EDITORIAL COMMENT

Triglyceride-Rich Lipoprotein Remnant Levels and Metabolism

Time to Adopt These Orphan Risk Factors?

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Among the major components of the standard plasma lipid profile, the total triglyceride (TG) level is generally considered the weakest risk factor for atherosclerosis, for which there are several likely contributing reasons. First, TG is readily metabolized and is not a major constituent of atherosclerotic plaque. Second, plasma TG varies greatly from day to day in the fasting state and even more in the generally unmeasured postprandial state. More importantly, there is considerable heterogeneity among TG-rich lipoproteins (TGRLs) ranging from highly atherogenic TGRL remnants to larger chylomicrons (and larger very low-density lipoproteins, VLDLs) too large to penetrate the artery wall (1) and hence ineffectual substrates for plaque progression (2). The preferential excess of these very large, nonatherogenic TGRLs above a plasma TG of approximately 600 to 800 mg/dl (3) paradoxically results in generally low (4), albeit not always absent (5), atherosclerosis risk at the highest TG levels. Thus, TG contrasts sharply with cholesterol, with its limited metabolism, well-established involvement in atherogenesis, and relative constancy in plasma level, such that risk even from somewhat heterogeneous low-density lipoprotein (LDL) particles is a strong log-linear function (6).

Despite these differences, however, total TG levels are important because the TGRLs that carry the majority of plasma TG carry more cholesterol molecules per particle than does LDL. Plasma TG levels, therefore, predict atherosclerosis because they are an indirect measure of the cholesterol content of potentially atherogenic TGRLs.

Lipolysis of TG in nascent TGRLs converts them to “remnant” lipoproteins, and atherosclerosis risk correlates both with remnant levels and metabolism. Each of these two parameters is highlighted by two reports in this issue of the Journal (7,8). First, TGRL remnant levels carry a strong risk because they have elevated cholesterol content and extended plasma residence time, and thus greater atherogeneity compared with their precursors. This effect is most clear in type III dyslipidemia, with greatly reduced remnant removal rates causing severely increased remnant levels and striking coronary artery disease (CAD) risk (9). Less dramatically elevated remnant levels strongly predict prevalent atherosclerosis in patients with (10) and without diabetes mellitus (11). The report of Fukushima et al. (7) in this issue of the Journal adds to this literature by showing that TGRL remnant levels predict CAD cross-sectionally and prospectively in diabetes.

There are several methods for estimating plasma levels of TGRL remnants. Classically, in the ultracentrifugal density <1.006 g/ml fraction either a cholesterol mass >0.3 times total plasma TG (cholesterol-rich VLDL by beta quantification) or the presence of a broadened band of beta mobility on agarose gel electrophoresis (beta-VLDL) signals excess remnant levels (9). These assays, however, are generally available only in lipid reference laboratories. Another method, suggested in the National Cholesterol Education Program-Adult Treatment Panel III guidelines (12) to capture risk associated with remnants, is the calculation of non–high density lipoprotein (HDL) cholesterol. This simply lumps cholesterol content of remnants together with that of all other particles besides HDL (12) and is to be used in place of LDL-cholesterol when the plasma TG exceeds 200 mg/dl. One obvious drawback of non–HDL-cholesterol measurement is that it cannot distinguish the broad variability of atherogenicity among the many non-HDL particles. Non–HDL-cholesterol is particularly misleading when excess (nonatherogenic) chylomicrons carry plasma TG over approximately 1,000 mg/dl.

Recently, a convenient immunologic method purported to isolate remnants has been developed. High-density lipoprotein and nascent chylomicrons are removed using immobilized antibodies against apolipoprotein (apo) A-I. Most LDLs and VLDLs are then removed using monoclonal antibody directed against a middle epitope of apo B-100. Chylomicron remnants (with apo B-48 but without the apo B-100 epitope) and some VLDL enriched in apo E remain unbound, constituting “remnant-like particles” (RLP), and their cholesterol content is measured as “RLP-C” (13). The mechanism(s) by which these apo-B-100–containing VLDLs (and some LDLs) fail to bind to the anti-apo B-100 monoclonal antibodies is unknown. The heterogeneity among RLPs is great and varies with patient phenotype (14). Remnant-like particle-cholesterol may not always add to risk information available from plasma TG because the two generally are highly correlated (15,16). Nevertheless, the RLP fraction appears enriched in true TGRL remnants (17) and can be positively associated with CAD risk independent of plasma TG (18). Although RLPs are of diverse origins and composition, RLP-C correlates strongly with the related lipid parameter of VLDL-cholesterol plus intermediate–density lipoprotein-cholesterol by ultracentrifugation or native gradient gel electrophoresis (19) and does...
not correlate with unrelated parameters such as LDL-cholesterol or LDL apo B (11). Remnant-like particle-cholesterol levels are elevated in most states of increased atherosclerosis risk, being higher in men than in women (11), in older than in younger subjects (11), in postmenopausal than in premenopausal subjects (11), postprandially (11), in obesity (20), in impaired glucose tolerance (21), in diabetes mellitus (22), and in end-stage renal disease and hemodialysis (11,22). More importantly, RLP-C is also elevated in atherosclerosis, as defined by carotid intima-media thickness (18) and by coronary angiography and clinical criteria (11,19). Most recently, Fukushima et al. (7) in this issue of the Journal report higher RLP-C in diabetics with CAD at baseline and in prospective follow-up, apparently independent of other standard risk factors, including total TG.

Remnants may be atherogenic by enhancing macrophage lipid content, inducing platelet aggregation, impairing endothelium-dependent coronary vasodilation, and activating endothelial adhesion molecule expression (11). Treatment with HMG-CoA reductase inhibitors (“statins”) lowers RLP-C levels (23), thus possibly constituting one of their anti-atherosclerotic mechanisms.

Triglyceride-rich lipoprotein lipolysis produces remnants and remnant uptake eliminates them. These two processes regulate remnant levels and thereby contribute importantly to atherosclerosis risk. In addition, remnant production and removal may have other effects on atherogenesis. In contrast to the considerable data linking TGRL remnant levels with atherosclerosis, the relationship between remnant metabolism and atherosclerosis is much less clear. For example, severe hypertriglyceridemia due to severely impaired lipolysis from complete deficiency of lipoprotein lipase (LPL) probably does not increase CAD risk (4). In contrast, heterozygous LPL deficiency, frequently associated with moderately increased TG as well as modest reductions in HDL-cholesterol, may increase CAD risk (5). Further, a common LPL gene variant with increased lipolytic activity is associated with lower CAD risk (24).

Despite the importance of LPL activity, many other factors such as particle content of apolipoprotein C-I, C-II, and C-III help regulate TGRL lipolysis (25), the overall rate of which might be a better CAD risk predictor. In this issue of the Journal, Sposito et al. (8) measured TG lipolysis in vivo by the turnover of an artificial chylomicronlike emulsion, radiolabeled both in the TG and cholesteryl-ester (CE) moieties, which appears to reflect metabolism of native chylomicrons. The disappearance, or fractional catabolic rate (FCR), of the TG component is a function both of lipolysis and of remnant particle removal, whereas the disappearance of the CE label is only a function of holoparticle clearance. Thus, one can estimate the rate of plasma TG removal by lipolysis by comparing the FCR of CE to that of TG. The expression \( 1 - (\text{CE FCR})/(\text{TG FCR}) \) is called the delipidation index (DI) (26) because it estimates in vivo lipolysis by correcting total TG disappearance (TG FCR) for its loss through remnant particle uptake (CE FCR).

Sposito et al. (8) studied 63 diabetic subjects with and 35 without angiographically proven CAD at baseline and then followed the former group for about four years with aggressive antianginal and statin therapies (LDL-cholesterol goal <100 mg/dl). The TG FCR, CE FCR, and DI were all reduced similarly in CAD subjects at baseline. For the first time, the ability of these parameters to predict future CAD was tested, and surprisingly, CE FCR (remnant holoparticle clearance) predicted neither angiographic nor clinical progression of CAD. Lipolysis (DI), however, strongly predicted both, even after inclusion of other standard risk factors in multivariate analysis. This suggests that reduced TGRL lipolysis might be more proatherogenic than reduced remnant clearance, despite the fact that TGRL lipolysis would be expected to reduce remnant levels whereas reduced remnant clearance would be expected to increase them. Given the aggressive treatment during follow-up, however, the lack of relationship between baseline CE FCR and CAD progression might simply be an artifact of statin-induced reductions in remnant levels. Unfortunately, we do not know if remnant levels are reduced in low-DI subjects (with or without CAD progression) because they were not measured in this or any other DI study of which we are aware.

If low lipolysis (DI) reduces remnant levels, how might it increase CAD progression? First and second, as it raises plasma TG levels (especially postprandially), it should impair arterial vasodilator capacity (27) and enhance procoagulant factors (28). Interestingly, TG levels reportedly predict CAD in statin-treated patients (29). Third, reduced lipolysis decreases generation of free fatty acids, which are natural activator ligands to alpha subclass peroxisome proliferator activated receptors (PPARs). This decreased PPARs activity should increase endothelial production of proinflammatory tumor necrosis factor-alpha and vascular cell adhesion molecule-1 (30). Fourth, because remnants must reach a certain degree of lipolysis to be recognized by remnant uptake receptors, reduced lipolysis and resulting increased plasma residence time would enhance TGRL availability for neutral-lipid exchange with LDL and HDL. Not only may the resulting decreases in LDL and HDL particle size promote atherosclerosis, but the clearance rate of HDL would be enhanced and its plasma levels decreased. Furthermore, excess CE enrichment of TGRL remnants would be proatherogenic, especially if the remnants were small enough to enter the arterial wall (31) and be taken up by the macrophage apo B-48 receptor (32). Unfortunately, detailed lipoprotein characterization to verify these expected compositional changes in remnants, LDL, and HDL has not been performed in any study of remnant metabolism of which we are aware.

In conclusion, RLP-C levels are a promising risk factor for which a practical and reproducible method of measurement is now available. Further studies are required to establish RLP-C levels as a clinically useful tool for diagnosis and follow-up of atherosclerosis risk. Randomized prospective trials of lipid-lowering treatment with reductions in clinical CAD events

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predicted by reductions in RLP-C levels independent of other risk factors would be particularly convincing. In contrast, the assessment of TGRL lipolysis (as well as LPL activity) is impractical even for large clinical trials, more so for routine clinical use. Nevertheless, the study of Sposito et al. (8) and another such report (33) have provided valuable insight into the likely contribution of TGRL lipolysis to CAD risk. Ideally, a feasible clinical assay of some parameter strongly correlated with TGRL lipolysis rates will be developed, perhaps even assessment of TG clearance by post-challenge TG level (or area under the curve) during a fat tolerance test or the measurement of some particular lipoprotein subclass. Triglyceride-rich lipoprotein lipolysis can be increased by fibrates and thiazolidinediones by different and perhaps complementary mechanisms (34), although this effect does not yet constitute a clinical indication for use of these agents.

The TGRL lipolysis rates and plasma levels of its resulting remnants appear to be important factors in atherosclerosis risk, and thus are potentially important targets in atheroprevention. These parameters are not yet, however, clinically useful tools for diagnosis and prevention of atherosclerosis.

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