Mini-Review: From Bench to Bedside

New Perspectives on Atherogenesis
Role of Abnormal Triglyceride-Rich Lipoprotein Metabolism

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Atherosclerosis, along with the resultant coronary artery disease (CAD), is a leading cause of mortality in industrialized countries. Significant attention has focused on the role of low-density lipoprotein (LDL) in the pathogenesis of CAD. A dyslipidemia characterized by a combination of abnormalities in the plasma levels of triglycerides and high-density lipoprotein (HDL) cholesterol, with or without elevated LDL cholesterol levels, affects many persons with premature CAD, however. In particular, both qualitative and quantitative abnormalities in circulating triglyceride-rich lipoproteins (TRLPs) may be a key factor in the development of CAD.

A number of advances have led to an increased appreciation of TRLP concentrations as independent predictors of risk for CAD. First, the meta-analysis by Hokanson and Austin demonstrated that increases in plasma triglyceride levels were associated with increased risk for CAD events, even after adjusting for numerous other predictive factors. The meta-analysis was supported by a more recent prospective study by Jeppesen et al., which demonstrated that triglyceride levels were independent predictors of ischemic heart disease in men. Second, a number of studies have demonstrated that TRLPs, whether assembled in and secreted from the intestine or the liver, can penetrate the artery wall and initiate or aggravate atherogenesis. Third, during the last decade, we have gained a much more detailed understanding of the metabolic relationship between high levels of TRLPs, low levels of high-density lipoprotein (HDL) cholesterol, and an abnormally small, cholesterol-depleted, dense LDL. This review will attempt to bring together information from recent cellular, biochemical, physiological, and molecular studies to provide an update of our understanding of both normal and abnormal TRLP metabolism and of the atherogenicity of TRLPs.

Normal TRLP Metabolism

Physical and Chemical Characteristics of TRLPs
All lipoproteins are macromolecular complexes composed of hundreds to thousands of core lipid molecules (triglycerides and cholesterol esters) that are covered by a surface monolayer of phospholipids, a small quantity of free cholesterol, and one or more apolipoproteins. The apolipoproteins play minor roles as amphipathic proteins at the interface of the aqueous plasma and the lipid-soluble core of each lipoprotein particle. They play major roles as regulators of the lipoprotein metabolism, however.

Apolipoprotein (apo) B48, which is synthesized in the intestine only, and apoB100, which is made in the liver, are the necessary structural proteins required for the assembly and secretion of chylomicrons and very low-density lipoproteins (VLDLs), respectively. In the absence of apoB, neither chylomicrons nor VLDLs are made and secreted. In addition, apoB100 is a ligand for the LDL receptor. ApoC-I, apoC-II, and apoC-III, which are synthesized mainly in the liver, are involved in several stages of the intravascular metabolism of TRLPs. ApoC-III is the most abundant apolipoprotein in TRLPs. ApoE is, like apoB100, a ligand for the LDL receptor, and it is also the ligand for the LDL receptor-related protein (LRP), the VLDL receptor, and recently identified apoE receptors.

Alapovic and colleagues developed the concept that apolipoprotein specificity can define subclasses of lipoproteins beyond the basic separation into those containing apoB and those containing apoA-I. Heterogeneity of the TRLPs based on apolipoprotein composition has been demonstrated by numerous laboratories, with particles carrying or lacking apoE, apoC-II, and apoC-III isolated by immunologic methods. Tomiyasu and coworkers have reported that the presence or absence of these apolipoproteins can impact the metabolism of apoB, the latter being a marker of the entire lipoprotein particle. The physiological determinants of the specific apolipoprotein content of any TRLP are undefined, although size and lipid composition seem to play important roles. It is also not clear whether apoB-containing lipoproteins are secreted with proportions different from those of other apolipoproteins, or if the apolipoprotein-specific subclasses are created entirely in plasma.

Regulation of TRLP Assembly and Secretion
Plasma levels of TRLP are determined by rates of secretion from the intestines and liver, as well as by rates of catabolism. The latter process includes both triglyceride hydrolysis and particle uptake by cells. Excellent reviews on the assembly and secretion of both intestinal and hepatic TRLP have been published recently, so only a brief description will be provided here. It seems that both apoB48 in the intestine and...
apoB100 in the liver are constitutively synthesized, and that their secretion is regulated mainly by the availability of the core lipids (triglycerides and cholesteryl esters) that they deliver into the circulation. The assembly of chylomicrons is stimulated by the delivery of dietary lipids into the cells of the small intestinal villi. Both lipogenesis and the delivery of fatty acids (from either peripheral lipolysis of stored adipose tissue triglyceride or as components of TRLP remnant core lipids) can stimulate the assembly of VLDL in the liver. Microsomal triglyceride transfer protein (MTP) plays a critical role in the transfer of lipoprotein lipids from the cytosol and/or endoplasmic reticulum membrane to nascent apoB. Indeed, absence of MTP results in the syndrome of abetalipoproteinemia and the absence of apoB lipoproteins. Recent studies indicating regulation of MTP gene expression by insulin, possibly via the transcriptional activity of sterol response element binding protein 1c (SREBP1c), suggests a molecular basis for the link between insulin resistance and increased VLDL secretion. Also of note, a series of recent studies in cultured liver cells and in transgenic animals indicates that the level of apoE in the hepatocytes impacts the assembly and secretion of TRLP.

**Regulation of TRLP Catabolism**

Catabolism of TRLPs begins with hydrolysis of core triglyceride by the enzyme lipoprotein lipase (LpL). LpL-mediated lipolysis is modulated by the relative amounts of apoC-II and apoC-III on the TRLPs. LpL is synthesized in fat and muscle and is secreted into the interstitial space. After secretion, LpL binds to glycosaminoglycans on the interstitial side of endothelial cells and then, via undefined pathways, undergoes transcytosis to the luminal surface of the capillary endothelium, where it is bound to heparan sulfate proteoglycans. It is LpL bound on the surface of vascular endothelial cells that interacts with TRLPs. ApoC-II is the necessary activator of LpL, and, although complete deficiency of apoC-II is extremely rare, heterozygotes for mutations in the apoC-II gene may have modest elevations in plasma TRLP levels. ApoC-III is an inhibitor of LpL, possibly by interfering with the binding of chylomicrons and VLDL to the heparan sulfate proteoglycans on endothelial cells. Importantly, persons lacking apoC-III have very low levels of TRLP accompanied by very efficient lipolysis of triglycerides. Furthermore, mice in which the apoC-III gene has been deleted also have very low triglyceride levels in blood and very efficient lipolysis of TRLP. LpL may also play a role as a bridging molecule and facilitate the uptake of remnant lipoproteins directly into cells.

Lipolysis of TRLP takes place in the capillary beds of adipose tissue, skeletal muscle, and the heart; the smaller chylomicron and VLDL remnants that remain are then able to permeate the fenestrated endothelium separating the hepatocyte surface and the space of Disse from the circulation. LDL receptors and the LDL receptor-related protein (LRP) on the surface of hepatocytes clear the remnant lipoproteins via an interaction with apoE on the apoB48-containing chylomicron remnant and with either apoB100 or apoE on VLDL remnants. Proteoglycans on the cell surface also play an important role in TRLP remnant uptake by the liver. Hepatic clearance seems to be the singular final step for chylomicron metabolism, whereas both hepatic clearance and conversion to intermediate-density lipoproteins (IDLs) and LDLs are alternative pathways for VLDL-remnant metabolism. Hepatic triglyceride lipase plays an important role in both the uptake and the conversion of TRLP remnants to IDL and LDL. Hepatic lipase seems to act both as a ligand and an enzyme in these processes.

The role of apoE in hepatic uptake of TRLP remnants has been very well studied. ApoE is a ligand for the LDL receptor; this is critical for intestinally derived TRLPs that carry apoB48, which lacks the LDL receptor binding domain present in apoB100. ApoE is also the ligand for LRP and binds to heparan sulfate proteoglycans; the latter seems to enhance interaction between TRLP remnants and LRP. Mice lacking apoE accumulate apoB48 particles and have severe hyperlipidemia and accelerated atherosclerosis. Rare instances of the absence of apoE in humans confirms the findings in mice. Additionally, persons homozygous for apoE2, which is defective in binding to the LDL receptor, are predisposed to develop type III dyslipoproteinemia, in which both intestinal and hepatic TRLP remnants accumulate.

The C apolipoproteins play particularly important roles in hepatic uptake of TRLP remnants. Early studies demonstrated that all 3 of these proteins inhibited the uptake of TRLP by the liver. In particular, apoC-I plays an inhibitory role in the binding of chylomicrons and VLDL to both the LDL receptor and LRP. Transgenic mice overexpressing apoC-I have moderate elevations in both plasma triglyceride and cholesterol levels and accumulate both apoB48- and apoB100-containing lipoproteins.

**Abnormal TRLP Metabolism**

**Abnormalities in TRLP Assembly and Secretion**

Increased rates of secretion of VLDL triglycerides are characteristic in persons with hypertriglyceridemia. Numerous studies of both VLDL triglycerides and VLDL apoB have indicated that the vast majority of people with elevated levels of VLDL have increased rates of secretion of TRLP into plasma. The most common physiological basis for these abnormalities seems to be insulin resistance, with a combination of increased free fatty acid flux from the periphery and insulin-stimulated lipogenesis driving VLDL production. Of interest are recent studies describing a potential link between hyperinsulinemia, SREBP1c gene expression, lipogenesis, and hyperlipidemia.

Increased VLDL secretion also characterizes familial combined hyperlipidemia, a common genetic form of dyslipidemia associated with coronary disease. Familial combined hyperlipidemia is most likely an oligogenic disorder, but no genetic variations have yet been definitively associated with increased assembly and secretion of VLDL. This is clearly a high priority for many investigative groups.

The assembly and secretion of TRLP from the intestine seem to be regulated by delivery of dietary cholesterol and triglycerides. However, recent studies identifying genes related to sterol absorption and the newly defined role of nuclear receptors LXR and FXR in intestinal bile and sterol...
metabolism suggest that novel insights regarding individual variability in the absorption of dietary sterols or fats, and subsequent chylomicron formation, may be forthcoming in the near future.

Abnormalities in TRLP Catabolism

The clearance of TRLP from plasma involves 2 phases; the initial step is lipolysis of nascent chylomicrons and VLDL, which is followed by hepatic uptake of remnants of those particles. In the case of VLDL, conversion to LDL can also occur. Persons lacking LpL enzymatic activity will present with severe hypertriglyceridemia and hyperchylomicronemia. Fortunately, although more than 50 mutations in the gene for LpL that could result in complete loss of activity have been identified, they rarely occur; the prevalence of patients with complete absence of LpL activity is about 1 per million. In contrast, the more common LpL mutations, such as D9N and N291S, result in only partial loss of enzymatic activity. These types of mutations and partial loss of LpL activity are present in about 2% to 5% of the population. Persons who are heterozygous for these mutations have moderate reductions in LpL activity and concomitant modest increases in plasma triglyceride levels, along with low levels of HDL cholesterol. In some studies, heterozygosity for the more common LpL mutations has been associated with increased risk of coronary heart disease. Smoking seems to interact with these mutations to increase risk for CAD.

As mentioned, increased levels of apoC-III would likely inhibit LpL-mediated hydrolysis of chylomicron and VLDL triglycerides. Indeed, although the absence of apoC-III is associated with low levels of plasma TRLP, elevated plasma levels of apoC-III are associated with increased levels of VLDL and decreased fractional catabolism in humans. Transgenic mice overexpressing apoC-III have severe hypertriglyceridemia, resulting mainly from inhibition of LpL-mediated triglyceride lipolysis.

The apoC-III content in plasma parallels the levels of VLDL and HDL. Additionally, because TRLPs can carry greater numbers of apoC-III molecules per particle than HDL particles, increased TRLP levels will lead to increased apoC-III concentrations. This made it difficult to move from the association of high apoC-III levels and increased TRLP to defining an etiologic role for apoC-III in hypertriglyceridemia. Several studies have, however, demonstrated increased rates of secretion of newly synthesized apoC-III into plasma in subjects with hypertriglyceridemia. These results strongly suggest that increased production of apoC-III could have played a primary, or at least an aggravating, role in the development of hypertriglyceridemia.

On the basis of the above facts, many investigators studying abnormalities in TRLP metabolism, particularly familial combined hyperlipidemia, have focused on both the apoC-III gene and the cluster of genes for apoA-I, apoC-III, and apoA-IV on the long arm of chromosome 11. Indeed, the first genetic polymorphism associated with hyperlipidemia, an Sst restriction enzyme site in the 3′ untranslated region (named the S2 allele), remains an important determinant of both hypertriglyceridemia and risk for familial combined hyperlipidemia. Several years ago, a series of polymorphic sites was identified in an insulin response element (IRE) in the apoC-III gene promoter, offering a potential basis for the link between hypertriglyceridemia, familial combined hyperlipidemia, and insulin resistance. Recent studies examining that association, however, are contradictory. The strong association of these elements in the apoC-III promoter with the presence of the S2 allele of the apoC-III gene may confound these studies. Other polymorphisms in the apoA-I–apoC-III region, together with the Sst polymorphism, seem to add significantly to the association of the S2 allele with hypertriglyceridemia. Until mechanistic studies define the manner in which these polymorphisms affect apoC-III and triglyceride metabolism, however, the role of these genetic variations will remain unclear.

The peroxisome proliferator-activated receptor α (PPAR-α) has been linked to the regulation of apoC-III gene expression, and this may provide a molecular mechanism for the triglyceride-lowering effects of fibrates, which are PPAR-α agonists. Importantly, PPAR-α agonists repress apoC-III gene expression in rodent livers. Because apoC-III inhibits LpL-mediated lipolysis of TRLP, suppression of apoC-III synthesis could be a key link between fibrate treatment and improved triglyceride catabolism observed in human studies. PPAR-α agonists also enhance fatty acid oxidation in the liver, which subsequently affects triglyceride synthesis and VLDL production. Thus, PPAR-α agonists could potentially reduce triglyceride levels by both decreasing secretion and accelerating fractional catabolism of VLDL triglycerides.

Abnormalities in Hepatic Uptake of TRLP Remnants

Removal of TRLP remnants by the liver is a complex process, and abnormalities in that process can derive from a number of pathways. Remnant removal can be affected by the ability of LpL, and possibly of hepatic lipase, to initially generate remnants that can be recognized by the liver; by the number and activity of hepatic remnant receptors, such as the LDL receptor and LRP; and by the interaction of TRLP apolipoproteins, particularly apoE and the C apolipoproteins, with hepatic receptors and/or cell surface proteoglycans. We will focus on the latter aspects of remnant removal.

As noted in the section on normal TRLP metabolism, apoE plays a key role in particle uptake by the liver. There is much less information available regarding the C apolipoproteins and abnormal hepatic uptake of TRLP remnants. As noted earlier, overexpression of apoC-I in mice leads to a pattern consistent with combined hyperlipidemia. Unexpectedly, the apoC-I–deficient mouse has reduced fractional catabolism of TRLP. Thus, the role of apoC-I in human dyslipidemias in which TRLP remnants accumulate is unclear. The potential adverse effects of increased synthesis of apoC-III on TRLP lipolysis were detailed above. Similarly, increased entry into plasma of apoC-III could impair hepatic uptake of TRLP remnants. The data from transgenic mice overexpressing apoC-III, however, suggest that this apolipoprotein has its major impact via reduced hydrolysis of TRLP triglycerides.
Atherogenicity of TRLPs
Abnormal transport and metabolism of TRLP, particularly in the postprandial period, have been linked to the presence of atherosclerosis, both in the coronary and carotid arteries.\textsuperscript{1,63–65} Chylomicrons and their remnants, as well as VLDL remnants, can penetrate into, and be trapped inside, the artery wall, where they can be taken up by macrophages and smooth muscle cells. Animal studies have shown that particle size determines the rate of particle entry into the artery. Both rabbit and mouse models of increased plasma apoB48-TRLP remnants develop atherosclerosis, and those remnants are actively taken up by arteries.\textsuperscript{66,67} Importantly, TRLPs have also been isolated from human artery segments.\textsuperscript{68} LpL, which would normally facilitate the catabolism of TRLPs in fat and muscle, may also be pro-atherogenic in the artery wall. Macrophages make LpL, and transgenic mice overexpressing LpL in macrophages have increased atherosclerosis.\textsuperscript{69} Whether this occurs because LpL increases penetration and retention of TRLP by the artery wall\textsuperscript{70} or because it facilitates endocytosis of TRLPs into macrophages is unclear.

Atherogenicity of TRLP Subclasses
The Cholesterol Lowering Atherosclerosis Study\textsuperscript{71} demonstrated that increased concentrations of apoC-III in HDL, a surrogate for efficient catabolism of TRLP, were associated with stabilization of atherosclerosis in subjects randomized to cholesterol/niacin treatment. The Monitored Atherosclerosis With Stabilization of Atherosclerosis in Subjects randomized to Cholesterol Lowering Treatment Approaches\textsuperscript{72} reported an association between higher apoB:C-III TRLPs and the progression of CAD. Koren et al\textsuperscript{73} identified a significant positive correlation between the global angiographic score and HDL cholesterol, the ratio of apoC-III in HDL to that in apoB lipoproteins, the total number of apoB-containing lipoproteins, and apoB:C: E lipoprotein levels. Sacks et al.,\textsuperscript{74} using the Cholesterol And Recurrent Events (CARE) trial data, stressed the importance of the number of apoB particles and, in particular, the apoC-III–enriched apoB particles in VLDL and LDL in predicting cardiovascular events.

Atherogenicity of TRLPs, Low HDL Cholesterol Levels, and Small, Dense LDL
Some of the atherogenicity of TRLPs derives from the nearly universal association of increased VLDL and chylomicron levels with low levels of HDL cholesterol and increased numbers of small, cholesteryl ester-depleted, dense LDL.\textsuperscript{4} This combination is characteristic of patients with the insulin resistance syndrome\textsuperscript{56} and strongly predicts an increased risk for CAD. The relative importance of each of these lipoprotein abnormalities as risk factors for CAD is, however, unclear. Although several population studies have suggested that small dense LDL are particularly atherogenic, patients with familial hypercholesterolemia have large, cholesteryl ester–rich LDL and increased CAD. Additionally, in the CARE trial, large LDL predicted CAD events.\textsuperscript{75}

Although the increased secretion of TRLP associated with insulin resistance can result in reduced HDL levels and the generation of small dense LDL, impaired TRLP catabolism resulting from reduced LpL activity can also “drive” these changes in HDL and LDL.\textsuperscript{76} A detailed discussion of the pathophysiological basis for the close association between increased levels of TRLP, reduced HDL cholesterol levels, and small, dense LDL is beyond the scope of this review, but it is also clear that all 3 lipid/lipoprotein abnormalities are closely associated through the action of cholesteryl ester transfer protein.\textsuperscript{77}

Conclusions
The causes of abnormal TRLP metabolism and hypertriglyceridemia are complex and varied. An important unanswered question is whether all forms of hypertriglyceridemia are equally atherogenic. Although it may be concluded from a number of studies in different experimental systems, as well as from naturally occurring extremes such as complete LpL deficiency, that very large, nascent (unmetabolized) TRLPs are less atherogenic than smaller remnant TRLPs, the ability to find some clear cut point between nonatherogenic and atherogenic TRLPs continues to evade us. The question of whether remnant TRLP should be classified as more or less atherogenic on the basis of apoC-III content has been addressed in a number of studies, some of which support such an approach.\textsuperscript{71–74} To advance from associations to causality, however, will take additional studies focusing on the etiology of increased apoB:C-III particles (increased production versus prolonged plasma residence time) and must be based on evidence for unique atherogenic properties of such TRLP. Until such data are available, it may be best to focus on the development of improved methods for determining the number of apoB-containing lipoprotein particles in the circulation, particularly the number of TRLP remnants, IDL, and LDL particles. Despite the complexity inherent in lipoprotein disorders, the simple message that continues to emerge from all available data is that reducing apoB-containing lipoproteins while increasing apoA-I–containing lipoproteins is beneficial.\textsuperscript{78}

References


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