

HDL-C vs HDL-P: How Changing One Letter Could Make a Difference in Understanding the Role of High-Density Lipoprotein in Disease

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Before about 2006, most papers focusing on high-density lipoprotein (HDL)² contained an introductory sentence that touted its protective role against cardiovascular disease (CVD), usually via the reverse transport of cholesterol away from the vessel wall. This was based on a mountain of epidemiological evidence showing an inverse correlation between plasma concentrations of HDL cholesterol (HDL-C) and CVD as well as thousands of *in vitro* and animal model studies demonstrating the cholesterol-carrying capacity of the particles. More recently, however, confidence in a direct atheroprotective role for HDL has wavered. HDL-targeted drugs such as niacin and 2 different cholesteryl ester transfer protein (CETP) inhibitors have failed in clinical trials, despite raising HDL-C (nicely reviewed in (1)). Furthermore, recent Mendelian randomization analyses suggest that genetic anomalies that raise HDL-C fail to impart the CVD protections predicted by epidemiologic findings (2). This has prompted some to regard HDL as a marker or byproduct of other, more directly atheroprotective, mechanisms. On the other hand, large studies have shown that the cholesterol efflux capacity of primarily HDL-containing [i.e., low-density lipoprotein (LDL)-depleted] serum is a robust predictor of CVD, even better than HDL-C (3), suggesting that HDL's mobilization of cellular cholesterol may indeed be atheroprotective.

So why has it been so difficult to determine whether HDL plays a direct protective role in CVD? Some argue that the picture has been clouded by the way HDL has been traditionally quantified. The

HDL-C measurement targets only a single HDL component, and a relatively minor one at that: a maximum of 20% of HDL mass is cholesterol or its ester. Ideally, one would like to know the actual number of HDL particles (HDL-P) circulating in plasma, not simply how much cholesterol they are carrying. This is a relatively easy determination for LDL. Because each particle contains a single copy of apolipoprotein B (apoB), LDL-P is closely correlated with apoB concentrations. HDL, on the other hand, can contain any combination of nearly 90 different proteins (4), and although much of its protein mass is composed of apoAI, there can be anywhere from 1 to 5 copies on a given particle. Furthermore, HDL lipid compositions vary widely from a few percent up to 50% of particle mass. Thus, HDL-C has an inherent bias toward the larger, more cholesterol/lipid-rich particles and underestimates smaller, lipid-poor forms. This is critical, because recent evidence indicates that HDL is actually a collection of numerous subspecies that play distinct functional roles, not only in lipid homeostasis but also in inflammation, innate immunity, and even glucose control. As an example, pre- β forms of HDL contain relatively little lipid/cholesterol, but excel in mobilizing cellular cholesterol via cell surface transporters. HDL-P would include such particles, but HDL-C likely does not.

Up to now, there have been 2 primary methods for measuring HDL-P. The most widely used relies on proton nuclear magnetic resonance (NMR) (5). In NMR, lipid methyl groups emit resonances that are unique to the chemical environments in lipoprotein particles of different diameter, with the signal intensity being proportional to their abundance. Therefore, the analysis not only quantifies overall HDL-P, but also breaks down the contribution of size subspecies—small, medium, and large HDL, for example. In a recent subanalysis of the Multi-Ethnic Study of Atherosclerosis (MESA), the NMR-derived HDL-P value outperformed HDL-C in predicting cardiovascular risk (6). Importantly, this relationship held when LDL-P (also measured by NMR) was

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² Nonstandard abbreviations: HDL, high-density lipoprotein; CVD, cardiovascular disease; HDL-C, HDL cholesterol; CETP, cholesteryl ester transfer protein; LDL, low-density lipoprotein; HDL-P, HDL particles; apo, apolipoprotein; NMR, nuclear magnetic resonance; MESA, Multi-Ethnic Study of Atherosclerosis; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; IM, ion mobility; CIM, calibrated IM.

factored into the analysis. In the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), NMR-derived HDL-P also showed a stronger association with CVD than HDL-C or apoAI among subjects receiving the statin (7). A second method for measuring HDL-P is based on ion mobility (IM) spectrometry, where ionized lipoproteins are separated by size and charge in a flow of inert gas subjected to modulated electric fields (8). This approach was used to examine subjects in the Malmö Diet and Cancer Study, revealing an HDL-P-associated protection as well as a pattern of both LDL and HDL subspecies that was associated with risk (9). These studies highlight the potential of HDL-P measurement for revealing new information about which types of particles, and potentially which metabolic processes, are key players in the protection from atherosclerosis.

Despite the success of these HDL-P measurement strategies, questions remain about their overall accuracy. The NMR method routinely reports HDL-P concentrations to be in the range of 32–34 $\mu\text{mol/L}$ (7, 10) in normal individuals, whereas the IM method reports 5–6 $\mu\text{mol/L}$ (9, 11), a substantial disparity. Both methods were initially validated by comparison to biochemical determinations of HDL-C, apoAI, and HDL size by nondenaturing gel electrophoresis of plasma samples (note: the NMR deconvolution algorithm is proprietary, and it is not explicitly clear from the literature how it was developed). Although it is clear that the HDL-P abundance and size readouts from both methods show strong correlations with these parameters, the absolute accuracy (i.e., how well the HDL-P value matches the true abundance of HDL particles in plasma) is less well established. This is illustrated by examining the stoichiometry of apoAI molecules per HDL particle determined by each method. Given that normal humans have apoAI concentrations of approximately 1.6 g/L, the NMR data suggest that each HDL particle averages <2 copies of apoAI. On the other hand, IM predicts some 13 apoAI molecules per particle. Both differ from biochemical measurements of isolated HDL samples indicating an average of 3–4 apoAI molecules per particle (12, 13).

The report by Hutchins et al. in this issue of *Clinical Chemistry* directly addresses this HDL-P accuracy problem (14). Building on the IM approach, they used a set of purified proteins of known size and concentration to internally calibrate the method. This allowed for an accounting of the significant variability in the production of gas-phase ions that underpins the analysis, permitting the conversion of the relative IM signal intensity to an absolute concentration. They dubbed this calibrated ion mobility (CIM). Using a signal deconvolution paradigm that reveals large, medium, and small

HDL particles, they validated the method using gold nanoparticles and reconstituted HDL particles of highly defined size, concentration, and stoichiometry. They observed impressive correlations between the CIM value and known particle concentrations for both analytes. But most importantly, the experimental values matched the benchmark values within 15%, indicating good accuracy as well. The validated method was used to determine HDL-P in a cohort of 40 controls and 40 individuals with carotid cerebrovascular disease. Overall, 3 HDL subspecies were identified, with masses and diameters consistent with biochemical measurements. Furthermore, the average HDL-P concentration was consistent with 3–4 molecules of apoAI per particle. In line with the reports described above, the CIM-derived HDL-P value was significantly better than HDL-C for identifying afflicted individuals, even in the quite small sample size of 80 individuals.

Aside from the direct calibration and accuracy gains, CIM has an advantage over the NMR technique in that it is a direct measure of holo-particle size (i.e., using all components) rather than relying on signal generated by 1 component that can vary highly among HDL species (lipid). However, this also contributes to a major drawback of the CIM method, namely the requirement that HDL must be first isolated from other serum components before the analysis. NMR can be applied directly to serum samples. Because antibodies and other large plasma proteins overlap with many HDL size species, the HDL samples analyzed by Hutchins et al. were first isolated from serum by density ultracentrifugation. This is an issue for 3 reasons. First, the labor-intensive separation pretty much precludes the use of this assay in most standardized and high-throughput clinical testing settings. Second, it is well documented that ultracentrifugation can result in the loss of certain apolipoproteins, and a redistribution of HDL subspecies cannot be completely ruled out (15). Third, extrapolation of the CIM value determined on purified HDL samples back to circulating plasma HDL-P concentrations requires careful accounting of volumes, dilutions, and sample loss during the separation—all of which can lead to increased variability. Nevertheless, the approach has significant potential for accurately analyzing samples in small clinical studies at a single site.

The major strength of all 3 HDL-P methods is the ability to track the numbers of HDL particles and monitor changes in their size subspecies in response to disease. This may point to specific metabolic processes that underlie the formation of each subspecies and thereby provide new information on the pathways that, either directly or indirectly, tie HDL to CVD. Furthermore, these methods should prove

useful to track reversion to healthier lipoprotein patterns induced by experimental therapeutics. From a basic science perspective, CIM should also prove highly useful for monitoring HDL particle size changes in vitro during perturbations such as lipase or lipid transfer protein additions, for example. Finally, it is hoped that the practitioners of all the HDL-P methods discussed here will collaborate, perhaps by adopting a set of universal calibration standards or even using CIM as a reference method, to resolve discrepancies among the methods so that direct comparisons can eventually be made across studies.

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References

- Kingwell BA, Chapman MJ, Kontush A, Miller NE. HDL-targeted therapies: progress, failures and future. *Nat Rev Drug Discov* 2014;13:445–64.
- Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study. *Lancet* 2012;380:572–80.
- Khera AV, Cuchel M, Llera-Moya M, Rodrigues A, Burke MF, Jafri K et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med* 2011;364:127–35.
- Shah AS, Tan L, Lu LJ, Davidson WS. The proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *J Lipid Res* 2013.
- Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 2006;26:847–70.
- Mackey RH, Greenland P, Goff DC, Jr., Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2012;60:508–16.
- Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation* 2013;128:1189–97.
- Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH et al. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. *Clin Chem* 2008;54:1307–16.
- Musunuru K, Orho-Melander M, Caulfield MP, Li S, Salameh WA, Reitz RE et al. Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. *Arterioscler Thromb Vasc Biol* 2009;29:1975–80.
- El HK, Arsenault BJ, Franssen R, Despres JP, Hovingh GK, Stroes ES et al. High-density lipoprotein particle size and concentration and coronary risk. *Ann Intern Med* 2009;150:84–93.
- Krauss RM, Wojnooski K, Orr J, Geaney JC, Pinto CA, Liu Y et al. Changes in lipoprotein subfraction concentration and composition in healthy individuals treated with the CETP inhibitor anacetrapib. *J Lipid Res* 2012;53:540–7.
- Huang R, Silva RA, Jerome WG, Kontush A, Chapman MJ, Curtiss LK et al. Apolipoprotein A-I structural organization in high-density lipoproteins isolated from human plasma. *Nat Struct Mol Biol* 2011;18:416–22.
- Kontush A, Chapman MJ. High-density lipoproteins: structure, metabolism, function and therapeutics. New York: Wiley & Sons; 2012.
- Hutchins PM, Rosein GE, Monette JS, Pamir N, Wimberger J, He Y, et al. Quantification of high density lipoprotein particle concentration calibrated by calibrated ion mobility analysis. *Clin Chem* 2014.
- Kunitake ST, Kane JP. Factors affecting the integrity of high density lipoproteins in the ultracentrifuge. *J Lipid Res* 1982;23:936–40.