Role of HDL, LCAT, and Lipid Transfer Protein in Lipoprotein Metabolism*

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High density lipoproteins (HDL) play an integral part in the lipid transport system. Numerous epidemiological studies have demonstrated an inverse relationship between HDL levels and the risk of developing coronary heart disease. The biochemical and physiological mechanisms behind this inverse relationship are unclear. It has been postulated that HDL, together with the enzyme lecithin cholesterol acyltransferase (LCAT) and a neutral lipid transfer protein, plays a key role in removing unesterified cholesterol from extrahepatic tissues, including the arterial wall, and in transporting it to the liver. This "reverse cholesterol transport process" is believed to prevent lipid accumulation in the artery wall and thereby prevent or retard atherogenesis. However, the specific steps and details of this reverse cholesterol transport process are controversial or remain to be clarified. The first step in this process involves the transfer of unesterified cholesterol from the surface of peripheral cells to the surface of HDL (Fig. 1). Recent evidence suggests that this transfer step may be enhanced by binding of HDL to the cell surface¹). It has been postulated that peripheral cells synthesize specific, cell-surface receptors for HDL in response to cholesterol accumulation and that these receptors may act to facilitate cholesterol transport from cells²). Because HDL particles can bind only a finite number of unesterified cholesterol molecules, step 2, or the conversion of unesterified cholesterol to cholesteryl ester by LCAT, is required to keep the chemical potential gradient

going. In order to remove the excess cholesteryl ester, step 3, or the transport of cholesteryl ester from HDL to acceptor lipoproteins, such as remnants of chylomicrons and VLDL, is required. Cholesteryl ester is transported from HDL to other lipoproteins by a plasma lipid transfer protein (LTP). The nature of this transfer protein or process remains controversial. The cholesteryl ester is transported to the liver by cholesteryl ester acceptor lipoproteins in step 4.

I wish to now discuss in some detail each of the key components of the "reverse cholesterol transport" process, HDL, LCAT, and LTP. Conventionally, high density lipoproteins are defined on the basis of their nonspecific physical properties, i.e., as those lipoproteins within the hydrated density range of 1.063–1.21 g/ml that have alpha-electrophoretic mobility. Furthermore, these lipoproteins generally have a floatation rate of 0 to 9 Svedbergs by analytical ultracentrifugation in a salt density of 1.20 g/ml and a size range of 80 to 120 Å. This lipoprotein class has been considered to be metabolically and functionally homogeneous. In fact, however, high density lipoproteins are a heterogeneous population of particles differing in size and in lipid and apoprotein composition. HDL particles differing in composition may differ in their metabolic characteristics and functional roles in lipoprotein metabolism.

The HDL fraction contains at least 8 different apoproteins (Table 1). Furthermore, other proteins, such as LCAT, LTP and sterol carrier protein (SCP), are also associated with HDL. Although quantitatively representing a small proportion of the total weight of HDL, these proteins may play a very important role in HDL and lipoprotein metabolism.

HDL particles may be derived from a number of

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Fig. 1 Reverse cholesterol transport.

Table 1 HDL protein composition

Protein	Weight (%)
A-I	67
A-II	20
C-I, C-II, C-III	6
D	3
E	2
B	2
LCAT, LTP, SCP	?

Fig. 2 HDL formation.

sources (Fig. 2). Nascent HDL particles may be produced by the liver or intestine in the form of discoidal particles or small spherical particles and apoprotein E containing discoidal particles may arise from macrophages. HDL particles may also arise from intravascular catabolism of chylomicrons or very low density lipoproteins (VLDL). Excess surface material derived from the action of lipoprotein lipase can form nascent HDL particles

Fig. 3 A-I/A-II ratio in HDL subfractions.

or combine with preexisting HDL particles. HDL receives unesterified cholesterol from the surface of lipoproteins and cell membranes. The cholesterol is esterified by LCAT and the cholesteryl ester transferred to the HDL core or transferred to acceptor lipoproteins by the lipid transfer protein (LTP).

Apoproteins A-I and A-II are the major polypeptides of HDL, comprising approximately 85% to 90% of the total HDL protein mass. HDL subfractions separated by density-gradient ultracentrifugation have differing A-I: A-II molar ratios; both the least dense and the most dense fractions have a higher A-I: A-II molar ratio than the intermediate fractions (Fig. 3). Quantitative immunochemical analysis of HDL subfractions precipitated with anti-A-II indicate that HDL particles containing both A-I and A-II, Lp (A-I + A-II), have a nearly constant A-I/A-II molar ratio of 2:1 regardless of the hydrated density of the subfraction³. Thus, the differing A-I: A-II molar ratio in HDL subfractions reflects the differing proportion of HDL particles with A-I without A-II, Lp (A-I). Additional studies of HDL heterogeneity suggest that the differences found in HDL composition reflect different proportions of distinct HDL subpopulations that differ in composition, rather than differences in the composition of HDL particles themselves.

In an effort to isolate and characterize HDL subpopulations which differ in apoprotein composition, lipoprotein particles were isolated directly from plasma by immunoaffinity chromatography (Fig. 4). Using immunoabsorbents containing

Fig. 4 Isolation of lipoproteins by immunoaffinity chromatography.

Fig. 5 Gradient gel electrophoresis of lipoproteins isolated by immunoaffinity chromatography and of HDL subfractions.

Fig. 6 HDL particle heterogeneity.

Table 2 Percentage of plasma protein associated with HDL particles

	Lp(A-I+A-II)	Lp(A-I)
Apo D	64±4	31±8
Apo E	62±7	7±5
LCAT	48±4	28±14

Fig. 7 Protein heterogeneity of HDL particles.

HDL⁴). Both particles were heterogeneous in size with the larger HDL particles predominantly in the HDL₂ fraction and the smaller particles in the HDL₃ fraction (Fig. 5). As illustrated diagrammatically in Fig. 6, Lp (A-I) contained two particle sizes with apparent mean molecular weights of 3.0×10^5 and 1.6×10^5 and Stokes diameters of 10.8 nm and 8.5 nm. Lp (A-I+A-II) contained three particle sizes with apparent mean molecular weights of 2.3×10^5 , 1.8×10^5 , and 1.2×10^5 and Stokes diameters of 9.6 nm, 8.9 nm, and 8.2 nm. The HDL₂ fraction isolated by ultracentrifugation contains the larger (10.8 nm) Lp (A-I) particles and the largest (9.6 nm) Lp (A-I+A-II) particles and perhaps some intermediate (8.9 nm) Lp (A-I+A-II) particles. The HDL₃ fraction contains the smaller (8.5 nm) Lp (A-I) particles and the smallest (8.2 nm) and intermediate (8.5 nm) Lp (A-I+A-II) particles and perhaps some of the largest (9.6 nm) Lp (A-I+A-II) particles. LCAT and apoproteins D and E were detected in both Lp (A-I) and Lp (A-I+A-II) (Table 2). Thus, these two types of HDL particles can exhibit heterogeneity not only in size but also in protein composition as illustrated in Fig. 7.

The kinetics and specificity of LCAT depends upon the nature and physical state of the substrate and apoprotein catalyst, and the availability and conformation of the acyl acceptor at the lipoprotein or vesicle surface. As a phospholipase A,

either anti-A-I or anti-A-II, the particles containing A-I and A-II, Lp (A-I+A-II) and A-I without A-II, Lp (A-I) were isolated directly from plasma without ultracentrifugation. Both types of particles possessed alpha-electrophoretic mobility and showed hydrated density in the range of plasma

Fig. 8 Reactions mediated by the LCAT enzyme.

LCAT catalyzes on the surface of HDL the hydrolysis of fatty acid from the Sn-2 position of phosphatidylcholine to form lysolecithin (Fig. 8). As an acyltransferase, it transfers the fatty acid to unesterified cholesterol, thereby forming cholesteryl ester. LCAT shows a relative lack of specificity in terms of acyl acceptor. Thus, in the presence of LDL, LCAT can catalyze the transfer of fatty acid to lysolecithin to form lecithin⁵⁾. The specificity of this lysolecithin acyl transferase (LAT) reaction results in preferential formation of disaturated phospholipid species⁶⁾. This fatty acid specificity of the LAT reaction may be physiologically significant in determining the fatty acid composition of phospholipid and cholesteryl ester of plasma lipoproteins and thereby altering the surface fluidity of lipoproteins and cell membranes.

The transesterification reaction of LCAT requires a protein catalyst for activity. Apoprotein A-I is believed to be the physiological cofactor for this reaction. Our recent findings, however, suggest that apoproteins other than apo A-I may serve as a physiological cofactor for the enzyme⁷⁾. Examination of a patient's plasma that completely lacked A-I, indicated that the cholesterol esterification rate, LCAT activity and LCAT mass were reduced to approximately 40% of that found in control subjects. Similarly, two subjects with A-I and C-III deficiency with a trace of A-I have a reduced rate

of cholesterol esterification and LCAT activity that is proportional to the reduced level of LCAT mass. These results suggest that apoproteins other than A-I can serve as cofactors for the cholesterol acyltransferase reaction mediated by LCAT.

The ability of apoprotein E or A-IV to activate LCAT was examined. Apo E was purified from the plasma of a subject with dysbetalipoproteinemia (phenotype E 2/2) and from a subject with hypertriglyceridemia (phenotype E 3/3); A-IV was purified from normolipidemic plasma. Lecithin-cholesterol apoprotein liposomes were prepared by the cholate dialysis procedure⁸⁾. LCAT activity obtained with liposomes containing apo E or A-IV was compared with liposomes containing purified A-I. Apo E-2, E-3, and A-IV, each activated purified LCAT using egg yolk phosphatidylcholine (PC) as the acyl donor. Apoprotein E-2 was $40 \pm 3\%$ as efficient as apoprotein A-I for LCAT activation and apoprotein E-3 was $32 \pm 2\%$ as efficient. Apoprotein A-IV activated LCAT $25 \pm 2\%$ as efficiently as apo A-I. Similar results were obtained using liposomes with α -palmitoyl- β linoleoyl phosphatidylcholine as the acyl donor. When phosphatidylcholine with two unsaturated fatty acids or dilinoleoyl phosphatidylcholine was used as the acyl donor, again, quite similar results were obtained. However, when phosphatidylcholine with two saturated fatty acids or dipalmitoyl-phosphati-

Fig. 9 Chromatography of plasma lipid transfer protein (LTP) on Sephacryl S-200.

dylcholine was used as the acyl donor, apo A-IV was slightly more efficient than apo A-I in activation of LCAT, whereas the apo E isoforms were about 30% as efficient as apo A-I in activation of the enzyme. Similar results were obtained with other disaturated phosphatidylcholines. Therefore, apoprotein E and A-IV may serve as physiological cofactors for the LCAT reaction.

Recently, there has been intense interest in the role of the lipid transfer proteins in lipoprotein metabolism. It was originally suggested that apoprotein D was responsible for the transport of cholesteryl ester between lipoproteins. Because this transport function for apoprotein D could not be confirmed⁹, we proceeded to isolate to homogeneity a neutral lipid transport protein from plasma of humans¹⁰. This plasma lipid transfer protein has an electrophoretic mobility similar to A-I in polyacrylamide gel containing 8 M urea and has an apparent molecular weight of $64,000 \pm 2,000$ on sodium dodecylsulfate gel electrophoresis and 64,000 by Sephacryl S-200® molecular sieve chromatography (Fig. 9). It has an isoelectric point of 5.0 ± 0.02 by both analytical isoelectrofocusing and chromatofocusing (Fig. 10). This purified transfer protein, named LTP-1, did not bind to a heparin column and was relatively resistant to elevated temperature (at 58°C., only 5% of the transfer activity was lost in 1 hour). Although the precise role of this protein in lipoprotein metab-

olism remains to be clarified, this purified transfer protein facilitated the net mass transfer of cholesteryl ester from HDL or LDL to VLDL, the net mass transfer of triglyceride from VLDL to LDL or HDL, and the mass transfer of phosphatidylcholine from VLDL to HDL. The transfer of neutral lipids among lipoproteins mediated by LTP-I is illustrated in Fig. 11. Thus, lipid transfer protein can cause extensive remodeling of the plasma lipoproteins. This process could significantly alter lipoprotein metabolism. For example, when lipid transfer protein causes transfer of excess triglyceride to cholesteryl-ester-rich particles like LDL, these triglyceride-rich, modified LDL particles may be hydrolyzed by triglyceride lipase to yield small LDL particles that are lipid-poor and rich in apoprotein B (Fig. 12). Lipid-poor, apoprotein-B-rich particles are characteristic of some forms of hyperlipidemia, such as familial combined hyperlipidemia, which is associated with an increased risk of premature coronary heart disease¹¹.

Although the lipid transfer protein has been postulated to play a fundamental role in reverse cholesterol transport and the compositional modification of lipoproteins, the cellular origin of human plasma lipid transfer protein is unknown. One cell type that may play an important role in lipoprotein metabolism and reverse cholesterol transport is the macrophage. Human macrophages secrete a multitude of biologically active products,

Fig. 10 Chromatofocusing and isoelectric focusing of plasma lipid transfer protein (LTP).

Fig. 11 Lipid transfer protein mediated cholesterol ester and triglyceride transfer among lipoproteins.

some of which, such as lipoprotein lipase and apoprotein E, play a central role in lipoprotein metabolism. Therefore, the possibility that human monocyte-derived macrophages (h-MDM) secrete lipid transfer protein was tested¹²⁾. Macrophages were isolated and cultured as illustrated in Fig. 13. Lipid transfer activity was assayed by the measurement of the transfer of labeled cholesterol ester,

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- CULTURE CONDITIONS:
- a) M ϕ plated @ 2×10^6 cells/dish
  - b) grown in 20% AS, fed 2x weekly
  - c) grown 7-14 days
  - d) triplicate dishes for each data point

Fig. 13 Macrophage isolation and culture. AS=autologous serum.

triglyceride, or phosphatidylcholine from HDL $_3$ to $d < 1.063$ g/ml lipoproteins. Labeled donor HDL was incubated with acceptor lipoproteins with or without a source of lipid transfer activity. Donor and acceptor lipoproteins were separated by heparin-MnCl $_2$ precipitation¹³. Conditioned medium from unstimulated macrophages in vitro facilitated the transfer of cholesteryl ester, triglyceride, and phosphatidylcholine between HDL and VLDL. The h-MDM lipid transfer activity was secreted into serum-free medium in a linear, time-dependent manner over a 24-hour period. When a macrophage activator, phorbol myristate acetate was added to h-MDM, lipid transfer activity secretion was stimulated by $66 \pm 18\%$ over unstimulated macrophages. When cycloheximide (10 μ M) was added, the appearance of lipid transfer activity in the incubation medium was completely inhibited.

The h-MDM lipid transfer activity was further characterized to compare it to the plasma lipid transfer protein and it was found to have remarkably similar physicochemical properties to the plasma-derived lipid transfer protein¹². The h-MDM-secreted lipid transfer activity eluted from Sephacryl S-200® just slightly later than bovine serum albumin with an apparent molecular weight of approximately 62,000. This is in good agreement with the molecular weight of the plasma-derived lipid transfer activity. Analysis of the activity profile of the h-MDM lipid transfer protein off chromatofocusing reveals a single symmetrical peak of activity near a pH of 5.0 ± 0.1 . This is identical to the isoelectric point of the plasma-derived lipid transfer protein. In addition to similar size and isoelectric point, both the h-MDM lipid transfer activity and the plasma-derived lipid transfer activity binds and elutes from phenyl-Sepharose® with water and neither have an affinity

Table 3 Comparison of macrophage- and plasma-derived LTP

Property	Macrophage-LTP	Plasma-LTP
Molecular weight	63,000	63,000
Isoelectric point	5.0	5.0
Hydrophobic (binds phenyl-Sepharose)	yes	yes
Affinity for Heparin-Sepharose	no	no
Facilitates transfer of:	CE, TG, PC	CE, TG, PC

for heparin-Sepharose® (Table 3). It is concluded, therefore, that the human monocyte-derived macrophage may be a cellular source of the lipid transfer protein (LTP-I) found in plasma. Furthermore, the human macrophage may play a major role in lipoprotein metabolism through its synthesis and secretion of LTP and may thereby facilitate reverse cholesterol transport.

HDL, together with LCAT and the neutral lipid transfer protein, play a central role in lipoprotein metabolism and cholesterol homeostasis. HDL accepts unesterified cholesterol from peripheral tissues. This cell surface transport process may be facilitated by high affinity receptors for HDL. HDL binding is enhanced by cholesterol-loading and decreased by cellular cholesterol depletion. Through LCAT action, unesterified HDL cholesterol is esterified and cholesteryl ester is transferred to acceptor lipoproteins by a lipid transfer protein (LTP-I) for direct or indirect transport to the liver, which permits additional transfer of unesterified cholesterol to HDL. Thus, LCAT and LTP-1 create a gradient of unesterified cholesterol concentration between peripheral tissues and HDL that permits a net efflux of cholesterol from tissues or "reverse cholesterol transport". HDL, LCAT, and LTP-1 also play

important roles in removing surface components (particularly phospholipid, unesterified cholesterol, and protein) from VLDL and chylomicron remnants during lipolysis. Furthermore, the lipid transfer protein provides a mechanism for the redistribution of neutral lipids between lipoproteins and between lipoproteins and plasma membranes. LCAT and LTP-1, together with HDL, play an essential role in human lipoprotein structure and metabolism.

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