Remnants of the Triglyceride-Rich Lipoproteins, Diabetes, and Cardiovascular Disease

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Diabetes is now a pandemic disease. Moreover, a large number of people with prediabetes are at risk for developing frank diabetes worldwide. Both type 1 and type 2 diabetes increase the risk of atherosclerotic cardiovascular disease (CVD). Even with statin treatment to lower LDL cholesterol, patients with diabetes have a high residual CVD risk. Factors mediating the residual risk are incompletely characterized. An attractive hypothesis is that remnant lipoprotein particles (RLPs), derived by lipolysis from VLDL and chylomicrons, contribute to this residual risk. RLPs constitute a heterogeneous population of lipoprotein particles, varying markedly in size and composition. Although a universally accepted definition is lacking, for the purpose of this review we define RLPs as postlipolytic partially triglyceride-depleted particles derived from chylomicrons and VLDL that are relatively enriched in cholesteryl esters and apolipoprotein (apo)E. RLPs derived from chylomicrons contain apoB48, while those derived from VLDL contain apoB100. Clarity as to the role of RLPs in CVD risk is hampered by lack of a widely accepted definition and a paucity of adequate methods for their accurate and precise quantification. New specific methods for RLP quantification would greatly improve our understanding of their biology and role in promoting atherosclerosis in diabetes and other disorders.

Diabetes and Residual Cardiovascular Disease Risk

Both type 1 (T1DM) and type 2 (T2DM) diabetes mellitus increase the risk of cardiovascular disease (CVD) (1,2). The causal relationship of LDL cholesterol (LDL-C) with increased CVD risk is well established, and drugs that lower LDL-C levels by increasing LDL receptor activity are widely used to prevent CVD. However, despite the benefit of statins and marked reduction of circulating LDL-C levels, patients with diabetes continue to have more CVD events than patients without diabetes, indicating significant residual CVD risk. A recent new classification system of T2DM, based on different patient characteristics and risk of diabetes complications, divides adults into five different clusters of diabetes (3). Such a substratification of T2DM, which takes insulin resistance and several other factors into account, could perhaps help tailor treatment to those who would benefit the most.

There are likely to be a number of reasons why cholesterol reduction in patients with diabetes is not sufficient to reduce CVD events to the levels found in patients without diabetes. Atherosclerotic lesions in patients with diabetes tend to be more inflammatory, i.e., have greater numbers of macrophages (4). Moreover, after cholesterol reduction, intravascular ultrasound studies show less regression of lesions in people with diabetes (5). Residual CVD risk appears to be at least partly linked to elevated plasma triglycerides and abnormal metabolism of triglyceride-rich lipoproteins (TRLs) (6,7), which are conventionally considered to consist of chylomicrons, VLDLs, and their respective remnant lipoproteins (remnant lipoprotein particles [RLPs]), many of which are present in intermediate-density lipoproteins (IDLs).

Normal fasting levels of plasma triglycerides are defined by current clinical guidelines as <1.69 mmol/L (<150 mg/dL). The definitions of elevated triglyceride levels vary, but fasting triglyceride levels of 1.69–2.25 mmol/L (150–199 mg/dL) are often considered moderately elevated and

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fasting triglycerides >2.26 or 2.83 mmol/L (200 or 250 mg/dL) are considered high and >5.65 mmol/L (500 mg/dL) severely elevated (8). Elevated plasma triglycerides can be due to increased triglyceride production, decreased lipolysis of triglycerides, and/or reduced clearance of TRLs (7). Moderate hypertriglyceridemia is common in subjects at increased risk of CVD, such as patients with T2DM. However, hypertriglyceridemia strongly associates with a host of other potential risk factors, including obesity, insulin resistance, increased levels of all apolipoprotein (apo)B particles, including RLPs and small-dense LDL, and low levels of HDL cholesterol (HDL-C) (6,9).

More than 40 years ago studies of familial forms of hypertriglyceridemia linked elevated triglyceride levels to an increased risk of CVD (10). Abundant epidemiological evidence associated triglyceride levels with CVD risk (11,12), although the risk was often attenuated when adjusted for potential confounders, such as HDL-C (13). Furthermore, clinical trials of fibrates and n-3 fatty acids (polyunsaturated fatty acids, also often referred to as fish oils) have, with the exception of REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial) and JELIS (Japan EPA Lipid Intervention Study), been mostly negative for CVD benefit (7). The STRENGTH (Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia) trial was recently stopped by the data monitoring board due to a low likelihood of success, but the results have yet to be published. These negative results could have resulted from inclusion of individuals without hypertriglyceridemia in most of the fibrate trials, use of inadequate doses or form (ester versus free fatty acid) in most of the n-3 fatty acid trials, or heterogeneity in the atherogenicity of the triglyceride-carrying lipoproteins, at least in terms of triglyceride as a marker of CVD risk, in the study cohorts. Therefore, there is a need to better define the relationship between plasma triglyceride levels, the apoB lipoprotein particles that carry triglycerides and cholesterol (chylomicrons, VLDL, IDL, and RLPs), and the relative atherogenicity of those lipoproteins versus LDL. Increased understanding of these difficult issues will offer critical insights needed to facilitate our search for additional approaches to CVD prevention and treatment options for hypertriglyceridemic patients, especially those with diabetes.

Genetic studies over the past several years provide strong evidence that elevated triglyceride levels are indeed an independent risk factor for atherosclerosis. Thus, genes involved in TRL metabolism such as lipoprotein lipase (*LPL*) and genes that control LPL's activity appear to strongly associate with CVD risk (14). A key early observation was that a null mutation in *APOC3*, the gene encoding apoC-III (apoC3), which acts in part by inhibiting LPL, associated with reduced triglyceride levels and reduced CVD risk in an Amish population (15). Subsequent studies have confirmed this observation and demonstrated that apoC3 as a CVD risk factor is largely independent of LDL-C (16,17). Other genes linked to elevated levels of triglycerides and CVD risk independently of LDL-C include *APOA5* and angiopoietin-like protein 4 *(ANGPTL4)*, an activator and an inhibitor of LPL, respectively (14). Loss-of-function mutations in *ANGPTL3*, another gene in the angiopoietin-like protein family, is associated with reduced CVD risk and lower triglyceride levels as well as lower levels of LDL-C and HDL-C (familial combined hypolipidemia) (4). It is now widely accepted that hypertriglyceridemia is a risk factor for CVD, although whether elevated triglycerides might be only a biomarker or a direct mediator of CVD remains unclear.

In patients with T1DM, fasting triglyceride levels are usually normal (<150 mg/dL) unless diabetes control is poor (9). There are uncertainties about the role of TRLs in CVD risk in these patients and in patients with T2DM and normal triglyceride levels. However, data from DCCT/EDIC (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) suggest that total serum apoC3 and apoC3 in HDL are important predictive biomarkers for CVD in adults with T1DM (18). Moreover, we recently showed that apoC3 is a CVD risk factor in a cohort of T1DM subjects with median triglyceride levels in the normal range (17). Although there was a strong positive correlation between triglyceride levels and serum apoC3 in this cohort, apoC3 appeared to be a stronger risk factor. These observations suggest that apoC3 and triglycerides promote atherosclerosis in T1DM subjects—and perhaps in other subjects with triglycerides generally in the normal range. One possible explanation is that accumulation of lipolytic products of chylomicrons and VLDL, i.e., cholesterol-enriched RLPs, is the reason for elevated CVD risk rather than the more triglyceride-rich precursors of RLPs, chylomicrons and VLDL. Moreover, this hypothesis implies that partial defects in the TRL lipolysis pathway, e.g., due to heterozygous loss of LPL or polygenic causes of reduced lipolysis, would be more atherogenic than total loss of LPL. Thus, very active lipolysis appears to lead to a rapid removal of both chylomicrons and remnants from the blood stream. Heterozygous deficiency of LPL leads to greater postprandial lipemia including the accumulation of more IDLs (19) and chylomicron remnants (20), whereas total LPL deficiency actually prevents the formation of RLPs (21). It is therefore possible that patients with LPL deficiency due to mutations that lead to very low activity levels could be harmed by strategies that activate the small amount of residual activity associated with these mutations. How these mechanisms relate to the residual CVD risk associated with diabetes remains to be established.

What Are Remnants of the TRLs?

The lipoproteins that predominantly transport triglycerides are chylomicrons and VLDL. Chylomicrons are large triglyceride-rich particles secreted by the intestines following ingestion of dietary fat. Their major apolipoprotein is apoB48, a truncated form of apoB100, and their primary role is the delivery of ingested energy, in the form of

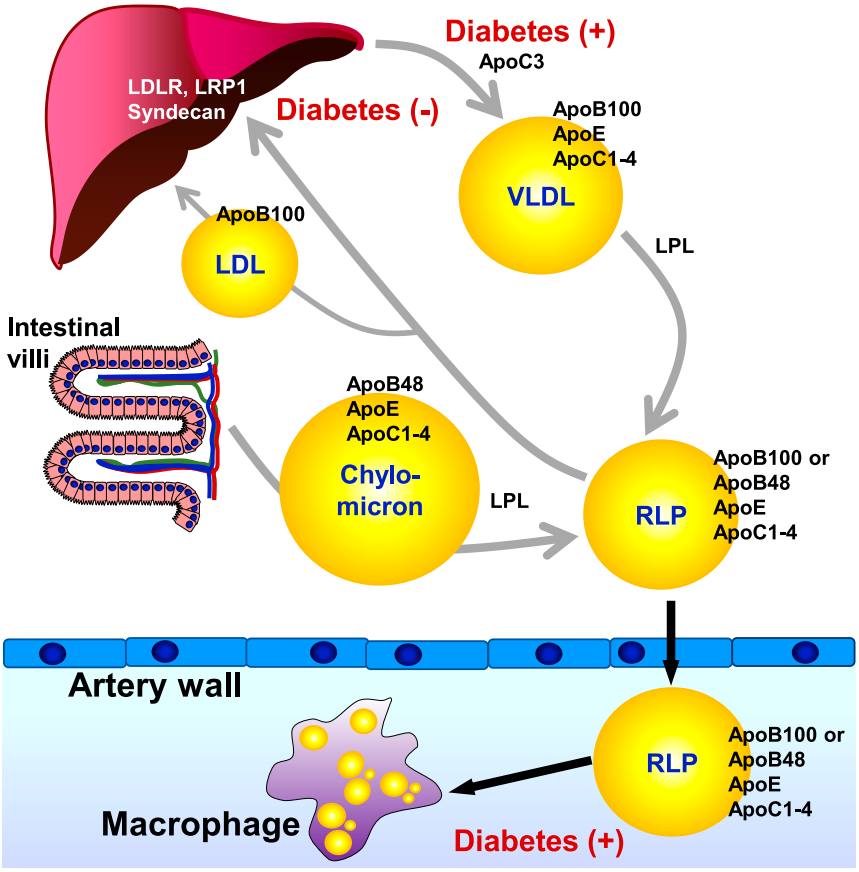

Figure 1—Overview of RLP metabolism and effects of diabetes. VLDL—which carries apoB100, apoE, and apoC3 in addition to other proteins—is produced by the liver. VLDL is converted to RLPs primarily by LPL. Some of the RLPs are converted to LDL, a significant fraction is cleared by the liver, and some RLPs accumulate in tissues, such as atherosclerotic lesions, and are taken up by macrophages. Chylomicrons are produced by the intestine after a meal. These large TRLs carry apoB48, apoE, and apoC3 in addition to other proteins and are converted to RLPs by LPL. Diabetes can alter TRL metabolism through several mechanisms, including increased VLDL secretion and reduced hepatic clearance of TRLs and RLPs due to increased apoC3 levels and maybe reduced cell surface levels of LRP1. Increased trapping of RLPs in the artery wall can drive atherosclerosis. LDLR, LDL receptor.

dietary triglycerides, to peripheral tissues for storage or utilization. In contrast to apoB100 present in VLDL and LDL, apoB48 does not bind to the LDL receptor. Instead, chylomicrons and their smaller remnants are cleared through interactions between apoE, an apo that is abundant in chylomicrons as well as other lipoprotein particles, and several hepatic receptors described below (Fig. 1). VLDLs, which are much smaller than chylomicrons (Fig. 2), are synthesized by the liver. Similar to chylomicrons, VLDL transport triglycerides from dietary sources, but most of VLDL triglyceride derives from lipolysis of adipose tissue and from the conversion of glucose to triglyceride in the liver. VLDL delivers these triglycerides to peripheral tissues in the fasted state. As noted above, VLDL contains apoB100, the major ligand for the LDL receptor, as its major apo. High levels of chylomicrons inhibit clearance of VLDL by substrate competition for LPL. As a result, although apoB48-containing chylomicrons and their remnants contribute the large majority of the rise of triglycerides in the postprandial state, TRLs containing apoB100 account for most of the increase in total apoB lipoproteins observed postprandially (22). Alternatively, rapid lipolysis of chylomicrons and hepatic uptake of exogenously derived triglyceride fatty acids could stimulate increased postprandial hepatic production of VLDL.

Hydrolysis of triglycerides in the core of chylomicrons and VLDL by LPL leads to the formation of triglyceride-depleted particles termed chylomicron remnants and VLDL remnants, respectively (Fig. 1). These RLPs also become depleted in apoCs and relatively enriched in apoE compared with their parent TRLs. The mechanism for these changes appears to be the reduction in volume and surface area of the nascent chylomicrons and VLDL by LPL resulting in the preferential movement of apoCs to HDL as well as the direct addition of apoE that is secreted by the liver (23,24). It is important to keep in mind that RLPs are dynamic products of the ongoing lipolysis of both apoB48-containing chylomicrons and apoB100-containing VLDL and, therefore, consist of a heterogeneous population of particles, varying in size and composition.

Early studies in rats demonstrated the importance of the liver in RLP removal, since hepatectomy resulted in

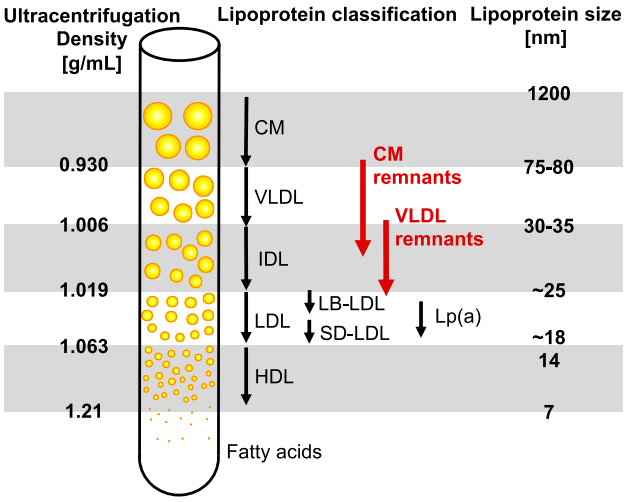

Figure 2—RLPs constitute a diverse population of particles, which cannot be effectively separated from other lipoproteins. Lipoprotein isolation by ultracentrifugation (left), lipoprotein classification (middle), and lipoprotein particle sizes (right). RLPs (chylomicron [CM] remnants and VLDL remnants) are present in chylomicron, VLDL, and IDL fractions isolated by density ultracentrifugation. The gray stripes represent density ranges (sequential ultracentrifugation). LB-LDL, large-buoyant LDL; Lp(a), lipoprotein(a); SD-LDL, small-dense LDL.

remnant accumulation (25). RLPs are cleared by the liver primarily by an apoE-dependent interaction with the hepatic LDL receptor and LDL receptor–related protein 1 (LRP1). The three apoE isoforms have different affinities for LRP1 and the LDL receptor. In subjects with T1DM and the apoE2/2 isoform, which has a low affinity for LRP1, most of the lipoproteins isolated in the LDL density range are composed of dense remnants and some lipoprotein(a) rather than terminal LDL particles (26), suggesting reduced clearance of RLPs. Furthermore, when apoE2/2 subjects become hyperlipidemic as a result of another primary or secondary abnormality of lipid metabolism, RLPs with both apoB100 and apoB48 accumulate in what is known as remnant removal disease, also known as type III hyperlipoproteinemia, broad band β disease, or dysbetalipoproteinemia (27). In mice, the proteoglycan syndecan 1 also plays an important role in the clearance of both hepatic and intestinally derived TRLs (28). A majority of chylomicron-derived RLPs are cleared by the liver. Between 25% and 75% of VLDL-derived RLPs undergo additional lipolysis and are converted to LDL (Fig. 1).

How Are RLPs Measured?

We define RLPs as postlipolytic partially triglyceride-depleted particles. If derived from chylomicrons, these RLPs contain apoB48, and if derived from VLDL they contain apoB100. As hydrolysis proceeds, the RLPs become progressively smaller and more triglyceride depleted and relatively enriched in cholesterol as a result. Additionally, the longer the RLPs remain in circulation, the greater their enrichment with cholesteryl esters due to the transfer of this lipid species from HDL via cholesterol ester transfer protein (CETP). At the same time, they also become enriched in apoE, as discussed above.

There is little consensus as to how to accurately measure RLPs. This in part is due to the fact that RLPs are defined by metabolic processes that alter the lipid and apolipoprotein composition of chylomicrons and VLDL. Therefore, the numbers and composition of RLPs are constantly changing as a result of ongoing hydrolysis, CETP lipid transfer, and uptake by hepatic receptors. Adding complexity, a majority of chylomicron remnants are removed by the liver, whereas, as noted earlier, 25–75% of VLDL remnants are not directly removed by the liver but, rather, are converted to LDL. Moreover, the accumulation of remnants is likely to differ in people with differing baseline triglyceride levels and is likely to increase with increasing triglyceride levels due to greater competition between chylomicrons and VLDL for both lipolysis and hepatic clearance. Because of the heterogeneity of RLPs and widely varying lipid and protein composition resulting from different degrees of hydrolysis, current physicochemical analytical techniques used for lipoprotein separation do not allow easy separation of chylomicron-derived RLPs from those derived from VLDL. Moreover, these methods cannot completely separate RLPs from their precursors, chylomicrons, or VLDL. Based on the definition used in this review, RLPs should *1*) be undetectable or dramatically reduced in individuals with LPL deficiency, *2*) have properties similar to the lipoprotein particles that accumulate in the disorder remnant removal disease (type III hyperlipoproteinemia, dyslipoproteinemia, broad band β disease), and *3*) be relatively enriched in apoE. The various methods by which RLPs have been identified and quantified so far are described in the next sections.

Ultracentrifugation

RLPs often are assessed by ultracentrifugation (29), which relies on relative flotation based on density. Due to their relative triglyceride depletion, RLPs are smaller and denser than their parent particles. Remnants derived from VLDL are found mainly in the IDL (d = 1.006–1.019 g/mL) range after ultracentrifugation, although some are also present in the VLDL density range (d < 1.006 g/mL). Remnants derived from chylomicrons are found in the chylomicron, VLDL, or IDL range (Fig. 2). Thus, there is overlap with other lipoproteins, and ultracentrifugation is therefore an imperfect way to isolate and quantify RLPs (Table 1).

Electrophoretic Mobility

RLPs also can have electrophoretic mobility different from that of their precursor lipoproteins (Fig. 2) due to differences in charge. Plasma from individuals with remnant removal disease exhibit a broad band of material that migrates between β-lipoproteins (LDL) and pre-β-lipoproteins (VLDL) on electrophoresis (30). Hence, the term broad β disease also has been used to describe remnant removal disease (31). β-Migrating VLDLs also are believed to be enriched in

Table 1—Methods currently used for quantifying RLPs

Method	What is measured	Pros	Cons
Sequential ultracentrifugation (d = 1.006–1.019 g/mL)	Lipids in d = 1.006–1.019 g/mL range	Easy	Contamination by small VLDL and absence of less dense RLPs
Electrophoresis	Separates lipoproteins based on their charge. When sufficient levels of RLPs accumulate, they appear as a broad band that migrates further than LDL (β-migrating lipoproteins)	Reasonable screening tool for remnant removal disease	Nonquantitative
Immunoaffinity gel using an anti-apoB100 monoclonal antibody and apoA1 antibody	Cholesterol in fraction not bound to the apoB100 and apoA1 antibody	Commercially available method	Epitope unknown, unknown entity measured
NMR	Six VLDL fractions and IDL	Can be quantified	Contaminated by nascent VLDL, which may contribute to a much greater extent than RLPs
Differential ion mobility analysis	Many lipoprotein particle populations can be analyzed	Can be calibrated to obtain lipoprotein particle concentrations	Precise size of RLPs distinct from other lipoproteins has not been established
ApoB48	ApoB48 in fasting plasma	Can be quantitative (e.g., by ELISA or targeted MS)	Nonremnant chylomicrons are included. VLDL-derived RLPs do not contain apoB48
Subtraction method	Cholesterol (RLPs = total cholesterol − HDL-C − LDL-C)	Fast and easy for clinical studies	Contamination by nascent VLDL, which may contribute to a much greater extent than RLPs

MS, mass spectrometry.

RLPs. Gradient gel electrophoresis resolves IDL into two major bands that overlap in size and density. Discontinuous nonequilibrium density gradient ultracentrifugation was able to isolate a series of fractions containing progressively smaller lipoproteins, which showed progressive enrichment in cholesteryl esters, depletion of triglycerides, and slower migration on agarose gels (29), consistent with enrichment of RLPs.

Immunoaffinity Method Using a Monoclonal Antibody

A widely employed immunoaffinity method of measuring RLPs uses a monoclonal antibody to apoB100 that apparently does not bind an apoE-rich population of VLDL containing apoB100 as well as TRLs containing apoB48 (32). This antibody together with a monoclonal antibody to apoA1 is used to bind most of the apoB100-containing lipoproteins (namely, LDL and VLDL) and apoA1-containing lipoproteins (namely, chylomicrons and HDL), leaving behind an unbound fraction of TRLs, including RLPs derived from VLDL and chylomicrons, both of which are enriched in apoE. Cholesterol or triglyceride is measured in the unbound fraction to quantify RLPs (33). Measurement of RLPs by this immunoaffinity method correlates both with triglycerides in the combined VLDL and IDL ultracentrifugal fractions and with RLPs determined electrophoretically. Changes in RLP cholesterol determined by this immunoaffinity method also correlate highly with increment in triglycerides postprandially (34). Although this method has been widely used to determine RLPs in human subjects, the nature of the epitope that allows for precipitation of apoB in VLDL, but not in remnant particles, is not fully characterized, and it is not clear whether the method detects all types of RLPs (Table 1). Although ELISAs have been developed for RLP measurements (35), the validity of these ELISAs has been questioned (36).

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR), which is based on computational deconvolution of the proton NMR signal of plasma lipid methyl groups characteristic of lipoproteins, has been used to quantify six subclasses of VLDL and IDL (37). It also has been used recently to measure RLPs (38). Although smaller VLDL subspecies and IDL are enriched in cholesterol relative to large VLDL, inclusion of all VLDL classes is unlikely to selectively quantify RLPs and leads to much greater estimates of "remnant lipoproteins" than other methods, presumably because it also contains nonremnant lipoproteins.

Differential Ion Mobility

Differential ion mobility analysis can directly determine the size of a broad range of lipoprotein particles (39) and can be calibrated to provide information on particle concentrations and sizes (40). The differential ion mobility analysis method is based on the principle that particles of a given size and charge behave in a predictable manner when carried in a laminar flow of air and subjected to an electric field (39). However, the utility of this approach for determining the sizes and particle concentrations of RLPs

has not been established. Differential ion mobility analysis may offer a powerful approach for quantifying RLPs because *1*) most remnant particles range in size between LDL and VLDL particles and *2*) this approach should readily distinguish between RLPs of different sizes.

Measurement of ApoB48 in Fasting Plasma

Specific measurement of apoB48 in fasting plasma as distinct from apoB100 has been used as a measure of chylomicron remnants derived from the intestines (41). However, measurements of fasting plasma apoB48 do not measure remnants derived from VLDL.

Subtraction Method

Another method used as a simple approximation of RLPs in nonfasting plasma relies on calculating the sum of VLDL, IDL, and chylomicron cholesterol, using the formula RLP = total cholesterol − HDL-C − LDL-C, but requires specific isolation of LDL by ultracentrifugation (42). However, the "RLP" fraction can also contain chylomicrons, depending on the time after the last meal and the meal's fat content, as well as IDL and nonremnant VLDL from which the RLPs were derived. While this calculation method is easy and readily applied to large population studies, it is not a specific measure of RLPs.

From the foregoing, it is clear that there is no ideal way of specifically quantifying RLPs. Development of accurate, reproducible methods for the quantification of RLPs would provide a powerful tool for determining the real role of this complex class of lipoproteins in the pathogenesis of atherosclerosis. Achieving this goal depends, however, on agreement of what the size, density, and the lipid and apolipoprotein content of the RLP are in people without the *APOE2/2* genotype.

Are Remnants of the TRLs Atherogenic?

Subjects with remnant removal disease are at a greatly increased risk for CVD (43,44). Remnant removal disease usually requires homozygosity for the *APOE2* genotype, resulting in impaired hepatic uptake of APOE-containing lipoproteins (45). In the majority of cases, remnant removal disease is an autosomal recessive disorder that associates with the *APOE2/E2* genotype. However, in the absence of additional genetic, hormonal, or environmental factors, RLPs do not accumulate to a degree sufficient to cause hyperlipidemia in *APOE2* homozygotes; in fact, hypolipidemia is commonly seen in this situation. RLP accumulation results when the *APOE2/2* genotype is accompanied by a second genetic or acquired defect that causes overproduction of VLDL such as obesity or diabetes (45,46), a decrease in RLP clearance, or a reduction in LDL receptor activity (e.g., hypothyroidism) (47). Thus, full phenotypic expression of remnant removal disease requires the presence of other environmental or genetic factors. Interestingly, the reduced uptake of RLPs by the liver results not only in accumulation of RLPs but also, for unclear reasons, in reduced conversion of VLDL and IDL to LDL (27,48). The high prevalence of premature coronary artery disease (43,44) in remnant removal disease is strongly supportive of a direct role for RLPs in atherogenesis. Patients with remnant removal disease, unlike those with familial hypercholesterolemia due to increased LDL, also have increased incidence of peripheral arterial disease (49), as do patients with diabetes.

Increased circulating RLPs are almost certainly related to increased CVD even in the absence of the specific remnant removal disorder. In patients with abnormally high triglyceride levels (e.g., obese patients with T2DM), RLPs have been claimed to be a risk factor for CVD distinct from LDL (6,50,51). Because RLPs are depleted in triglycerides as compared with chylomicrons and VLDL, elevated plasma triglyceride levels are not necessarily an indication of elevated RLPs. Hence, it is possible that increased concentrations of RLPs contribute to atherogenic risk even in subjects with normal triglyceride levels.

Cholesterol is the major lipid that accumulates in atherosclerotic lesions. RLPs have a greater cholesterol content per particle than LDL (7). Chylomicrons and VLDL particles are likely too large to effectively cross the endothelium that lines the artery wall and noneroded lesions of atherosclerosis, in contrast to the much smaller RLPs. Although RLPs are larger than LDL, they likely are small enough to cross the endothelial barrier, where they could be trapped by proteoglycans in the subendothelial space (7). Already in the early 1970s Zilversmit proposed that TRLs and remnants accumulate at arterial foci in proportion to the local concentrations of sulfated polysaccharides (21). In contrast to unmodified LDL, native RLPs promote rapid cholesterol accumulation by macrophages (52,53), suggesting that RLPs are much more potent on a per particle basis than LDL at producing macrophage foam cells in vivo. This might be due to increased uptake of the apoE enriched remnants by non-LDL receptor pathways (52). In summary, while these are good reasons to invoke RLPs in promoting atherosclerosis, uncertainty still exists as to their precise role in promoting atherosclerosis (Fig. 1, bottom).

Do RLPs Play a Role in Residual CVD Risk in Diabetes?

The major differences in lipid and lipoprotein profiles between T1DM and T2DM have previously been reviewed (54). The hallmark of dyslipidemia in T2MD is high plasma triglycerides and low HDL-C. LDL-C concentrations are normal or only slightly elevated, but the LDL particles are characteristically small and dense with triglyceride enrichment (55). T1DM dyslipidemia primarily occurs in the setting of dysglycemia. With diabetic ketoacidosis, absolute insulin deficiency results in hypertriglyceridemia with low HDL-C and LDL-C levels (56). Well-treated patients with T1DM tend to have normal lipid profiles (54).

T2DM is characterized by increased apoB secretion and elevated plasma VLDL triglycerides (9) (Fig. 1). RLPs, measured by ultracentrifugation and the antibody method alluded to earlier, have also been reported to be increased

in T2DM independent of the presence of dyslipidemia (57). The increased VLDL secretion is regulated largely by increased hepatic triglycerides rather than by chronically increased insulin signaling (58). Furthermore, diabetes prevents clearance of TRLs by reducing hepatic uptake of apoB-containing lipoproteins, including RLPs (59), and by increasing levels of apoC3 through relative insulin deficiency or perhaps hepatic insulin resistance (17). Furthermore, hepatic insulin resistance has been shown to result in impaired hepatic LRP1 translocation from intracellular vesicles to the plasma membrane, which could contribute to impaired hepatic clearance of TRLs (60,61) (Fig. 1). Accordingly, in postmenopausal women, diabetes has been shown to decrease the catabolism of chylomicron-derived RLPs, resulting in accumulation of RLPs in plasma (62). Some early studies in diabetic rats injected with radiolabeled chylomicrons demonstrated the accumulation of the cholesteryl ester but not the triglyceride components in plasma, suggesting that chylomicron-derived RLPs accumulate in diabetes (63). Elevated levels of RLPs (detected by electrophoresis) also were observed in cholesterol-fed diabetic dogs (64). However, the role of RLPs per se in diabetic dyslipidemia is unclear, in part due to the difficulty in specifically measuring RLPs. For the same reasons, the potential causative role for RLPs in mediating increased CVD risk associated with diabetes also remains unclear.

How could RLPs accelerate CVD in the setting of diabetes? Early studies identified increased binding of chylomicrons/chylomicron remnants to the artery wall despite a lack of detectable accumulation of RLPs in plasma (65). These findings are consistent with those of a subsequent study showing that retention of injected radiolabeled chylomicron remnants by the arterial intima in diabetic rabbits and rats correlated with hyperglycemia due to insulin deficiency (66). These early studies suggested that entrapment of RLPs within the subendothelial space could in part explain the increased prevalence of atherogenesis in diabetes. Furthermore, macrophage uptake of RLPs from diabetic patients, isolated by immunoaffinity gel using the method described above (33) followed by ultracentrifugation, correlated with the degree of glycemic control in those subjects (67). However, although review articles suggest a role of RLPs in the relationship of diabetic dyslipidemia with the accelerated CVD seen in diabetes (6,13), definite studies supporting the role of RLPs are needed because most studies do not differentiate RLPs from TRLs, for which there is strong evidence of an association with CVD (13). Moreover, very little is known about RLP levels in normolipidemic subjects with or without diabetes, other than those with the apoE2/E2 isoform discussed earlier.

Recent studies have revealed that apoC3 predicts CVD risk in two independent cohorts of subjects with T1DM and fasting plasma triglyceride levels close to the normal range (17,18). Increased plasma apoC3 was associated with a significant increase in CVD risk; this risk was independent of diabetes duration and HbA_{1c} as well as LDL-C and HDL-C but not of plasma triglycerides (17). Moreover, apoC3, apoE, and apoB accumulated in the atherosclerotic lesions of diabetic mice, consistent with a role for RLPs in atherogenesis (17) (Fig. 1). Macrophages isolated from the peritoneum of diabetic mice contained increased levels of cholesteryl ester, and this accumulation was prevented by suppression of hepatic apoC3 expression (17). Taken together, these observations raise the possibility that apoC3 is an important risk factor for CVD in patients with diabetes and that RLPs containing apoC3 and apoE may play a key role in atherogenesis.

Conclusion and Remaining Questions

A causative role of circulating triglyceride levels and CVD has been debated for at least 50 years. Recent genetics studies and analyses of subgroups in fibric acid trials are supportive of the triglyceride/CVD hypothesis (7). In addition, beneficial effects of n-3 fatty acids in REDUCE-IT (68) and JELIS (69) have been interpreted in this light. However, n-3 fatty acids are likely to affect processes other than triglyceride levels, and in REDUCE-IT, benefits occurred in subjects whose triglyceride levels were not elevated. Moreover, aside from reducing triglycerides (primarily in VLDL), it is likely that these treatments also reduce RLPs.

Despite decades of experimental data linking RLPs to atherogenesis in animal models and humans (70), the impact of RLPs as causal mediators in CVD associated with hypertriglyceridemia in patients with T2DM is still uncertain. A major limitation of addressing the role of RLPs in CVD is the difficulty in accurately quantifying levels of these lipoproteins, and therefore the number of studies of RLPs' causal role in diabetic CVD is limited. Once a suitable and specific method becomes available, it will be important to also assess their role as a CVD risk factor in subjects with normal levels of cholesterol and triglycerides. Furthermore, it is becoming clear that not all hypertriglyceridemia is associated with elevated levels of RLPs and that RLPs may be elevated even in people with normal triglycerides and cholesterol (e.g., those with T1DM). New methods are critically needed that can specifically and accurately quantify RLPs derived from different metabolic pathways and in subjects with relatively normal lipid levels to test the long-standing hypothesis that RLPs mediate residual CVD risk associated with diabetes and in other disorders.

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References

1. Sarwar N, Gao P, Seshasai SR, et al.; Emerging Risk Factors Collaboration. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular

disease: a collaborative meta-analysis of 102 prospective studies. Lancet 2010; 375:2215–2222
2. de Ferranti SD, de Boer IH, Fonseca V, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Diabetes Care 2014;37:2843–2863
3. Ahlqvist E, Storm P, Käräjämäki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6:361–369
4. Yahagi K, Kolodgie FD, Lutter C, et al. Pathology of human coronary and carotid artery atherosclerosis and vascular calcification in diabetes mellitus. Arterioscler Thromb Vasc Biol 2017;37:191–204
5. Nicholls SJ, Tuzcu EM, Kalidindi S, et al. Effect of diabetes on progression of coronary atherosclerosis and arterial remodeling: a pooled analysis of 5 intravascular ultrasound trials. J Am Coll Cardiol 2008;52:255–262
6. Xiao C, Dash S, Morgantini C, Hegele RA, Lewis GF. Pharmacological targeting of the atherogenic dyslipidemia complex: the next frontier in CVD prevention beyond lowering LDL cholesterol. Diabetes 2016;65:1767–1778
7. Sandesara PB, Virani SS, Fazio S, Shapiro MD. The forgotten lipids: triglycerides, remnant cholesterol, and atherosclerotic cardiovascular disease risk. Endocr Rev 2019;40:537–557
8. Brahm A, Hegele RA. Hypertriglyceridemia. Nutrients 2013;5:981–1001
9. Ginsberg HN. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. Diabetes Care 1991;14:839–855
10. Goldstein JL, Hazzard WR, Schrott HG, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. I. Lipid levels in 500 survivors of myocardial infarction. J Clin Invest 1973;52:1533–1543
11. Miller M, Stone NJ, Ballantyne C, et al.; American Heart Association Clinical Lipidology, Thrombosis, and Prevention Committee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292–2333
12. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA 2007;298:299–308
13. Chapman MJ, Ginsberg HN, Amarenco P, et al.; European Atherosclerosis Society Consensus Panel. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J 2011;32:1345–1361
14. Toth PP. Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. Vasc Health Risk Manag 2016;12:171–183
15. Pollin TI, Damcott CM, Shen H, et al. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. Science 2008;322: 1702–1705
16. Pechlaner R, Tsimikas S, Yin X, et al. Very-low-density lipoprotein-associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III. J Am Coll Cardiol 2017;69:789–800
17. Kanter JE, Shao B, Kramer F, et al. Increased apolipoprotein C3 drives cardiovascular risk in type 1 diabetes. J Clin Invest 2019;130:4165–4179
18. Basu A, Bebu I, Jenkins AJ, et al.; Diabetes Control Complications Trial/Epidemiology of Diabetes Interventions Complications Research Group. Serum apolipoproteins and apolipoprotein-defined lipoprotein subclasses: a hypothesis-generating prospective study of cardiovascular events in T1D. J Lipid Res 2019;60:1432–1439
19. Ooi EM, Russell BS, Olson E, et al. Apolipoprotein B-100-containing lipoprotein metabolism in subjects with lipoprotein lipase gene mutations. Arterioscler Thromb Vasc Biol 2012;32:459–466
20. Sprecher DL, Knauer SL, Black DM, et al. Chylomicron-retinyl palmitate clearance in type I hyperlipidemic families. J Clin Invest 1991;88:985–994
21. Zilversmit DB. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. Circ Res 1973;33:633–638
22. Schneeman BO, Kotite L, Todd KM, Havel RJ. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. Proc Natl Acad Sci U S A 1993;90:2069–2073
23. Havel RJ, Kane JP, Kashyap ML. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. J Clin Invest 1973;52:32–38
24. Mjos OD, Faergeman O, Hamilton RL, Havel RJ. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. J Clin Invest 1975;56:603–615
25. Redgrave TG, Small DM. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. J Clin Invest 1979;64:162–171
26. Murdoch SJ, Boright AP, Paterson AD, et al.; DCCT/EDIC Research Group. LDL composition in E2/2 subjects and LDL distribution by Apo E genotype in type 1 diabetes. Atherosclerosis 2007;192:138–147
27. Chait A, Brunzell JD, Albers JJ, Hazzard WR. Type-III hyperlipoproteinaemia ("remnant removal disease"). Insight into the pathogenetic mechanism. Lancet 1977;1:1176–1178
28. Stanford KI, Bishop JR, Foley EM, et al. Syndecan-1 is the primary heparan sulfate proteoglycan mediating hepatic clearance of triglyceride-rich lipoproteins in mice. J Clin Invest 2009;119:3236–3245
29. Musliner TA, Giotas C, Krauss RM. Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects. Arteriosclerosis 1986;6:79–87
30. Patsch JR, Jackson RL, Gotto AM Jr. Evaluation of the classical methods for the diagnosis of type III hyperlipoproteinemia. Klin Wochenschr 1977;55:1025–1030
31. Hazzard WR, Porte D Jr, Bierman EL. Abnormal lipid composition of chylomicrons in broad-beta disease (type 3hyperlipoproteinemia). J Clin Invest 1970; 49:1853–1858
32. Campos E, Nakajima K, Tanaka A, Havel RJ. Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. J Lipid Res 1992;33: 369–380
33. Leary ET, Wang T, Baker DJ, et al. Evaluation of an immunoseparation method for quantitative measurement of remnant-like particle-cholesterol in serum and plasma. Clin Chem 1998;44:2490–2498
34. Nakajima K, Tokita Y, Sakamaki K, et al. Triglyceride content in remnant lipoproteins is significantly increased after food intake and is associated with plasma lipoprotein lipase. Clin Chim Acta 2017;465:45–52
35. Miyauchi K, Kayahara N, Ishigami M, et al. Development of a homogeneous assay to measure remnant lipoprotein cholesterol. Clin Chem 2007;53:2128–2135
36. Schaefer EJ. Limitations of automated remnant lipoprotein cholesterol assay for diagnostic use. Clin Chem 2009;55:2061–2062; author reply 2062–2063
37. Otvos J. Measurement of triglyceride-rich lipoproteins by nuclear magnetic resonance spectroscopy. Clin Cardiol 1999;22(Suppl.):II21–II27
38. Balling M, Langsted A, Afzal S, Varbo A, Davey Smith G, Nordestgaard BG. A third of nonfasting plasma cholesterol is in remnant lipoproteins: lipoprotein subclass profiling in 9293 individuals. Atherosclerosis 2019;286:97–104
39. Caulfield MP, Li S, Lee G, et al. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. Clin Chem 2008;54:1307–1316
40. Hutchins PM, Ronsein GE, Monette JS, et al. Quantification of HDL particle concentration by calibrated ion mobility analysis. Clin Chem 2014;60:1393–1401
41. Nakajima K, Nagamine T, Fujita MQ, Ai M, Tanaka A, Schaefer E. Apolipoprotein B-48: a unique marker of chylomicron metabolism. Adv Clin Chem 2014; 64:117–177
42. Nordestgaard BG. A new start for triglycerides and remnant cholesterol-nonfasting. Clin Chem 2017;63:1418–1419
43. Morganroth J, Levy RI, Fredrickson DS. The biochemical, clinical, and genetic features of type III hyperlipoproteinemia. Ann Intern Med 1975;82:158–174
44. Koopal C, Retterstøl K, Sjouke B, et al. Vascular risk factors, vascular disease, lipids and lipid targets in patients with familial dysbetalipoproteinemia: a European cross-sectional study. Atherosclerosis 2015;240:90–97
45. Mahley RWRS Jr. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In *The*

Metabolic Basis of Inherited Disease. Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. New York, McGraw-Hill, 1989, p. 1195
46. Koopal C, Marais AD, Visseren FL. Familial dysbetalipoproteinemia: an underdiagnosed lipid disorder. Curr Opin Endocrinol Diabetes Obes 2017;24:133–139
47. Feussner G, Ziegler R. Expression of type III hyperlipoproteinaemia in a subject with secondary hypothyroidism bearing the apolipoprotein E2/2 phenotype. J Intern Med 1991;230:183–186
48. Chait A, Hazzard WR, Albers JJ, Kushwaha RP, Brunzell JD. Impaired very low density lipoprotein and triglyceride removal in broad beta disease: comparison with endogenous hypertriglyceridemia. Metabolism 1978;27:1055–1066
49. Koopal C, Geerlings MI, Muller M, et al.; SMART Study Group. The relation between apolipoprotein E (APOE) genotype and peripheral artery disease in patients at high risk for cardiovascular disease. Atherosclerosis 2016;246:187–192
50. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. Lancet 2008;371:1800–1809
51. Krauss RM, Lindgren FT, Williams PT, et al. Intermediate-density lipoproteins and progression of coronary artery disease in hypercholesterolaemic men. Lancet 1987;2:62–66
52. Van Lenten BJ, Fogelman AM, Jackson RL, Shapiro S, Haberland ME, Edwards PA. Receptor-mediated uptake of remnant lipoproteins by cholesterol-loaded human monocyte-macrophages. J Biol Chem 1985;260:8783–8788
53. Whitman SC, Miller DB, Wolfe BM, Hegele RA, Huff MW. Uptake of type III hypertriglyceridemic VLDL by macrophages is enhanced by oxidation, especially after remnant formation. Arterioscler Thromb Vasc Biol 1997;17:1707–1715
54. Subramanian S, Chait A. Dyslipidemia in diabetes. In *Encyclopedia of Endocrine Diseases*. 2nd ed. Huhtaniemi I, Martini L, Eds. Oxford, U.K., Academic Press, 2019, p. 186–198
55. Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia 2015;58:886–899
56. Vergès B. Lipid disorders in type 1 diabetes. Diabetes Metab 2009;35:353–360
57. Yoshino G, Hirano T, Kazumi T. Atherogenic lipoproteins and diabetes mellitus. J Diabetes Complications 2002;16:29–34
58. Moon BC, Hernandez-Ono A, Stiles B, Wu H, Ginsberg HN. Apolipoprotein B secretion is regulated by hepatic triglyceride, and not insulin, in a model of increased hepatic insulin signaling. Arterioscler Thromb Vasc Biol 2012;32:236–246
59. Haas ME, Attie AD, Biddinger SB. The regulation of ApoB metabolism by insulin. Trends Endocrinol Metab 2013;24:391–397
60. Laatsch A, Merkel M, Talmud PJ, Grewal T, Beisiegel U, Heeren J. Insulin stimulates hepatic low density lipoprotein receptor-related protein 1 (LRP1) to increase postprandial lipoprotein clearance. Atherosclerosis 2009;204:105–111
61. Gordts PL, Nock R, Son NH, et al. ApoC-III inhibits clearance of triglyceride-rich lipoproteins through LDL family receptors. J Clin Invest 2016;126:2855–2866
62. Dane-Stewart CA, Watts GF, Barrett PH, et al. Chylomicron remnant metabolism studied with a new breath test in postmenopausal women with and without type 2 diabetes mellitus. Clin Endocrinol (Oxf) 2003;58:415–420
63. Redgrave TG, Snibson DA. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. Metabolism 1977;26:493–503
64. Wilson DE, Chan IF, Elstad NL, et al. Apolipoprotein E-containing lipoproteins and lipoprotein remnants in experimental canine diabetes. Diabetes 1986;35:933–942
65. Staprans I, Pan XM, Rapp JH, Feingold KR. Chylomicron and chylomicron remnant metabolism in STZ-induced diabetic rats. Diabetes 1992;41:325–333
66. Proctor SD, Pabla CK, Mamo JC. Arterial intimal retention of pro-atherogenic lipoproteins in insulin deficient rabbits and rats. Atherosclerosis 2000;149:315–322
67. Tomono S, Kawazu S, Kato N, et al. Uptake of remnant like particles (RLP) in diabetic patients from mouse peritoneal macrophages. J Atheroscler Thromb 1994;1:98–102
68. Bhatt DL, Steg PG, Miller M, et al.; REDUCE-IT Investigators. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med 2019;380:11–22
69. Handelsman Y, Shapiro MD. Triglycerides, atherosclerosis, and cardiovascular outcome studies: focus on omega-3 fatty acids. Endocr Pract 2017;23:100–112
70. Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979;60:473–485