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Response

Lipoprotein Subclass Profiling Reveals Pleiotropy in the Genetic Variants of Lipid Risk Factors for Coronary Heart Disease

A Note on Mendelian Randomization Studies

We thank Würtz and colleagues for their positive comments on our recent paper (1) on remnant cholesterol (remnant-C) as a causal risk factor for ischemic heart disease. We naturally agree that lipoprotein metabolism is very complex, with many subclasses of lipoproteins and different lipid contents of these subclasses, making it difficult, if not impossible, to find genetic instruments completely without pleiotropic effects on the other lipoprotein subclasses. The various lipoprotein subclasses derived from the endogenous pathway are a continuum from large very-low-density lipoproteins excreted from the liver, which are then degraded into intermediate-density lipoproteins by lipolysis and exchange of lipids and apolipoproteins in plasma, which are then degraded further into low-density lipoproteins. The differentiation into these specific subclasses is somewhat arbitrarily defined from the early ultracentrifugation studies (2). Therefore, it seems unlikely that there are genetic variants that exclusively affect levels of just 1 lipoprotein subclass. In our study (1), we used combinations of genetic variants to minimize pleiotropy but of course did not completely avoid it; this limitation was also discussed in our paper and nicely demonstrated by Würtz and colleagues in their letter. Although the allele score of *TRIB1* (tribbles homolog-1), *GCKR* (glucokinase regulatory protein), and *APOA5* (apolipoprotein A-V) genetic variants used in our study as an instrument for nonfasting remnant-C alone also had small effects on the cholesterol content in the other lipoprotein subclasses, it was clearly associated with a much larger effect on nonfasting remnant-C levels as demonstrated in our Figure 4. This finding makes it unlikely that our results should be explained by pleiotropic effects only.

Würtz and colleagues have performed an elegant lipoprotein subclass profiling study by using nuclear magnetic resonance (NMR) spectroscopy in 10,547 fasting samples from young adults from the general population of Finland. This method is undoubtedly more detailed in differentiating between subclasses of lipoproteins and their lipid content than our simple calculation of nonfasting remnant-C as nonfasting total cholesterol minus low-density lipoprotein cholesterol minus high-density lipoprotein cholesterol; however, it is exactly the simplicity of our calculation that makes it clinically useful. Clinicians anywhere can use our method to estimate levels of nonfasting remnant-C in patients if they have measured a standard nonfasting lipid profile and, importantly, at no extra cost; lipoprotein subclass profiling with NMR spectroscopy, even though it is very informative, is not suitable for clinical purposes, because of the expenses and requirements of very specialized equipment, and not least because of the complicated interpretation of the results.

In our study (1), we included adults aged 20 to >100 years who were nonfasting at the time of blood sampling. This segment of the population has a broader range and is closer to the everyday state of the entire population (including with respect to other risk factors for ischemic heart disease) than the fasting adolescent and young adult segment examined by Würtz and colleagues; this choice of patient population may have influenced the generalizability of their results. Thus, although they demonstrated pleiotropy of the genetic instruments used by us, they probably did not illustrate the entire variation of lipoprotein subclass profiles as a function of these genetic variants. It is entirely possible that even more pleiotropy could be detected if samples taken at different times after a meal were analyzed, and if, for example, samples were analyzed separately for men and women, young and old. Even more complications can be added if one also considers the influence of remnant-C on other processes likely in the biological pathway; these include from elevated remnant-C to atherosclerosis to ischemic heart disease (i.e., as with inflammation caused by elevated remnant-C and not by elevated low-density lipoprotein cholesterol) (3).

After lowering of low-density lipoprotein cholesterol to recommended levels, there is still a substantial residual risk of ischemic heart disease. Some of this risk is probably explained by elevated levels of cholesterol in the other lipoprotein subclasses than low-density lipoprotein (i.e., remnant-C levels). It is therefore important that clinicians acknowledge and intervene on these other lipid

risk factors in addition to low-density lipoprotein cholesterol, and our simple way of estimating levels of nonfasting remnant-C makes this possible.

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The Addition of Niacin to Statin Therapy Improves High-Density Lipoprotein Cholesterol Levels But Not Metrics of Functionality

To the Editor: The role of niacin in the era of widespread statin use has been called into question by 2 recent clinical trials, AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health) and HPS2-THRIVE (Treatment of HDL to Reduce the Incidence of Vascular Events), indicating no added benefit when niacin was added to standard low-density lipoprotein (LDL) reduction therapy with simvastatin and ezetimibe (1).

Mass-based assessment of high-density lipoprotein cholesterol (HDL-C) levels may not fully capture substantial variability in HDL functional properties. Cholesterol efflux capacity, a marker of the ability of HDL to accept cholesterol from macrophages and thus facilitate reverse cholesterol transport, served as a stronger predictor of atherosclerotic burden than HDL-C levels in 2 independent cohorts (2). This inverse association with coronary disease prevalence was confirmed using a related assay in 2 additional cohorts by Li et al. (3); an unexpected direct association with prospective events awaits further confirmation. Furthermore, the HDL inflammatory index, a surrogate for HDL's antioxidant capacity, was impaired in patients in the midst of an acute coronary syndrome (4).

We conducted a study designed to assess the impact of niacin added to statin therapy on HDL-C levels, cholesterol efflux capacity, and the HDL inflammatory index. Samples were derived from a previously described randomized controlled trial (NCT00307307) (5). In brief, patients with carotid atherosclerosis were randomized to simvastatin 20 mg daily plus either placebo or extended-release (ER) niacin, titrated up to 2 g daily. HDL-C levels and functional parameters were assessed at baseline and after 6 months of therapy.

Cholesterol efflux capacity was quantified using a previously validated cell-based assay that quantifies the ability of apolipoprotein B–depleted plasma to accept radiolabeled cholesterol from J774 macrophages ex vivo (3). Similarly, the HDL inflammatory index measured the capacity of apolipoprotein B–depleted plasma to inhibit the oxidation of purified LDL-C (4). All assays were performed in duplicate. To control for interassay variation, sample values were normalized to a pooled plasma control run on each plate.

The association between HDL functional parameters and baseline biomarkers was assessed using Pearson correlation coefficients. Paired Student *t* tests were used to analyze the effect of pharmacotherapy on HDL parameters. These changes were compared with placebo using an analysis of covariance test using the patient's baseline value and treatment group as covariates.

Baseline and 6-month plasma samples were available in 39 patients, 19 in the simvastatin plus ER niacin group and 20 who received simvastatin plus placebo. Fifteen of the 19 patients (79%) randomized to receive ER niacin achieved the target dose of 2 g daily, with a mean achieved daily dose of 1.8 g. Baseline characteristics were similar to those of the study population as a whole and revealed no significant difference between treatment groups. The mean age of study participants was 71 years; 64% were male, 26% had diabetes, 69% had hypertension, 23% had a history of coronary artery disease, and 67% were taking a statin at baseline. Average total cholesterol was 185 mg/dl, with mean HDL-C and LDL-C of 46 mg/dl and 116 mg/dl, respectively. Median values for triglycerides and C-reactive protein were 135 mg/dl and 1.4 mg/l, respectively.

Substantial variation was noted across study participants in HDL functional parameters. Mean normalized cholesterol efflux capacity was 0.95, with a range of 0.57 to 1.54; average HDL inflammatory index value was 1.15, with range of 0.70 to 2.81. The mean