review

# Hepatic uptake of chylomicron remnants

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**Abstract** Chylomicrons are formed in the intestine and transport dietary triglyceride to peripheral tissues and cholesterol to the liver. The enzyme lipoprotein lipase, with apolipoprotein (apo)C-II as a co-factor, hydrolyzes chylomicron triglyceride allowing the delivery of free fatty acids to muscle and adipose tissue. As a result, a new particle called a chylimicron remnant is formed. This particle is enriched in cholesteryl ester and fat-soluble vitamins and contains apoB-48 and apoE. It is rapidly removed from the circulation by the liver. ApoE is the moiety required for rapid hepatic removal. Its activity is inhibited by C apolipoproteins, especially apoC-I. Hepatic removal appears to be accomplished by several overlapping mechanisms. The particle must first achieve a size that allows it to be "sieved" through the endothelial fenestre allowing entrance into the space of Disse. Here, it may *1*) be removed directly by LDL receptors; *2*) acquire additional apoE that is secreted free into the space, and then be removed directly by the LDL receptor-related protein (LRP); or *3*) it may be sequestered in the space. Sequestration occurs by binding of apoE to heparan sulfate proteoglycans and/or binding of apoB to hepatic lipase. Sequestered particles may be further metabolized allowing apoE, and lysophospholipid enrichment, followed by transfer to one of the above receptors for hepatic uptake. The above formulation is based upon animal studies. In humans, delayed removal of chylomicron remnants has been documented in diabetes, renal failure, and familial combined hyperlipemia and is the abnormality resulting in type III hyperlipidemia. Case control studies have identified delayed remnant removal as an independent risk factor for atherosclerotic cardiovascular disease. Thus, understanding the further details of the processes, and how it can be regulated in humans, is an important challenge for the future.—**Cooper, A. D.** Hepatic uptake of chylomicron remnants. *J. Lipid Res*. 1997. **38:** 2173–2192.

**Supplementary key words** chylomicron remnants • chylomicron • LDL receptor • LDL receptor-related protein • hepatic lipase • heparan sulfate proteoglycan

Consideration of lipoprotein metabolism has often been divided into endogenous and exogenous pathways. The endogenous pathway has received the bulk of attention, primarily because of the well-documented association between the levels of two components, low density lipoprotein (LDL) and high density lipoprotein (HDL), and the occurrence of atherosclerosis in a population. In contrast, metabolism of exogenous particles is so rapid that it is difficult to appreciate or measure alterations in this pathway. However, in quantitative terms, as much lipid probably fluxes through the exogenous pathway per day as through the endogenous pathway. It has recently become appreciated that the two pathways are not as distinct as previously thought. Thus, affecting one certainly influences the other. Moreover, evidence has accumulated suggesting that the rate of clearance of diet-derived lipids may be an independent risk factor for atherosclerosis. Accordingly it is timely to re-examine the details of the metabolism of dietary lipids.

Metabolism of lipid in the diet involves a large number of steps. More complex lipids are degraded into their constitutive molecules in the lumen of the intestine. These are absorbed by the enterocyte where they undergo resynthesis into complex lipids, and are ultimately assembled into chylomicrons. The chylomicrons are secreted into the lymph. Once in the general circulation they undergo hydrolysis of the triglyceride by lipoprotein lipase, with the formation of chylomicron remnants. Finally, the remnants are removed from the circulation. This review will focus on the last step in this pathway, the removal of remnant particles from the circulation by the liver.

As will emerge, this has been a confusing and sometimes contentious area of research that has been discussed in several previous review (1–3). As the result of considerable effort in a number of laboratories using modern molecular techniques, as well as traditional biochemistry and physiology, an increasingly clear understanding of the mechanisms involved has evolved. The current formulation suggests that there are several redundant systems capable of removing chylomicron remnants with varying degrees of efficiency. Even in the presence of genetic and metabolic abnormalities that

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; VLDL, very low density lipoprotein; LRP, LDL receptor-related protein; RAP, receptor-associated protein; FFA, free fatty acid.

cause profound changes in other aspects of lipoprotein and lipid metabolism, the function of remnant removal is reasonably well preserved. This suggests that from an evolutionary point of view this is an important process and that accumulation of these particles for prolonged periods or in high concentrations may have particularly deleterious consequences. There is, in fact, accumulating evidence for this notion that will be discussed below.

For the sake of clarity the review will not attempt to be completely inclusive but will try to summarize substantial amounts of information concisely. I will, however, try to do justice to the many individuals who have made contributions to the large body of literature on the subject and apologize in advance for any omissions or inaccuracies relating to the work of my colleagues in this field.

## BACKGROUND

The first evidence for a selective pathway for the hepatic removal of diet-derived cholesterol was reported by the author's mentor, the late R. Gordon Gould and his colleagues (4). These investigators made the observation that cholesterol feeding rapidly reduced the rate of cholesterol synthesis in the liver, but not in other tissues. Subsequent studies by the late De Witt Goodman (5), and Stein et al. (6), demonstrated the rapid uptake and metabolic effects of cholesterol from chylomicrons by the liver. At about the same time a key role for lipoprotein lipase, an enzyme shown to be present in adipose tissue and muscle, as the "clearing factor" for dietary triglyceride was described (7). Separate tissue sites for the removal of dietary triglyceride and cholesterol were confirmed in elegant experiments by Bergman et al. (8) that demonstrated the hepatic removal of cholesterol and the removal of triglyceride by tissues outside the splanchnic circulation. The two-step model for chylomicron metabolism was directly established by Trevor Redgrave (9) who demonstrated the accumulation of cholesteryl ester-rich particles in the blood of functionally hepatectomized rats and coined the term chylomicron remnants for these particles. Their composition was characterized by investigators in several laboratories. The essential changes in chylomicrons include, in addition to the depletion of up to 85% of the triacylglycerol, loss of apolipoprotein (apo) A-I and C apolipoproteins and the acquisition of apoE, as well as the inconsistent presence of several other poorly characterized proteins (9, 10). The other important finding was that rather than the full-length form of apoB (apoB-100), apoB-48 is the sole form of apoB present (11).

Somewhat later, the rapid specific and saturable uptake of chylomicron remnants, but not chylomicrons, by the liver was characterized (12–16). The essential role of apoE in their removal by the liver was also shown in a number of laboratories, in several different systems (17–19). It has also become established that C apolipoproteins can inhibit the apoE effects (19, 20). The mechanism for this last effect is not fully established but it appears that all of the C apoproteins can do this and may act in part by displacing the apoE from the particle.

With this information in hand by the early 1980s, it seemed that the mechanism of remnant removal would be solved rapidly. Using the LDL receptor pathway that had recently been described in the Nobel prize-winning work of Brown and Goldstein (21) as the model for lipoprotein removal, numerous laboratories set out to purify a remnant or apoE receptor. This proved to be difficult and the remnant removal pathway has proven to be more complex than anticipated.

Recently, I proposed a model (22) whereby a series of interacting and independent and overlapping pathways can account for the generally rapid removal of the particle despite abnormalities in any of the single components. We will discuss, in turn, the receptors, receptor candidates, and the ancillary molecules that have been identified as having roles or potential roles in the hepatic removal process and, in the final sections, try to integrate these into a coherent model as well as discuss the impact of abnormalities on the processes in animal models and in humans.

A brief consideration should be given to the nature of the particles used in studies of remnant metabolism. If chylomicrons are used as the precursor, they can be converted into remnants either in vitro, by incubation with post-heparin plasma (12) or by perfusion through an isolated heart (23), or in vivo, by being allowed to re-circulate in a functionally hepatectomized animal (9). In all instances the nature of the particle produced can be quite variable depending on the amount of starting material, the amount of albumin present, and the amount of other serum lipoproteins available for exchange. If, for example, there is inadequate albumin, the particles formed have a large amount of free fatty acid and an unusual morphology. An alternative approach has been to use model particles. β-Migrating very low density lipoprotein (VLDL) is an apoE-rich lipoprotein species that accumulates in the blood of many species upon administration of a cholesterol- and fat-rich diet. This fraction, at least in the dog, is composed of particles derived from both the liver and the intestine (24). In most studies the particles have behaved in a manner similar to chylomicron remnants prepared by other methods. Redgrave and his colleagues (25–28) have characterized the behavior of a series of triglyceride and cholesterol emulsions. Emul-

sions that contained between 16 and 22% cholesterol behaved quite similarly to chylomicron remnants in in vivo studies. These particles are useful only for in vivo studies as they lack apolipoproteins, which they presumably acquire in the circulation. Borensztajn and his colleagues (29–35) have demonstrated that chylomicrons rendered protein-free by trypsin digestion behave like remnants after they have been treated with phospholipase. However, it remains unclear whether these particles require further modification after reaching the circulation or the liver. Thus, the nature of the true remnant particle that is formed and removed in vivo is not known and it is likely that this will always be a question that has a somewhat speculative answer. Accordingly, studies with remnants must be evaluated in the context of the particles chosen for study.

Similarly, the nature of the particle that accumulates in humans has not been described definitively. In fact, it is likely that there is a spectrum of particles formed with varying triglyceride to cholesterol and apoB-48 to apoE to apoC ratios. The predominant species would most likely depend on the rate of lipolysis, the efficiency of removal, and the amount of cholesteryl ester transfer from HDL. At one extreme are the cholesterol-rich, triglyceride-poor β-migrating VLDL-size particles of type III hyperlipidemia. At the other extreme, the particle may be so efficiently removed that it is very difficult to isolate and characterize. Hopefully, a comprehensive examination of the particles in humans will be undertaken.

## LIGANDS AND COMPETITORS

### ApoE

The role of apoE, as a determinant on the lipoprotein for its rapid removal by the liver, was established in a series of liver perfusion experiments by Sherrill, Innerarity, and Mahley (17), and later by Shelbourne et al. (19), and Windler, Chao, and Havel (20). Our laboratory (18) demonstrated that apoE was the determinant for binding of chylomicron remnants to liver membranes. It was later discovered (36) that of the three common isoforms of apoE, apoE-III and apoE-IV bound to the LDL receptor and that apoE-II did so with reduced affinity. This was consistent with the discovery that patients with type III hyperlipidemia were almost all of the apoE2,2 genotype (37). Similarly, patients who lack apoE accumulate remnants (38). Surprisingly, not all apoE2,2 patients have lipid abnormalities and, in fact, on average they have lower serum cholesterol levels than individuals with the other apoE isoforms (39). In contrast, patients with abnormality of apoE caused by a series of other mutations, including $Arg_{142}$—Cys, $Arg_{145}$—Cys, $Lys_{146}$—Gln, Glu and apoE-Leiden, a duplication of residues 121–127, all have type III hyperlipidemia (40). Further, these tend to be expressed as a dominant disorder, despite the fact that the apolipoproteins have some residual ability to bind to the LDL receptor and the LDL receptor-related protein (LRP), two candidates for a remnant receptor. Mahley (40) has proposed that this is due to the fact that apoE-II is able to bind normally to heparan sulfate proteoglycans while the other mutated forms do not. This might allow sequestration of the apoE-II particles in the liver despite their lack of binding to the receptors. Woollett et al. (41) have provided evidence that supports the hypothesis that the lower LDL levels in the apoE-II individuals are due to the fact that their remnants do not compete with LDL for the LDL receptor, thus allowing more efficient clearance of LDL. These results are consistent with the existence of more than one pathway for remnant removal, as will be discussed below. They suggest that as long as one of the pathways is functional, remnants will not accumulate, unless the system is stressed by another metabolic condition as presumably occurs in the apoE2,2 homozygotes that express type III hyperlipidemia.

### ApoCs

During the conversion of chylomicrons to chylomicrons remnants the most obvious change in protein composition is the acquisition of apoE. There is also, however, loss of apoA-I and of the C apolipoproteins. The loss of apoC-II is generally believed to account for the termination of lipolysis by lipoprotein lipase, which requires this as a co-factor. In their initial liver perfusion studies both Shelbourne et al. (19) and Windler et al. (20) suggested that the C apolipoproteins might play a direct inhibitory role because the ratio of apoE to apoC s seemed to correlate better with the uptake of the lipoprotein than did the absolute apoE content. Similarly, studies of LRP-mediated uptake of β-VLDL, by Weisgraber et al. (42), suggested that apoC-I per se might be an inhibitor of binding to the receptor. A direct role for the C apolipoproteins has been difficult to document because in all studies they displace apoE from the particle and this could account for their inhibitory role. Recent results from studies of transgenic mice that overexpress the apoCs have provided some elucidation for this. Overexpression of any of the apoCs does lead to triglyceride accumulation. For apoC-III this may be due largely to the inhibition of lipoprotein lipase (43). However, the hypertriglyceridemia that occurs in mice that overexpress apoC-II was unexpected as LPL activity should have been enhanced (44). There

was in these animals, in fact, some displacement of apoE from the triglyceride-rich lipoproteins and evidence of delayed remnant removal. The mice overexpressing apoC-I had only a modest hypertriglyceridemia, but they had a more marked increase in cholesterol levels than the transgenic mice overexpressing apoC-II or apoC-III (45). These animals also had a clear delay in remnant removal. Together, these results demonstrate a complex role of the C apolipoproteins in remnant formation and removal and suggest a special role for apoC-I in directly delaying remnant removal. Such a mechanism might be important in assuring that chylomicrons have adequate time in the circulation to deliver triglyceride to peripheral tissues before hepatic uptake can begin.

### Phospholipolysis

Borensztajn, Kotlar, and McNeill (29, 30) initially noted that chylomicrons treated with phospholipase $A_2$ were taken up rapidly by the liver while intact chylomicrons were not. In reconstitution experiments they were able to demonstrate that the lysophospholipid fraction that could be generated by treatment of chylomicrons with hepatic lipase was required for particle uptake by the liver (33). Further, hepatic lipase treatment of chylomicrons caused more rapid hepatic uptake than did lipoprotein lipase treatment, even though the particles had the same content of apolipoprotein (46). They have argued on the basis of these and several other sets of experiments that the hepatic uptake of chylomicron remnants may be independent of the apolipoprotein composition and content, and cite the fact that dietary lipid is removed with reasonable efficiency even in mice that lack apoE (47). Together their data certainly provide evidence that generation of lysophospholipid may be an important component of the changes that culminate in the transformation of a chylomicron into a chylomicron remnant. We have noted that remnants prepared in hepatectomized rats are, in fact, rich in lysolecithin (L. G. Fong and A. D. Cooper, unpublished results). However, in the absence of apolipoproteins and apolipoprotein secretion by the liver, remnants that have lost all of their apolipoproteins are not removed by the liver (48).

## SITES OF UPTAKE

The original report of Gould et al. (4) stressed the finding that, in the dog, dietary cholesterol affected cholesterol synthesis only in the liver. Quarfordt and Goodman (49) reported that, in the rat, about 80% of chylomicron cholesteryl ester appeared rapidly in the liver with most of the rest remaining in the plasma. No other tissue accumulated an appreciable amount of radioactivity. However, in a recently reported study in the rabbit, Hussain et al. (50) found significant extrahepatic uptake of radioactivity from β-VLDL. Much of this was, interestingly, in the bone marrow. In our studies in mice (51), the spleen generally demonstrates a high specific activity for uptake, although because of its size its total uptake is not high. Given the presence of the LDL receptor and LRP, as well as the ability of macrophages to secrete lipoprotein lipase and apoE, it is not surprising that these cells are able to accumulate chylomicron remnants. The implications of this in foam cell formation is an interesting question that is currently being studied (52).

## RECEPTORS

### LDL receptor

The study of remnants has been profoundly influenced by the work of Brown and Goldstein (21) and their elucidation of the LDL receptor pathway. The initial characterization of the LDL receptor was carried out with fibroblasts (53). This led to the postulate that the clearance of LDL took place largely in non-hepatic tissue and that the liver had another receptor system for the removal of remnant lipoproteins. This was further strengthened by the finding that LDL could not compete for chylomicron remnant binding to cells or to liver membrane preparations (54). Moreover, the ligand binding region of apoB was not present on apoB-48, the form of apoB synthesized by the intestine. These facts, together with the absence of overt accumulation of chylomicron remnants in the plasma of patients with familial hypercholesterolemia (55), supported the hypothesis that the LDL receptor did not play a role in chylomicron remnant removal.

Evidence leading to revision of this formulation evolved rapidly. First, it was established from studies of bovine (56) and rat liver membranes (57) that LDL receptors were indeed present in the liver. Moreover, they could be induced to high levels in the rat liver by the synthetic estrogen analog ethinyl estradiol (57, 58). Eventually it was established that the liver was the principle site of LDL degradation (59, 60). Perhaps the seminal observation that potentially linked the LDL receptor to remnant binding and ultimate removal was that the LDL receptor actually has a far higher affinity for particles containing several molecules of apoE than for a single molecule of apoB (61). By this time a number of liver cell receptors for lipoproteins had been described based on cell (62) and membrane binding stud-

ies (63), as well as protein purification (64). The criteria used to define these as remnant binding sites independent of the LDL receptor included lack of competition with LDL and the lack of a requirement for $Ca^{2+}$, which was generally assessed by the ability to bind remnants in the presence of EDTA. The absence of LDL receptors in the liver of adult animals, or at least dogs and rats, was also reported (65).

In contrast, early cell culture studies carried out by Floren and colleagues (66) demonstrated that fibroblasts could bind and degrade chylomicron remnants, presumably by the LDL receptor. Studies of monocytes (67) and macrophages (68) also demonstrated that most of the binding uptake and degradation of chylomicron remnants by these primary cells, as well as derived cells with the macrophage phenotype, utilized the LDL receptor. Other cell culture studies (69–71) demonstrated LDL receptors in liver-derived cells from several sources and noted that it was more difficult to induce down-regulation of the LDL receptor in the liver-derived cell types than in most non-hepatic-derived cells.

In order to elucidate the nature of the various receptors and their relation to the LDL receptor, we prepared polyclonal antibodies to the LDL receptor of the rat liver (72). The antibodies have a very high affinity for the LDL receptor and are monospecific. Fortunately, they cross-react with the LDL receptor from all of the species, except rabbits, tested thus far. They compete for the binding of both apoB and apoE to the LDL receptor with about the same affinity as LDL and do not cross-react with the LRP or VLDL receptor. Using these antibodies (72) it was observed that in cultured rat liver cells, isolated by collagenase digestion and centrifugal elutriation, virtually all of the specific binding and almost all of the specific degradation could be inhibited by the anti-LDL receptor antibodies and was thus, presumably, due to the LDL receptor. In contrast, binding to cell membranes prepared from these cells, as well as cell membranes from the intact liver or purified plasma membranes prepared from intact liver, had more than one binding site. Depending upon the metabolic state of the animal, between 20 and 50% of the binding of chylomicron remnants was likely to be due to the LDL receptor. This binding could be blocked by either the anti-LDL receptor antibodies or EDTA. The same amount of residual binding could not be inhibited by either. Thus, liver membranes have apoE binding sites that are not the LDL receptor. It is likely that in the absence of the LDL receptor at least some of these molecules can play a role in remnant removal. One membrane apoE binding protein was identified as the mitochondrial $F_1$ ATPase (64). Another membrane binding protein has been identified as the fatty acid-induced LDL binding protein (Bihain and colleagues, see below). It is also likely that some of the membrane binding observed is due to both the LDL receptor-related protein (LRP) and to hepatic lipase, although this has not been established experimentally. The roles of these other proteins will be addressed below.

Immunohistochemical (73) studies of rat liver demonstrated the presence of LDL receptors, albeit at low abundance in the liver of normal rats, and immunologic studies demonstrated the presence of LDL receptors even in the liver of cholesterol-fed adult dogs (74, 75). Together, the above could be construed as suggesting that the LDL receptor is always present in the liver of normal animals and that it is capable of binding and initiating the cellular uptake of chylomicron remnants. The next task was to estimate what role if any the receptor plays in vivo.

Studies were carried out in rabbits, taking advantage of the naturally occurring genetic abnormality in the LDL receptor in the Watanabe heritable hyperlipidemic rabbit. These animals have marked elevations in the serum levels of both cholesterol and triglyceride. The initial study of remnant removal in these animals did not reveal a defect in the rate of removal of a trace of remnant lipoproteins (76). However, several subsequent studies using different types of remnant preparations (77) and different protocols (78, 79) have reported delayed removal of remnants in these animals.

Recently much in vivo experimentation has been done in mice. This is, in part, because of the availability of genetically altered animals and, in part, because the size of the animal allows an investigator to obtain adequate quantities of potential inhibitors to achieve significant blood levels. Studies from our laboratory have utilized a protocol in which a potential inhibitor, such as anti-LDL receptor antibodies, is injected into mice followed at an appropriate interval by injection of radiolabeled lipoproteins. The rate of disappearance of radioactivity from the blood is then determined and hepatic uptake is measured at the end of the experiment. Because of the rapid removal of some of the lipoproteins tested, a number of assumptions have to be made for kinetic analysis. These are discussed in detail elsewhere (80) and generally lead to conservative estimates. The antibody decreases the rate of removal of LDL to the same extent as methylation of LDL does, suggesting that the antibody almost completely inhibits the LDL receptor for LDL in vivo. Because of the higher affinity of apoE for the receptor, it may not be as effective in inhibition of the removal of apoE-containing lipoproteins. Treatment of mice with the antibody increased the half-life of remnant removal from 1 to 2 min. There was a concomitant reduction of the hepatic accumula-

tion of remnants by 50% in the first 5 min after injection. These results suggest that the LDL receptor plays a major role in the initial removal of remnants from the circulation of mice but that in its absence there can be a continuous, fairly rapid removal of the particles (80, 81).

Studies in a variety of transgenic and knockout mice generally support a role for the LDL receptor in remnant removal. Although LDL receptor knockout mice do not have high levels of triglyceride on a low-fat diet, they are highly sensitive to fat feeding and develop hypertriglyceridemia on such a diet (82, 83). Blocking the LRP, the other member of the LDL receptor family that is a prime candidate for a remnant receptor, by adenovirus-mediated transfer of the LRP inhibitor, receptor-associated protein (RAP), does not result in hyperlipemia unless the LDL receptor is absent (84). Animals that have low levels of LRP because of the knockout of RAP, which has a chaperonin function, also do not have hyperlipemia unless LDL receptors are absent (85). Although the disappearance from the circulation of a trace of injected remnants in LDL receptor knockout mice is normal, the particles seemed to accumulate in the sinusoidal spaces rather than enter the hepatocyte (86, 87), as they do in the normal animal. Consistent with this is a recent report (88) and that documents that the serum concentration of retinyl esters is substantially elevated and their disappearance from the circulation considerably delayed after the feeding of labeled vitamin A to LDL receptor knockout mice. Thus, when the quantity of particles to be removed exceeds the ability of receptors to internalize them, they may become trapped in the space of Disse in the liver, the so called "sequestration" or "capture" space. When the quantity of particles that has been removed also exceeds the capacity of this space and the ability of the LRP to clear them from this space, the LDL receptor defect becomes manifest as a delay in their removal.

Taken together these reports all suggest an important role for the LDL receptor in chylomicron remnant removal in at least two animal species, the mouse and the rabbit. They also clearly demonstrate that this is not the exclusive mechanism for remnant removal by the liver and that other molecules can compensate very well, if not completely, for the LDL receptor if it is absent. The very high affinity of the particles for the LDL receptor suggest that even if this is not the principle pathway of remnant removal in humans, enhancing its level could help correct defects in remnant removal. At least one study (89) is in accord with this concept, which is certainly worthy of further testing in the future.

### LDL receptor-related protein (LRP)

A major advance in our understanding of the receptors involved came as the result of elegant cloning experiments done by Herz et al. (90). They constructed a probe from the cysteine-rich regions of the LDL receptor, a region with a high degree of homology to the terminal portion of complement that was located in the ligand binding region of the receptor. Upon screening a liver cDNA library, they identified a cDNA that encoded a large protein (600 kD) that had numerous regions of homology to the LDL receptor. Interestingly, while the LDL receptor's ligand binding domain is composed of seven imperfect repeats of the cysteine-rich complement homology domains, the LRP has five arrays of varying numbers of the repeats. Further areas of homology between the two proteins were found in the epidermal growth factor precursor homologous regions, of which three repeats are found in the LDL receptor while the LRP has 17 of these repeats. The LRP is probably the progenitor molecule of the LDL receptor family as it is found in species as primitive as *C. elegans*. The protein was isolated and purified independently from placenta by others (91), looking for the receptor for activated $\alpha_2$ macroglobulin. It was subsequently found that the receptor also binds vitellogenin (92), *Pseudonomas* exotoxin A (93), and fibrinolytic-anti-fibrinolytic complexes (94), among other substances.

A number of lines of research established a potential role for the protein in lipoprotein and specifically remnant removal. Ligand blotting experiments demonstrated that both apoE- (95) and apoB- (96) containing lipoproteins bound to a protein of the appropriate molecular weight when it had been transferred to nitrocellulose paper. It was, however, difficult to document a direct role of the LRP in the hepatic uptake of chylomicron remnants. In cell culture experiments, using LDL receptor-deficient cells, no remnant binding could be measured (A. D. Cooper, unpublished data). It was then discovered (97) that the presence of additional apoE in the medium resulted in demonstrable metabolic effects of β-VLDL in LDL receptor-deficient fibroblasts. These effects could be prevented by an anti-LRP antibody that blocked binding on ligand blots and by chloroquine, a drug that blocks lysosomal degradation of ligands that have been taken up by the endocytic pathway.

As the LRP was further studied and other competitors became available the picture was clarified somewhat. In vivo, activated $\alpha_2$ macroglobulin was found to slightly slow and reduce the hepatic uptake of β-VLDL (98) and of chylomicron remnants (81) when injected into mice, simultaneously with labeled lipoproteins. When injected into rats, however, there was no effect of $\alpha_2$ macroglobulin on remnant removal (99).

There is some disagreement regarding the degree to which remnants and $\alpha_2$ macroglobulin share the same

binding sites (100). The LRP co-purifies and is associated, intracellularly, with a 39 kD protein called the receptor-associated protein (RAP) (101). This protein appears to have a chaperonin function and is necessary for the proper intracellular processing and membrane insertion of the LRP (85). The RAP was found to block all of the binding sites on the LRP (100). However, this protein also inhibits the binding of ligands to the VLDL (102) and LDL receptor (103), albeit with a lower affinity, particularly for the latter.

The most definitive evidence that LRP can play a role in remnant removal comes from the elegant studies of Ishibashi et al. (83) and Willnow and colleagues (84, 85). They demonstrated that infection of mice that lack LDL receptors with an adenovirus that expressed RAP resulted in accumulation of remnant-like lipoproteins. Similarly, mice that are engineered to lack RAP have reduced levels of LRP and, when they are crossed with LDL receptor-deficient mice, the progeny have high levels of remnant-like lipoproteins. These studies provide strong support for the two-receptor hypothesis of remnant removal.

A recent study by Jong et al. (104) confirmed the importance of the LRP for remnant removal when the LDL receptor is absent and apoC-I is overexpressed. Interestingly, the presence of RAP in apoC-I overexpressing mice had little effect when the LDL receptors were present, suggesting that the apoC-I effect may be primarily on binding to the LRP.

These results, together with our more recent inhibitor studies, have allowed us to provide some quantitative estimates regarding the relative contributions of the two receptors to the removal process in the mouse (51) (**Fig. 1**). The extremes are based upon the assumption that the anti-LDL receptor antibody blocks all of LDL receptor-mediated remnant removal (low LDL receptor contribution) or that $\alpha_2$ macroglobulin blocks all of LRP-mediated removal (high LDL receptor contribution). To the extent that these assumptions are incorrect, as they most likely are, and that some of the RAP effects are due to blocking both receptors, the actual values lie in between. The precise estimate is less important than the concept that most of the rapid removal of chylomicron remnants is due to a combination of the two receptors. If the LDL receptor has the highest affinity and if the LRP is dependent upon the secretion of additional apoE, then, in principle, the rate of remnant removal could be influenced by a variety of metabolic factors. For example, cholesterol feeding would be expected to decrease the number of LDL receptors while increasing the amount of apoE being secreted. The net effect could be to slow remnant removal or it could be neutral, as has been observed in the rat (105). How this applies to humans remains to be elucidated.

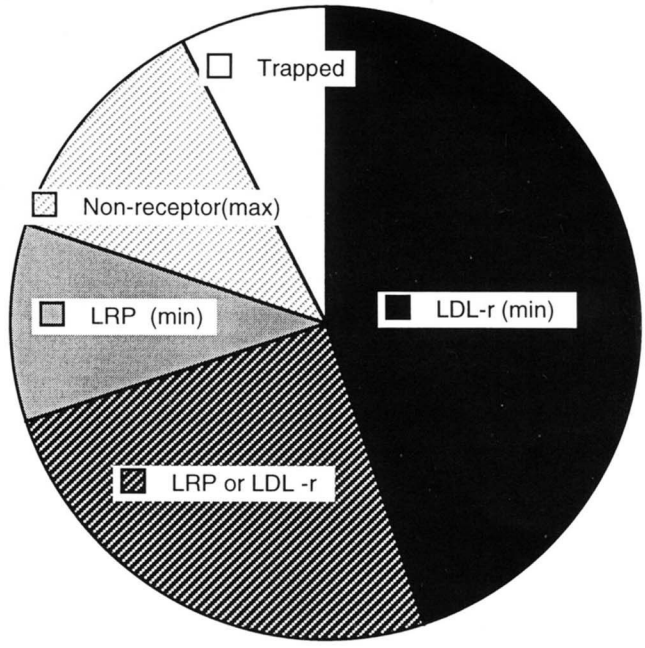


**Fig. 1.** Estimates of the contribution of receptors to chylomicron remnant removal in the mouse. The data used to prepare these estimates are drawn from refs. 51, 80, 81. They represent the amount of remnant uptake by the liver dependent upon the various receptors, 5 min after injection. LDL-r (min) is the amount dependent upon the LDL receptor, assuming that the anti-LDL receptor antibody completely eliminates LDL receptor-dependent uptake. LRP (min) represents the amount dependent upon the LRP assuming that activated $\alpha_2$-macroglobulin completely eliminates LRP-mediated uptake. LRP or LDL-r is the additional uptake inhibited by RAP. This could be due to either receptor. Trapped is the portion of total uptake that is accounted for by the plasma space as estimated by $^{125}$I-labeled albumin. Non-receptor (max) is the amount not accounted for by the other measurements. It could represent sequestered particles.

## CANDIDATE RECEPTORS

### Asialoglycoprotein receptor

The asialoglycoprotein receptor is a hepatocyte-specific molecule that has served as a paradigm for studies of receptor-mediated endocytosis (106). Its normal ligand is not fully established. Because of its organ specificity, its high affinity, and high capacity for the removal of proteins lacking a terminal sialic acid, it was a logical candidate for a remnant receptor. It was, in fact, shown to remove lipoproteins carrying a ligand for the receptor (107) but it did not cause enhanced catabolism of desialated LDL (108). Moreover, in both membrane binding studies and perfused liver studies from our laboratory (109) there was no competition between chylomicron remnants and asialoglycoproteins. A report by Windler et al. (110) contradicted this, but the conditions of their experiments in terms of the concentrations used raised questions regarding the physiologic relevance of their conclusions. In a subsequent report

they suggested that this was a phenomenon seen only in the rat.

### VLDL receptor

The report of another member of the LDL receptor family that binds normal VLDL raises an interesting possibility of a role for this protein in remnant metabolism (111). The protein can lead to the endocytosis of lipoproteins and can bind chylomicron remnants in the presence of additional aopE or lipoprotein lipase (112). Its localization to the heart, skeletal muscle, and adipose tissue, however, precludes a role in hepatic uptake; the receptor could contribute to the non-hepatic uptake of remnants.

### Fatty acid-induced LDL binding protein

Bihain and Yen (113) have characterized a candidate for another chylomicron remnant receptor, the lipolysis-stimulated receptor (LSR). This membrane protein is activated by free fatty acids (FFA) (113), and is distinct from the LDL receptor and the LRP (113, 114). Like the LDL receptor, the LSR binds both apoB and apoE (113, 114). The LDL receptor has affinity which is greater for triglyceride-rich lipoproteins while the converse appears to be true for the LSR (113, 114). The affinity of the LSR for VLDL isolated from subjects with type III hyperlipidemia (apoE2/2 phenotype) is decreased when compared to that isolated from normal individuals (114). The physiological role of the LSR in chylomicron remnant removal has remained speculative. During in vitro assays, maximal activation of the LSR requires the addition of FFA at concentrations that exceed albumin binding capacity and therefore not normally observed under physiological conditions. FFA bound to albumin at the physiological molar ratio of 1 to 1 are sufficient to consistently activate the LSR (113) to only 10% of the maximal activity. Thus, a relatively high concentration of FFA would have to be produced in the liver sinusoid when intestinally derived lipoproteins are released after a meal. Consistent with this, the apparent level of LSR expression in rat liver plasma membranes correlates negatively with plasma triacylglycerol concentrations measured in the physiological postprandial state (115). LSR is inhibited by lactoferrin (114, 115) and the 39-kDa receptor-associated protein (116), both at concentrations reported to inhibit chylomicron remnant clearance in vivo (84, 117). Definitive validation of its role in remnant removal awaits molecular cloning of the LSR gene(s).

### Lactoferrin-inhibited receptor

van Berkel and colleagues (118–122) continue to argue for the existence of another distinct receptor system that mediates the uptake of chylomicron remnants. Their argument is based upon the very low concentration of LDL receptors in the adult rat liver, the lack of competition of $\alpha_2$-macroglobulin with β-VLDL both in vitro and in vivo in the rat, and the profound effect of lactoferrin upon remnant removal compared to a lack of effect of the compound upon the removal of other ligands for the other known receptors.

## OTHER MOLECULES

### Hepatic lipase

It has been suspected for some time that this enzyme has a role in remnant metabolism. This is based both on its tissue localization and the finding that patients with a congenital lack of this enzyme, among several other lipoprotein abnormalities, accumulate remnant-like particles in their blood (123). Borensztajn and his colleagues, (30, 31, 33–35, 46, 124) moreover, have presented a substantial amount of data that suggest that phospholipolysis is an essential step in remnant formation and may be sufficient to render a lipoprotein susceptible to removal by the liver (see above). Hepatic lipase, unlike the related lipoprotein lipase, has considerable phospholipase activity. Finally, Sultan et al. (125) reported that injection of anti-hepatic lipase antibodies resulted in the accumulation of remnant-like lipoproteins in rats.

Cell culture studies have provided somewhat contradictory results regarding the effect of hepatic lipase on remnant uptake by cells. Ji and colleagues (126) and Lauer et al. (127) reported that when the hepatoma-derived cell line, McA-RH7777 was transfected with the cDNA for hepatic lipase, the binding and uptake of β-VLDL was increased. They attributed this to enhanced transport by the LRP pathway. Similarly, Diard et al. (128) observed that active and heat-inactivated hepatic lipase accelerated the uptake of chylomicron remnants by cultured hepatocytes and their binding to liver membranes.

In contrast, studies by our group (129) using CHO cells transfected with the cDNA for rat hepatic lipase failed to induce an increase in the binding or uptake of chylomicron remnants, although the uptake of LDL by the LDL receptor was enhanced. As the expressed hepatic lipase was secreted and the extent of its binding to the surface of the CHO cells we used initially was not known, a CHO cell line was created that expressed a surface-bound form of hepatic lipase by creating a chimera of hepatic lipase and the signal for the glycophosphoinositol anchor of decay accelerating factor. Again,

there was enhancement of LDL but not chylomicron remnant uptake (130). These results suggest that the presence of hepatic lipase alone on the cell surface may not be sufficient to enhance the binding and uptake of chylomicron remnants and that liver-derived cells may have other molecules that act in concert with the lipase. This is consistent with recent results of Ji et al. (131–133) regarding the need for glycosaminoglycans for this effect.

Havel and his colleagues (134) reported that, in perfused rat liver, anti-hepatic lipase antibodies caused a decrease in remnant removal. Their further studies suggest that this is due to decreased binding of the particles to the cell surface and that there is actually an increase in endocytosis of the particles when hepatic lipase is blocked. This would be consistent with the hypothesis that hepatic lipase plays a role in the sieving or sequestration of the particles in the space of Disse.

The mouse has much less hepatic lipase than most other mammals and the hepatic lipase it produces is only poorly anchored to the cell surface in the liver. Thus perhaps it was not surprising that the hepatic lipase knockout mouse did not have abnormalities in remnant removal (135). Despite this, in our experiments (51) with mice injected with anti-hepatic lipase antibody, there was a modest delay in remnant clearance from the blood and a decrease in hepatic uptake. This effect was not additive to that of anti-LDL receptor antibodies, suggesting it affected LDL receptor-mediated uptake. The antibody delayed uptake even though the lipolytic activity of the enzyme was not inhibited. Together these results suggest that even the modest amount of hepatic lipase present in the mouse liver may serve as a binding site, in conjunction with other molecules, to temporarily sequester the particles. A mechanism for this has been provided by Choi et al. (136) who, in a preliminary report, demonstrated the presence on apoB of binding sites for hepatic lipase. The affinity of these sites is low, so that alone they may not facilitate much binding, but they could enhance the affinity of a particle for the cell surface by allowing binding to several sites. When there is simultaneous binding to several sites, so-called multifooted binding, the affinity increases by the product, not the sum, of the individual binding reactions. This is clearly seen with apoE where one molecule has the same affinity for the LDL receptor as apoB but the affinity of particles bearing increasing numbers rises progressively (36). Similarly, it has been long established that the binding of two Fab′ fragments of an immunoglobulin is the square of that of the single $F(ab')_2$. Thus, multifooted binding of discrete components of a lipoprotein to two or three low affinity sites could result in high affinity binding of the whole particle.

### Lipoprotein lipase

Shortly after the description of chylomicron remnants, the late Jim Felts and his colleagues suggested (12) that lipoprotein lipase might be acquired by the particles during their formation and serve as the moiety that initiated hepatic removal of the particle. Little further support for this proposal was produced until it was noted by Beisiegel, Weber, and Bengtsson-Olivecrona (137) that lipoprotein lipase enhanced chylomicron binding to the LRP. Indeed lipoprotein lipase can facilitate the uptake of lipoproteins by the LRP (138, 139) and the LRP may play a role in the catabolism of this protein (140). There is little evidence to suggest, however, that remnants ever have a sufficient quantity of lipoprotein lipase to initiate their removal using this as the ligand, in vivo. However, a recent report suggests that in post-heparin plasma lipoprotein lipase dimers are associated with triglyceride-rich lipoproteins (141).

### Heparan sulfate proteoglycans

Based upon considerable original work (40, 79, 131–133, 142) Mahley and his colleagues have made a compelling case for a role for heparan sulfate proteoglycans (HSPGs) in remnant removal. Specifically it has been suggested that the interaction between apoE and HSPGs is required for the uptake of remnants by the LRP pathway. These investigators have observed that when β-VLDL are incubated with cells in the presence of additional apoE, there is a marked increase in the uptake of the lipoprotein. This can be abolished by removing the HSPGs from the surface of the cell with heparinase and is not observed in mutant CHO cells that lack HSPGs. The addition of mutant apoEs that do not bind to HSPGs do not produce this phenomenon. In vivo injection of heparinase into the portal vein of mice reduced the hepatic uptake of $^{125}$I-labeled β-VLDL that are enriched with apoE-III. Lactoferrin, which is one of the few potent inhibitors of remnant uptake in vivo, blocks the interaction of apoE with HSPGs. Together these results support the hypothesis that HSPGs are a component of the sieving or sequestration process of remnants by the liver. How the transfer from the HSPG to the LRP or LDL receptor occurs is not yet clear. Such binding could, however, be important in allowing remnants to acquire additional apoE for uptake by the LRP.

## LIVER ARCHITECTURE

The liver has a unique architecture. Molecules can gain direct access to the surface of each hepatocyte. To

do this they must pass through the fenestrae between the endothelial cells that line the hepatic sinusoids. The space between the endothelium and the hepatocyte is called the space of Disse. As pointed out by Fraser, Dobbs, and Rogers (143), entry of intact large chylomicrons to the space of Disse is probably precluded by their size. Smaller chylomicrons and chylomicron remnant-sized particles can, however, gain entry. This phenomenon was originally referred to as sieving by Wisse et al. (144) and may help to explain some of the differences in metabolism that have been reported to exist between large and small chylomicrons (145). The sieving phenomenon also gives a structural plausibility to concepts of "secretion capture", sequestration, and multifooted binding. The space of Disse is enriched in apoE (146) and is of such a dimension that it is plausible, at least in some locations, for a particle to bind simultaneously to an endothelial cell, perhaps by an apoB interaction with hepatic lipase and an apoE interaction with the LDL receptor on the hepatocyte surface.

In studies from our laboratory published in preliminary form (147), using hepatoma cells that had been engineered to express several of the proteins relevant to remnant uptake and grown in vivo, the ability to remove LDL was markedly enhanced and approached that of the liver, but remnant uptake, while increased, remained about 40-fold less efficient than in the liver. This is consistent with the hypothesis that particle size and the sinusoidal structure remained important determinants of remnant uptake.

## MODEL FOR INDEPENDENT AND OVERLAPPING PATHWAYS FOR THE HEPATIC REMOVAL OF CHYLOMICRON REMNANTS

A model for chylomicron uptake that attempts to integrate the information accumulated to date is shown in **Fig. 2.** Many details remain unclear, but the elements of the hypothesis should be amenable to testing. Chylomicrons undergo lipolysis in the circulation catalyzed by lipoprotein lipase. During this process they lose their complement of C apolipoproteins which allows them to gain apoE. Their premature removal by the liver is prevented by several factors. The ratio of apoE to C apolipoproteins is one determinant of this. In addition, a particle must gain entrance to the hepatocyte surface by virtue of having achieved a size that allows it to traverse the fenestrae in the endothelium. Once in the space of Disse, between the endothelium and the hepatocyte, the particle may be taken up directly by the LDL receptor. The LDL receptor can recognize chylomicron remnants and plays an important role in their removal from the circulation, at least in mice. This is probably the pathway with the highest affinity and appears to result in the most rapid endocytosis. The number of LDL receptors is regulated and highly variable and thus rarely does this pathway account for all of remnant removal. It is not clear whether there is direct removal via the LRP. The in vivo data suggest this is limited, perhaps because most particles need to be further modified before they are ligands for this receptor. If LDL receptors are down-regulated, saturated, or absent, remnants become sequestered in the space of Disse. This can occur by virtue of interactions between apoE and heparan sulfate proteoglycans and between apoB and hepatic lipase. After sequestration the particles may undergo further modification. They are then transferred to one of the endocytic receptors for internalization. If they acquire sufficient apoE, which is secreted free into the space of Disse, they become ligands for the LRP. As LDL receptors return to the surface these can also be used for internalization. When both receptors are blocked, rapid removal of particles can occur only until the "sequestration" space is filled.

Even in the absence of apoE there is remnant removal, which although it is slow can clear the particles with some efficiency. This might occur by sequestration mediated by hepatic lipase-apoB interaction, followed by further lipolysis, and then uptake either by pinocytosis or by the LRP which can use hepatic lipase and lipoprotein lipase as ligands. It has been suggested that proteoglycans themselves can mediate slow endocytosis.

### VLDL remnants

The mechanism of VLDL metabolism is believed to be generally similar to that of chylomicrons. Several additional complexities exist, however. In most nonprimate species, hepatic VLDL particles may contain either the full-length apoB-100 transcript or the edited apoB-48 protein. In primates, in contrast, hepatic VLDL contain only apoB-100. This may affect their propensity to be metabolized along the divergent pathways described below. Secondly, if the particles undergo a high degree of hydrolysis of triglyceride, perhaps by combined action of LPL and hepatic lipase, as well as further enrichment with cholesteryl ester catalyzed by the cholesteryl ester transfer protein, they become LDL particles. This class is generally free of apoE, and thus, has a lower affinity for the LDL receptor and little, if any, affinity for the LRP or VLDL receptor. As they progress along the delipidation cascade, the particles fall into the intermediate density lipoprotein (IDL) class and, because they contain some apoE and decreasing amounts of apoCs, they are potential participants in the "remnant" removal pathway. There is relatively little firm data regarding what regulates the extent to

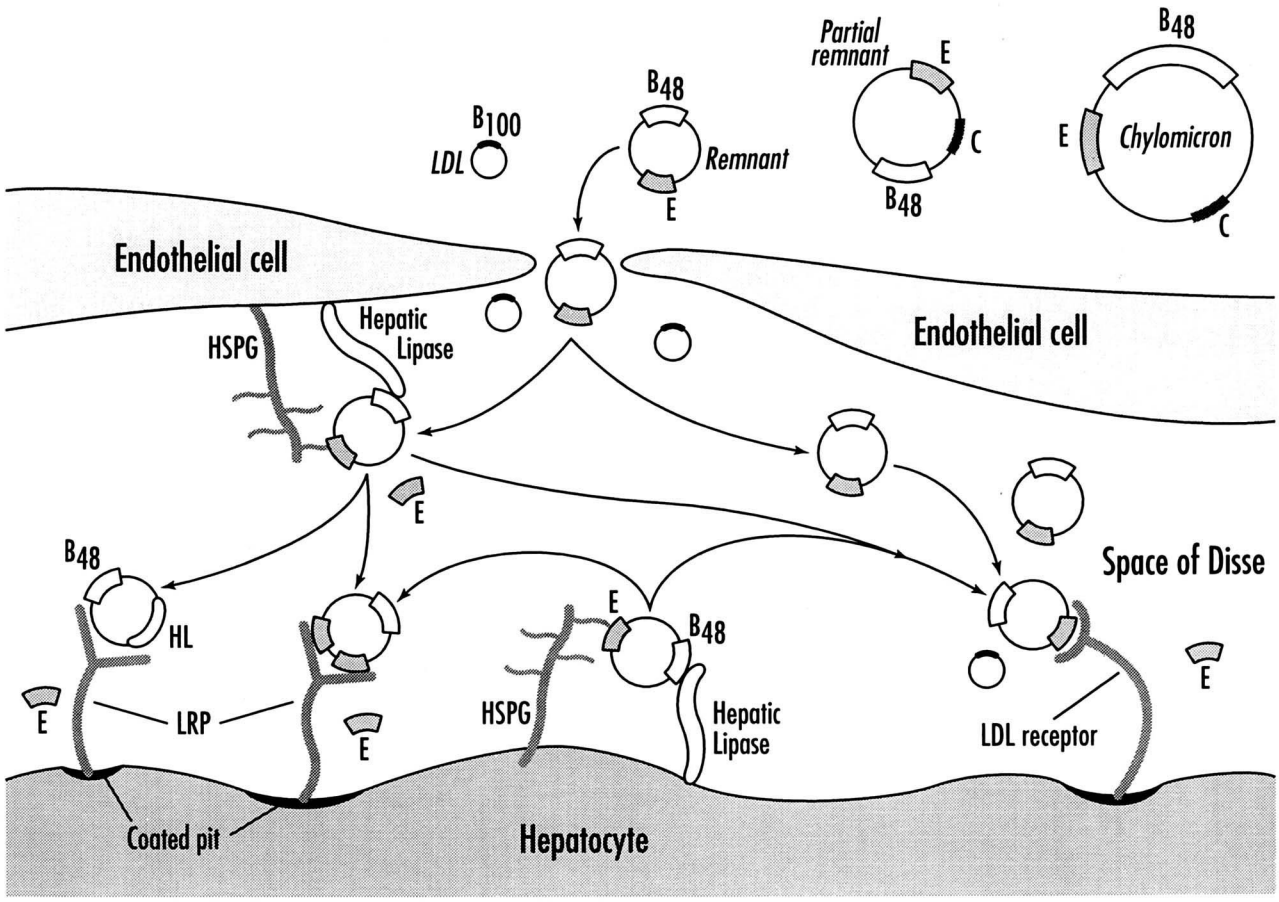


**Fig. 2.** Pathways of chylimicron remnant removal by the liver. This illustrates the possible pathways a chylomicron remnant may follow upon reaching the liver. If it is of the appropriate size it enters the space of Disse. It can: *1*) be taken up directly by the LDL receptor; *2*) acquire additional apoE and be taken up directly by the LRP; *3*) become sequestered by binding to heparan sulfate proteoglycans, mediated by apoE and/or hepatic lipase, mediated by apoB. Sequestered particles can then be transferred to the LDL receptor or LRP after further modifications. In the absence of apoE, hepatic lipase might serve as a ligand for the LRP. Note the dimension of the space of Disse has been expanded to allow clarity but is actually more like that of a finestre.

which they participate in the two pathways (LDL vs. remnant) but the presence of competitors could play an important role as a determinant of utilization of the receptor pathway.

Highly apoE- and cholesterol-enriched β-VLDL are clearly metabolized in a manner quite similar to chylomicron remnants (see above). Arbeeny and Rifici (148) reported that VLDL remnants prepared from rat serum VLDL were removed largely by the LDL receptor. Studies from our laboratory (149), in contrast, found that VLDL remnants prepared from the hepatic perfusate of rat liver behaved almost identically to chylomicron remnants. The model proposed above could account for this disparity, in that the more apoE-rich particles, or those that can acquire apoE well, are quickly removed by the sequestration-transfer pathway, as well as the LDL receptor, while those that are smaller behave like small chylomicrons and become ligands primarily or exclusively for the LDL receptor pathway (145). This formulation implies that interaction between the remnant removal and LDL removal pathways are of considerable importance in determining steady-state lipoprotein levels.

## REMNANT REMOVAL IN ANIMAL MODELS OF DISEASE

Cholesterol feeding has been reported to decrease the rate of chylomicron remnant uptake in vivo by Redgrave and Snibson (150) and Ross and Zilversmit (151). Studies from our laboratory (105) suggested that this was due primarily to expansion of the pool size of lipoproteins, of both endogenous and exogenous origin, that compete for remnant removal and not to an

intrinsic decrease in the capacity of the liver to remove the particles. Estrogen administration in pharmacologic doses has been found to increase the rate of removal of apoE-rich lipoproteins (152) an effect consistent with the effect of large doses of estrogen upon LDL receptor levels in the liver (58). Hepatocytes cultured in the presence of insulin have an enhanced ability to take up chylomicron remnants (153, 154). Again this is consistent with the ability of insulin to stimulate LDL receptors (155, 156). In diabetes there is a delay in remnant removal both in the rat (153) and in humans (see below). The molecular basis for this has not been elucidated. Similarly, thyroid hormone, which has a number of effects upon lipid metabolism, has been reported to alter remnant metabolism. The process is increased with thyroid hormone excess (157) and decreased with deficiency (150).

After bile duct obstruction, cholesterol metabolism is markedly altered because of interruption of the enterohepatic circulation of bile salts and cholesterol. Remnant removal is reduced while LDL uptake is not (158, 159). This seems to be due to an effect of the abnormal serum that accumulates in this circumstance and does not occur with only partial bile duct obstruction, a condition where the abnormal lipoprotein, LpX, does not appear (160, 161).

Siperstein and colleagues (162, 163) discovered, in the 1960s, that the feedback regulation of cholesterol synthesis by dietary cholesterol was deficient in malignant liver. Bell, Sargeant, and Watson (164) found that this was not due to an inability of HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, in these cells to respond to sterols. Our group then noted (165, 166) that in vivo the uptake of chylomicron remnants by hepatic tumors was decreased. This could have been due to the abnormal architecture and blood flow to this tissue. However, the abnormality precedes the development of malignancy (167) which occurs despite a normal level of LDL receptors and LRP in the premalignant tissue (A. D. Cooper, unpublished observation). Thus a change in the expression of other cell surface proteins that assist in remnant binding may be responsible.

## CHYLOMICRON REMNANT METABOLISM IN HUMANS

Much less is known about remnant metabolism in humans than in animal models. This is due to the methodological difficulties in identifying and labeling remnants. There are several possible solutions to the problem. One is to follow apoB-48 appearance and disappearance in various lipoprotein fractions after a meal. This is tedious and may not distinguish between large remnants and small chylomicrons. Hazzard and Bierman (168) were probably the first to administer a vitamin A-containing meal and follow the vitamin A level, as a measure of the rate of clearance of dietary lipoproteins. From this they concluded that in type III hyperlipidemia remnant removal was defective. The retinyl ester method was further refined by Berr and Kern (169) who validated it, in particular by demonstrating that, at least acutely in humans, plasma retinyl esters did not exchange among lipoprotein fractions. Using this, Berr (170) has concluded that in normal humans the $t_{1/2}$ of remnant removal is on average 18.8 min. This is much faster than any other lipoprotein fraction and is in proportion to studies in other animals when circulation times are considered. He was also able to demonstrate saturation kinetics in humans with a $K_m$ and $V_{max}$ of 0.88 nM, and $0.25 \times 10^{-3}$ nmol/min per g liver for apoB-48, respectively. This is quite consistent with an estimate of 0.5 nM for the $K_d$ in the rat. Nakandakare and colleagues (171) used artificial emulsions of the type described by Redgrave et al. (172) and reported somewhat more rapid removal times. They suggested the reason for the discrepancy was related to the amount of material infused.

Remnant removal has been studied in a variety of disease, nutritional, and pharmacological states. Perhaps most important is the question of whether chylomicron remnants per se contribute to atherosclerosis. Much of the early epidemiological and experimental evidence in favor of this hypothesis was reviewed by Zilversmit in 1979 (173). In carefully done studies, Patsch et al. (174) have identified the magnitude of postprandial hypertriglyceridemia as an important risk factor for cardiovascular diseases. Remnant removal is delayed in patients on dialysis (175), a condition associated with accelerated atherosclerosis, and remnant removal is more rapid in octogenarians (176), suggesting this causes a survival advantage. Particularly significant are several case control studies. Karpe with Olivecrona and his colleagues (177) established that there was a longer residence of larger chylomicron remnants in the blood of patients with documented coronary artery disease when compared to that in carefully selected disease-free controls. It was especially notable that this was true even in patients with a normal fasting triglyceride level. These data extended an older case control study that demonstrated a higher apoB-48:B-100 ratio in patients with cardiovascular disease than in controls (178). This association remains significant even when corrected for other lipid and non-lipid risk factors. A recent study from Israel (179) using similar case control methods also produced virtually identical results identifying de-

layed remnant removal in patients with atherosclerosis and no other risk factors. Thus, a variety of approaches in different populations have consistently identified delayed chylomicron remnant removal as an independent risk factor for premature atherosclerosis.

Based on a series of systematic studies, Reaven and Chen and their co-workers (180–188) have suggested that delayed remnant removal is part of the atherogenic phenotype encompassed in syndrome X, which includes hypertriglyceridemia, low HDL, small dense LDL, insulin resistance, and, in many instances, mild hypertension, with obesity as a frequent exacerbating factor. They have documented that in both normal individuals and in patients with NIDDM the area under the retinyl palmitate curve is increased in those ingesting a high carbohydrate as compared to a high fat diet. Further, in tests of normotriglyceridemic individuals, those with NIDDM and elevated fasting plasma insulin concentrations had higher peak and slower decay of retinyl palmitate than those without NIDDM. The delayed remnant clearance in the NIDDM patients was partially corrected by treatment with metformin. As remnants may be one of the direct mediators of atherogenesis in this prevalent syndrome, finding ways to reduce their residence time and serum concentration is of potential therapeutic importance. Further support for this concept has been recently put forward by Georgopoulos and colleagues (189–192) based upon similar studies.

The model for hepatic remnant removal proposed above is based upon animal studies and has been tested in humans to only a limited degree. The role of the LDL receptor in remnant removal in humans has been addressed in several studies. The study of Rubinsztein et al. (55) in patients with homozygous familial hypercholesterolemia found only a small increase in the vitamin A level after a test meal. Another set of studies (193) reporting the lack of a role of the LDL receptor in remnant removal, used "inferred" LDL receptor levels based upon the patients' underlying lipid disorder. Two studies measured remnant removal in groups of patients after cholesterol feeding, a perturbation that should have down-regulated LDL receptors. In the study by Clifton and Nestle (194) there was a decrease in the rate of remnant removal after cholesterol feeding only in hypertriglyceridemic patients. Neither they, nor Ginsberg et al. (195), detected changes in remnant removal in normolipidemic individuals fed a cholesterol-enriched diet. Together these results are consistent with more than one mechanism of remnant removal, with a role for the LDL receptor becoming appreciable only when the other pathway(s) are partially saturated.

The determinants of the other pathways have not been clearly identified. Attempts to identify surrogate markers that might predict the rate of remnant removal have met with only limited success. In careful studies by Lewis et al. (196, 197) only the fasting triglyceride level was a predictor of the area under the retinyl ester curve after a vitamin A-containing meal. In other hyperlipidemias, overproduction of endogenous particles that could compete for chylomicron remnant removal have been reported to result in a delay in the process. In familial combined hyperlipidemia, an increase in the area under the retinyl ester curve has been reported (198) and this can be returned towards normal by treatment with simvastatin (89). Similarly, fibrates have been reported to correct the delay in remnant removal in familial hypertriglyceridemic subjects (199). Cortner et al. (200) have measured remnant removal rates using isotopic tracers and computer simulation in normal and hyperlipidemic individuals. Consistent with the above studies, they found delays in remnant removal in patients with type III hyperlipidemia and familial hypertriglyceridemia.

The above studies are consistent with the model proposed but further and more directed experimentation in humans will be required before the process is fully understood. Such information may help to define better therapeutic regimens directed at normalizing the delayed remnant removal in conditions where this occurs. This, in turn, will lead to a direct testing of the hypothesis that these particles are atherogenic and that their residence time in the circulation contributes significantly to the pathogenesis of atherosclerosis.

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