RESPONSE TO COMMENT ON MATHEW ET AL.

Therapeutic Lifestyle Changes Improve HDL Function by Inhibiting Myeloperoxidase-Mediated Oxidation in Patients With Metabolic Syndrome. Diabetes Care 2018;41:2431–2437

Anna V. Mathew[1] *and Subramaniam Pennathur*[1,2]

Diabetes Care 2019;42:e26–e27 | https://doi.org/10.2337/dci18-0053

We thank Holzer and Marsche (1) for their interest in our work on the impact of therapeutic lifestyle changes (TLC) on HDL function in patients with metabolic syndrome (2). They suggest that a modified isolation technique for HDL2 and HDL3 proposed by their group removes proteins such as albumin (most abundant plasma protein) and apolipoprotein (apo)B (the major constituent of LDL), thereby enhancing HDL purity. It is well known that HDL protein composition is highly heterogeneous, and different HDL isolation techniques—such as sequential ultracentrifugation (KBr or D_2O/sucrose), size exclusion chromatography, immune affinity capture, ion exchange chromatography, or isoelectric focusing—yield variable combinations of ~95 proteins, as reviewed (3) and as updated periodically on the website http://homepages.uc.edu/~davidswm/HDLproteome.html. These proteins include albumin and apoB, which have been routinely identified in multiple studies as HDL-associated proteins, including in our previous work, implying that these are real associations and not an artifact (3–5). Indeed, each isolation technique "biases" the analysis toward enrichment of certain protein subsets. For example, a D_2O/sucrose buffer, which utilizes physiologic ionic strength compared with classical KBr ultracentrifugation (used in our study), yields a distinct subset of HDL proteome content in part related to the high ionic strength of KBr. Thus, we contend that it is more important to be consistent with the method of isolation and not to change methodology, especially when comparing proteome content across different studies. In our study (2), the primary goal was to understand the effect of TLC on the entire HDL fraction in terms of its function, the impact of oxidative stress, and differential protein content. The secondary goal was to compare patients in the current study to previous patient populations we have analyzed (cardiovascular disease and autoimmune disease, among others) to understand the biological impact of metabolic syndrome and the effect of TLC. Therefore, we focused on the entire density range of 1.063 to 1.210 g/mL without fractionation of the individual HDL subfractions (HDL2 and HDL3). We used a standard, reliable, and reproducible KBr ultracentrifugation protocol previously published by our group (4,5) and therefore believe these studies are highly reproducible in our hands. Additionally, we routinely monitor the apoA-I content of the fractions by immunoassays (55–65% protein by quantitative Western blots) to confirm purity. The HDL proteome content described in our recent article (2) is well aligned with that of our previous studies (4,5) and that of others (3). Importantly, we would like to emphasize that the raw spectral counts provided by shotgun proteomic analyses in this study are not quantitative, and therefore inferring percentage protein content from raw spectral counts can be misleading. Appropriate quantitative techniques such as targeted proteomics using isotope dilution, selected reaction monitoring studies using isotopically labeled peptides, or antibody-based immunoassay studies are needed to obtain quantitative results. As we acknowledge in our article (2), we believe the lack of significant proteome changes is more likely related to the small sample size rather than isolation protocols. We agree that future studies should focus on larger cohorts and systematically test HDL protein heterogeneity and its link with HDL function.

Funding. This work was supported in part by grants from the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (P30DK081943 and DK089503) and National Heart, Lung, and Blood Institute (K08HL130944).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Holzer M, Marsche G. Comment on Mathew et al. Therapeutic lifestyle changes improve HDL function by inhibiting myeloperoxidase-mediated oxidation in patients with metabolic syndrome. Diabetes Care 2018;41:2431–2437 (Letter).

[1]*Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI*
[2]*Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI*

Corresponding author: Subramaniam Pennathur, spennath@umich.edu

Diabetes Care 2019;42:e25. DOI: 10.2337/dc18-2026
2. Mathew AV, Li L, Byun J, et al. Therapeutic lifestyle changes improve HDL function by inhibiting myeloperoxidase-mediated oxidation in patients with metabolic syndrome. Diabetes Care 2018;41:2431–2437
3. Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. J Lipid Res 2013;54:2575–2585
4. Vaisar T, Pennathur S, Green PS, et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007;117:746–756
5. Wang G, Mathew AV, Yu H, et al. Myeloperoxidase mediated HDL oxidation and HDL proteome changes do not contribute to dysfunctional HDL in Chinese subjects with coronary artery disease. PLoS One 2018;13:e0193782