitial lipoproteins are inadequate substrates for LCAT. When semipurified LCAT was added to lymph lipoproteins, the enzyme decreased free cholesterol and phospholipid content and density of lymph HDL while increasing its ester and apoE content of the lower density fraction (54). These data suggest that HDLc may be formed in plasma through the action of plasma LCAT on HDL particles derived from lymph. Hepatic uptake of HDLc would constitute a pathway for catabolism of cholesterol derived from peripheral cells (76, 77).

The biological activity of interstitial LDL has been studied by Reichl et al. (78). Lymph LDL was recognized by the peripheral LDL receptors and suppressed HMG-CoA reductase activity in a manner similar to plasma LDL.

Peripheral apoprotein synthesis during the formation of interstitial lipoproteins has been studied by injecting labeled amino acids locally into the dog's paw, and measuring the incorporation of label into peripheral lymph apoproteins (57). The specific activity of apoE in the lymph collected at the injected side was 10 to 100 times higher than the specific activity of apoE in the peripheral lymph of the contralateral leg or in the plasma. Most of the newly synthesized apoE was associated with the larger discoidal fractions of lymph HDL. No apoA-I or apoA-IV synthesis could be demonstrated.

Reichl et al. (42, 47) studied peripheral cholesterol flux by injecting labeled cholesterol into humans and determining the specific activity of plasma and lymph cholesterol at different intervals over a period of 9–10 months. At later intervals, the specific activity of lymph cholesterol was higher than that of plasma. This increase in cholesterol specific activity was mainly due to increased free cholesterol specific activity in both HDL and LDL. The authors concluded that both HDL and LDL in lymph can accept free cholesterol from the tissues (47).

Role of interstitial fluid lipoproteins in reverse cholesterol transport

Several investigators have postulated that interstitial lipoproteins, especially HDL, play an essential role in reverse cholesterol transport (2, 79). The major HDL apoproteins, apoA-I, apoE, and apoA-IV, are all good

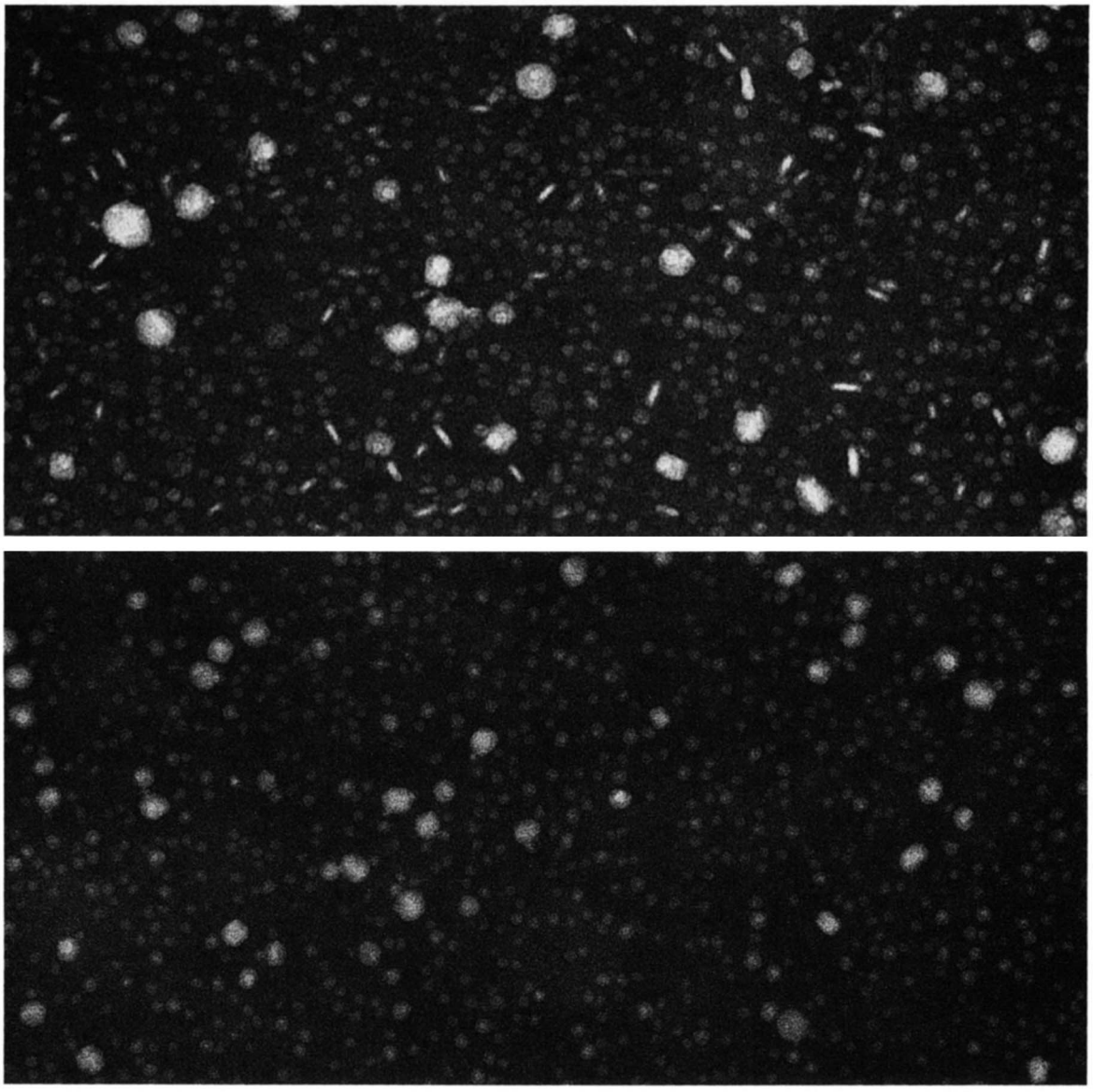

Fig. 5. Electron micrographs of negatively stained HDL fractions (d < 1.063–1.21 g/ml) from hindlimb lymph (top) and blood plasma (bottom) from the same euthyroid dog. Note that the lymph HDL (top) typically contains numerous (about 50, this image) discoidal particles on edge, which are absent from plasma HDL (bottom). Smaller HDL (< 100 Å diameter) are the predominant particle of both lymph and plasma HDL. The much larger (> 200 Å diameter) and more electron lucent particles are contaminating LDL. × 180,000. (Unpublished data.)

acceptors of cellular cholesterol and are present in high concentration in lymph HDL (80).

Interstitial fluid HDL contains both spherical and discoidal particles. The spherical lymph HDL particles are larger than plasma HDL and contain predominantly apoA-I. We believe that they are formed by peripheral modification of filtered plasma HDL by the transfer of free cholesterol and phospholipid from peripheral cells (Table 7). All lymph discoidal HDL particles contain large amounts of free cholesterol and phospholipid, although they differ in apoprotein composition. One type of disc contains mainly apoA-IV, which is probably derived from filtered "free" apoA-IV. The other particle contains peripherally synthesized apoE in addition to apoA-IV. Both particles also contain some apoA-I. The relative participation of these particles in reverse cholesterol transport is yet unknown and may vary, depending on the relative cholesterol flux.

Ultimately, interstitial lipoproteins reach the plasma compartment and are exposed to enzymes and plasma lipoproteins. We postulate that the apoE-containing discoidal HDLs are converted to spherical particles by the LCAT reaction, forming HDLc, which then can be preferentially removed by the liver (77). We have no infor-

TABLE 8. Protein and cholesterol concentrations of lymph and plasma HDL from control, *euthyroid hypercholesterolemic, and hypothyroid hypercholesterolemic dogs*

	Protein (mg/dl)			Cholesterol			Cholesterol/Protein Ratio	
	Plasma	Lymph	R[a]	Plasma	Lymph	R[a]	Plasma	Lymph
Euthyroid (cholesterol-fed)[b]	335	25.4	0.08	165	14.8	0.09	0.49	0.58
Hypothyroid (cholesterol-fed)[c]	47	6	0.13	19.9	4.7	0.23	0.42	0.78
Control[c]	260	20	0.08	102	9	0.09	0.39	0.45

[a] R, lymph/plasma concentration ratio.
[b] Unpublished observations, Sloop, C. H. et al.
[c] Data from Tables 1 and 2 of reference 55.

mation on the metabolism of the apoA-IV-containing discoidal particles; however, they may be rapidly removed by the liver. The spherical interstitial lipoprotein particles with high apoA-I and increased free cholesterol content may also be further esterified by LCAT in the plasma compartment. Thus, reactions occurring in the interstitial space and plasma influence the degree of reverse cholesterol transport and the organism's susceptibility to atherosclerosis.

SUMMARY

While a wide variety of techniques has been used to collect samples of interstitial fluid, most of our detailed knowledge about the composition of interstitial fluid lipoproteins has come from lymph collection studies. The considerable variability of lymph data probably reflects the effect of variable metabolic modification and different capillary permeabilities on the lipoprotein composition of interstitial fluid.

All density classes of plasma lipoproteins are present in lymph. In peripheral lymph, the lymph/plasma concentration ratios of lipoproteins vary from 0.03 for VLDL-sized particles to 0.2 for HDL. Lymph from more permeable vascular beds, such as lung and myocardium, contains proportionately more lipoproteins. Their lymph/plasma concentration ratios vary from 0.1 to 0.6.

In general, lymph lipoproteins are more heterogeneous in size than their plasma counterparts. Lymph HDL and LDL contain larger and smaller particles than their plasma equivalents. Lymph lipoproteins have unusual shapes (square packing and discoidal), chemical compositions, and molecular charge, which suggest de novo formation and/or extensive peripheral modification. Lymph HDL and LDL are enriched in free cholesterol. Lymph HDL also has increased cholesterol/protein and phospholipid/protein (especially sphingomyelin) ratios (Sloop, C. H., L. Dory, and P. S. Roheim, unpublished observations). Lymph HDL apoprotein composition differs from that of plasma, with an increase in apoE and apoA-IV content relative to apoA-I. These discoidal HDL particles may be products of an initial stage of reverse cholesterol transport. We believe further study of their metabolic fate would give important information concerning the later stages of reverse cholesterol transport.

UNRESOLVED ISSUES CONCERNING INTERSTITIAL LIPOPROTEINS

Obviously, data on the composition and metabolism of interstitial fluid lipoproteins are limited and fragmentary. Interstitial fluid has been collected by a variety of techniques but from a limited number of vascular beds, primarily subcutaneous connective tissue and skin.

Many unanswered questions remain concerning the events of lipid transport within the local environment of the cell and the metabolism of interstitial lipoproteins. This section discusses some of the areas that we believe are most important.

Role of "free" and peripherally synthesized apoproteins in interstitial fluid lipid transport

Most experimental studies of cholesterol transport to and from cells are carried out under conditions that allow direct contact between lipoproteins and peripheral cells. There is abundant evidence, however, that molecules the size of lipoproteins are excluded from at least 50% of the interstitial space under physiological conditions (4). This fact, together with the small diffusion coefficients of lipoprotein-sized molecules, prompts us to postulate that smaller complexes may be involved in the transfer of lipids between peripheral cells and interstitial fluid lipoproteins.

TABLE 9. Free-to-esterified cholesterol ratios of plasma and lung lymph lipoproteins[a]

	LDL	HDL
Plasma	0.31	0.15
Lymph	0.54	0.36

[a] From page 1361 of reference 66.

Such complexes would distribute in a larger fraction of the interstitial space and would diffuse more efficiently within that space. Apoprotein E, which is synthesized by peripheral cells, could combine with phospholipid and plasma membrane cholesterol to form a complex that could then diffuse to HDL particles in the sol phase of the interstitial fluid. Free apoproteins derived from plasma could form similar complexes. The small size of these particles would increase their filtration from plasma and their distributed volume within the interstitial space. The lipophilic characteristics of these apoprotein complexes should enable them to transport lipid between the cell and the interstitial fluid lipoproteins. It may be possible to compare the composition of small molecular weight apoprotein complexes in lymph and plasma under conditions that prevent ultracentrifugal artifacts. Such studies could be made under conditions of varying cholesterol efflux and would provide evidence for the participation of those particles in reverse cholesterol transport.

Role of LDL in reverse cholesterol transport

Low density lipoproteins are known to transport cholesterol to peripheral cells. Some information, however, suggests that LDL may also be involved in reverse cholesterol transport, a process usually associated with HDL. Several authors have shown that lymph LDL undergoes considerable modification within the interstitial space and has increased cholesterol content (47, 66). It is difficult to envision a process of cholesterol efflux and influx involving the same particle. Possibly, in animals where it is present, cholesterol transfer protein could shift newly acquired cholesterol from HDL to LDL particles that have undergone modification within the interstitial space. We are not aware of any studies on the cholesterol transfer protein (CTP) activity within the interstitial fluid of any animal.

Enzymes of lipid metabolism within the interstitial space

Although the concentration of CTP within the interstitial space has not been reported, we would expect that it would be relatively high. Measured activity, however, of other small enzymes of lipid metabolism in interstitial fluid is surprisingly low. Reported LCAT and LPL activities are about 0–10% that of plasma in peripheral tissue. This low concentration of LPL was not expected, in view of its synthesis by the adipocyte and its presumed transfer across the interstitial space to the surface of the capillary endothelial cell. The reason for such low activities is unknown and the physiologic implication of these observations has not yet been clarified. The low LCAT and LPL activities in interstitial fluid, however, lessen modification of newly formed particles, which makes it easier to isolate "nascent" interstitial lipoproteins.

Metabolism of lipoproteins formed in the interstitial space

Interstitial fluid lipoproteins ultimately reach the plasma, where they are diluted and undergo modification. Their metabolic fate remains unknown and presents an important experimental challenge. The relatively small quantities of interstitial fluid available make it difficult to isolate subfractions in quantities necessary for metabolic studies in whole animals. We hypothesize that specific receptors for interstitial fluid lipoproteins may exist and are important to cholesterol homeostasis. Tissue culture and organ perfusion studies should clarify this question.

Role of reverse cholesterol transport in the susceptibility to atherosclerosis

The cholesterol content of the cell is determined by the balance of cholesterol influx and efflux. The mechanisms of cholesterol influx have been extensively studied; however, little is known about the initial stages of reverse cholesterol transport that occur in the interstitial space. Studies of interstitial fluid lipoprotein metabolism in atherosclerosis-susceptible and nonsusceptible animals could demonstrate whether changes in reverse cholesterol transport influence the organism's susceptibility to atherosclerosis.

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