Regulation of Plasma Cholesterol by Lipoprotein Receptors

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Summary. The lipoprotein transport system holds the key to understanding the mechanisms by which genes, diet, and hormones interact to regulate the plasma cholesterol level in man. Crucial components of this system are lipoprotein receptors in the liver and extrahepatic tissues that mediate the uptake and degradation of cholesterol-carrying lipoproteins. The number of lipoprotein receptors, and hence the efficiency of disposal of plasma cholesterol, can be increased by cholesterol-lowering drugs. Regulation of lipoprotein receptors can be exploited pharmacologically in the therapy of hypercholesterolemia and atherosclerosis in man.

Recent advances in the genetics and cellular biology of cholesterol metabolism have provided new insights into the control of plasma cholesterol levels in man. It is now apparent that normal humans possess efficient mechanisms for the removal of cholesterol from plasma (*1*). This disposal process depends on receptors located on the surface of cells in the liver and extrahepatic tissues. The receptors bind circulating lipoproteins that transport cholesterol in the bloodstream, thereby initiating a process by which the lipoproteins are taken up and degraded by cells, yielding their cholesterol for cellular uses (*2*).

The lipoprotein receptors are components of an integrated transport system that shuttles cholesterol continuously among intestine, liver, and extrahepatic tissues (*1*). An interesting feature of the system is that the lipoproteins are degraded as they deliver their cholesterol to tissues, while the cholesterol survives, eventually to be excreted from the tissues bound to new lipoprotein carriers. Exit of cholesterol from the body occurs only when the sterol is transported to the liver for excretion into the bile.

Because of the continuous cycling of cholesterol into and out of the bloodstream, the plasma cholesterol concentration is not a simple additive function of dietary cholesterol intake and endogenous cholesterol synthesis. Rather, it reflects the rates of synthesis of the cholesterol-carrying lipoproteins and the efficiency of the receptor mechanisms that determine their catabolism. Recent experiments show that the number of lipoprotein receptors in the liver, and hence the rate of removal of cholesterol from plasma, is under regulation and that the number of receptors can be increased by certain cholesterol-lowering drugs (*3, 4*). This new information eventually may reveal why different individuals respond differently to dietary cholesterol.

In this article we review the general features of the lipoprotein transport system worked out in studies from many laboratories over the past 25 years (*5*). We then focus on the lipoprotein receptors of liver and extrahepatic tissues and their role in regulating plasma cholesterol levels. Finally, we raise the possibility that pharmacologic manipulation of the hepatic and extrahepatic receptors may have therapeutic importance in the treatment of hypercholesterolemia and atherosclerosis in man.

The Lipoprotein Transport System

The lipoprotein transport system carries two classes of hydrophobic lipids, triglycerides (esters of glycerol and long-chain fatty acids) and cholesteryl esters (esters of cholesterol and long-chain fatty acids). Before they can be used by cells, the triglycerides and cholesteryl esters must be hydrolyzed to liberate fatty acids and unesterified cholesterol, respectively (*1*). Triglycerides are delivered primarily to adipose tissue and muscle where the fatty acids are stored or oxidized for energy. Cholesteryl esters are transported to all body cells where the unesterified sterol is used as a structural component of plasma membranes. These esters also supply cholesterol for synthesis of steroid hormones and bile acids.

For transport in plasma, triglycerides and cholesteryl esters are packaged into lipoprotein particles in which they form a hydrophobic core surrounded by a surface monolayer of polar phospholipids. The surface coat also contains unesterified cholesterol in relatively small amounts together with proteins called apoproteins (*5*). Through interactions with enzymes and cell surface receptors, the apoproteins direct each lipoprotein to its site of metabolism.

The lipoprotein transport pathway can be divided conceptually into exogenous and endogenous systems that transport lipids of dietary and hepatic origin, respectively (Fig. 1). Both systems begin with the secretion of triglyceride-rich lipoproteins—intestinal chylomicrons in the exogenous system and hepatic very-low-density lipoproteins (VLDL) in the endogenous system. Each of these particles contains an apoprotein called apoB, which maintains its structural integrity and which remains with the particle throughout its interconversions in the plasma. Recent studies indicate that the apoB of chylomicrons is not identical to that of VLDL (*6*).

Exogenous lipid transport. A typical American adult absorbs about 100 grams of triglyceride and 250 milligrams of cholesterol from the diet daily (Fig. 1). The intestine incorporates these lipids into chylomicrons, huge lipoproteins (diameter, 800 to 5000 angstroms) that are secreted into the lymph and from there enter the bloodstream. Inasmuch as chylomicrons are too large to cross the endothelial barrier, they must be metabolized while still in the bloodstream. For this purpose the chylomicrons bind to lipoprotein lipase, an enzyme (E.C. 3.1.1.34) that is fixed to the luminal surface of the endothelial cells that line capillaries of adipose and muscle tissues (Fig. 2A). The chylomicrons contain an apoprotein (C-II) that activates the lipase, which liberates free fatty acids and monoglycerides. The fatty acids enter the adjacent muscle or adipose cells where they are either oxidized or reesterified for storage (*1*). As the triglyceride core is depleted, the chylomicron

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shrinks. The excess surface material, primarily phospholipids and free cholesterol, is transferred to another plasma lipoprotein, high-density lipoprotein (HDL) (*7*). A similar transfer reaction occurs in the endogenous lipoprotein transport system (discussed below).

The depleted chylomicron is released from the capillary wall and reenters the circulation. The particle, now known as a chylomicron remnant (or simply, remnant), retains its cholesteryl ester and apoB along with another important apoprotein, apoE. The remnant (diameter, 300 to 800 Å) is carried to the liver where it binds to receptors on the surface of hepatic cells (Fig. 2B). The remnants are immediately internalized by receptor-mediated endocytosis and degraded in lysosomes.

The two-step pathway of chylomicron metabolism (triglyceride removal in extrahepatic tissues followed by cholesteryl ester uptake in the liver) (*8*) is quite efficient. In man, the half-time for the clearance of chylomicrons and their remnants from the plasma is 4 to 5 minutes (*9*). Thus, the plasma level of cholesterol rises very little, if at all, after a single high cholesterol meal.

The liver, which rapidly takes up dietary cholesterol in the form of chylomicron remnants, disposes of the sterol in the bile, either as unesterified cholesterol or as bile acids. No other quantitatively significant breakdown products of cholesterol are formed in man (Fig. 1). Much of the cholesterol and bile acid secreted by the liver is reabsorbed in the intestine and again delivered to the liver for excretion, thus forming an enterohepatic circulation. During each cycle a portion of the cholesterol and bile acid escapes reabsorption and is lost in the feces. With the typical American diet, which is high in cholesterol, about 1100 mg of sterol is lost from the body each day. In the steady state, about 850 mg of this sterol is derived from endogenously synthesized cholesterol and approximately 250 mg from previously absorbed dietary cholesterol (*1, 9, 10*).

Endogenous lipid transport. The liver converts carbohydrates and fatty acids into triglycerides, which it packages into lipoproteins for transport to adipose tissue. These lipoproteins also contain cholesterol, which will be delivered to extrahepatic cells. When dietary cholesterol is available, the liver uses that source of sterol, derived from the receptor-mediated uptake of chylomicron remnants, for lipoprotein synthesis. When dietary cholesterol is insufficient, the liver synthesizes its own cholesterol (*11*) by increasing the activity of a rate-controlling enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (E.C. 1.1.1. 34; HMG CoA reductase) (*12*).

For export of triglycerides and cholesterol, the liver incorporates the lipids into VLDL (300 to 800 Å) (Fig. 1). The VLDL particles interact with lipoprotein lipase in capillaries, releasing most of their triglycerides (Fig. 2A). The interaction of VLDL with lipoprotein lipase is less efficient than the interaction of chylomicrons with this enzyme (*1, 9*). Thus, the half-life of the VLDL particle in the circulation of humans is 1 to 3 hours (*13*), in contrast to the previously cited half-life of 4 to 5 minutes for chylomicrons (*9*). As the size of the VLDL particle diminishes owing to its interaction with lipoprotein lipase, its density increases, and the particles are converted to intermediate density lipoproteins (IDL) (*13*) (Fig. 1). The excess surface materials, mostly phospholipids and cholesterol, are transferred to HDL. The HDL particles interact with the plasma enzyme, lecithin–cholesterol acyltransferase (E.C. 2.3.1.43; LCAT), which esterifies the excess cholesterol with fatty acids derived from the 2-position of lecithin, the major phospholipid of plasma (*14*). The newly synthesized cholesteryl ester is transferred back to the IDL particles from HDL, apparently through the action of a plasma cholesteryl ester exchange protein (*15*). The net result of the coupled lipolysis and exchange reactions is the replacement of most of the triglyceride core of VLDL with cholesteryl esters.

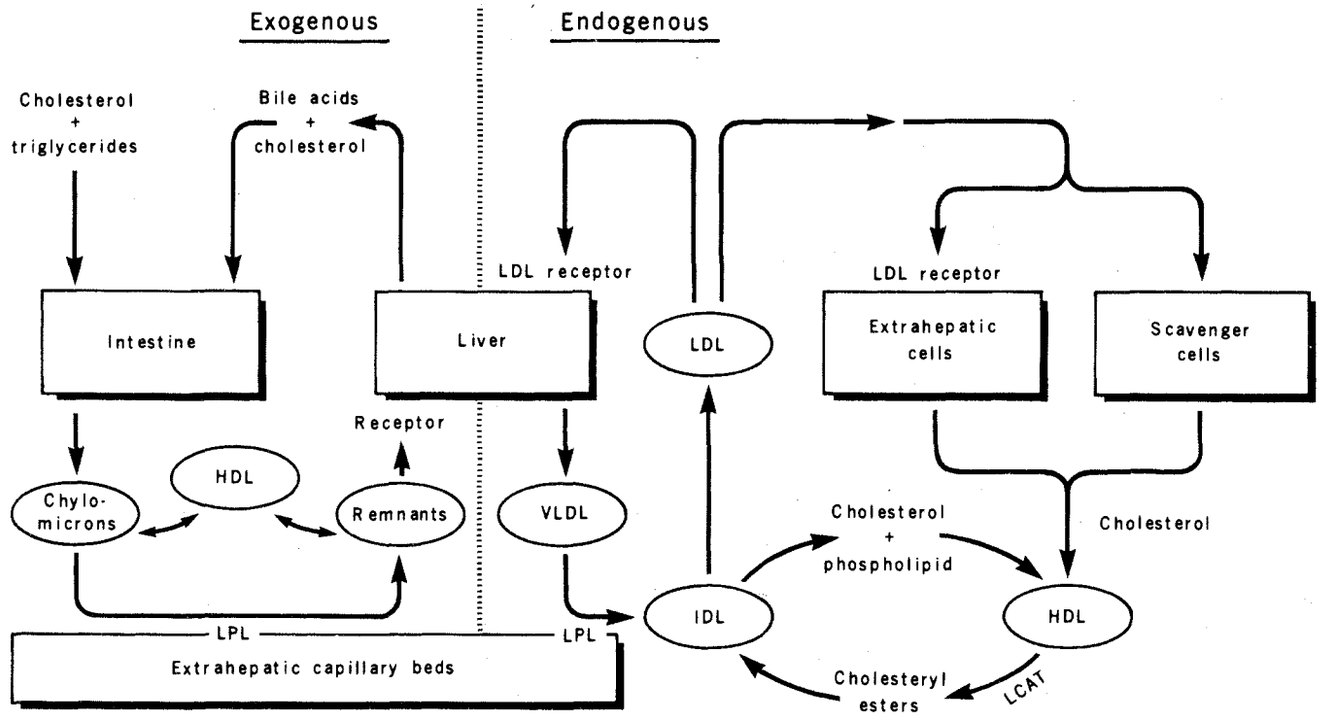

Fig. 1. Model for lipoprotein transport in man, illustrating the division between the exogenous and endogenous cycles. Both cycles begin with the secretion of triglyceride-rich particles (*chylomicrons* and *VLDL*) that are converted to cholesteryl ester-rich particles (*remnants*, *IDL*, and *LDL*) through interaction with *LPL*. Abbreviations are as follows: *LPL*, lipoprotein lipase; *VLDL*, very-low-density lipoproteins; *IDL*, intermediate-density lipoproteins; *LDL*, low-density lipoproteins; *HDL*, high-density lipoproteins; *LCAT*, lecithin-cholesterol acyltransferase.

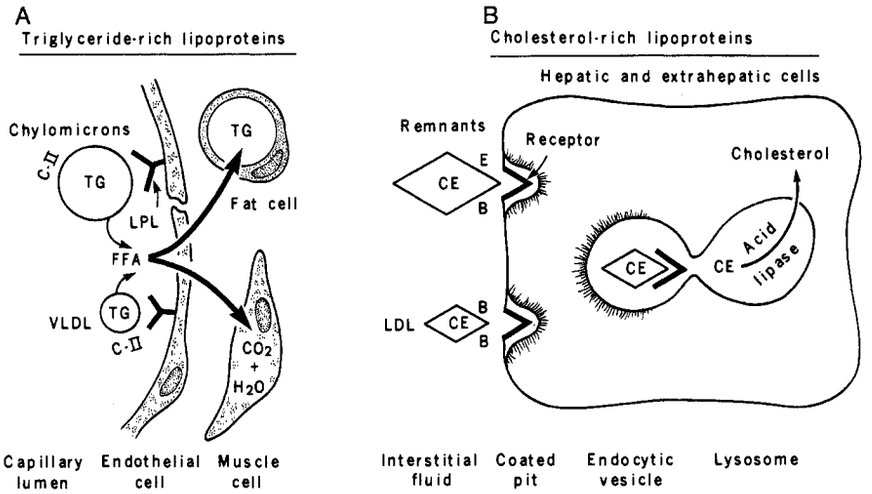

Fig. 2. Comparison of the mechanisms by which triglyceride-rich lipoproteins (A) and cholesterol-rich lipoproteins (B) deliver their core lipids to target tissues. Triglycerides are hydrolyzed by an extracellular enzyme (*LPL*) that is attached to endothelial cells and operates at the endothelial surface. Cholesteryl esters are hydrolyzed by an intracellular enzyme, acid lipase, that is located in lysosomes and cleaves the esters that enter cells via receptor-mediated endocytosis. Abbreviations are as follows: *TG*, triglycerides; *FFA*, free fatty acids; *LPL*, lipoprotein lipase; *VLDL*, very-low-density lipoproteins; *CE*, cholesteryl esters; *LDL*, low-density lipoproteins. The apoproteins responsible for the interactions (*C-II*, *B*, and *E*) are indicated.

After lipolysis, the IDL particles are released from the capillary wall into the circulation. They then undergo a further conversion in which most of the remaining triglycerides are removed and all of the apoproteins except apoB are lost. The resultant particle, which contains almost pure cholesteryl ester in the core and apoB at the surface, is LDL (diameter, 220 Å) (*5*). The site of the final conversion of IDL to LDL is unknown, but there is speculation that it occurs in the liver sinusoids (*16*). During this conversion a portion of the cholesteryl ester of IDL is removed, but the mechanism is unknown (*17*). In addition, some of the IDL particles are catabolized by the liver without being converted to LDL.

In normal subjects the cholesterol in LDL constitutes about two-thirds of the total plasma cholesterol. The LDL particles are removed from the plasma with a fractional catabolic rate of about 45 percent of the plasma pool per day (*18, 19*). The LDL delivers cholesterol to extrahepatic cells and to liver. Delivery is accomplished when the LDL binds to high-affinity receptors located in regions of the plasma membrane called coated pits (Fig. 2B). These pits invaginate into the cell and pinch off to form endocytic vesicles that carry the LDL to lysosomes (*20, 21*). Fusion of the vesicle membrane with the lysosomal membrane exposes the LDL to a panoply of hydrolytic enzymes that degrade the apoB to amino acids. The cholesteryl esters are hydrolyzed by an acid lipase and the liberated cholesterol leaves the lysosomes for use in cellular reactions. As a result of this uptake mechanism, extrahepatic cells have low rates of cholesterol synthesis, relying instead on LDL-derived cholesterol (*2, 20*).

In addition to its degradation via the high-affinity LDL receptor pathway, plasma LDL can be degraded by less efficient mechanisms that require high plasma levels to achieve significant rates of removal. One of these mechanisms occurs in scavenger cells or macrophages of the reticuloendothelial system (*22*). When the plasma level of LDL rises, these scavenger cells degrade increasing amounts of LDL. When overloaded with cholesteryl esters, they are converted into "foam cells," which are classic components of atherosclerotic plaques. In man and experimental animals, estimates of the proportion of plasma LDL degraded by the LDL receptor system range from 33 to 66 percent (*22–25*). The remainder is degraded by the scavenger cell system and perhaps by other mechanisms not yet elucidated.

In the steady state, tissues excrete cholesterol into the plasma in amounts equal to the amounts taken up from LDL. Such excretion results from cell death as well as membrane turnover in living cells. The cholesterol leaving cells is believed to be adsorbed onto HDL (*14*) (Fig. 1); HDL, which is synthesized in liver and intestine, circulates in man with the longest half-time of all lipoproteins, 5 to 6 days (*1*). It functions primarily in cholesterol and phospholipid exchange and esterification reactions within plasma (*14*). Excreted cholesterol that binds to HDL is esterified by plasma LCAT, after which the cholesteryl esters are transferred to VLDL and IDL (*1, 14, 15*). Since VLDL and IDL are converted to LDL, the LCAT reaction completes a cycle by which body cells acquire cholesterol from the catabolism of LDL and ultimately return cholesterol to new particles of LDL (Fig. 1). Much of the cholesteryl ester in human LDL represents cholesterol that has been recycled from tissues in this way (*1*).

The crucial role of genetic factors in the orchestration of the lipoprotein transport system is underscored by clinical observations in individuals with inborn errors of metabolism affecting this scheme. At least six genetically determined disorders disrupt the endogenous or exogenous transport system in ways that produce an increase in the plasma level of one or more lipoproteins. Collectively, these defects are responsible for about 20 percent of all myocardial infarctions occurring in persons under 60 years of age [for reviews, see (*1, 26*)].

Points of overlap between the exogenous and endogenous lipid transport systems. Although the exogenous and endogenous lipid transport systems can be viewed as functionally distinct, there are three points at which common mechanisms are used. These overlap points provide sites at which dietary fat intake can influence the metabolism of endogenous lipoproteins. One of these occurs at the enzyme lipoprotein lipase. The same enzyme hydrolyzes exogenous triglycerides carried in chylomicrons and endogenous triglycerides carried in VLDL (*1*). A second point of overlap involves the acceptance by HDL of phospholipids and cholesterol from exogenous chylomicron remnants as well as from endogenous IDL (*14*).

A third, and perhaps most important, point of overlap involves the extrahepatic and hepatic lipoprotein receptors. The LDL receptors on extrahepatic parenchymal cells normally take up LDL, while lipoprotein receptors in the liver take up chylomicron remnants rapidly and LDL slowly. Yet, recent studies in vitro suggest that these two functionally distinct binding reactions may be performed by a single type of receptor that can bind both chylomicron remnants and LDL (*3, 27, 28*). The overlapping specificities of these two receptor systems creates a sorting problem in vivo: How are chylomicron remnants directed selectively to the liver and how is LDL directed primarily to the periphery? In the remainder of this article we provide an analysis of the extrahepatic and hepatic LDL receptors.

Extrahepatic LDL Receptors

Low-density lipoprotein receptors were discovered during studies of an extrahepatic cell, the cultured human skin fibroblast (*2, 29*). These cells supply themselves with cholesterol by receptor-mediated endocytosis of LDL present in the serum of the culture medium. The cholesterol liberated from LDL within the lysosomes is used for membrane synthesis. It also triggers three regulatory responses that assure cholesterol homeostasis: (i) suppression of HMG CoA reductase, which turns off cholesterol synthesis by the cell; (ii) activation of an acyl-CoA: cholesterol acyltransferase (E.C. 2.3.1.26; ACAT), which reesterifies excess cholesterol for storage as cholesteryl ester droplets; and (iii) suppression of the synthesis of LDL receptors, which prevents an overaccumulation of cholesterol via the receptor pathway. Cultured cells adjust the number of receptors to provide cholesterol just sufficient to support cell growth and membrane turnover. As the rate of growth declines, the number of receptors declines (*29*). The number of receptors in fibroblasts can be increased by certain hormones that stimulate cell growth, including insulin, thyroxine, and platelet-derived growth factor (*30*).

Function of the extrahepatic LDL receptor. Low-density lipoprotein receptors provide the major source of cholesterol to most types of animal cells that grow in tissue culture (*31*) as well as to a number of extrahepatic tissues in the body (*20, 32*). The receptors are particularly important in adrenocortical cells, where the LDL-derived cholesterol provides one source of substrate for the synthesis of steroid hormones (*20*).

The functional significance of the LDL receptor became apparent when it was recognized that cells from patients with the genetic disorder familial hypercholesterolemia (FH) have a defect in the gene coding for the receptor (*33*). Subjects heterozygous for this mutant gene produce about half the normal number of LDL receptors; patients who are homozygous produce either no functional receptors or only a very small number. Even though the unesterified cholesterol on the surface of LDL can be exchanged with cell membrane cholesterol in the absence of a receptor, the net rate of flux is too slow to supply sufficient cholesterol for growth (*34*). The mutant FH cells grow in tissue culture because they adapt to the unavailability of LDL-cholesterol by increasing HMG CoA reductase activity and cholesterol synthesis. In the body, the deficiency in LDL receptors is harmful because it produces a block in LDL degradation. As a result, LDL-cholesterol accumulates in plasma to concentrations about threefold above normal in FH heterozygotes and six- to tenfold above normal in homozygotes (*33*). The excessive LDL-cholesterol in plasma rapidly leads to deposition of the sterol in the artery wall, inducing premature atherosclerosis (*2*).

Specificity of the extrahepatic LDL receptor. The only apoprotein known to be present in LDL is apoB, a protein of about 250,000 daltons, for which there are two copies per LDL particle (*1, 5*). Early studies showed that the LDL receptor binds LDL by interacting with apoB (*2, 29*) and that modification of the apoB by acetylation of lysine residues destroys its ability to bind to the receptor (*35*).

An important advance came when it was recognized that the LDL receptor can bind lipoproteins that contain apoE in addition to those that contain apoB. This conclusion emerged from an observation made in a collaborative study between Mahley's laboratory in Bethesda and our laboratory in Dallas (*36*). This study showed that HMG CoA reductase activity could be suppressed and the ACAT enzyme could be activated in cultured fibroblasts from normal subjects, but not FH homozygotes, when the cells were incubated with a lipoprotein called HDL_c (*36*). This cholesteryl ester-rich lipoprotein is isolated from the plasma of swine and dogs that have been fed high cholesterol diets. It does not contain apoB. One form of HDL_c, called apoE-HDL_c, contains apoE as the sole protein (*37*). Mahley and co-workers (*38*) showed that the affinity of the fibroblast LDL receptor for apoE-HDL_c is 10- to 25-fold higher than its affinity for LDL, but at saturation the number of apoE-HDL_c particles bound is only one-fourth that of LDL. These data have been explained by a model in which each apoE-HDL_c particle binds to four LDL receptors, whereas each LDL particle binds to only one receptor site (*38*).

In normal humans apoE is found primarily in the triglyceride-carrying lipoproteins and their remnants—that is, chylomicron remnants, VLDL, and IDL (*1*). In vitro these apoE-containing particles bind with high affinity to fibroblast LDL receptors; in vivo they do not normally deliver cholesterol to extrahepatic tissues and are either rapidly catabolized in the liver or converted to LDL (*1, 39*). This finding raised the possibility that the apoE-containing lipoproteins are taken up in the liver by virtue of their ability to bind to a hepatic receptor that resembles the fibroblast LDL receptor.

Properties of the extrahepatic LDL receptor. The following properties of the fibroblast LDL receptor have been useful in comparing it with the hepatic lipoprotein receptor: (i) affinity for apoE-containing particles is 10- to 25-fold higher than for apoB-containing lipoproteins (*38*); (ii) binding of lipoproteins containing apoB or apoE is abolished by modification of the lipoprotein's lysine residues by acetylation (*35, 40*) or reductive methylation (*40*), or by arginine modification with cyclohexanedione (*41*); (iii) binding requires a divalent cation and is abolished by EDTA (*29*); (iv) binding is destroyed by treatment of cells with Pronase (*29*); (v) the receptor does not bind HDL_3, the major fraction of human HDL, which does not contain apoB or apoE (*2, 37*); and (vi) binding of LDL is abolished by treatment of cells with an antibody prepared against the bovine adrenal LDL receptor (*42*). Each of these properties of the LDL receptor of intact fibroblasts is preserved when the cells are homogenized and the binding reaction is performed with isolated membranes (*43*).

Extrahepatic LDL receptors have been solubilized with detergents and purified 350-fold from the bovine adrenal cortex (*44*), and an antibody to the receptor has been prepared (*42*). The purified receptor retains all of the properties mentioned above, including a higher binding affinity for canine apoE-HDL_c than for LDL (*45*). The receptor-detergent complex has a molecular weight of about 160,000.

Hepatic Lipoprotein Receptors

As discussed above, chylomicron remnants, but not chylomicrons, are rapidly removed from the circulation upon passage through the liver (*8*). This uptake has been duplicated in vitro in rat hepatocytes, perfused rat livers, and isolated liver membranes (*3, 27, 28, 46, 47*). Rat hepatocytes take up remnant particles intact and hydrolyze the protein and cholesteryl esters in lysosomes (*46, 47*). The lipoprotein-derived cholesterol suppresses HMG CoA reductase (*48*).

Several lines of evidence indicate that the hepatic uptake of chylomicron remnants is mediated by a receptor that resembles the extrahepatic LDL receptor and that recognizes the apoE of the remnant: (i) in dogs, intravenously administered ^{125}I-labeled apoE-HDL_c is rapidly cleared by the liver and clearance is retarded by modification of the lysine residues of the apoprotein (*49*); (ii) up-

take of ^{125}I-labeled apoE-HDL$_c$ by perfused rat livers is rapid, saturable, and susceptible to competition by chylomicron remnants (*50*); and (iii) in a human genetic disease, familial dysbetalipoproteinemia (type 3 hyperlipoproteinemia) (*1*), a mutant allele at the locus specifying the structure of apoE (*51*) causes chylomicron remnants and IDL to accumulate in plasma owing to their faulty recognition by hepatic lipoprotein receptors (*52*).

To study the hepatic lipoprotein receptors in vitro, our laboratory has recently developed methods to measure the binding of iodinated lipoproteins to isolated liver membranes of rats, young dogs, and rabbits (*3, 27, 28*). To confirm that the in vitro binding site has a physiologic function, the membrane binding reactions have been performed in parallel with studies of the uptake of the lipoproteins by the livers of the intact animal. The power of the correlations has been increased by treatment of the animals with drugs that alter the number of receptors. These drugs produce parallel effects on the receptor number as measured in vitro and on hepatic uptake as monitored in vivo.

Hepatic LDL receptors in the rat. The rat is unique among animal species in that in the rat, high doses of 17α-ethinyl estradiol cause the virtual disappearance of all lipoproteins from plasma (*53*). The decrease in plasma LDL is due in part to a markedly accelerated rate of LDL uptake and catabolism by the liver (*27, 54*). The membrane binding assay showed that the livers of estradiol-treated rats have a tenfold increase in the number of lipoprotein receptors (*27*). These receptors shared all the properties of the extrahepatic LDL receptors listed above, including susceptibility to inhibition by an antibody prepared against the LDL receptor of bovine adrenal cortex (*27, 42*). The livers of normal rats expressed the same receptor, albeit in much lower amounts. The receptor recognized rat lipoproteins containing apoB or apoE (LDL, VLDL, IDL, and chylomicron remnants) as well as canine apoE-HDL$_c$. In parallel studies, Havel's laboratory (*27*) showed that the lipoproteins that exhibited enhanced binding to membranes from estradiol-treated rats in vitro were taken up at accelerated rates by perfused livers in vivo. Autoradiographic studies with ^{125}I-labeled LDL indicated that the uptake occurred via receptor-mediated endocytosis (*55*).

The studies in rat liver also showed that VLDL particles that contained a large amount of C apoproteins bound poorly to the hepatic receptor in vitro and had a relatively low rate of uptake by the liver in vivo (*27, 56, 57*). Partial lipolysis of VLDL, which leads to a loss of C apoproteins, produced a particle that showed enhanced binding in vitro and enhanced hepatic uptake in vivo (*27, 56*). Since the C apoproteins are present on chylomicrons and are largely removed from the particle when it is converted to a remnant, this mechanism may explain the finding that chylomicron remnants, but not chylomicrons, are taken up by the liver.

Hepatic LDL receptors in the dog. In young beagle dogs an increase in hepatic LDL receptors, as measured in vitro, has been shown to be correlated with an increase in removal of ^{125}I-labeled LDL from the circulation as measured in vivo (*3*). To produce this increase, dogs were treated with the combination of colestipol, a bile-acid binding resin, and mevinolin, an inhibitor of cholesterol synthesis (*58*). By trapping bile acids in the intestine, colestipol causes the liver to convert more cholesterol to bile acids and hence increases the hepatic demand for cholesterol. Mevinolin blocks the compensatory increase in cholesterol synthesis that would ordinarily occur under these conditions and thus forces the liver to rely on lipoprotein cholesterol for the synthesis of bile acids. In response to this treatment the dog liver produced a threefold higher number of LDL receptors as measured in vitro (*3*). This change was associated with a twofold increase in the fractional clearance rate of intravenously administered ^{125}I-labeled LDL from the plasma. This increased efficiency of LDL clearance, plus a 50 percent reduction in the synthetic rate of LDL produced by mevinolin, contributed to a remarkable 75 percent drop in the plasma level of LDL-cholesterol in the treated dogs (*3*).

Hepatic LDL receptors in the rabbit. Of all animal species studied, only the rabbit has the propensity to develop a massive increase in plasma cholesterol within days after being placed on a high cholesterol diet (*59*). Most of the elevated cholesterol is contained in cholesteryl ester-rich particles called β-VLDL. These particles accumulate in plasma because their removal rate fails to increase in proportion to the increased production of chylomicrons and VLDL that occurs during cholesterol feeding (*28, 59*). Lipoprotein binding and turnover studies with ^{125}I-labeled β-VLDL demonstrated that the reason for the failure of clearance was twofold (*28*). First, the hepatic receptors, although of high affinity, are of low capacity. When the rate of production of β-VLDL exceeds a threshold, the receptors become saturated, the removal rate reaches a maximum, and the β-VLDL level shoots up dramatically. Second, the number of hepatic receptors declines by 60 to 80 percent after long-term cholesterol feeding. This suppression may serve as a protective mechanism to help guard hepatocytes against further accumulation of cholesterol. The rabbit liver receptor that mediates rapid uptake of β-VLDL shares all of the properties of the fibroblast LDL receptor (*28*).

Hepatic and Extrahepatic LDL Receptors in vivo

From the above discussion it is clear that the biochemical properties of the extrahepatic and hepatic LDL receptors in vitro are remarkably similar (Table 1). Both receptors promote the uptake of lipoproteins by endocytosis. Both receptors are subject to metabolic regulation. Both receptors bind apoE-containing lipoproteins, such as chylomicron remnants, with higher affinity than LDL (Table 1). However, in the body the extrahepatic receptors take up LDL, whereas the hepatic receptors take up chylomicron remnants with great efficiency and LDL with much less efficiency.

One reason for this paradox may stem from the large size of the remnant particles, which can cross the endothelium of extrahepatic tissues only slowly. Hence they are found in only trace amounts in interstitial fluid and lymph, precluding access to extrahepatic cells (*39*). The rapid hepatic uptake of remnants implies that these particles have some way of crossing the endothelium that lines hepatic sinusoids to gain access to the receptors on hepatocytes. Whether this mechanism involves bulk flow through the fenestrated sinusoidal endothelium, which permits passage of molecules up to 1000 Å in diameter (*56*), or whether it requires specific transendothelial transport is unknown.

If the hepatic receptors have a high affinity for apoE-containing lipoproteins, and if these lipoproteins can gain access to these receptors in the liver sinusoids, the question arises as to why apoE is ever found in the circulation. Why are not all apoE-containing particles cleared immediately by the liver? The answer may lie in the observation that apoE is not always in an active form: its ability to bind to receptors can be regulated by

several factors. One of these, discussed above, is the presence of C apoproteins, which impair the ability of apoE to bind to receptors in liver (*27, 56, 57*) and extrahepatic tissues (*60*). A second factor is the formation of disulfide-linked dimers between apoE and apoA-II, a normal constituent of human HDL and chylomicrons (*61*). The coupled apoE is unable to bind to receptors, but it can be activated by reduction of the disulfide bond (*61*). In addition, apoE exists in several forms (*51*) bearing different carbohydrate residues (*62*), and it is possible that binding activity is modulated by changes in carbohydrate. Finally, the high affinity of apoE-containing particles for the receptor depends on the ability of four apoE molecules on the same particle to bind to receptors simultaneously (*38*). This requires that each particle contain at least four apoE molecules in close proximity on the lipoprotein's surface. Such criteria may not be met for all lipoproteins that contain apoE.

These observations raise the possibility that apoE may circulate in plasma in an inactive form without binding to either hepatic or extrahepatic receptors. When it is transferred to certain lipoproteins, such as chylomicron remnants, the apoE could be activated, directing the lipoprotein immediately to the liver. Thus, when ^{125}I-labeled apoE-HDL$_c$ was injected intravenously into dogs, some of the lipoprotein was cleared rapidly by the liver (*49*). After a few minutes hepatic uptake slowed. Possibly the remaining ^{125}I-labeled apoE had been transferred from apoE-HDL$_c$ to another lipoprotein particle in which it was no longer active in binding to hepatic receptors.

Is There a Separate Hepatic Receptor for Chylomicron Remnants?

The above mechanism may explain how the same type of LDL receptor can mediate the uptake of chylomicron remnants and LDL in the liver and the uptake of only LDL in the periphery. There is one observation that suggests that the human liver may produce a separate chylomicron remnant receptor in addition to its LDL receptor. Patients with the receptor-negative form of homozygous FH show a marked reduction in the fractional catabolic rate for plasma LDL. These individuals show no decline in plasma LDL levels upon administration of bile acid–binding resins (*63*), suggesting that their livers are unable to synthesize LDL receptors. Yet, these subjects have no clinical evidence for impaired catabolism of chylomicron remnants (*1, 26*).

The observations in FH raise the possibility that the human liver may be capable of producing two types of lipoprotein receptors. One of these, the LDL receptor, binds LDL plus remnants. The other, the remnant receptor, binds only chylomicron remnants. If two such receptors were operative, then the degradation of chylomicron remnants and LDL could be regulated independently. Dietary, drug, and hormonal factors that regulate the number of hepatic LDL receptors could alter the rate of removal of LDL from plasma, and hence regulate plasma LDL levels, without greatly affecting the removal of chylomicron remnants.

A new way to resolve the question of one or two hepatic lipoprotein receptors has become available with the discovery by Watanabe (*64*) of an animal model of familial hypercholesterolemia. These Watanabe hereditary hyperlipidemic (WHHL) rabbits are homozygous for a genetic defect in the LDL receptor that is expressed in cultured fibroblasts (*64*) in a manner identical with that of FH homozygotes (*65*). Like their human counterparts, the WHHL rabbits have massive hypercholesterolemia, even on a cholesterol-free diet, and they develop severe atherosclerosis. The livers of WHHL rabbits lack LDL receptors (*65*). Studies are now under way to determine whether the livers of these rabbits are able to take up chylomicron remnants at a normal rate.

Regulation of LDL Receptors in Man: Therapeutic Implications

The characterization of the LDL receptor in vitro has paved the way for the development of a method to quantify the receptor-mediated catabolism of plasma LDL in intact humans. This method is based on the observation of Mahley *et al.* (*41*) that cyclohexanedione treatment of LDL blocks its ability to bind to LDL receptors in fibroblasts. The same is true in liver (*27*). Shepherd *et al.* adminis-

Table 1. Comparison of the extrahepatic and hepatic LDL receptors. The extrahepatic LDL receptors are those that have been characterized in cultured mammalian cells, adrenal gland of animals, and freshly isolated human mononuclear cells. The hepatic LDL receptors are those that have been characterized in livers of rats, dogs, and rabbits. In addition to the LDL receptors described in this table, the liver may also produce a separate receptor for chylomicron remnants (see text).

Characteristic	Extrahepatic LDL receptors	Hepatic LDL receptors
Binding properties exhibited by both receptors in vitro	Affinity for apoE > apoB Binding abolished by: Ethylenediaminetetraacetic acid Modification of lysine or arginine residues of apoproteins Pronase treatment of membranes Antibody against LDL receptor of bovine adrenal cortex Receptor initiates endocytosis	
Major lipoproteins bound in vivo	LDL	Chylomicron remnants and LDL
Factors that increase (↑) or decrease (↓) receptors		
Genetic	(↓) Familial hypercholesterolemia (humans, WHHL rabbits)	(↓) Familial hypercholesterolemia (humans*, WHHL rabbits)
Hormonal	(↑) Thyroxine (↑) Insulin (↑) Platelet-derived growth factor (↑)Adrenocorticotropic hormone in adrenal cortex	(↑) Thyroxine (humans)*
Nutritional	(↑) Cholesterol deprivation (↓) Excess cellular cholesterol	(↓) Cholesterol feeding (rabbits)
Pharmacologic		(↑) 17α-Ethinyl estradiol (rats) (↑) Bile acid–binding resins (humans, dogs, rabbits) (↑) Inhibitors of cholesterol synthesis (dogs)

*Receptor-mediated catabolism of LDL has been shown to be altered in humans through measurements of ^{125}I-labeled LDL turnover in plasma (*18, 19, 66*). The magnitude of the changes suggests involvement of hepatic as well as extrahepatic receptors, but this inference lacks direct experimental support.

tered ^{125}I-labeled LDL and ^{131}I-labeled cyclohexanedione-treated LDL simultaneously to normal humans (*23*). Because it could bind to LDL receptors, the labeled LDL was cleared from plasma faster than the labeled cyclohexanedione-LDL. The difference between the clearance rates for the two lipoproteins was taken as a measure of the receptor-dependent clearance of LDL. In FH heterozygotes, the difference in disappearance rates between labeled LDL and labeled cyclohexanedione-LDL was diminished, confirming the reduced number of LDL receptors in these patients in vivo (*23*). Thompson, Myant and coworkers (*66*) then found that FH homozygotes showed no difference between the removal rates of ^{125}I-labeled LDL and ^{131}I-labeled cyclohexanedione-LDL, a finding consistent with an absence of LDL receptors in vivo.

One of the exciting applications of this double isotope method is its use in the assessment of the effects of drugs on the number of LDL receptors in intact humans. The method has revealed that a bile acid–binding resin (when administered to FH heterozygotes) (*4*) and L-thyroxine (when administered to a hypothyroid subject) (*66*) selectively enhance the receptor-mediated removal of LDL from plasma. This increase in LDL receptor activity explains the long-known ability of these two agents to lower plasma cholesterol levels. These results in humans have been supported by animal and cell culture experiments showing that bile acid–binding resins increase hepatic LDL receptors in rabbits (*4*) and dogs (*3*) and that L-thyroxine increases LDL receptors in fibroblasts (*30*) and probably in the liver.

Lipoprotein Receptors, Hypercholesterolemia, Atherosclerosis

Of the six lipoproteins that participate in lipid transport (Fig. 1), three (chylomicron remnants, IDL, and LDL) produce fulminant atherosclerosis when they become elevated in human plasma (*2, 67*). Two others (chylomicrons and VLDL) appear to be neutral, while one (HDL) is actually associated with a reduction in symptomatic atherosclerosis (*68*). The mechanisms by which the offending lipoproteins produce atherosclerosis are unknown. Clearly they involve complex interactions among lipoproteins, blood platelets, arterial endothelium, arterial smooth muscle cells, and macrophages. These detrimental interactions are enhanced by many contributory genetic and environmental factors, including cigarette smoking, high blood pressure, and diabetes mellitus, as well as by local factors that render certain segments of arteries more susceptible than others (*69*).

Each of the three atherogenic lipoproteins must be metabolized ultimately by lipoprotein receptors, either in the liver or in extrahepatic tissues. The hepatic lipoprotein receptors allow large amounts of dietary cholesterol to be delivered to the liver, thereby ensuring low levels of dietary cholesterol in plasma. For this reason, normal humans are somewhat resistant to direct effects of dietary cholesterol intake on plasma cholesterol levels. Yet, there are circumstances in which this efficient lipoprotein transport system breaks down. One fascinating example is the disease familial dysbetalipoproteinemia, in which a mutation in the gene for apoE (*51*) results in the production of chylomicron remnants and IDL particles that cannot bind normally to hepatic receptors (*52*). Since this defect involves the exogenous cholesterol transport system (Fig. 1), the degree of hypercholesterolemia in these patients is sensitive to the level of cholesterol in the diet (*1*).

The high affinity of the LDL receptors allows large amounts of endogenous cholesterol to be transported to liver and extrahepatic cells without the need for a high level of LDL in plasma. Yet this endogenous transport system, too, can be disrupted by genetic defects. The most well studied example is familial hypercholesterolemia, in which a defect in the LDL receptor prevents LDL from being metabolized normally (*33*). Since most LDL arises from endogenous cholesterol (Fig. 1), patients with this disorder have severe hypercholesterolemia even when they consume a cholesterol-free diet (*1*).

In many "normal" individuals in Western countries, the LDL levels are above those that appear to be appropriate for the receptor system (*2*). These levels are in a range that predisposes the individuals to atherosclerosis. Epidemiologic evidence suggests that excessive dietary intake of fat, cholesterol, and calories is responsible for the increased blood cholesterol. Yet, the lipoprotein that is elevated is a product of the endogenous system, namely LDL. The lipoprotein transport system of Fig. 1 suggests a mechanism by which excessive dietary fat and calories could produce this abnormality: the delivery of excessive calories and fat to the liver stimulates VLDL production with subsequent conversion to LDL in amounts that saturate the LDL receptors.

The ability of a given individual to cope with these dietary challenges may be dictated, in part, by the hormonal and genetic factors that control the number of lipoprotein receptors (Table 1). The more receptors that an individual produces, the lower the plasma cholesterol level will be. If all other factors that affect atherosclerosis are held constant (for example, smoking, blood pressure, and platelet function), the individual with the most lipoprotein receptors will be the least vulnerable when faced with a high cholesterol diet.

The interplay of genetic and environmental factors in controlling the number of lipoprotein receptors may explain, in part, the common "polygenic" or "multifactorial" forms of hypercholesterolemia that are so prevalent in Western societies (*70*). This genetic-environmental interaction may also explain why large-scale epidemiologic studies that do not consider genetic and hormonal variability fail to show a direct relation between dietary cholesterol intake and plasma cholesterol levels. Differences in individual responses to dietary cholesterol may underlie much of the confusion and controversy that surrounds the formulation of nutritional recommendations for the population as a whole (*71*). A diet that is well tolerated by some individuals may be extremely detrimental to others. Knowledge of the lipoprotein transport system, and its receptors, should facilitate the development of methods to predict which individuals are at risk from dietary cholesterol and which individuals will be resistant.

The experiments reviewed in this article also offer hope that lipoprotein receptors may be susceptible to enhancement by drugs. This pharmacologic manipulation takes advantage of the knowledge that the receptors are normally under feedback regulation and that the number of receptors in the liver can be increased when the liver's demand for cholesterol is increased.

Such an effect can be produced by the synergistic interaction of a bile acid–binding resin (such as cholestyramine or colestipol) and an inhibitor of cholesterol synthesis (such as compactin or mevinolin) (*3*). The potency of this drug combination in lowering plasma cholesterol levels is unprecedented [see (*72*)]. It may soon be possible for the first time to provide a scientific answer to the all-important question: Will the lowering of plasma cholesterol by a physiologic mechanism, such as stimulation of receptor-mediated catabolism of cholesterol-rich lipoproteins, protect humans against atherosclerosis?

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