Remnant Lipoproteins: Measurement and Clinical Significance

In this issue of the Journal, Chan et al. (1) make the significant observation, by three distinct measures, that the concentrations of remnant lipoproteins (RLPs) in plasma are increased in patients with obesity. This increase in RLPs could explain, in part, the increased cardiovascular risk associated with obesity (2). To date, although LDL- and HDL-cholesterol are clearly accepted as independent risk factors for premature atherosclerosis, the role of plasma triglycerides as a risk factor remains somewhat controversial.

Plasma triglycerides are clearly a measure of triglyceride-rich lipoproteins (TRLs), which derive from both the intestine and the liver. Measurement of plasma triglycerides, however, does not distinguish the various subtypes of TRLs, which clearly have various degrees of atherogenicity. Triglyceride-rich RLPs are formed in the circulation when chylomicrons of intestinal origin [with apolipoprotein B-48 (apo B-48)] and VLDL of hepatic origin (with apo B-100) are converted by lipoprotein lipase (and to a lesser extent by hepatic lipase) into smaller and more dense particles. Compared with their nascent precursors, RLPs are depleted of triglycerides, phospholipids, and apo C and are enriched in cholesteryl esters and apo E and are believed to be more atherogenic than the larger TRLs (3).

Several lines of evidence have implicated RLPs in premature atherosclerosis. In fact, the prototypic disorder of remnant metabolism, type III dyslipidemia, is associated with accelerated atherosclerosis (4). Other evidence incriminating remnants as proatherogenic factors include the following: increased intermediate-density lipoprotein (IDL) concentrations have been associated with an increased incidence or recurrence of coronary artery disease (CAD) (3). Increased IDL is also found in diseases associated with accelerated atherosclerosis, such as type III dyslipidemia, type 2 diabetes mellitus, end stage renal disease (ESRD), and familial combined hyperlipidemia. Several previous reports have linked various measures of remnants to CAD in controlled angiographic follow-up trials (5).

Further emphasis on RLPs was highlighted by the National Cholesterol Education Panel report, which recommended calculation of non-HDL cholesterol as a measure of RLPs (6). Although non-HDL cholesterol would clearly provide a measure of RLPs, it also is a measure of triglyceride-rich particles, which are not atherogenic. Nonetheless, it is clearly the first step in emphasizing the relevance of RLPs in evaluating cardiovascular risk.

Because of the heterogeneity of TRLs, measurement of RLPs has been difficult (3). RLPs can be identified, separated, and quantified according to differences in density, charge, size, specific lipid components, apolipoprotein composition, and/or apolipoprotein immunospecificity. Accurate quantification of plasma remnants is complicated because they are difficult to differentiate from their triglyceride-rich precursors, their rapid catabolism produces low concentrations in plasma, and at any given time, they are heterogeneous in size, density, and composition because they are present at various stages of catabolism.

The traditional method to separate RLPs is ultracentrifugation and measurement of cholesterol in the IDL fraction (3, 5). Numerous cross-sectional studies have shown that patients with CAD or diabetes or who have experienced myocardial infarctions have increased plasma IDL-cholesterol compared with controls (3). Plasma IDL concentrations have been related to the extent and severity of angiographically assessed coronary atherosclerosis in cross-sectional and longitudinal studies (3, 5). Measurement of IDL-cholesterol by ultracentrifugation is not available in most clinical laboratories. Furthermore, the precision of the assay is far from optimal, and between-laboratory variability can be high.

RLPs can also be separated according to charge by agarose gel electrophoresis (double pre-B lipoproteins) or capillary isoelectric focusing, or to their size as midband lipoproteins (between VLDL and LDL bands) by 3% polyacrylamide or gradient gel electrophoresis. In addition, the concentrations of retinyl esters after ingestion of a fat-rich meal containing vitamin A have been used as markers of the presence of apo B-48-containing RLPs of intestinal origin. RLPs can also be quantified on the basis of their apolipoprotein composition. These include measurement of the amount of apo B associated with plasma lipoproteins containing apo E (LpB:E) or quantification of apo E in apo B-containing lipoproteins (LpB:E), as well as measurement of apo C III or apo B-48. Although these measures appear to be reliable indices of RLPs and correlate with atherosclerosis, they suffer from certain deficiencies, as detailed in a recent review by Cohn et al. (3).

Recently, an immunoaffinity chromatography method was introduced for assaying RLP concentrations according to their apolipoprotein content and immunospecificity (7). This methodology provides a quantitative measure of remnant status and can easily be adapted to the clinical laboratory. In this assay, RLPs are separated from plasma by immunoaffinity chromatography with a gel containing an anti-apo A1 and a specific apo B-100 monoclonal antibody (JL-H antibody, which does not recognize apo B-48 lipoproteins). The former antibody recognizes all HDL and any newly synthesized chylomicrons containing apo A1, whereas the latter antibody recognizes all apo B-100-containing lipoproteins except for certain particles enriched in apo E. HDL, LDL, large chylomicrons, and most VLDL are thus retained by the gel. The unbound RLPs are made up of remnant-like VLDL containing apo B-100 and TRLs containing apo B-48. This assay was initially approved by the Food and Drug Administration for diagnosis of type III dyslipidemia, where a molar ratio of RLP-cholesterol (RLP-C) to total triglyceride >0.23

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(>0.10 when results are expressed in mg/dL) confirms a diagnosis (8); recently, it has also received approval for assessing CAD risk. The intra- and interassay CVs of this assay are <6% and 10%, respectively (9, 10). With this assay, plasma concentrations of RLP-C have been shown to be higher in patients with CAD, diabetes, or ESRD; in patients with coronary artery restenosis postangioplasty; and in cases of sudden cardiac death (3).

More importantly, RLP-C is increased at least threefold in patients with type III dyslipidemia (9). Increased RLP-C is a significant predictor of myocardial infarction in patients with vasospastic angina and has recently been shown to be strongly associated with angiographically verified progression of focal coronary atherosclerosis (11). The atherogenicity of RLPs is supported by the observations that RLPs can promote lipid accumulation by mouse peritoneal macrophages, stimulate whole-blood platelet aggregation, and impair endothelium-dependent vaso-relaxation. RLPs promote endothelial dysfunction, a key early event in atherogenesis, up-regulating endothelial production of intercellular adhesion molecule, vascular cell adhesion molecule, and tissue factor. Kugiyama et al. (12) have shown that high RLP concentrations predict coronary events in CAD patients independently of traditional risk factors. Recently, Karpe et al. (13) showed a significant correlation of RLP-C to carotid intimal medial thickness in a cohort of healthy 50-year-old men; this association was independent of plasma triglycerides and HDL-cholesterol. Masuoka et al. (14) showed that RLP-C concentrations were strongly associated with coronary artery stenosis in normotriglyceremic patients. Thus, this assay is a valid measure of RLPs that are proatherogenic and, in three prospective studies (12–14), has correlated with atherosclerosis or cardiovascular events.

In their report in this issue, Chan et al. (1) used four measures of remnant metabolism: RLP-C, apo B-48, apo C-III, and the fractional catabolic rate of a remnant emulsion. Although all four measures were abnormal in the obese patients, when the results were stratified for triglyceride concentrations, the best measure was RLP-C, which was increased in the patients with both high and normal triglycerides (<1.7 mmol/L). The authors documented significant correlations between RLP-C and both apo B-48 and apo C-III. Triglycerides accounted for <65% of the variance in these three markers. The correlation between RLP-C and apo B-48 suggests that chylomicron remnants account for only 36% of RLP-C. This study further underscores the validity of RLP-C as a measure of remnant metabolism.

RLP-C can be modulated by lipid-lowering therapy. Karpe et al. (11) examined the effect of gemfibrozil therapy on median RLP-C in the 2-year randomized, placebo-controlled Lipid and Coronary Angiography Trial (LOCAT). Gemfibrozil treatment reduced median RLP-C concentrations by 34% and was associated with a decrease in vein-graft stenosis. We have recently shown in a randomized crossover trial that simvastatin and atorvastatin effectively reduced RLP-C in patients with combined hyperlipidemia (15). Our results also suggest a strong correlation between reductions in plasma triglycerides and reductions in RLP-C. However, the reduction in triglycerides accounted for only ~44% of the reduction in RLP-C.

Thus, each of these measures of remnant status identifies a different class of TRLs, but each of these TRLs represents a component of the overall remnant risk and has been shown, in many studies, not only to be atherogenic but also to predict atherosclerotic risk. Furthermore, this risk may be modulated by established therapies such as statin, fibrates, and possibly niacin therapy. In conclusion, the RLP-C assay is precise, is easily adaptable to the clinical laboratory, and is a valid measure of atherogenic TRL remnants, especially in patients with metabolic abnormalities such as obesity, the metabolic syndrome, type 2 diabetes, and ESRD.

The holy grail is to find a precise, accurate, cost-effective, standardized assay for RLPs that can easily be incorporated in the clinical laboratory. Currently, although the RLP-C assay fulfills most of these criteria, it is desirable that its standardization be optimized and that more studies be conducted to show that it predicts cardiovascular events independently. In addition, clinical findings by one technique may not be extrapolated to all other techniques.

References

