The formation of LDL: mechanisms and regulation

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Low density lipoproteins (LDL) in blood plasma and their immediate precursors are generally thought to be the major atherogenic lipoproteins. The initial indications that LDL are formed from very low density lipoproteins (VLDL) were obtained more than 25 years ago (1, 2), yet even today the precise mechanism of LDL formation from VLDL is unknown. During the last 15 years the processes of interconversion of lipoproteins and their component lipids and proteins have been studied intensively (3). Essentially all of the approaches used in these investigations, ranging from in vitro studies of lipolysis products to multicompartmental kinetic analysis of tracers injected into humans, have been applied to study the formation of LDL. The complexity of VLDL metabolism has become particularly evident during the last 5 years and some of the truisms concerning LDL formation have had to be reconsidered. In this brief review, I describe those aspects of VLDL metabolism that are central to the mechanism of LDL formation. Recently discovered factors that influence the extent to which VLDL particles are converted to LDL and the question of VLDL-independent LDL formation will then be considered. Finally, some contemporary approaches to these issues will be addressed.

PROPERTIES OF NASCENT VLDL

Before they are secreted from the cell, triglyceride-rich lipoproteins are packaged into secretory vesicles of the Golgi apparatus: chylomicrons in absorptive cells of the small intestine and VLDL in hepatocytes (4). Although some of the proteins of these lipoproteins can be made in other cells (5–8), the particles recognized in the blood are thought to be made only at these two sites. The size of both chylomicrons and VLDL varies in part owing to the rate of triglyceride synthesis in the cells of origin (3). Because VLDL were originally defined operationally from their ultracentrifugal flotation rates (9), many investigators still use the term "intestinal VLDL" to describe the intestinal particles with diameters less than ~800 Å. Nascent hepatic lipoproteins larger than 800 Å (10) are not ordinarily called chylomicrons, however. In most mammals, triglyceride-rich lipoproteins of intestinal and hepatic origin can now be distinguished by the species of apolipoprotein B that they contain (11). For this reason and, as described later, because only nascent hepatic particles can form LDL, I believe that the term VLDL should be restricted to hepatic particles and the term chylomicrons to intestinal particles, irrespective of the size of either.

Like chylomicrons, the size of nascent VLDL made at a given time and even their size within individual secretory vesicles in hepatocytes is variable (see 12). Diameters may vary by 3-fold (and particle volumes therefore by 27-fold). This heterogeneity is caused mainly by variable content of triglycerides; the extent to which the composition of the nascent particle surface varies with size is unknown. However, regardless of size, each VLDL particle contains a constant mass of apolipoprotein (apo) B-100 (the hepatic form of this protein) (3). Obviously, the larger particles contain not only more triglycerides in their core, but also more polar lipids and probably proteins other than apoB-100 on their surface. As discussed later, these aspects of VLDL heterogeneity are relevant to LDL formation.

The heterogeneity of nascent VLDL has been studied most commonly by isolating them from perfusates of isolated livers. Like plasma VLDL, their diameter exceeds 250 Å. Particles with densities in the range of LDL have been observed in perfusates of isolated livers of several species, especially livers from cholesterol-fed animals (13). These particles are derived to a large extent from "washout" of preformed LDL, which can contaminate the extracellular, extravascular compartment of the liver (space of Disse) when they are present in high concentrations in the animal, but in some abnormal circumstances they seem to be synthesized and secreted directly (13–15). LDL in blood plasma are

Abbreviations: VLDL, LDL, IDL, very low density, low density, and intermediate density lipoproteins, respectively; WHHL, Watanabe heritable hyperlipidemic.

generally considered to contain no proteins other than apoB-100. As discussed later, a functional definition might restrict the term LDL to particles with this characteristic. Nascent particles with this characteristic may be secreted from livers of cholestatic rats (14). Perfused livers of such rats also secrete very little VLDL (16), presumably because they are in a catabolic state. The "LDL" secreted from livers of cholesterol-fed animals also contain much apoE and may be part of a spectrum of nascent particles that are secreted from livers that are loaded with cholesteryl esters. These particles are denser than VLDL in part because they also contain a large amount of cholesteryl esters, the density of which is considerably higher than that of triglycerides.

Rat livers may also secrete apoprotein B in particles denser than 1.063 g/ml (16, 17). The apoB in these particles is predominantly a lower molecular weight form, resembling intestinal apoB (B-48) (17). The same protein seems to be present in nascent VLDL of rats (11), a peculiarity that complicates research on LDL formation in this species.

REMNANTS AND THE TWO-STEP PROCESS OF LDL FORMATION

The remnant concept, which arose from studies of chylomicron metabolism in several species (18), is now well accepted. The idea of a lipolytic cascade by which VLDL are converted to LDL is even older (1). During the last ten years, the remnant concept has been applied to VLDL as well as chylomicrons, chiefly from studies in the rat (18, 19). The concept is based upon two types of observations. First, the initial step in the catabolism of chylomicrons and VLDL occurs in extrahepatic tissues, is mediated by lipoprotein lipase, and results in hydrolysis of most of the component triglycerides and some of the phospholipids (20). Second, cholesteryl esters, the other major core component of chylomicrons and VLDL, together with some of the other components, are taken up into the liver, evidently by endocytosis into hepatocytes (21). This is thought to constitute the second step in the metabolism of triglyceride-rich lipoproteins because chylomicrons and VLDL that have been subjected to the action of lipoprotein lipase (in functionally hepatectomized rats or in vitro) are taken up into the liver much more rapidly than chylomicrons (19). The basis for the rapid uptake of remnant particles into the liver has been partially elucidated. The process seems to be receptor-mediated (19, 21) and is greatly facilitated by apolipoprotein E, and inhibited by C apoproteins (19). Although much of the research on hepatic processing of VLDL remnants has been done in the rat, in which these particles contain B-48 (or a protein of similar size) as well as B-100, the process seems to be similar in rabbits (see below), in which apoB-100 is virtually the sole protein of VLDL (22).

Although the remnant concept has been applied to the metabolism of VLDL as well as chylomicrons, the receptor that mediates hepatic uptake of VLDL that have been processed by lipoprotein lipase evidently is distinct from the receptor that mediates endocytosis of chylomicron remnants into hepatocytes. In Watanabe heritable hyperlipidemic (WHHL) rabbits, which virtually lack hepatic LDL receptors (23), particles resembling VLDL remnants accumulate in the blood (as well as LDL), whereas chylomicron remnants do not (22). In these animals, hepatic uptake of labeled cholesteryl esters and proteins of chylomicrons is unimpaired (24), whereas that of apoB of VLDL is greatly reduced (25). In dogs, manipulations that substantially alter the activity of hepatic LDL receptors have essentially no effect upon the uptake of cholesteryl esters of chylomicrons (26). This observation is consistent with the unimpaired metabolism of chylomicron remnants in WHHL rabbits.

The impaired uptake of putative VLDL remnants into the liver of WHHL rabbits strongly suggests that, like LDL, VLDL remnants bind to the LDL receptor and are taken up by endocytosis and catabolized in lysosomes. In rats treated with ethinyl estradiol, whose hepatocytes express large numbers of LDL receptors, particles resembling VLDL remnants have been isolated from multivesicular bodies (27), a prelysosomal compartment of the endocytic pathway (28, 29). Multivesicular bodies in these rats are larger than normal and packed with these remnant-like particles (27), consistent with processing via the LDL receptor.

MECHANISM OF FORMATION OF LDL

The remnant concept has been applied slowly to VLDL metabolism in humans because it was thought until recently that virtually all VLDL particles are converted to LDL in normal persons (3). This conclusion was based upon kinetic analysis of the conversion of radioiodinated apoB-100 of VLDL to LDL. In the rat, it has been evident for some time that only a fraction of the B apoprotein of VLDL is converted to LDL (18). The fraction is probably quite small, although there have been few attempts to quantify the conversion by the methods that have been widely used in humans (30). The significance of a species difference between rats and humans in the extent to which VLDL are converted to LDL is clouded by the presence of a protein resembling apoB-48 in rat VLDL, but there is increasing

evidence that conversion of VLDL to LDL may also be incomplete in humans (31, 32). Humans, however, have been the focus of most research on the mechanism of LDL formation from VLDL.

After injection of radioiodinated VLDL into normolipidemic humans, a precursor-product relationship is usually observed between labeled apoB of VLDL ($d < 1.006$ g/ml) and IDL ($1.006 < d < 1.019$ g/ml), and in turn between labeled apoB of IDL and LDL ($1.006 < d < 1.063$ g/ml) (33). VLDL are not only heterogeneous in size and composition but may also contain nascent particles together with particles that have been variably lipolyzed (i.e., "remnants") (34). IDL probably contain few nascent particles and may be considered to be composed predominantly of remnants. IDL contain appreciably more triglycerides, phospholipids, and cholesterol than LDL, and they also contain proteins other than apoB-100, mainly apoE and C apoproteins (34). Therefore, conversion of IDL to LDL must be accompanied by loss of core and surface components. Loss of triglycerides could occur by exchange with cholesteryl esters in other particles (35), but this would yield particles with more cholesteryl esters than LDL. Hence, loss of triglycerides is likely to occur by further enzymatic hydrolysis. Loss of surface phospholipids may also involve hydrolysis, but each surface component other than apoB can be lost solely by transfer to other particles, as occurs during initial hydrolysis of nascent chylomicrons and VLDL (18). The heparin-releasable hepatic lipase is a likely candidate to participate in hydrolysis of ester bonds in IDL lipids. Triglycerides of small VLDL and IDL from humans are preferred substrates for hepatic lipase (36). In cynomolgus monkeys, injection of antiserum to hepatic lipase, which is thought to reside on the surface of hepatic endothelial cells (37), delays the conversion of apoB of IDL to that of LDL (38). In rats fed ordinary chow, injection of antiserum to hepatic lipase (39) and in cholesterol-fed rats, reduced hepatic lipase activity associated with hypothyroidism (40) are accompanied by increased levels of IDL. Consistent with these observations, measurements of splanchnic balance of radioiodinated IDL in humans indicate that labeled LDL is released into the hepatic vein as IDL is taken up (41). Remodeling of surface components could follow lipase action as a result of altered composition of the particle surface. An alternate possibility consistent with the data on splanchnic balance would be a diacytotic process related to uptake of IDL into endosomes where remodeling of the particle occurs, followed by "retroendocytosis" to release the modified particle as LDL (42). If such a process occurs, it is unlikely to be mediated by the LDL receptor because conversion of VLDL to LDL is enhanced rather than inhibited in WHHL rabbits (25).

Although incubation of VLDL with postheparin plasma or its component lipases under appropriate conditions can hydrolyze almost all VLDL triglycerides (35), particles with all of the properties of LDL have not been produced. In part, this may reflect the use of less than perfect populations of precursor particles, only some of which can be converted to LDL. It may also reflect the absence of certain required components in the in vitro systems.

A role for apoE in the conversion process has been suggested by the reduced concentration of LDL in familial dysbetalipoproteinemia, in which point mutations of apoE are thought to lead not only to impaired interaction with hepatic receptors but also to impaired conversion of remnant particles to LDL (43). ApoE, among other apolipoproteins, inhibits hydrolysis of emulsified triglycerides by hepatic lipase (44), and evidence has been presented that mutant apoE may inhibit the conversion of the beta-VLDL (which are thought to represent remnants of VLDL) to LDL in vitro by lipoprotein lipase or hepatic lipase (45). This could reflect a greater affinity of apoE for the surface of remnant particles and consequent impairment of lipase action. Alternatively, the defective receptor-binding properties of the mutant protein may impair interaction with a receptor in the liver that is required for the conversion process. A kindred has been reported in whom three siblings had dysbetalipoproteinemia with accumulation of particles resembling chylomicron and VLDL remnants and no detectable apoE in their plasma lipoproteins (46). Their LDL cholesterol levels were higher than those of dyslipoproteinemic patients homozygous for apoE-2, an observation consistent with either of the possibilities just described. In any event, apoE seems not to be required for LDL formation.

Two brothers have been described with severe deficiency of hepatic lipase and their hyperlipoproteinemia was characterized chiefly by remnant-like particles with densities in the range of IDL and LDL (47). When heparin was injected into them, apoE remained with the remnant-like particles, whereas in normal subjects it was transferred from VLDL to high density lipoproteins (48). These observations are consistent with a role for hepatic lipase in the conversion of VLDL to LDL, but not necessarily with a role in the hepatic uptake of VLDL remnants. It should be pointed out that uptake of chylomicron remnants[1] or VLDL remnants (49) into perfused rat livers is not impaired after hepatic lipase on cell surfaces has been washed out by infusion of heparin. Also, several mammalian species (guinea pigs, cows, rabbits) seem to have little hepatic lipase (50–53), yet they seem to process chylomicron and VLDL rem-

[1] Windler, E. E. T., and R. J. Havel. Unpublished observations.

nants effectively. LDL formation seems to be a minor pathway of VLDL metabolism in these species (25, 54).

REGULATION OF LDL FORMATION

VLDL remnants have at least two potential fates: receptor-mediated endocytosis and conversion to LDL. Current evidence suggests that both processes may occur in all mammals, albeit to different relative extents. In rabbits, the two processes seem to be competitive. Thus, in WHHL rabbits, absence of functional LDL receptors in the liver not only leads to reduced hepatic uptake of VDL remnants but seems to lead to increased formation of LDL from VLDL as well (25). Therefore, LDL formation may be an inverse function of the activity of hepatic LDL receptors. If so, the concentration of LDL should increase disproportionately as hepatic receptor activity falls because increased formation and impaired removal occur concurrently.

The differing extent to which VLDL are converted to LDL in various species may reflect the prevailing activity of LDL receptors, but at least one other factor seems to be involved. ApoB-100 of large nascent VLDL, ~300–700 Å in diameter, obtained from a patient with genetic lipoprotein lipase deficiency, was rapidly removed from blood plasma of normolipidemic recipients with little or no conversion to apoB-100 of LDL (32). Consistent with this observation, only a small fraction of the apoB of large VLDL obtained from normolipidemic subjects or patients with endogenous hyperlipemia appears in LDL (55), although low conversion could reflect in part the presence of intestinal particles containing apoB-48 in the particle population.

Two reasons can be adduced for failure of LDL formation from large VLDL. First, the remnants that are produced from large VLDL may be taken up into hepatocytes with high efficiency, perhaps as a result of the large number of molecules of apoE that the larger remnants contain (18). Such remnants could bind to multiple receptors (56) which may promote binding (57), endocytosis, or both. By contrast, smaller remnants produced from smaller VLDL (such as IDL) may contain such a limited complement of apoE (i.e., one or two molecules) that binding and endocytosis occur slowly, providing a greater opportunity for the particles to be processed to LDL (during which they lose the limited amount of apoE).

A second factor that could limit formation of LDL from large VLDL is the large number of cholesteryl ester molecules in their core, which may considerably exceed the number found in LDL (32, 35, 58). In humans, these esters are thought to be acquired from other lipoproteins, especially from the cholesteryl ester transfer complex as they are synthesized by lecithin:cholesterol acyltransferase (59). This factor may come into play particularly when residence times of the VLDL are long, as in various hypertriglyceridemic states (32, 35, 58). Even if the triglycerides of such particles are removed completely and all apoproteins other than apoB-100 leave the surface, they are likely to have densities lower than 1.019 g/ml as a result of their increased content of cholesteryl esters and of surface lipids relative to apoB-100. They could, therefore, yield functional LDL that float with IDL or even with VLDL separated in the usual way.

The extent to which VLDL are converted to LDL may therefore vary according to the size of the VLDL produced. When the rate of hepatic triglyceride synthesis is increased but that of apoB is not, LDL levels may fall as a result of more efficient remnant uptake or failure to form "LDL" particles of sufficiently high density. This phenomenon could contribute to the reciprocal relationship between VLDL-triglyceride and LDL-cholesterol levels observed in hyperlipemic humans (60). As VLDL particles in most mammals are larger than those of humans, the inverse relationship between VLDL size and LDL formation could also be related to the low fractional conversion of VLDL to LDL in these species (32). Finally, the lack of formation of LDL from chylomicrons may reflect, in part, the size and composition of their remnants rather than the apoB species that they contain.

VLDL-INDEPENDENT LDL FORMATION

Kinetic studies in patients with familial hypercholesterolemia have suggested that LDL must arise in part from sources other than VLDL. Double-label experiments suggest that synthesis of apoB of LDL exceeds that of VLDL by about twofold in homozygotes (61) and specific activity relationships of apoB of VLDL, IDL, and LDL after injection of ^{125}I-labeled VLDL suggest that most LDL in homozygotes and some LDL in heterozygotes is derived neither from VLDL nor IDL (62, 63). Homozygous WHHL rabbits also have high rates of LDL synthesis (about five times normal) (64). However, perfused livers of WHHL homozygotes secrete normal numbers of VLDL particles (as judged from rates of accumulation of apoB-100 in liver perfusates) and little or no newly synthesized apoB has been observed with a density of IDL ($1.010 < d < 1.019$ g/ml) or LDL ($1.019 < d < 1.063$ g/ml) (65). In normal rabbits only a small fraction (~10%) of apoB-100 of VLDL seems to appear in LDL (25, 66), so that a fivefold increase in conversion is quite feasible. Evidence that conversion is increased has been obtained in WHHL

homozygotes, although the magnitude of the increase has not been determined (25).

Even in normal rabbits, a precursor-product relationship between apoB of IDL derived from radioiodinated VLDL and apoB of LDL has not been observed (66). IDL are kinetically heterogeneous in rabbits and multicompartmental analysis suggests that a fraction of IDL with rapid turnover is the precursor of LDL.[2] The turnover of the slow component resembles that of LDL and a component of IDL containing no apoE has been found by an immunoabsorption technique. The extent to which kinetic heterogeneity of precursor particles can account for apparent independent secretion of LDL in humans is not clear, but IDL do seem to exhibit kinetic heterogeneity in humans with homozygous familial hypercholesterolemia (63). Human LDL are composed of subspecies that can be separated by gradient ultracentrifugation or electrophoresis (67, 68). Therefore kinetic heterogeneity of particles that contain wholly apoB-100 may also have to be taken into account in evaluating the formation of LDL.

CONCLUSIONS

LDL are formed in two steps, both of which may involve lipolytic removal of lipids and transfers of lipid and protein components to and from other lipoproteins. In humans as well as other mammals, not all VLDL are converted to LDL; a variable fraction of VLDL remnants formed in the first step of VLDL catabolism is taken up into hepatocytes via the LDL receptor. The extent of conversion is a function of several factors, which have to do with certain properties of nascent VLDL secreted from hepatocytes and the activity of hepatic LDL receptors. Apolipoprotein E is a major determinant of hepatic uptake of VLDL remnants but it may not be essential to LDL formation. The heparin-releasable hepatic lipase, on the other hand, seems to participate in LDL formation, but not in remnant uptake. Particles resembling LDL can be secreted from mammalian livers under certain circumstances, but in most cases the apoprotein complement of these particles resembles that of nascent VLDL; their higher density reflects enrichment with cholesteryl esters and their smaller size. Recent studies have raised the possibility that some VLDL and IDL may contain hepatic apoB as the sole protein component. Such particles may resemble LDL functionally and could account for some of the kinetic heterogeneity of LDL precursors. Kinetic studies that have suggested that some LDL are secreted from the liver at a density > 1.019 g/ml may have to be reevaluated in light of this heterogeneity.

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