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Response to ‘The complex HDL proteome: A matter of purification?’

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In their letter, Holzer et al. recommend an additional purification step after ultracentrifugation for HDL isolation, suggesting that certain identified proteins may not associate with HDL particles and are therefore contaminants. Distinguishing HDL-associated from contaminating proteins has been an ongoing challenge since the initial application of proteomics to HDL. HDL was first isolated by density-gradient ultracentrifugation and defined as the fraction with a density of 1.210–1.063 g/ml.¹ This method remains the ‘gold standard’ for HDL isolation.² However, densities of lipoprotein classes are not discrete, and a density threshold of 1.063 g/ml does not cleanly separate HDL from LDL. Small dense LDL particles may overlap in density with buoyant HDL particles, resulting in the presence of trace apoB and apo(a) in isolated HDL. Serum proteins may also associate with HDL, including α -1-antitrypsin, which notably has been shown to influence HDL function.³ Alternatively, HDL may be defined and isolated by apolipoprotein A-I immunoaffinity, electrophoretic gel mobility, or a combination of gel-filtration followed by lipid capture, each method having different advantages and defining a different spectrum of particles.⁴ In our study, we used the gold standard density-gradient ultracentrifugation and optimized it to minimize contamination from both plasma and LDL [figure]. This enabled us to analyze over 500 samples from a large cohort and investigate the relationship between CKD and the HDL proteome in a cross-sectional study for the first time. Although our key findings are not affected by contaminants, they require further study to replicate and demonstrate association with clinical outcomes.

References

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Figure. SDS-PAGE gel of representative isolated HDL. Five micrograms of HDL isolated using the optimized sequential gradient-density ultracentrifugation method was separated on SDS-PAGE gel (4–12% Tris-Glycine Novex Mini-Gel, Invitrogen) and stained with Sypro Ruby protein stain.