Sites and mechanisms of uptake and degradation of high density and low density lipoproteins

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Abstract In spite of much progress in understanding the mechanisms of lipoprotein metabolism at the cellular level, much less is known quantitatively about what actually occurs in vivo. A new tool for understanding the catabolic fates of lipoproteins in vivo is the use of noncatabolizable ligands for labeling proteins. These ligands are incorporated into cells with the labeled protein. The protein is catabolized but the ligand remains intact and trapped at the sites of hydrolysis as a cumulative marker of the extent of protein uptake. This tool was first applied to LDL in pigs, rats, and rabbits. In all three, hepatic catabolism predominated. Adrenal gland was the most active per unit wet weight. The trapped label methodology was also applied to the apoA-I component of HDL in rats. [^{3}H]Cholesteryl *ethers* were also incorporated as noncatabolized, intracellularly trapped tracers of cholesteryl esters. Liver was the predominant site of HDL cholesteryl ether uptake, consistent with a role for HDL in reverse cholesterol transport; adrenal was the most active per unit wet weight. In adrenal, ovary, and liver, the cholesteryl ether tracer was taken up at a much greater rate than apoA-I, suggesting a mechanism for cholesteryl ester uptake independent of holo-particle uptake. Such a mechanism was verified in primary cultures of rat adrenal cells and hepatocytes.—**Pittman, R. C., and D. Steinberg.** Sites and mechanisms of uptake and degradation of high density and low density lipoproteins. *J. Lipid Res.* 1984. **25:** 1577–1585.

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I. EVOLVING CONCEPTS OF LIPOPROTEINS AS ENTITIES

Our concepts of lipoprotein molecules have gone through a considerable evolution over the past 25 years—and they are still evolving. Recognition that the cholesterol, triglycerides, phospholipids, and other lipids in plasma were transported as components of reasonably well-defined lipoprotein particles represented an important step forward. While it was recognized that each major class of lipoproteins was somewhat heterogeneous, there seemed at least to be a modal composition and a degree of kinetic homogeneity that justified dealing with each class as an entity. This notion received important reinforcement from studies of LDL uptake and metabolism which indicated that LDL particles were bound and internalized as entities, that is, as holoLDL particles. On the other hand, it was recognized rather early that chylomicrons and VLDL were *not* metabolized as intact entities; most of the triglyceride was taken up peripherally while most of the cholesteryl ester was taken up by the liver. Still, the concept of a basic unit structure was retained with respect to the "remnants" generated from chylomicrons and VLDL. These products, not degraded further by lipoprotein lipase, were presumed to be metabolized as unit particles.

It is now clear that the composition of plasma lipoproteins is constantly in flux as a result of enzymatic reactions and transfer processes. Furthermore, the metabolic pattern for the various protein and lipid components is highly fluid, if not chaotic. In fact, the more we learn about the complex array of exchange reactions and selective transfers that go on, the more difficult it becomes to deal meaningfully with the kinetics of lipoprotein transport. The nature of the problem becomes particularly evident when we consider the kinetics of HDL metabolism. First, we have to recognize that there are several subclasses of HDL. Second, we recognize that there are very active exchange processes within each subclass and also between HDL and other classes of lipoproteins. For example, the cholesteryl esters of HDL exchange with cholesteryl esters and/or triglycerides of the VLDL and LDL fractions; the phospholipids exchange; the apoproteins exchange. Third, components of HDL can enter the HDL plasma pool on a net mass flux basis independently of the other components; for example, free cholesterol by direct uptake from cells, cholesteryl ester through the action of LCAT, and apoprotein and lipid components from the intravascular metabolism of triglyceride-rich lipoproteins. To create

Abbreviations: VLDL, LDL, and HDL, very low, low, and high density lipoproteins, respectively; LCAT, lecithin:cholesterol acyltransferase; TC, tyramine-cellobiose.

a complete picture one would need data on the metabolism of each and every one of these components, not only in HDL itself but also in each of the other lipoprotein classes involved in the exchange and metabolic processes. Future efforts to characterize lipoprotein metabolism will have to take account of these complex exchanges and structural alterations. The models will be ever more elaborate and validations more difficult, but not impossible.

II. LOW DENSITY LIPOPROTEIN

A. Sites of irreversible uptake and degradation

Until the early 1970's it was generally assumed that essentially all LDL must be returned to the liver for uptake and degradation. This was a logical assumption since only the liver can metabolize and excrete any large quantity of cholesterol; the amount utilized by the adrenals and gonads for steroidogenesis represents only a small fraction of total plasma LDL cholesterol turnover. The quantitative significance of the peripheral tissues in LDL degradation was first suggested by two mutually reenforcing lines of evidence. Sniderman et al. (1) showed in pigs that even after total hepatectomy LDL continued to be removed from the plasma compartment and degraded, demonstrating that nonhepatic tissues have the capacity to degrade LDL at a very significant rate. At about the same time, Bierman, Stein, and Stein (2) reported a significant rate of LDL degradation by cultured arterial smooth muscle cells, and over the next few years it was shown that many different peripheral cell types in culture can take up and degrade LDL at significant rates (reviewed in Ref. 3). Studies in perfused liver (4) and cultured hepatocytes (5, 6) demonstrated some degree of LDL degradation. However, none of these early studies told us what actually occurs in the intact, unperturbed animal. It was clear that some method must be found to evaluate rates of lipoprotein uptake, tissue by tissue if possible, under in vivo conditions.

Some attempts to estimate rates of lipoprotein uptake and degradation by individual tissues in vivo have been based on measurements of initial rates of entry from plasma into tissue using a pulse injection and killing the animal after a very short time interval. A short time interval is chosen, in part, because the degradation products of conventionally iodinated proteins rapidly exit the cells and are lost from the tissue. If *all* the labeled protein entering the tissue were ineluctably programmed for degradation, then the initial influx rate would indeed provide a measure of degradation rate. But some *does* reenter the plasma (or lymph) and the measurement yields an *overestimate* of degradation. If, on the other hand, the time interval is prolonged to avoid this problem, then some fraction of the protein taken up will have been degraded and the degradation products lost back to the plasma, leading to an *underestimate* of the true degradation rate. There is no reliable way, then, to evaluate studies measuring tissue content of radioactivity after injection of conventionally iodinated lipoproteins, whether short-term or long-term.

Faced with this dilemma we developed a new approach to the problem—the use of "trapped ligands" (7, 8). The concept, illustrated in **Fig. 1,** is straightforward: a molecule is chosen that will resist degradation by lysosomal enzymes and that will not move readily across the lysosomal membrane. That molecule, appropriately tagged with a radioisotope, is covalently attached to the protein of interest. If the tagged protein now enters a cell and is delivered to a lysosome, it should undergo the usual degradation *except for* the nondegradable, covalently attached ligand. That will remain behind intact in the lysosome. Sucrose is an example of such a molecule. It cannot be degraded because there is no significant sucrase activity in lysosomes and sucrose cannot readily move across the lysosomal membrane. The validity of the approach and the feasibility of its application were first demonstrated using proteins labeled covalently with [^{14}C]sucrose coupled to the protein using cyanuric chloride as the coupling reagent (7–12). The same principle has been used by Baynes and Thorpe for studies of sites of albumin catabolism (13); these authors covalently linked labeled raffinose, a trisaccharide that is not degradable, to the protein.

The use of ^{14}C-labeled sucrose as the "trapped ligand" has proved quite satisfactory for most purposes. However, the specific activities available become limiting when attempts are made to study tissues of very low catabolic activity, such as the aorta, or to study proteins present at very low concentrations in the plasma, such as insulin. For these reasons an alternative trapped ligand was sought that could be labeled with radioiodine to very high specific radioactivities. We showed that radioiodinated tyramine-cellobiose could be substituted for sucrose (14) and demonstrated the validity of its use in studies of LDL and HDL (15, 16). Further modifications in methods will be needed for studies of smaller proteins, however, because of some self-condensation of the ligand during the synthesis.

Table 1 summarizes the information available in three animal species with respect to sites of LDL degradation (8–11). Clearly there is a common pattern, the liver being in every case the dominant site of degradation. However, as much as 50% of total body LDL degradation occurred *extra*hepatically. If 50% of LDL degradation in man takes place extrahepatically, the "load" of extra

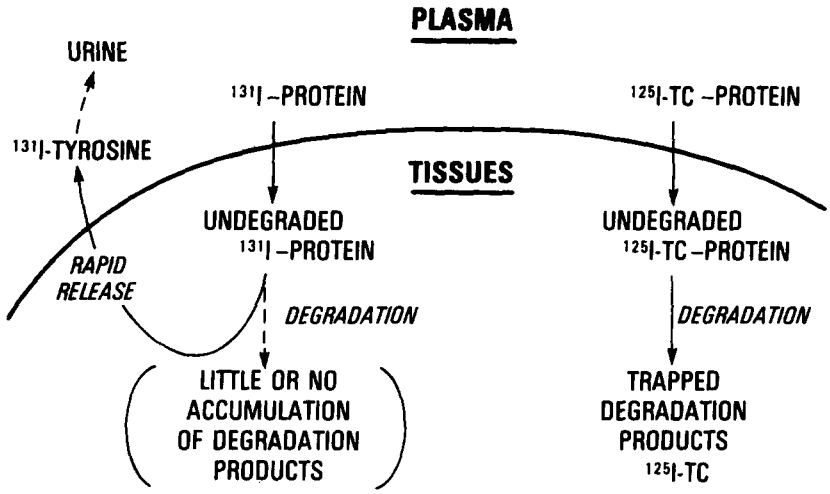

Fig. 1. Schematic representation of the principle of the "trapped ligand" approach to quantifying protein degradation in individual tissues in vivo (see next for details).

cholesterol that needs to be returned to the liver is of the order of one gram daily. This assumes, of course, that the uptake of cholesterol can be calculated from the primary data, the uptake of the apoprotein B, assuming that the LDL particles are taken up intact.

Another way of assessing the activity of tissues in LDL degradation is to compare them in terms of a rate per unit wet weight. In those terms, the adrenal is the most active of the tissues studied in all three animal species (see Table 1). The other highly active tissues in these terms are the liver, the spleen, and the ovary (and presumably the testis in male animals). These three most active tissues are, notably, tissues that further metabolize cholesterol to steroid hormones or to bile acids. Studies of the distribution of LDL receptors show that these are tissues in which the cells have a high density of specific LDL receptors (17), correlating nicely with their high activity in LDL uptake under in vivo conditions.

The peripheral LDL uptake most relevant to those interested in atherosclerosis is, of course, the uptake by the aorta and other large arteries. Using conventional labeling methods, it has been established that most of the cholesterol in atherosclerotic lesions represents deposition of cholesterol from plasma lipoproteins (18). The initial rates of penetration of lipoproteins, including LDL, have been measured (19) but these values do not necessarily correlate with the rates of irreversible uptake and degradation. Analysis of the problem at this level is just beginning. Using the iodinated TC method, Carew et al. (20) found that the rate of intimal degradation of homologous LDL in the rabbit, expressed in terms of clearance of an equivalent volume of plasma, averaged about 8 nl/cm^2 hr (20). Expressed in the same terms, the degradation rate in the media-adventitia was about 12 nl/cm^2/hr. Since the tissue mass represented by the intima is only about $^1/_{40}$th of the total, the degradation per unit wet weight of tissue is 25–30 times faster in the intima than in the media-adentitia. In related work (21), the *total* LDL flux into the artery was estimated using conventionally iodinated LDL and short-term studies (30 and 60 min). The overall entry rate was estimated to be 70 ± 29 nl/cm^2/hr, considerably greater than the irreversible degradation, implying that a large fraction of the LDL entering the wall of the artery is returned to the plasma compartment rather than undergoing irreversible degradation.

Because the use of iodinated TC adds so much in sensitivity, it is already possible to do studies of relative

TABLE 1. Tissue sites of LDL degradation in vivo

		Swine	Rat	Rabbit
Ranked by contribution to total degradation	1st	Liver (50%)	Liver (67%)	Liver (58%)
	2nd	Gut (15%)	Gut (10%)	Gut (6%)
Ranked by relative activity per gram wet weight (liver = 100)	1st	Adrenal (360)	Adrenal (119)	Adrenal (911)
	2nd	Liver (100)	Liver (100)	Spleen (137)
	3rd	Spleen (29)	Ovary (91)	Liver (100)
	4th	(Gonads not studied)	Spleen (76)	Ovary (21)

rates of irreversible LDL degradation at different points in the arterial tree (22) by dissection or gross radioautography. One can even do radioautography at the light microscope level for identification of the cell types involved in that degradation (22; E. von Hodenberg, T. E. Carew, R. C. Pittman, and D. Steinberg, unpublished results) (**Fig. 2**). Further applications of this refined approach to examination of lipoprotein degradation sites can be expected.

B. Mechanisms of LDL uptake

1. By way of the high-affinity LDL receptor. The critical importance of the Brown-Goldstein high-affinity LDL receptor for uptake of LDL is best illustrated by considering patients who lack it, i.e., those with the homozygous form of familial hypercholesterolemia (23). These patients, having little or no functional LDL receptor activity, develop LDL concentrations several times the normal and their LDL fractional catabolic rate is reduced to about one-third normal. From this alone, even without direct further experimentation, one could infer that about two-thirds of LDL degradation normally must occur via the LDL receptor. The question has been approached experimentally in animals and in humans by comparing the fractional catabolic rates of native LDL with that of LDL modified in such a way as to block its recognition by the receptor (24). In animals, the fractional catabolic rates for the blocked forms of LDL have been 25–35% of the rates for native LDL, consistent with about two-thirds of LDL degradation occurring normally by way of LDL receptors. Some early studies in man suggested a lower figure (25), but the use of glucosylation to block LDL receptor recognition yielded a higher figure comparable to that found in animal species (26).

The contribution of receptor-mediated uptake to total uptake can be assessed in individual tissues in vivo by combining the trapped ligand approach with the use of blocked LDL to prevent receptor recognition. One can simultaneously inject into an animal native LDL labeled with ^{125}I-labeled TC and reductively methylated LDL labeled with ^{131}I-labeled TC. ^{125}I from the native TC-LDL trapped in any tissue reflects both receptor-mediated and receptor-independent uptake; the amount

Fig. 2. Radioautograph of an aortic section of a 3-year-old LDL-receptor-deficient rabbit (WHHL) injected 24 hrs previously with ^{125}I-TC-labeled rabbit LDL. (Magnification 120×) Depicted is the intimal surface of an established lesion area showing predominant localization of ^{125}I-TC in foam cells just underneath the intact endothelium. The aorta was fixed by pressure perfusion using modified Karnovsky's fixative, embedded in Epon, and sectioned 1-μm thick. The section was dipped in Kodak NTB liquid emulsion and exposed in the cold for 60 days before developing and fixing with toluidine blue.

of trapped ^{131}I, from the blocked form of LDL, reflects only uptake by receptor-independent pathways. This approach has been used to assess the receptor-dependent uptake in tissues of the rat (11). Analogously, receptor dependency can be examined by comparing the rates of uptake of TC-labeled LDL by various organs of normal animals and animals deficient in LDL receptors. This has been done in assessing receptor-dependent uptake in the rabbit (10). The tissue showing the highest proportion of receptor-dependent uptake was the rabbit adrenal, over 90%. The percentage uptake due to receptor in the liver of both species was, not surprisingly, very similar to the percentage of receptor-dependent uptake calculated from the plasma decay curves. This is expected since the liver is responsible for 50–75% of total body LDL degradation and is the major determinant of the plasma decay curves.

2. LDL receptor-independent pathways. The tissues have a very large capacity to degrade LDL by pathways independent of the LDL receptor. This is evident when we consider that the total turnover of LDL in patients who lack the LDL receptor (27) or in animals that lack the LDL receptor (28), while low expressed in fractional terms, is just as great (or greater) than it is in normal animals when expressed in terms of the absolute flux of LDL. This is possible, of course, only because the plasma concentrations are markedly elevated and "drive" the uptake by LDL receptor-independent pathways. These alternative pathways thus far are poorly understood, but there are at least three categories to be considered: *a*) fluid or bulk endocytosis; *b*) nonspecific adsorptive endocytosis; *c*) high-affinity uptake by alternative receptor-mediated pathways.

It is of interest that the tissue sites of LDL degradation in the WHHL rabbit, deficient in LDL receptors, are not much different from the sites of degradation in the normal rabbit (10). There seems no a priori reason to expect that nonspecific mechanisms would happen to be most effective in the same array of tissues in which receptor-mediated uptake is most effective. Further studies are needed to characterize these LDL receptor-independent processes. There are some unusual binding sites for LDL in the liver, sites that are saturable but not calcium-dependent (10, 29), and these binding sites may be a clue to pathways for LDL uptake that have not yet been fully characterized. Whether these less characteristic pathways for LDL uptake are affected by LDL composition and whether they can be regulated in response to dietary or drug interventions remains to be determined.

Other types of high affinity mechanisms for LDL uptake are the subject of intense investigation at the present time. The LDL molecule can be modified chemically (24, 30, 31) or biologically (32) in certain ways that make it no longer recognized by the receptor for native LDL but readily recognized by alternative saturable, specific receptors. These latter have so far been demonstrated only in endothelial cells (33) and in macrophages (24, 30–32). Uptake in these two tissues is obviously of potential importance in atherogenesis.

III. HIGH DENSITY LIPOPROTEIN

A. Irreversible degradation

A great deal of interest centers on the metabolism of HDL because of its demonstrated high negative correlation with risk of coronary heart disease (34, 35). However, as discussed above, the study of HDL metabolism is beset with problems of interpretation arising from the readiness with which its components exchange and the complexity of its origins and fate. Models that take into account the exchanges of HDL apoproteins with apoproteins in other lipoprotein fractions have been developed and data for *overall* apoprotein plasma decay kinetics have been reported in normal man and in certain disease states, including Tangier disease in which turnover of A-I is enormously increased (36–38). The role of individual tissues in HDL degradation, however, is much more difficult to approach. The use of the trapped ligand approach cannot yield unambiguous data because there is no way to know the lipoprotein source of the label trapped in any given tissue.

Since the apoproteins of HDL exchange so readily and thus present interpretational problems, can we do better by studying the transport of HDL in terms of its core lipids? In many species the answer, unfortunately, is no. In the rabbit, the rate at which cholesteryl esters of HDL exchange for cholesteryl esters or triglycerides of LDL or VLDL is so fast relative to the transport of HDL, that measurements of the flux of labeled cholesteryl esters in the HDL fraction would reflect *both* exchange and net flux, as is the case for the apoprotein. Studies utilizing the trapped ligand approach, using cholesteryl ethers for example (39, 40), do not tell you where *HDL* cholesteryl esters per se go.

One way to avoid all these complications is to use an animal model in which these transfer processes do not take place or do so at acceptably low rates. For studies of HDL, the rat is ideal in this connection: *1*) there is very little apoA-I outside the HDL fraction (in the fasting state) so that the fate of labeled A-I reflects metabolism of HDL itself; *2*) there is little or no cholesteryl ester exchange activity in the plasma (41) so that the fate of labeled cholesteryl esters (or ethers) introduced into the HDL fraction again reflects almost exclusively the metabolism and metabolic fate of HDL

per se. While this simplification permits us to obtain readily interpreted data in the rat, there is obviously no assurance that the results will apply to other species.

The fate of HDL apoA-I was first studied by purifying the apoprotein from rat HDL, covalently labeling it with ^{125}I-tyramine-cellobiose, and reassociating it with rat HDL in vitro (15). The resultant labeled HDL was then biologically screened by injection into a first recipient rat to remove any damaged material and to insure that only HDL-associated apoA-I was followed in the second recipient rat. Analysis by gel chromatography confirmed that essentially all of the readioactivity in the plasma drawn from the screening animal (1 hr after injection) was associated with the HDL fraction. Plasma from the screening animal (or reisolated HDL) was injected into second recipient rats and they were killed 24 hr later. By this time more than 85% of the injected dose had been irreversibly degraded (calculated from analysis of the decay curve). In other studies, both the apoA-I and cholesteryl esters were studied. ^{3}H-labeled cholesteryl *ethers* were incorporated into the lipid core by in vitro reconstitution and ^{125}I-TC-apoA-I was added as described above (16). Most of the key findings with respect to cholesteryl ether metabolism were confirmed using biologically prepared cholesterol ester-labeled HDL (16). The salient results of these studies (**Table 2** and **Table 3**) can be summarized as follows.

1. Unexpectedly, the kidney made the greatest contribution to apoA-I catabolism. However, this was associated with essentially no uptake of cholesteryl ethers or esters (16). Also it was shown, using immunofluorescence, that there was a great deal of apoA-I in the proximal tubular epithelium but very little elsewhere in the kidney (15), strongly implying tubular reabsorption and degradation of filtered apoA-I unassociated with intact HDL. Evidently apoA-I exists at some small concentration in free form in equilibrium with HDL-associated apoA-I.

2. The liver was the major site of HDL cholesteryl ether uptake, consistent with the proposed role of HDL in reverse cholesterol transport. The liver was also a major site of apoA-I catabolism, only exceeded by the kidney which, as discussed above, represents a special case.

TABLE 2. Percentage contribution of various tissues to total clearance of homologous, doubly labeled HDL

Organ	% of Total Body Uptake	
	^{125}I-TC-ApoA-I	[^{3}H]Cholesteryl-Linoleyl Ether
Liver	37	64
Kidney	18	0.5
Skin	13	8.4
Muscle	7.3	6.8
Small intestine	5.2	5.5
Other tissues	13	15

TABLE 3. Specific activities of various tissues in uptake of the cholesterol ester and apoA-I components of doubly labeled HDL

Organ	Fraction of the Plasma Pool Cleared Per Gram Tissue (10^{-3} hr^{-1} g^{-1})	
	^{125}I-TC-ApoA-I	[^{3}H]Cholesteryl-Linoleyl Ether
Adrenal	9.1	60
Ovary	9.8	39
Liver	6.5	15
Spleen	6.3	5.7
Kidney	12	0.4
Other tissues	(0.1–2.8)	(0.1–3.0)

3. The tissues with the greatest activity in HDL uptake on a unit weight basis were the adrenals and the gonads, with the liver close behind. These findings are consistent with the special role described for HDL in the rat as a source of cholesterol for steroidogenesis (42–45).

4. In the adrenal, gonads, and liver, the uptake of cholesteryl ether was out of proportion to the uptake of apoA-I. In the adrenal, it was 6- to 8-fold greater than that of apoA-I, about 4-fold greater in the ovary, and 2- to 3-fold greater in the liver. This disproportionate uptake was confirmed using biologically labeled HDL to be certain it was not an artifact of the reconstitution procedure. The disproportionate uptake was also demonstrated using primary cultures of rat adrenal cells and rat hepatocytes incubated with reconstituted doubly labeled HDL. No other lipoproteins were added and thus there was no possibility of transfer of either protein or lipid out of the HDL fraction. The ratio of the uptake of cholesteryl ether to that of apoA-I was even greater in the cell culture studies than in vivo. The exact mechanism of this selective transfer of cholesteryl ester into these cells remains to be established. It occurs using HDL devoid of apoE (46) and it has been demonstrated using artificially constructed HDL-like particles to which only apoA-I is attached (unreported results). Studies in adrenal cultures show that there is a net transfer of cholesteryl ether to the cells and that the transfer is not strictly an exchange phenomenon. Whatever the molecular mechanism, the phenomenon seems to be well established. Earlier studies by Schreiber, Nakamura, and Weinstein (47) showed that the increase in steroid hormone production induced by HDL was greater than could be explained on the basis of the uptake and degradation of HDL protein. However, the apoproteins were randomly iodinated and exact balance could not be struck. A possibly related phenomenon has been described by Fielding (48). They showed that in the perfused heat there is a disproportionate delivery of cholesteryl ester without concomitant uptake of the chylomicron protein. Finally, studies by Stein et al. (49)

in vivo utilizing their cholesteryl ether method but using conventionally iodinated apoprotein showed an apparently disproportionate uptake of the ether in the adrenal at short time intervals.

As discussed above, the lipoprotein metabolism of the rat is somewhat different from that of some other animals, such as man. Its major lipoprotein fraction is HDL; apparently cholesterol is delivered to peripheral tissues largely through HDL since the concentration of LDL is so much lower. Some other animals deliver the needed cholesterol precursor to the adrenal by way of the LDL receptor instead. Does the phenomenon observed in rats relate at all to metabolism of HDL in man or other species in which LDL is the dominant circulating lipoprotein? Preliminary studies in this laboratory indicate that a similar preferential uptake of cholesteryl ester occurs in cultured human cells, hepatoma G-2 cells, and normal cultured skin fibroblasts (M. S. Rosenbaum, and R. C. Pittman).

B. Relationship to reverse cholesterol transport

A role for HDL in reverse cholesterol transport, first suggested by Glomset (50), has been strengthened by cell culture studies in a number of laboratories (51–54). Recently, Oram, Brinton, and Bierman (55) have described specific binding of HDL to cultured human fibroblasts that appears to be associated with removal of cholesterol from the cells. Binding in their studies is *not* associated with internalization and degradation; instead, the binding seems to be related to transfer of cholesterol out of the cell. When the cells are loaded with cholesterol by incubation with LDL, for example, the number of binding sites for HDL increases, consistent with a role for these receptors in facilitating reverse cholesterol transport.

It is not clear how the receptor described by Oram et al. relates to the binding associated with *delivery* of cholesteryl esters to cells. It is conceivable that they are related, particularly since both appear to involve binding through apoA-I. Could they be two faces of a single HDL receptor binding event, leading to mobilization of stored cholesterol under some circumstances and to delivery of cholesteryl ester under others? Could this simply depend upon the nature of the cell involved? Or are we dealing with two quite separate receptors, both of which bind apoA-I? One could visualize these two mechanisms as two components of a reverse cholesterol transport system. Peripheral cells, containing an excess of cholesterol, would bind HDL through its apoA-I and transfer free cholesterol into it. This HDL-associated free cholesterol would then be esterified in the extracellular compartment by LCAT, thereby increasing the cholesteryl ester content of the HDL. This increased amount of ester could then be delivered selectively to the liver by the mechanism described above without concomitant uptake of apoA-I. Thus, an HDL molecule (or some parts thereof) could function through several cycles for transfer of cholesterol from the periphery to the liver.

In order to evaluate the contributions of the several pathways discussed above and to assess their importance in reverse cholesterol transport, it will be necessary to measure fluxes through various components of the systems involved. Unfortunately, there are some important pieces of information that are missing. For example, we can now state the rate at which cholesteryl esters are delivered to the liver of the rat in HDL. However, we do not have any reliable values for the net rate at which cholesterol and cholesteryl esters are secreted from the rat liver in HDL. Nor do we have reliable data on possible fluxes of cholesterol as *free* cholesterol. The problem of rapid isotopic exchange makes it very difficult to know whether there is some net transfer over and above the rate of uptake of lipoprotein particles.

C. Mechanisms of HDL uptake and degradation

As we have indicated above, there is evidence from a number of laboratories that the binding of HDL to cell membranes is, at least in part, through apoA-I. However, HDL can also bind through apoE, which is recognized by the same receptor that recognizes LDL apoprotein B (56). The amount of apoE in HDL differs from species to species. Much more is present in rat HDL. Rat HDL is taken up much more rapidly by cells that express the LDL receptor, fast enough to suppress cholesterol synthesis in many instances (57). Mahley, Weisgraber, and Innerarity (58) have identified a particularly apoE-rich class of HDL, HDL_c, that increases in concentration during cholesterol feeding. This form of HDL, and presumably other HDL molecules containing a significant amount of apoE, can be rapidly taken up into the liver either because of interaction with the LDL receptor or with the receptor that recognizes apoE on chylomicrons and chylomicron remnants (59). Mahley and coworkers have postulated a mechanism whereby apoE plays a role in reverse cholesterol transport by directing cholesterol-rich HDL to liver by virtue of an increased apoE content (60). The amount of apoE in the HDL fraction in man is not enough to provide even one molecule of apoE per HDL particle (60). This heterogeneity adds still greater complexity to our attempts to characterize kinetics and transport properties. Thus far it has not been possible to identify a mutation in which HDL receptors are absent. Some families with hyperalphalipoproteinemia have been described (61), but no evidence for receptor deficiency has been presented. A great deal of clarification might come if such a kindred were discovered.

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