

EDITORIAL COMMENT

## Remnant Cholesterol

### “Non-(HDL-C + LDL-C)” as a Coronary Artery Disease Risk Factor\*

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In this issue the *Journal*, Varbo et al. (1) demonstrate that irrespective of the fasting state, the cholesterol transported by remnant lipoproteins is a robust predictor of coronary artery disease (CAD) risk. Their findings provide clarification to the debate regarding triglyceride-rich lipoproteins and atherosclerosis (2). Overall, a consensus is emerging on the basis of clinical and population data and genetic studies that fasting and nonfasting plasma triglycerides are predictive of both CAD and stroke risk, although the relationship is attenuated after correction for associated variables, including high-density lipoprotein cholesterol (HDL-C). However, triglycerides per se are not considered to be direct culprits in the atherogenic process, hence the appropriate focus in this report on the cholesterol component of triglyceride-rich lipoproteins.

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Several population studies have demonstrated that non-HDL-C, a measure of the cholesterol content of all apolipoprotein B (apo B)-containing lipoproteins (including remnants), is a better marker of CAD risk than low-density lipoprotein cholesterol (LDL-C) alone (3–5). Similarly among statin-treated subjects, on-treatment levels of LDL-C, non-HDL-C, and apo B were each associated with risk for future major cardiovascular events, but the strength of this association was greater for non-HDL-C than for LDL-C or apo B, a marker of atherogenic particle number rather than cholesterol content (6). The National Cholesterol Education Program Adult Treatment Panel III recommended non-HDL-C as a secondary target in patients with triglycerides >200 mg/dl (2.2 mmol/l) (7).

To specifically address the hypothesis that nonfasting remnant cholesterol is causally related to coronary heart

disease risk, blood samples were collected at various times throughout the day, the majority 1 to 5 h after a meal. Remnant cholesterol was calculated as total cholesterol – HDL-C – LDL-C, essentially non-(HDL-C + LDL-C). As shown in Figure 3 in the paper, the observational hazard ratio (HR) for CAD was greater for subjects in the highest versus lowest quintile of remnant cholesterol (HR: 2.3; 95% confidence interval [CI]: 1.7 to 3.1) or remnant cholesterol/HDL-C (HR: 2.6; 95% CI: 2.1 to 3.2) compared with those in the upper versus lower quintile of LDL-C (HR: 1.8; 95% CI: 1.4 to 2.2). This may not be surprising given that remnant cholesterol levels displayed much greater variability than did LDL-C. HDL-C was also strongly predictive of CAD risk, with an HR of 2.5 (95% CI: 2.1 to 3.0) for subjects in the lowest versus highest HDL-C quintile, consistent with other data (5).

The investigators then used Mendelian randomization to support a causative role for remnant cholesterol in CAD. To do this, they chose a small number of single-nucleotide polymorphisms (SNPs) shown to be strongly associated with each of remnant cholesterol, remnant cholesterol/HDL-C, LDL-C, and HDL-C. The notable findings were that the numbers of risk alleles for remnant cholesterol, remnant cholesterol/HDL-C, and LDL-C were even more strongly associated with CAD risk than measured lipid levels. This observation concurs with data from previous genetic studies (8,9) and likely relates to the fact that the genetic determinants of a lipid trait are a more accurate representation of a subject's lifetime exposure compared with a single biochemical measure. In contrast, the number of risk alleles for reduced HDL-C showed no relationship to cardiovascular events.

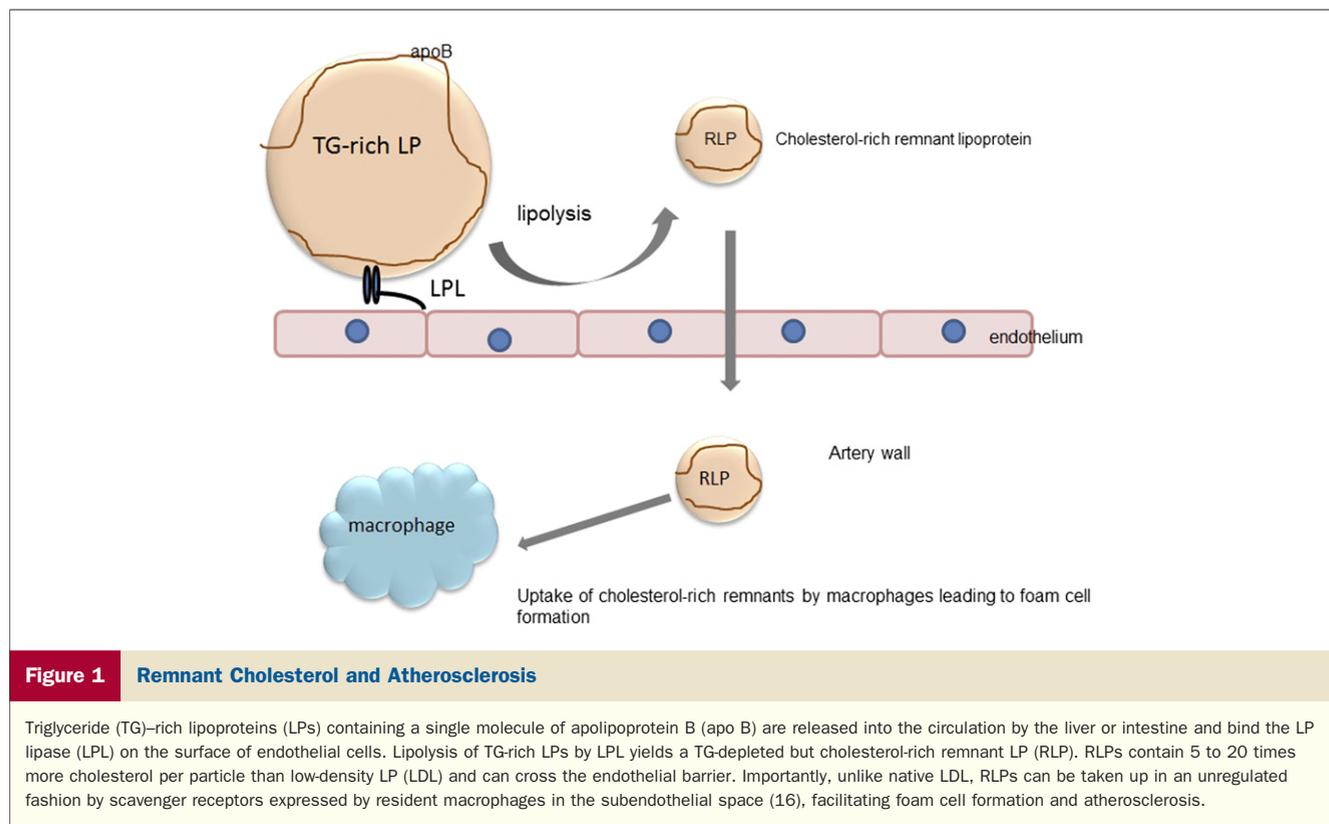
The strength of the analysis includes the large datasets, consisting of over 73,000 Copenhagen subjects, enrolled in 1 of 2 prospective studies or a case-control study with a total of 12,000 diagnosed with CAD. The results are plausible given that non-HDL-C is a robust marker of CAD risk (5). The novel features of this analysis include the use of nonfasting lipid measurements and the focus on the non-LDL-C component of non-HDL-C.

A 9- to 12-h fast is the usual stipulation for plasma lipid measurements. A nonfasting measurement would be more convenient for both patients and physicians and is already the standard recommendation in Denmark. Although plasma triglycerides vary considerably in the pre-prandial versus post-prandial state in subjects with even mild hypertriglyceridemia, this study demonstrates that there are relatively small diurnal fluctuations in remnant cholesterol. Of note, the Emerging Risk Factors Collaboration also demonstrated this, and that fasting status does not substantially alter the relationship of plasma lipids and lipoproteins to cardiovascular risk (5).

Varbo et al. (1) have exploited the principle of Mendelian randomization to support their findings. Mendelian randomization is based on the premise that because a genetic

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**Figure 1** Remnant Cholesterol and Atherosclerosis

Triglyceride (TG)-rich lipoproteins (LPs) containing a single molecule of apolipoprotein B (apo B) are released into the circulation by the liver or intestine and bind the lipoprotein lipase (LPL) on the surface of endothelial cells. Lipolysis of TG-rich LPs by LPL yields a TG-depleted but cholesterol-rich remnant LP (RLP). RLPs contain 5 to 20 times more cholesterol per particle than low-density LP (LDL) and can cross the endothelial barrier. Importantly, unlike native LDL, RLPs can be taken up in an unregulated fashion by scavenger receptors expressed by resident macrophages in the subendothelial space (16), facilitating foam cell formation and atherosclerosis.

variant is assigned randomly at birth, it is not susceptible to confounding environmental factors. If such a variant selectively influences a given trait and exhibits a concordant effect on an outcome associated with the trait, it can be inferred that the trait and outcome are causally related (10). However, a requirement for Mendelian randomization is lack of pleiotropy. A limitation of the present study is that some pleiotropic effects are evident for all 3 genes showing associations with remnant cholesterol and coronary heart disease risk. In large genome-wide association studies, *TRIB1* was also associated with LDL-C and HDL-C, *APOA5* with HDL-C and LDL-C (8), and *GCKR* (encoding the glucokinase receptor) with fasting glucose and diabetes risk (11).

Another limitation of this Mendelian randomization study is that the analyses were confined to a small number of SNPs and genes, including 3 SNPs (*TRIB1*, *GCKR*, and *APOA5*) for remnant cholesterol; 4 SNPs in a single gene, lipoprotein lipase (*LPL*) for “remnant cholesterol and HDL-C”; SNPs in 2 genes, *ABCA1* and *LIPC* (encoding hepatic lipase) for HDL-C; and SNPs in 3 genes (*LDLR*, *APOB*, and *PCSK9*) for LDL-C. Because different biological pathways lead to variability in these lipid fractions, the interpretation of the results should be limited to the processes regulated by the genes included in the analysis. Consistent with the present findings, previous large Mendelian randomization studies have not supported a causal relationship between common genetic variants associated with HDL-C (near *ABCA1*, *LCAT*, *LIPC*, or *LIPG*) and

cardiovascular disease (12,13), but it remains possible that SNPs altering expression or function of other genes in the HDL synthesis pathway such as *APOA1* might associate with altered CAD risk (14).

Importantly from a clinical point of view, there is abundant evidence that remnant lipoproteins are atherogenic. Remnants are known to cross the endothelial barrier (15) and have been identified in human arteries. Because of their larger size, they carry 5 to 20 times as much cholesterol per particle as LDL. Importantly, unlike native LDL, remnants can be taken up in an unregulated fashion by scavenger receptors expressed by resident macrophages in the subendothelial space (16), thus promoting foam cell formation (Fig. 1). What is clear from the study of Varbo et al. (1) is that irrespective of the fasting state, remnant cholesterol is an important component of “non-HDL-C” and a risk factor for CAD.

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