Pathophysiology of Reverse Cholesterol Transport
Insights from Inherited Disorders of Lipoprotein Metabolism

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On the basis of its kinetic behavior, the total cholesterol of body tissues can be divided into three major pools, which differ in their mean rates of exchange with plasma cholesterol. Each pool can be expanded by addition of endogenously synthesized cholesterol, cholesterol from the diet, or both. The only route of excretion of quantitative significance is via the hepato-biliary system, as cholesterol itself and as bile acids. Although endogenous synthesis is under feedback control, it cannot be completely suppressed; on the average, a minimum of about 20 mg is produced per kilogram of body weight per day. As cholesterol absorption by the jejunum is not actively regulated, the maintenance of a near-constant body content of cholesterol must be largely dependent on the efficiency of its elimination via the liver.

The movement of cholesterol from extra-hepatic tissues to the liver was termed reverse cholesterol transport by Glomset. Current evidence suggests that there may be several pathways of reverse transport, perhaps reflecting the importance to the organism of the preservation of a steady state of cholesterol balance and of the diversity of requirements of different tissues for cholesterol. It is likely that some pathways operate more efficiently in some tissues than in others. Accordingly, although mathematical modeling of plasma cholesterol specific radioactivity decay curves gives useful information on the overall kinetics of body cholesterol, it provides no information on regional differences, which may be of critical importance in relation to atherogenesis. We have recently reviewed the current information on the processes that contribute to reverse cholesterol transport and have discussed them in relation to physiologic and anatomic considerations.

Transport of Dietary Cholesterol to Liver

All food of animal origin contains cholesterol, up to 50% of which is absorbed. Absorbed cholesterol enters intravascular lymph as a component of chylomicrons, the main apoprotein (apo) of which is apo B-48. Once chylomicrons enter the blood circulation, their triglyceride is hydrolyzed by endothelial-bound lipoprotein lipase (LPL). Concomitantly, some of their surface material is transferred to the high density lipoproteins (HDL), and their complement of apoproteins changes. The resulting cholesteryl ester-rich remnant is efficiently cleared by the liver. In studies in dogs, which have no lipid-transfer protein activity (see later) and in which the kinetics of chylomicron cholesteryl esters (CE) are therefore easier to follow than in humans, about 70% of chylomicron CE were cleared within 15 minutes of their intravenous injection, and about 70% of this cholesterol was found in the liver.

The uptake of chylomicron remnants by hepatocytes occurs by a specific receptor-mediated process, the essential ligand for which is apo E. In humans, the E3 and E4 isoforms of apo E have greater affinities for the receptor than has E2, homozygosity for which predisposes to Type III hyperlipidemia, in which there is an accumulation of remnants in plasma. For reasons that are not clear, the removal rate of chylomicron remnants is reduced in patients with familial lecithin:cholesterol acyltransferase (LCAT) deficiency. On the other hand, remnant clearance is normal in familial hypercholesterolemia, suggesting that the apo B-100/E receptor, which is responsible for the catabolism of low density lipoprotein (LDL), does not contribute significantly to the process.

An alternative pathway of transfer of chylomicron cholesterol to the liver seems to operate in the absence of normal degradation of chylomicrons by LPL. Chylomicrons from patients with chylomicronemia due to the genetic absence of LPL contain 4.5% by weight of cholesterol as compared with 0.5% to 1.5% in normal subjects. The rate of transfer of CE from HDL to chylomicrons is not known, but that from HDL to very low density lipoprotein (VLDL), under conditions in vitro that are comparable to those in vivo, is approximately 12 μg/ml/6 hr. This should be the order of magnitude of the removal rate of CE from the expanded pool of chylomicrons in familial hyperchylomicronemia. This may occur by nonspecific endocytosis of chylomicrons by macrophages, as evidenced by the formation of eruptive xanthomas. The macrophages of newly formed eruptive xanthomas contain predominantly triglyceride (mean, 18 μg/mg wet weight), but also significant amounts of cholesterol (mean, 11 μg/mg). After elimination of chy-
lomiconemria in four diabetic patients by diet and insulin, Parker et al.\textsuperscript{18} found that the cholesterol content of eruptive xanthomas decreased an average of 0.36 $\mu$g/mg/day, demonstrating that cholesterol can be removed from the macrophages of such lesions by acceptor lipoproteins. There is no evidence of widespread cholesterol accumulation in patients with familial chylomiconemia, and they do not develop premature atherosclerosis.\textsuperscript{16}

**Transport of Cholesterol from Peripheral Tissues to Liver**

Dietary cholesterol that is not removed by the chylomicron remnant pathway of the liver must enter the cholesterol pools of peripheral tissues, along with the cholesterol of LDL. On physiologic and anatomic grounds, reverse cholesterol transport from peripheral cells can be viewed as being composed of three stages: from tissues to plasma; between plasma lipoproteins; and from plasma lipoproteins to hepatocytes.\textsuperscript{5} As no quantitative data are available for the earliest stage in humans, we shall first consider the terminal intrahepatic events.

**Cholesterol Transport from Plasma to Liver**

In humans, much of the delivery of cholesterol from plasma to the liver may be mediated by apo B-containing lipoproteins. Measurements of artery-hepatic vein differences of plasma cholesterol concentrations in humans have provided evidence for extraction of LDL CE by splanchnic tissues.\textsuperscript{19} In the same study, no extraction of cholesterol from other lipoproteins was detected. On the contrary, a positive arteriovenous difference was observed for VLDL unesterified cholesterol (UC). The rate of extraction of LDL cholesterol was approximately 1 g/hour, which is five times the calculated turnover of LDL CE\textsuperscript{20} and is also much greater than the rate of delivery of CE to VLDL from HDL in vitro (see below). Although this discrepancy awaits explanation, these results suggest strongly that the splanchnic region does extract LDL CE. Similar studies of arteriovenous differences of LDL apo B mass and radioactivity detected no removal of LDL apo B.\textsuperscript{21} This latter result does not weaken the evidence from other sources that the liver plays a major role in the receptor-mediated removal of LDL particles from the circulation, as the arteriovenous difference in particle number would be expected to be below the limit of detection.\textsuperscript{21} But the fact that LDL CE uptake is detectable in the absence of the demonstrable removal of LDL apo B suggests that CEs are removed from LDL particles during passage through the splanchnic region. This would be consistent with the evidence that in humans, circulating LDL particles become progressively depleted of CE, and consequently more dense, with time.\textsuperscript{22} The mechanism by which CEs could be removed from LDL is not clear, but it might be similar to that by which CEs are transferred from HDL to the liver in rabbits and rats (see later).\textsuperscript{23,24} As the conversion of light LDL to heavy LDL is impaired in subjects with familial hypercholesterolemia,\textsuperscript{22} the process may be facilitated by transient binding of LDL to hepatic apo B-100,E receptors.

It has been shown in animals that nascent VLDL contain cholesterol mostly in its unesterified form.\textsuperscript{25} In contrast, the ratio of CE to UC in circulating VLDL in humans is 1:2.\textsuperscript{26} The fatty acid composition of VLDL CEs indicates that they are mostly products of the intravascular LCAT reaction.\textsuperscript{27,28} As VLDL UC is a poor substrate for LCAT (the optimum substrate being HDL UC\textsuperscript{27}), most VLDL CE must be acquired from other lipoproteins. The net transport of CE from HDL to VLDL and of triglycerides in the reverse direction is mediated by a lipid-transfer protein.\textsuperscript{29–36} As no net transfer of CE from LDL to VLDL occurs,\textsuperscript{37,38} VLDL appears to be the preferential acceptor of CE generated by the LCAT reaction and also the principal donor of triglycerides to other lipoproteins in the postabsorptive state. Radiolabeled CEs of HDL are rapidly transferred to VLDL after intravenous injection into humans. From such studies, it has been estimated that the flux of CE from HDL is approximately 50 mg/kg/day,\textsuperscript{39} which is of the same order of magnitude as the in vivo rate of esterification of HDL UC\textsuperscript{40,42} and three to four times the rate of whole-body cholesterol synthesis.\textsuperscript{3} It seems, therefore, that much of the delivery of HDL CE to hepatocytes in humans occurs indirectly, during the removal of CE transported in other lipoproteins.

When VLDL particles are acted upon by LPL, they become depleted of about 75% of their triglyceride.\textsuperscript{41} As they decrease in size, a portion of their surface constituents becomes redundant and is released.\textsuperscript{42} When VLDL particles of average M, 23M are converted to intermediate density lipoproteins (IDL) of M, 7M in vitro, they lose 25% of their CE, the fate of which has not been determined.\textsuperscript{41} Compositional studies of apo B-containing particles of comparable size isolated from plasma have not shown a reduction of CE per IDL particle relative to VLDL but have demonstrated that IDL particles lose about 50% of their CE when converted to LDL in vivo.\textsuperscript{43} In contrast, when this IDL-to-LDL conversion is mimicked in vitro, the resulting LDL particle retains the full complement of CE of its precursor.\textsuperscript{41} It appears, therefore, that under physiologic conditions, tissues may mediate the removal of CE from IDL particles. Neither the site nor the mechanism of this removal is known. Measurements of arteriovenous differences of apo B mass and radioactivity have demonstrated that approximately 30% of IDL particles are removed by the tissues of the splanchnic bed in humans.\textsuperscript{21} About half of this is due to conversion of IDL to LDL.\textsuperscript{21} Taken together, these findings suggest that 15% of the CEs originally transferred from HDL to VLDL are removed as a component of IDL by organs of the splanchnic region.

Clinical evidence indicates that whether complete or partial absence of apo B-containing lipoproteins from plasma leads to accumulation of cholesterol in tissues or to atherogenesis. On the contrary, the opposite appears to be the case. Neither of the two genetic syndromes characterized by a complete absence of apo B-containing lipoproteins in plasma\textsuperscript{44} nor that in which there is a selective defect in apo B-100 synthesis\textsuperscript{45} shows signs of atherosclerosis or xanthomas. Patients with abetalipoproteinemia synthesize cholesterol at a rate of 15 to 25 mg/kg/day\textsuperscript{46} and must have a mechanism for delivering a portion of this to the liver via a route that does not require apo B-containing particles. The plasma of such patients contains lipoproteins that are rich in apo E.\textsuperscript{47}
Most of these are in the density range of HDL2, but particles of similar density to LDL are also present. It has been suggested that the primary function of these particles is to bind to apo B-100,E receptors in peripheral tissues and thereby to deliver cholesterol to those cells. It may be that another function is to deliver cholesterol from peripheral tissues to the liver. In animals, an apo E-rich plasma lipoprotein of similar density is present, the concentration of which increases when the animals are fed cholesterol and when their tissues are acutely loaded with cholesterol by intravenous infusion of exogenous LDL. The same particle decreases in concentration when the animals are infused with exogenous lipoprotein transfer protein. It has been shown that this particle (HDL3) is rapidly cleared in the perfused rat liver via the same receptor that binds chylomicron remnants. There is evidence that a similar lipoprotein accumulates in subjects with familial deficiency of lipoprotein transfer protein activity and in normal subjects during consumption of cholesterol-rich diets. It is probably also present in low concentration in humans under normal circumstances. It increases when human plasma is incubated in the presence of LCAT in vitro. A similar particle is formed when HDL3 is incubated with cholesterol-loaded cultured macrophages. Thus, the total evidence from diverse sources suggests that, during the intravascular phase of reverse cholesterol transport, an apo E-rich and CE-rich particle is formed from HDL3, which is ultimately cleared by hepatic receptors recognizing apo E. This route of cholesterol transport is probably quantitatively less important in humans than in species with no lipoprotein transfer protein activity but may increase in importance when an increased load of cholesterol is delivered to certain peripheral cells and when CE cannot, because of absence of apo B-containing lipoproteins or transfer-protein activity, be efficiently transported out of HDL. It is to be noted, however, that the apo E-rich lipoproteins in abetalipoproteinemia differ from those in normals in that the CE/UC ratio is close to unity, as compared to approximately 3 in normal subjects and 4 in cholesterol-fed dogs.

Other pathways of delivery of CE from plasma to the liver may also be mediated by HDL. Evidence has been presented for the existence of additional receptors recognizing apo A-I and apo A-IV in the plasma membranes of liver cells, and it is possible that these may mediate the endocytosis of HDL particles, although the presence of specific protein binding sites for apo E-free HDL in human liver has recently been questioned by the results of radiation inactivation studies. Other work has shown that the rate of uptake of HDL CE by the liver in rats is several times greater than that of HDL apo A-I, suggesting that CE dissociates from HDL particles at the surfaces of hepatocytes, hepatic macrophages, and/or hepatic endothelial cells. This process might be facilitated by transient binding of HDL to the plasma membranes of such cells, irrespective of whether or not such binding is mediated by specific protein receptors. A similar process has been studied in cultures of human hepatoma cells. Dissociation of CE may also take place within certain hepatic cells, as there is evidence that HDL can be endocytosed by a nonlysosomal pathway in macrophages and hepatic sinusoidal cells.

The pathways of delivery of CE to the liver clearly cannot operate in patients with familial LCAT deficiency. Yet such patients do not accumulate large quantities of cholesterol in their plasma, as might be expected if the delivery of CE to the liver were rate-limiting for the final stage of reverse transport. The mechanism of transfer of UC from lipoproteins to the liver is not clear. The livers of LCAT-deficient patients contain about five times more UC than do those of normal subjects. Nevertheless, the molar ratio of phospholipid to UC in the livers of such patients (3.5) exceeds that in plasma lipoproteins. If a similar ratio is present in the plasma membranes of hepatocytes in such patients, it would maintain a net flux of UC from plasma to the liver. Kinetic evidence has been presented for a similar process in normal humans. The phospholipase activity of hepatic endothelial cells may aid this process by raising the UC/phospholipid ratio at the surface of HDL. The continuous conversion of cholesterol to bile acids and the secretion of UC into bile may have a similar effect, by lowering the UC/phospholipid ratio in hepatocytes.

Intravascular Events in Reverse Cholesterol Transport

Three processes probably contribute to the intravascular phase: the LCAT reaction; movement of lipids between different lipoproteins, mediated in part by transfer proteins; and hydrolysis of lipoprotein glycerides and phospholipids by LPL of peripheral tissues and by hepatic endothelial lipase. These have already been mentioned in connection with the role of apo B- and apo E-containing lipoproteins in delivering CE to the liver. Here we shall deal with other intravascular effects of these processes.

Essentially, all of the esterification of cholesterol that takes place intravascularly is mediated by the LCAT reaction. The optimum lipoprotein substrates appear to be small HDL particles, although the UC utilized in the reaction is acquired by HDL from other lipoproteins and from cell membranes. As UC moves rapidly (without a catalyst) between different lipoproteins, between erythrocytes and lipoproteins, and between cell surfaces and lipoproteins, these different potential sources will compete for transfer of UC to HDL down the chemical gradient generated by the LCAT reaction. Hence, it is possible that an elevated content of UC in apo B-containing lipoproteins might impede the transport of UC from peripheral cells to HDL, although any such effect is likely to be small owing to the much greater size of the rapidly exchangeable pool of tissue UC. In an analogous way, elevated production of small HDL particles in vivo might be expected to enhance the transport of cholesterol from tissues. On the other hand, persons with familial LCAT deficiency do not show signs of significant cholesterol accumulation in tissues before the age of 40, and even then with only limited, mostly renal and hematologic, consequences. Although such patients accumulate cholesterol in arteries, premature atherosclerosis appears to be only moderately increased, and these persons do not develop cholesterol-laden xanthomas. In contrast, in patients with HDL
absence due to major defects of the apo A-I, C-III, A-IV gene cluster, which predispose to severe premature atherosclerosis, the UC/EC ratio in plasma is not raised, indicating that lipoproteins other than HDL can act as substrates for LCAT in vivo. 

The role of the lipid-transfer proteins also needs to be considered. Nichols and Smith first observed the net transfer of CE from HDL to VLDL that occurs when human plasma is incubated at 37°C. It has since been shown that phospholipids and triglycerides also move between lipoproteins, and that all these processes are facilitated by lipid-transfer proteins. Both molecular exchange and net transfer of lipids occurs. Of particular importance is the fact that CEs exchange bidirectionally between HDL and VLDL, and that HDL CEs are also exchanged for VLDL triglyceride. Bidirectional exchange of CEs between HDL and LDL in human plasma occurs in vitro at a rate of 39 to 116 μg/ml/hr and produces no net mass transfer between the particles. The exchange of CE between VLDL and HDL may occur at a similar rate. As nascent VLDL contain very little CE, the transfer must initially be predominantly from HDL to VLDL. It has also been suggested that an equilibrium between these two lipoproteins is not attained in vivo because of the much greater fractional catabolic rate of VLDL than of HDL, resulting in a continuous mass transfer of CE from HDL to VLDL. The net transfer of CE from HDL to VLDL in vitro has been estimated to be 15 to 30 μg/ml/hr. The potential importance of this process in reverse cholesterol transport has already been discussed in connection with the role of apo B-containing lipoproteins in the delivery of CE to the liver. Concomitantly with the movement of CE out of HDL, there is a net mass transfer of triglycerides into HDL from triglyceride-rich lipoproteins, also catalyzed by transfer protein activity. This process, acting in conjunction with the hydrolysis of triglyceride in HDL by hepatic endothelial-bound lipase, may provide one mechanism for the regeneration of small HDL particles that are poor in CE and hence good substrates for LCAT. Even without the action of lipases, lipid-transfer proteins may help to generate small HDL particles that are good substrates for LCAT. If HDL CE is exchanged for triglyceride mole for mole, the HDL particle should increase in size, because the partial molar volume of triglycerides is some 30% greater than that of CE. However, when artificial triglyceride- and phospholipid-rich emulsions were incubated with HDL and a plasma fraction rich in lipid-transfer proteins, both larger and smaller HDL were generated.

It is possible that LPL anchored to the luminal surfaces of endothelial cells may affect reverse cholesterol transport in other ways. It has already been mentioned that when VLDL and chylomicron triglycerides are hydrolyzed, surface components of the lipoproteins (including UC, phospholipid, and apo protein) are transferred to the HDL density range. The possible effect of this process on cholesterol transport by HDL is a subject to which we shall return.

**Cholesterol Transport from Tissues to Plasma Lipoproteins**

Cholesterol enters cells as a component of lipoproteins in both its esterified and its unesterified forms. In vitro it leaves cells predominantly, and perhaps entirely, as UC. This appears also to be true under physiologic conditions. Most of the esterification of UC derived from cell membranes probably occurs only after it has entered the bloodstream, and not in interstitial fluid; in dogs the CE/UC ratio in peripheral lymph is much lower than in plasma, and in both humans and dogs, cholesterol esterifying activity is much lower in lymph than in plasma. Moreover, in subjects who had been injected intravenously with [14C]-cholesterol several weeks earlier, the specific radioactivity of lymph UC was higher than that of lymph CE and plasma cholesterol.

Essentially two types of transfer of UC from cells to plasma lipoproteins may occur. First, there may be lateral diffusion within membrane-like structures that appear to connect various cells with the abluminal surface of the vascular endothelium. This type of transport will occur only in those tissues where such structures are present and only when the chemical potential of UC on plasma lipoproteins is lower than that of the plasma membranes of the cells in those tissues. Second, the transport of UC can be carrier-mediated. This is dependent on the maintenance of a difference in the chemical potential of UC between cell membranes and the carrier particles, and will operate even when there is no difference in the chemical potential of UC between the extravascular carriers and plasma lipoproteins. Experiments in vitro have shown that sustained removal of cholesterol mass from cells can take place only in the presence of acceptors. Of the various potential acceptors that have been tested by addition to cultured cells, HDL and complexes of apo A-I with phospholipids were among the most efficient. More recently, Castro and Fielding found that when radiolabeled cholesterol effused from cultured human fibroblasts into medium containing human serum, almost all entered a minor component of HDL composed of very small apo A-I-containing particles. These particles differed from the majority of plasma HDL in having pre-beta electrophoretic mobility in agarose gel. A similar subclass of plasma HDL has been studied by other investigators. It is rich in phospholipid, contains little or no core lipid, and contains apo A-I (one or two molecules) as the only recognized apoprotein. The first evidence for the existence of a pre-beta migrating subclass of apo A-I-containing particles was obtained in studies of human amniotic fluid. Similar particles have since been demonstrated in dog peripheral lymph and human lymphedema fluid.

The physical properties of the small pre-beta apo A-I-containing particles make them strong candidates for the role of principal primary acceptors of UC in the interstitial fluid of many tissues in vivo. The sieving character of the interstitium dictates that the volume of distribution of large macromolecules is less than that of small molecules. Umbilical cord is one of the few human tissues that can be studied in vitro under conditions similar to those in vivo. In the interstitium of this tissue, albumin (Stokes radius, 3.5 nm) is excluded from approximately 40% and IgG (5.0 nm) from approximately 60% of the space of distribution of sucrose. Particles the radius of most plasma HDL is (5.0 to 7.0 nm) should be excluded from about 70% of this interstitium. Results similar to
these figures have been obtained in postmortem and preoperative specimens of human arteries. Because the smallest apo A-I-containing particles found in lymph\(^{[96,97,98]}\) and lymphedema fluid\(^{[99]}\) have a radius of less than 3.5 nm, they should have a volume of distribution that exceeds that of albumin. Hence, of all the extravascular lipoproteins, the smallest apo A-I-containing particles should have the greatest probability of reaching cell surfaces, and if only for that reason are the most likely acceptors of UC from cell membranes.

Processes have been identified in vitro that increase the size of small HDL\(^{[50,53,55,99]}\) that convert larger HDL into smaller ones\(^{[78,77,96,100]}\) and that dissociate small apo A-I-containing particles from large HDL.\(^{[101,102,103]}\) Any process that increases the size of an acceptor will skew the distribution of its probability of diffusion toward regions of the interstitium into which only larger particles are permitted. If the transfer of UC to these acceptors is associated with an increase in their propensity to form larger particles, this should increase the efficiency of carrier-mediated transport of cholesterol within the interstitium in the direction of lymph capillaries and/or post-capillary venules. Studies in dogs have shown that cholesterol feeding results in enrichment of peripheral lymph HDL with UC, associated with a size shift from smaller to larger particles.\(^{[90,104]}\) In this species, apo A-IV may play a role in extravascular fluids similar to that of apo A-I.\(^{[91]}\) More information is needed on this apoprotein in humans.

Several types of peripheral cells have on their surfaces binding sites with high affinity for HDL and other apo A-I-containing particles.\(^{[105-108]}\) In studies with cultured transformed adipocytes, binding sites were expressed only when the cells released cholesterol; in growing cells, when cholesterol was not released, the binding sites could not be detected.\(^{[109]}\) In cultured smooth muscle cells, macrophages’ and fibroblasts’ binding of HDL has been shown to increase when the cells are loaded with cholesterol, leading to the suggestion that the number of binding sites may play a homeostatic role in regulating the efflux of UC from tissues.\(^{[93,107]}\) More recently evidence has been presented to show that binding of HDL to cell surfaces may augment the translocation of UC to the plasma membrane\(^{[110]}\) before desorption into the extracellular fluid and adsorption by HDL particles that are free in solution.\(^{[111]}\) Proteins that bind apo A-I have been isolated from the plasma membranes of several types of nonhepatic cells,\(^{[101,112]}\) and it has been proposed that these represent specific receptors for a subclass of HDL particles. Other studies, however, have questioned the existence of specific receptor proteins for HDL in peripheral cell membranes,\(^{[113]}\) and more information is needed in this area.

**Clinical Disorders of High Density Lipoprotein Metabolism**

In the preceding paragraphs, we outlined processes of reverse cholesterol transport that probably take place in the liver, in the circulation, and in the interstitium of tissues, all of which appear to involve the participation of different subclasses of HDL. At this point it is appropriate to consider clinical observations that are pertinent to the pathophysiology of reverse cholesterol transport and its role in atherogenesis.

The negative correlation between plasma HDL cholesterol concentration and CHD risk has now been demonstrated in numerous epidemiologic studies. Other evidence supporting a link between HDL and atherogenesis has been provided by angiographic studies, by examination of autopsy material, by studies of kindreds with familial hyperalphalipoproteinemia or familial hypoalphalipoproteinemia, and by genetic and pharmacologic manipulation of HDL in animals (reviewed in reference 114). Recent results of clinical studies of drugs\(^{[115,116,117]}\) and hormones\(^{[118]}\) with HDL-raising activity have also supported the notion that coronary atherogenesis is strongly influenced by HDL metabolism.

There are clear indications that the association between HDL and CHD is not simple, and that we must think in terms of specific components of HDL metabolism that are related to the atherogenic process and not consider merely the plasma HDL cholesterol level. Clinical studies have provided evidence that different disorders characterized by low plasma HDL cholesterol levels have rather different effects on cholesterol transport.\(^{[119]}\) Homozygous apo A-I, C-III deficiency and homozygous apo A-I, C-IV deficiency are associated with the virtual absence of HDL, severe premature coronary atherosclerosis, corneal opacities, and (in one case) xanthomas, but not with CE accumulation in reticuloendothelial cells.\(^{[72,73]}\) In a family with a virtual absence of HDL and apo A-I, but normal apo C-III levels, the clinical features were planar xanthomas, premature CHD, and corneal opacities.\(^{[120]}\) Reduced apo A-I synthesis, low plasma HDL cholesterol levels, and premature atherosclerosis are also present in normotriglyceridemic familial hypoalphalipoproteinemia.\(^{[121]}\)

In Tangier disease, in which an apparently normal apo A-I is catabolized at an abnormally high rate\(^{[122]}\) due possibly to defective HDL processing in macrophages,\(^{[123]}\) the prevalence of CHD is only modestly increased in spite of HDL concentrations that are less than 5% of normal.\(^{[124]}\) However, CE accumulates in reticuloendothelial cells of the skin, tonsils, liver, spleen, and elsewhere; some patients develop corneal opacities.\(^{[44]}\)

Another genetic disorder, in which plasma apo A-I and HDL cholesterol concentrations are low but which does not predispose to premature CHD (or result in deposition of CE in the reticuloendothelial system), is apo A-I homozygous.\(^{[125]}\) As in Tangier disease, the low apo A-I levels in this disorder are due to enhanced catabolism, but in this case the result is the formation of an abnormal apo A-I.\(^{[126]}\)

In familial LCAT deficiency, in which very low levels of HDL are present mostly as discoidal nascent particles devoid of CE,\(^{[127]}\) premature atherosclerosis is only modestly increased, the most prominent features being corneal opacities and the accumulation of cholesterol in kidney, liver, spleen, and bone marrow.\(^{[65]}\)

In fish-eye disease, in which plasma HDL cholesterol is about 10% of normal, the most characteristic feature is severe corneal opacities, with no evidence of premature CHD or marked deposition of CE in reticuloendothelial cells.\(^{[128]}\) In a Swedish family, this condition appears to reflect a disorder of LCAT activity,\(^{[128]}\) although in another
patient, evidence of a failure of HDL to retain CEs has been documented.129

These clinical observations suggest that different genetic defects of HDL metabolism disrupt reverse cholesterol transport in different tissues to widely varying degrees. In conditions associated with the absence of apo A-I synthesis,72,73,120 cholesterol homeostasis in the walls of arteries and in subcutaneous connective tissue (where cholesterol transfer from cells to plasma proceeds through a sieving medium) is severely affected, presumably because the availability of the small acceptors required for reverse transport under these conditions is reduced. In the cornea (which is an avascular tissue), it is probable that reverse transport depends on a gradient of chemical potential of UC between cell surfaces and plasma. Since apo A-I is a potent activator of LCAT, it is possible that in the absence of this apoprotein the activity of the enzyme in plasma does not support this type of transport sufficiently. On the other hand, because the function of lymphoid tissue dictates that even very large particles have access to its cells, large non-apo A-I particles may be adequate acceptors here. In less severe deficiencies of apo A-I synthesis,121 atherosclerosis, but not xanthomas or corneal opacities, develop, suggesting that arterial tissues are particularly sensitive to decreases in the availability of small apo A-I-containing particles. In contrast to conditions in which there is decreased apo A-I synthesis, two disorders in which the primary genetic abnormality results in an increased fractional rate of clearance of HDL particles from plasma122,125 appear to produce only a small increase in CHD or no increase at all. This may be so because the synthesis of small apo A-I-containing lipoproteins is sufficient in these conditions to sustain adequate reverse cholesterol transport in the walls of arteries. The characteristic accumulation of CE in the cornea of Tangier patients may be due to failure of these cells to exocytose internalized HDL.123

Familial LCAT deficiency supports the conclusion reached on the basis of in vitro studies and of the CE/UC ratios in extravascular fluids that esterification of cholesterol in the extravascular compartment is not rate-limiting for carrier-mediated reverse transport from the majority of cells. Although the composition of plasma HDL in these patients differs from normal, in many respects it is similar to that of the HDL of normal peripheral lymph. The tissues that are most severely affected by LCAT deficiency are those in which the predominant transport mechanism is probably dependent on a gradient of chemical potential of UC between cell surfaces and plasma (e.g., erythrocytes, cornea, glomeruli). Although atherosclerosis is probably accelerated in these patients, it is not grossly so, indicating that reverse transport from arteries probably depends predominantly on a carrier-mediated process. The absence of premature CHD in patients with fish-eye disease is compatible with this conclusion.

Thus, it seems likely that several mechanisms of reverse cholesterol transport operate in parallel, perhaps because of the crucial biological importance of this process, and that of all tissues the arterial wall may depend most on reverse transport mediated by the small apo A-I-containing HDL particles. This conclusion is consonant with the evidence for the presence of apo A-I-containing particles with pre-beta electrophoretic mobility in relatively high concentrations in human aortic intima and atherosclerotic lesions130,131 and with reports that the cholesterol content of human vascular tissues in vivo is a negative function of plasma HDL cholesterol concentration.132,133,134

The availability of small apo A-I-particles, and hence the rate of atherogenesis, is likely to be determined in part by the rate of apo A-I synthesis.135 Two experimental observations can be cited in support of this notion. First, the susceptibility of nonhuman primate species to diet-induced atherosclerosis at similar levels of plasma total cholesterol was found by Sorci-Thomas and coworkers136 to be inversely related to apo A-I mRNA abundance in liver and intestine, and to the rate of secretion of nascent HDL by liver. Second, in a recent study, repeated infusions of the d = 1.063 to 1.25 g/ml fraction of plasma were found to inhibit the formation of fatty streaks and the accumulation of cholesterol in the aortas of cholesterol-fed rabbits.137

Reference has already been made to other metabolic processes that may generate small apo A-I particles from larger plasma HDL. These include the combined effects of lipid transfer proteins and hepatic endothelial lipase in depleting HDL particles of core material and of an HDL conversion factor.138 Gebhardt et al.99 provided evidence that phospholipase activity may generate pre-beta HDL from alpha-migrating HDL in amniotic fluid. The possibility that the phospholipase activities of hepatic lipase and LCAT might have similar effects on HDL is worthy of consideration.

Fusion of discoidal lipoproteins with plasma HDL may also be important in generating small pre-beta HDL particles. Such a process has been demonstrated in vitro during incubations of artificial discoidal complexes and plasma HDL.102 Current information suggests that two types of discoidal phospholipid-apolipoprotein particles may be produced in peripheral tissues: apo E discs and the surface remnants of triglyceride-rich lipoproteins. Apo E discs are synthesized by several peripheral cell types and are secreted into interstitial fluid at a rate that is a positive function of the cholesterol content of the cells.103 By augmenting the production of small A-I particles from HDL in interstitial fluid, and at the same time furnishing the additional apo E required for the receptor-mediated clearance of mature HDL from the circulation by hepatocytes, the production of such discs may provide an important regulatory mechanism for cholesterol homeostasis in tissues.

The interaction with HDL of discoidal surface remnants liberated from triglyceride-rich lipoproteins during their hydrolysis by LPL might also generate small apo A-I-containing particles.122 Evidence in support of such a process has been provided by observations that the lipolysis of triglyceride-rich lipoproteins is associated with the transfer of surface phospholipid to the HDL density range.142 An increase in HDLc,143 and, of particular interest, an increase in the concentration of pre-beta HDL particles.144 Since vascular endothelium is rich in LPL,145 lipolysis might generate small apo A-I-particles at the blood-arterial wall and blood-capillary interfaces or within transport vehicles in endothelial cells,146 particles that
would then be available to enter the interstitial fluid of tissues. The possibility that catabolism of triglyceride-rich lipoproteins augments reverse cholesterol transport in this way is supported by the equilibrium state of lipids in plasma lipoproteins. Using phase diagram analysis of published data on lipoprotein composition, Miller and Small concluded that the lipolysis of chylomicrons and VLDL in plasma must be accompanied by a net influx of UC into the system. In very recent studies (Miller NE, Nanjee N, Colvin PL, unpublished observations), evidence has been obtained that heparin-induced lipolysis of triglyceride-rich lipoproteins may indeed augment the efflux of cholesterol from tissues into plasma in rabbits.

A significant role of lipolysis in releasing small phospholipid-rich apo A-I-containing cholesterol acceptors from HDL is also compatible with the negative correlation that has been observed in humans between the fractional catabolic rate (FCR) of apo A-I and both adipose tissue LPL activity and VLDL apo B FCR (Figure 1). By releasing apo A-I from mature plasma HDL particles containing apo E in addition to three or four molecules of apo A-I, lipolysis would result in the recycling of apo A-I, and slow that component of apo A-I catabolism that occurs as a consequence of the endocytosis of mature HDL particles via hepatic apo E receptors. This is illustrated in the hypothetical scheme shown in Figure 2. The clinical observation that genetic disorders that produce complete absence of LPL activity are not associated with accelerated atherosclerosis may be attributed to the fact that patients with these conditions also have concentrations of LDL cholesterol that are extremely low.

A link between the lipolysis of triglyceride-rich lipoproteins and reverse cholesterol transport would provide a potential explanation for the epidemiologic associations between HDL cholesterol, plasma triglycerides, and CHD. There is a large body of data demonstrating that plasma HDL cholesterol concentration is inversely related to the plasma total and VLDL triglyceride concentrations. In several prospective studies, CHD risk has been found, on univariate analysis, to be associated positively with plasma

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Figure 1. Relationships of plasma apoprotein (apo) A-I fractional catabolic rate (FCR) to the FCR of very low density lipoprotein (VLDL) apo B ($r = -0.52$, $p = 0.05$) and adipose tissue lipoprotein lipase (LPL) activity ($r = -0.67$, $p = 0.01$) in humans. Data from Magill et al.

Figure 2. Putative effect of surface remnants released during the lipolysis of triglyceride-rich lipoproteins (TGRL) on the production of small apoprotein (apo) A-I-containing particles, with consequent augmentation of reverse cholesterol transport and recycling of apo A-I molecules between plasma and interstitial fluid. E = apoprotein E, CE = cholesteryl ester, UC = unesterified cholesterol, INT. FLUID = interstitial fluid, LCAT = lecithin:cholesterol acyltransferase.
triglyceride concentration, as well as negatively with HDL cholesterol. In most cohorts, only HDL cholesterol retained its association with CHD on multivariate analysis: i.e., at a constant HDL cholesterol, triglyceride concentration no longer influenced risk, whereas at a constant triglyceride level, HDL cholesterol continued to have a strong negative impact on CHD. In two cohorts, however, Framingham women and Speedwell men, HDL cholesterol and plasma triglycerides retained joint predictive power on multiple logistic regression. These observations suggest that one commonly occurring defect in HDL metabolism that increases atherogenesis may be secondary to a disorder of plasma triglyceride transport.

For two reasons, the putative defect in triglyceride transport is unlikely to be hepatic overproduction of VLDL triglyceride. First, measurements of the production rates of VLDL apo B and VLDL triglyceride have provided no evidence that HDL cholesterol is influenced by VLDL synthesis in humans. Second, in monogenic familial hypertriglyceridemia, in which enlarged triglyceride-enriched VLDL accumulate in plasma due to hepatic oversynthesis of triglyceride, CHD prevalence has not been found to be increased. On the other hand, there are indications that variations in the FCR of triglyceride-rich lipoproteins may be pertinent. First, HDL cholesterol has been shown to be positively correlated with the FCRs of VLDL, chylomicrons, and an intravenously infused lipid emulsion, and also with the activities of LPL in muscle and adipose tissue. Second, the disturbance of HDL composition that is associated with CHD (i.e., a greater reduction of HDL cholesterol than of apo A-I) is similar to that found in subjects with low LPL activity and a low FCR of triglyceride-rich lipoproteins. Third, in an angiographic study, the severity of coronary artery disease was found to be negatively correlated more strongly with postheparin LPL activity than with HDL cholesterol. This result was consonant with previous reports dating from the 1950s showing that the rate of clearance of plasma turbidity after intravenous heparin given postprandially was lower in CHD cases than in healthy controls. These early observations were made before LPL had been discovered and at a time when the clarifying effect of heparin was thought to reflect a change in the physical state of plasma lipids.

According to this thesis, persons with low HDL cholesterol levels will include some individuals in whom the catabolism of triglyceride-rich lipoproteins is delayed due to low LPL activity, and some persons in whom HDL cholesterol is low on account of other mechanisms. Each of these groups has a high overall incidence of CHD: in one case due to the putative effect of lipolytic rate on reverse cholesterol transport, and in the other case due to the inclusion in the normotrigeicidemic group of subjects whose HDL cholesterol levels are low on account of low rates of apo A-I synthesis in liver or intestine. Hypertriglyceridemic subjects will also be composed of two groups: those with low LPL activities and low HDL cholesterol levels already considered; and those in whom hepatic VLDL triglyceride synthesis is increased, but in whom LPL activity and plasma HDL cholesterol are normal. Of these hypertriglyceridemic groups, only the former has an increased risk of CHD. These hypothetical subdivisions are illustrated in Figure 3.

The concept that both apo A-I synthesis and lipolysis may, by generating cholesterol acceptors, promote HDL-mediated reverse cholesterol transport from arterial tissues, and thereby retard atherogenesis, is compatible with the known effects of estrogen, cholestyramine, and gemfibrozil on lipoprotein metabolism, and with the evidence that these agents reduce coronary disease via a mechanism which, in statistical terms, is mediated in part via an increase in plasma HDL cholesterol.
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