Hepatic Catabolism of Remnant Lipoproteins: Where the Action Is

Richard J. Havel, Robert L. Hamilton

Brown and Goldstein described the classical pathway of low-density lipoprotein (LDL) catabolism in human fibroblasts, initiated by LDL-binding to the LDL receptor (LDLR) and followed by endocytosis and lysosomal catabolism of its components. The initial steps in the hepatic catabolism of chylomicron remnants and large very-low-density lipoprotein (VLDL) remnants have turned out to be more complex, involving initial binding to other cell surface molecules, including heparan sulfate proteoglycans (HSPGs), apo E, and hepatic lipase (HL), followed by transfer to endocytic receptors (LDLR and LDLR-related protein [LRP]). Apo E was first demonstrated on hepatocyte surfaces in rat liver. At the light microscopic level, the bulk of hepatic apo E was in the space of Disse. At the electron microscopic level, this apo E was found exclusively on microvilli, occasionally associated with an evident lipoprotein particle. Virtually no apo E was in the electron-lucent matrix. HL is also associated mainly with basolateral microvilli of hepatocytes in rat liver and in rabbit liver transplanted with human HL. Apo E-deficient mice are dysbetalipoproteinemic, with massive accumulation of remnants in the blood. In mice doubly deficient in apo E and HL, accumulation of lipoproteins in the blood is even greater and includes vesicular lipoproteins, suggesting further impairment of endocytosis, together with selective uptake of cholesteryl esters, presumably by scavenger receptor B1.

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The location of the primary binding sites for remnants on microvilli makes sense because these tiny finger-like projections are the first cellular structures that remnants encounter after they enter the space of Disse via the fenestrae (~100 to 200 nm diameter) of endothelial cells that line hepatic sinusoids. Wisse et al have postulated a dynamically active space of Disse undergoing forced sieving of lipoprotein particles through a process of sinusoidal "endothelial massage" by compression from bypassing blood cells. It is not widely appreciated that LDLR and LRP are also associated with the basolateral microvilli of rat hepatocytes. Thus, initial binding of remnant particles and their subsequent endocytosis involve several macromolecules located on the plasma membrane of hepatocytic microvilli (Figure). Because endocytosis presumably occurs by invagination of coated pits at the base of microvilli, remnant lipoproteins must migrate there together with these receptors along the plane of the microvillar membrane.

Both HL and apo E are thought to be bound to the surface of liver cells by HSPGs. Although heparin releases some apo E from cell surfaces in rat liver, heparin is much less effective in releasing apo E as compared with the negatively charged polyelectrolyte, suramin. Some cell surface apo E may be bound to LRP or LDLR, for which it is a high-affinity ligand. In this regard, suramin, but not heparin, can release α2-macroglobulin from its high-affinity binding to LRP. In human hepatoma cells, surface apo E is bound not only to HSPGs but also to chondroitin sulfate proteoglycans in an even larger fraction. Moreover, in these cultured cell monolayers, apo E is bound with selective uptake of cholesteryl esters, presumably by scavenger receptor B1.

The distinct effects of human apo E isoforms on lipoprotein metabolism have been the subject of elegant studies by Maeda et al at the University of North Carolina. They have generated mice that express human apo E2, apo E3, or apo E4 in a physiologically regulated manner by replacing the coding sequences of the mouse apo E2 gene with each of the 3 human alleles. Mice expressing human apo E2 are dyslipoproteinemic. When the apo E2 mice were bred with mice expressing a human LDLR minigene regulated by the endogenous mouse promoter but modified to increase mRNA stability, the resulting increased LDLR expression corrected the dysbetalipoproteinemia, as expected. Contrary to expectation, however, Knouff et al found that VLDL levels were doubled and rates of VLDL clearance were halved in mice expressing apo E4 as compared with those observed in mice expressing apo E3. Because the delayed clearance could not be explained by alterations of the VLDL particles, they postulated that it reflected intrinsic differences in the animals themselves, perhaps related to altered interactions with the "hepatic microenvironment."

In a recent report in this journal, Malloy et al have tested this hypothesis in apo E4 and apo E3 mice crossed with mice expressing the human LDL receptor minigene, as in the earlier studies with human apo E2 mice. Once again, the results were
Top left, Receptor-mediated uptake and intracellular processing of triglyceride-rich remnants and cholesteryl ester-rich LDL in rat liver (1988 version), showing passage of lipoproteins through fenestrae in the sinusoidal endothelium, followed by binding to endocytic receptors (Y) on hepatocytic microvilli projecting into the space of Disse (SD). The endocytic receptors migrate with their cargo to coated pits at the microvillar bases where they undergo endocytosis to form primary endosomes. After loss of the clathrin coat and endosome-fusion, the lipoproteins dissociate from the receptors at the acidic pH within the endosome. The lipoproteins are transported within the maturing endosomes toward the basolateral pole of the cells, forming multivesicular bodies (MVB) (late endosomes), whereas the excess surface membrane resulting from endosome-fusion forms recycling endosomes that carry the receptors back to the basolateral surface of the cell (modified from Figure 1 in Havel RJ, Hamilton RL. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. Hepatology. 1988;8:1689–1704.). Top right (2003 version), Enlargement of a basolateral microvillus and adjacent endothelial cell (E), showing that chylomicron and large VLDL remnants pass through endothelial cell-fenestrae to the space of Disse where they bind initially to proteoglycan-bound apo E and hepatic lipase as well as LDLR, all of which are anchored to the microvillar membrane. Proteoglycan-bound hepatic lipase binds and hydrolyzes remnant-lipids, increasing exposure of the endocytic receptor-binding domain of apo E. Hepatic lipase can also act as a ligand for the endocytic receptors. Additional proteoglycan-bound apo E on microvilli acquired by the remnants increases the affinity of the remnant particles for LDLR. The extent to which proteoglycans, hepatic lipase, and surface apo E themselves undergo endocytosis with the remnant particles is unknown. Normally, the surface density of LDLR greatly exceeds that of LRP, but only LDLR can readily bind remnants without further modification in the space of Disse. Bottom left, Thin section electron micrograph of normal rat liver illustrating the basolateral surface of hepatocytes (H) projecting numerous microvilli into the space of Disse. Plasma, including lipoproteins up to ~200 nm diameter in the sinusoid, exchanges freely with the space of Disse through fenestrae (arrows) in the endothelium (E). ×18 000 diameters. Bottom right, Ultracyotthin section of normal rat hepatocyte space of Disse showing microvilli decorated with 5-nm colloidal gold complexed with affinity-purified rabbit polyclonal anti-LRP from rat liver. ×90 000 diameters.
unexpected. When the animals were fed cholesterol- and fat-rich diet, the apo E4 mice, but not apo E3 mice, had pronounced hypercholesterolemia because of accumulation of cholesterol-rich, but triglyceride- and apo E-poor, remnants. These particles contained mainly apo B-48 and apo A-I, but little apo E, and their concentration decreased profoundly after a 12-hour fast, suggestive of an intestinal rather than hepatic origin. Apo E concentrations were comparably reduced in plasma but increased in liver in the crossbred apo E4 and apo E3 mice. Other data suggested that chylomicron secretion and hepatic VLDL production were unaltered in apo E4 mice. Despite the increased expression of hepatic LDLRs, the apo E4 mice did not clear radiolabeled VLDL from apo E-deficient mice faster than mice expressing apo E4 but lacking the human LDL receptor minigene, which they did clear apo E-enriched radiolabeled VLDL at a more rapid rate. Apo E is transferred from HDL to nascent triglyceride-rich lipoproteins, particularly those from the intestine, rendering the particles competent to bind to endocytic receptors. It has also been proposed that further enrichment occurs in the space of Disse through acquisition of surface apo E from hepatocytes. Malloy et al propose the novel hypothesis that apo E4 becomes “trapped” in the liver to a greater extent than apo E3 because of its increased affinity for LDLR. As a result, postprandial triglyceride-rich lipoproteins, which remain deficient in apo E, could be readily converted to remnants by lipoprotein lipase but would have low affinity for hepatic lipoprotein receptors. This is consistent with the observed accumulation of remnants deficient in triglycerides and apo E in the apo E4 mice expressing increased levels of LDLR.

How might the postulated “trapping” take place? HDL that contain apo E are taken up rapidly by the liver of rats treated with 17-α-ethinyl estradiol, which induces high expression of hepatic LDLRs. Cell surface apo E may also be endocytosed more rapidly under conditions of increased LDLR expression. Other possibilities should be considered. In rat liver, apo E is abundant in multivesicular bodies. These late endosomes are the immediate prelysosomal compartment (Figure). Furthermore, in perfused rat livers, labeled HDL particles were unaltered in apo E4 mice. Despite the increased expression of hepatic LDLRs, the apo E4 mice did not clear radiolabeled LDL from apo E-deficient mice faster than mice expressing apo E4 but lacking the human LDL receptor minigene, which they did clear apo E-enriched radiolabeled LDL at a more rapid rate. Apo E is transferred from HDL to nascent triglyceride-rich lipoproteins, particularly those from the intestine, rendering the particles competent to bind to endocytic receptors. It has also been proposed that further enrichment occurs in the space of Disse through acquisition of surface apo E from hepatocytes. Malloy et al propose the novel hypothesis that apo E4 becomes “trapped” in the liver to a greater extent than apo E3 because of its increased affinity for LDLR. As a result, postprandial triglyceride-rich lipoproteins, which remain deficient in apo E, could be readily converted to remnants by lipoprotein lipase but would have low affinity for hepatic lipoprotein receptors. This is consistent with the observed accumulation of remnants deficient in triglycerides and apo E in the apo E4 mice expressing increased levels of LDLR.

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In their article, Malloy et al cite studies of postprandial lipemia in normolipidemic humans by Bergeron et al, which indicated prolonged residence times of triglyceride-rich lipoproteins particles containing apo B-48 and apo B-100 in apo E 4/3 heterozygotes as compared with apo E3 homozygotes. Bergeron et al suggested that apo E on chylomicron remnants of persons with an apo E4/3 phenotype may be less accessible to hepatic lipoprotein receptors than apo E in those persons with an apo E3/3 phenotype. They proposed that consequent increased conversion of VLDL (containing apo B-100) to LDL might cause the increased LDL levels observed in apo E4/3 heterozygotes. As Malloy et al point out, this proposal is consistent with kinetic studies in E4/4 homozygotes indicating reduced direct removal of VLDL remnants accompanied by increased conversion of VLDL to smaller particles. In the studies of Bergeron et al, however, there was no evidence that the remnants that accumulated postprandially in apo E4/3 heterozygotes were depleted of apo Es. Accordingly, the trapping hypothesis should stimulate further studies of these interactions and the influence of apo E isoforms in remnant catabolism.

References


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In the January 2004 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, in Figure 1 of the Editorial by Dr. Heistad (*Arteriosclerosis, Thrombosis, and Vascular Biology*, 2004; pp 1–3), the impact factor is provided for T&H, but the legend should indicate correctly Thrombosis and Haemostasis.

In the February 2004 issue, in the Editorial by Havel and Hamilton (Hepatic Catabolism of Remnant Lipoproteins: Where the Action Is; pp 213–215), an oversight in the production process allowed the publishing of an incomplete figure. The complete, correct figure, as it should have published, is shown below. All online versions of this Editorial have been corrected to include the complete figure. The Publisher apologizes for the error.

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**Corrections**

Top left, Receptor-mediated uptake and intracellular processing of triglyceride-rich remnants and cholesteryl ester-rich LDL in rat liver (1988 version), showing passage of lipoproteins through fenestrae in the sinusoidal endothelium, followed by binding to endocytic receptors (Y) on hepatocytic microvilli projecting into the space of Disse (SD). The endocytic receptors migrate with their cargo to coated pits at the microvillar bases where they undergo endocytosis to form primary endosomes. After loss of the clathrin coat and endosome-fusion, the lipoproteins dissociate from the receptors at the acidic pH within the endosome. The lipoproteins are transported within the maturing endosomes toward the biliary (apical) pole of the cells, forming multivesicular bodies (MVB) (late endosomes), whereas the excess surface membrane resulting from endosome-fusion forms recycling endosomes that carry the receptors back to the basolateral surface of the cell (modified from Figure 1 in Havel RJ, Hamilton RL. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology.* 1988;8:1689–1704.).

Top right (2003 version), Enlargement of a basolateral microvillus and adjacent endothelial cell (E), showing that chylomicron and large VLDL remnants pass through endothelial cell-fenestrae to the space of Disse where they bind initially to proteoglycan-bound apo E and hepatic lipase as well as LDLR, all of which are anchored to the microvillar membrane. Proteoglycan-bound hepatic lipase binds and hydrolyzes remnant-lipids, increasing exposure of the endocytic receptor-binding domain of apo E. Hepatic lipase can also act as a ligand for the endocytic receptors. Additional proteoglycan-bound apo E on microvilli acquired by the remnants increases the affinity of the remnant particles for LRP. The extent to which proteoglycans, hepatic lipase, and surface apo E themselves undergo endocytosis with the remnant particles is unknown. Normally, the surface density of LRP greatly exceeds that of LDLR, but only LDLR can readily bind remnants without further modification in the space of Disse. Bottom left, Thin section electron micrograph of normal rat liver illustrating the basolateral surface of hepatocytes (H) projecting numerous microvilli into the space of Disse. Plasma, including lipoproteins up to ~200 nm diameter in the sinusoid, exchanges freely with the space of Disse through fenestrae (arrows) in the endothelium (E). ×18 000 diameters. Bottom right, Ultracyrothin section of normal rat hepatocyte space of Disse showing microvilli decorated with 5-nm colloidal gold complexed with affinity-purified rabbit polyclonal anti-LRP from rat liver. ×90 000 diameters.