# Frail HDLs and Stiff Arteries in Type 2 Diabetes in Juveniles

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Lowering of LDL cholesterol plasma levels with statins reduces coronary heart disease (CHD) event rates by up to 50% (1), implying a residual cardiovascular risk of the same magnitude despite treatment. Moreover, statins increase the risk of type 2 diabetes mellitus (T2DM), especially in patients showing components of the metabolic syndrome (2). Hence, there is considerable need for novel therapeutic regimens improving CHD prevention without increasing the risk of T2DM.

HDLs are an interesting target for this objective. Most observational studies and meta-analyses thereof demonstrated the inverse relationship of HDL cholesterol (HDL-C) levels with the CHD risk (3) as well as T2DM and its vascular complications (4,5). HDL particles exert various potentially antiatherogenic (6–8) and antidiabetogenic activities (4). Atherosclerotic lesions were decreased or even reversed in animals by transgenic overexpression or application of exogenous apolipoprotein (apo) A-I, which constitutes the most abundant protein of HDL (6). Animal experiments also provided evidence that HDL improves the function and survival of pancreatic β-cells and glucose uptake into muscle, liver, and adipose tissue (4). In humans, artificially reconstituted HDL particles reduced coronary plaque volume (9,10) and improved glycemia (11). In contrast to these promising results, addition of fenofibrate, niacin, torcetrapib, or dalcetrapib to statins failed to reduce cardiovascular risk beyond that provided by statin treatment alone despite increasing HDL-C (12–15). Moreover, alterations in HDL-C, either associated with mutations in the human genome or provoked in genetic mouse models, did not consistently translate into opposite changes of cardiovascular risk and atherosclerotic plaque load, respectively (16,17).

Because of these controversial data, the suitability of HDL as a therapeutic target has been increasingly questioned. However, it is important to emphasize that interventional trials and Mendelian randomization studies targeted HDL-C, which neither exerts nor reflects any of the potentially antiatherogenic activities of HDL (6). In a prototypic HDL particle, two to five molecules of apoA-I and ~100 molecules of phosphatidylcholine form an amphipathic shell in which several molecules of unesterified cholesterol are imbedded and it surrounds a core of cholesterol esters (18). Molar differences in the content of these major protein and lipid constituents produce considerable heterogeneity of HDL in shape, size, density, and charge (Fig. 1). The macroheterogeneity of HDL is further compounded by quantitatively minor proteins, lipids, or microRNAs (19–21), many of which contribute to the potentially antiatherogenic and antidiabetogenic properties of HDL. Additional HDL microheterogeneity is a consequence of various inflammatory diseases, including T2DM or CHD, which lead to the loss of or structural modification of typical HDL constituents or the acquisition of atypical HDL constituents (22). Several alterations of HDL structure and composition have been associated with the loss of potentially vasoprotective functions, such as stimulation of cholesterol efflux (7) and endothelium-dependent vasodilation (8), independently of plasma HDL-C levels. Importantly, the plasma concentrations of many microcomponents of HDL amount to only ≤1 μmol/L. Hence, they are three to four orders of magnitude lower than those of HDL-C (usually >1 mmol/L) and one to two orders of magnitude lower than those of apoA-I (50 μmol/L) or HDL particles (10–20 μmol/L). Accordingly, these microcomponents are nonrandomly distributed among HDL subclasses and are not recovered by measurements of HDL-C, apoA-I, or HDL subclasses.

Many laboratories worldwide currently are searching for functional biomarkers of HDL, which are more closely related to atherosclerosis and cardiovascular outcomes than HDL-C. One promising approach in this direction has been undertaken by Gordon et al. (23), who investigated the association of HDL subclasses and their proteomes with the presence of T2DM and obesity in adolescents and with pulse wave velocity (PWV), a noninvasive measure of vascular stiffness and hence a surrogate of atherosclerosis. Among 12 HDL subfractions identified by the authors, large HDL particles showed greatest differences between T2DM patients and nondiabetic controls. Compared with those in healthy controls, these subfractions were deprived of several proteins, including apoA-I, apoA-II, apoE, apoM, and paraoxonase 1 (PON1) in T2DM patients. These results in humans closely correspond to recent findings by Kothapalli et al. (24) showing increased arterial stiffness in apoE-deficient mice and favorable effects on arterial elasticity exerted by apoE-containing HDL particles. In addition to changes in protein composition, the phospholipid content of large HDL subfractions showed a significant inverse correlation with PWV. In agreement with protective functionality, large HDL particles were enriched in the sphingosine-1-phosphate–binding lipocalin apoM (25) and the antioxidative enzyme PON1 (26) in nondiabetic subjects. In contrast, cholesterol concentrations in smaller HDL particles showed positive correlations with PWV, suggesting adverse effects on vascular health. Of note, HDL-C did not show any significant correlation with PWV.

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DOI: 10.2337/db13-0784


**See accompanying original article, p. 2958.**

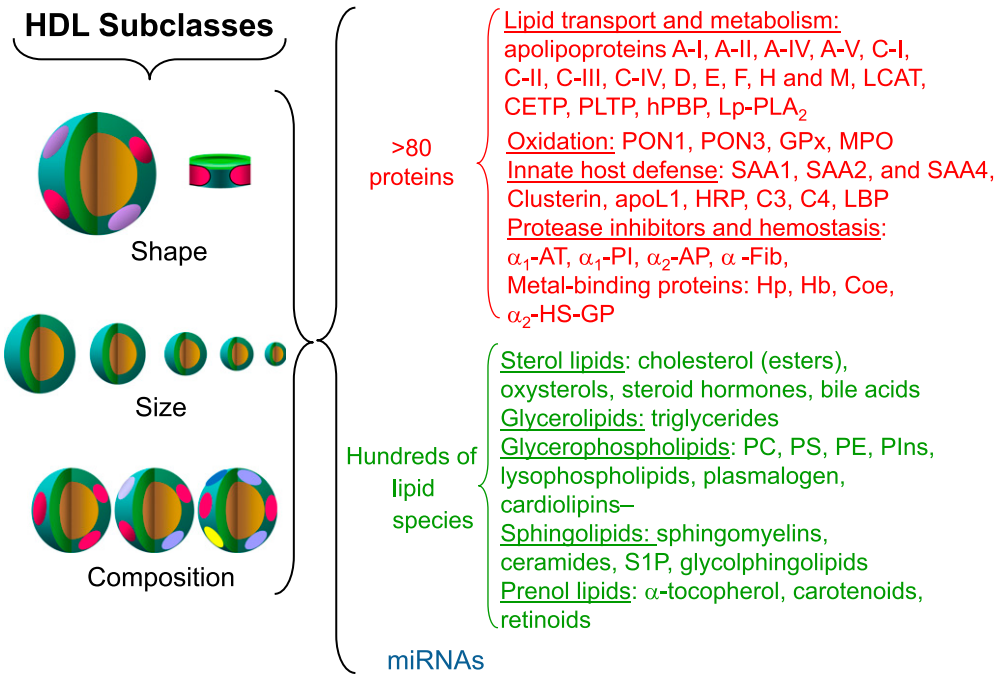


**FIG. 1. Structural and compositional heterogeneity of HDL. HDLs are macromolecular complexes containing >80 proteins and peptides, hundreds of diverse lipid species, and transporting microRNA (miRNA). This structural complexity gives rise to considerable heterogeneity with respect to size, shape, density, and charge. AP, antiplasmin; AT, antitrypsin; CETP, cholesteryl ester transfer protein; Coe, ceruloplasmin; Fib, fibrinogen; GPx, glutathione peroxidase; Hb, hemoglobin; Hp, haptoglobin; hPBP, human phosphatidylethanolamine; HRP, haptoglobin-related protein; HS-GP, HS-glycoprotein (fetuin A); LBP, lipopolysaccharide-binding protein; LCAT, lectin-cholesterol acyltransferase; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; MPO, myeloperoxidase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, proteinase inhibitor; PIns, phosphatidylinositol; PLTP, phospholipid transfer protein; PS, phosphatidylserine; SAA, serum amyloid A; S1P, sphingosine 1-phosphate.**

The study has several strengths. By investigating young patients with diabetes and controls, Gordon et al. (23) minimized the effect of confounders complicating data interpretation in diabetic adults. By using gel filtration rather than ultracentrifugation, the authors eliminated artifacts arising from protein and lipid displacement by shear forces or high ion concentrations. It is noteworthy that the authors retrieved several proteins, including apoM and PON1, in large HDL particles that were previously assigned to small HDL particles by ultracentrifugation (27). The risk of recording false-positive results after using the comprehensive proteomic approach was limited by the stringent selection of those proteins for statistical analyses, which were identified by previous proteomic examination of HDL.

General limitations of these explorative studies are the cross-sectional design and the small number of patients. As acknowledged by the authors, statistical association does not imply causality. In this respect, it will be interesting to test the effect of large HDL fractions on endothelial functions, which were previously found to be modulated by PON1 or apoM (8,25,26). The expansion to prospective studies will require methodological advancements permitting analyses of hundreds or even thousands of samples. To this end, a refined proteomic and lipidomic examination of large HDL particles might help to identify proteins or lipids that can be specifically targeted by high-throughput technologies such as immunoassays or single-reaction monitoring mass spectrometry. It also should be emphasized that the enzymatic assay used by the authors, which quantifies choline rather than phospholipids, neither discriminates between phosphatidylcholines, lysophoshatidylcholines, plasmalogens, and sphingomyelins nor records noncholine phospholipids such as phosphatidylethanolamines, phosphatidylserines, and sphingosine-1-phosphate. Another limitation of the study is that the mass spectrometry approach measured only relative concentrations. Interestingly, however, this semiquantitative approach unraveled reduced peptide signals in HDL from T2DM patients. It is possible that posttranslational protein modifications altered the mass of peptides and thereby prevented their recording by the assigned molecular mass. This explanation is congruent with previous findings showing enhanced glycation, nitration, chlorination, sulfoxidation, or carbamylation in HDL from diabetic subjects (22). Such modifications may offer new opportunities for use as biomarkers (7,8,22).

In conclusion, the study by Gordon et al. (23) provides new insights into the molecular heterogeneity of HDL and its association with T2DM and atherosclerosis. Apart from reproducing and extending these findings in larger observational studies, it will be important to resolve the structure, function, and metabolism of large HDL fractions. Further structure–function studies may help to select molecules or modifications within HDL, which can be used as biomarkers for identification, treatment stratification, and monitoring of patients at increased risk for cardiovascular diseases or diabetes mellitus.

## ACKNOWLEDGMENTS

A.v.E. is supported by grants from the Swiss National Science Foundation (3100A0-116404/1, 3100A0-130836/1), the FP7 Programme of the European Commission (RESOLVE, 305707), and the Zurich Center of Integrative Human Physiology, and has received honoraria for lectures and advisory activities from Hoffmann-La Roche, Merck Sharpe & Dohme, and AstraZeneca. J.-R.N. is supported by grants

from the German Foundation for Pathobiochemistry and Molecular Diagnostics, the Italian Ministry for Education, University, and Research (IDEAS RBID08777T), Novartis Germany, Actelion Pharmaceuticals Germany, and Siemens Healthcare Diagnostics. No other potential conflicts of interest relevant to this article were reported.

## REFERENCES

1. Baigent C, Blackwell L, Emberson J, et al.; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 2010;376:1670–1681
2. Waters DD, Ho JE, DeMicco DA, et al. Predictors of new-onset diabetes in patients treated with atorvastatin: results from 3 large randomized clinical trials. J Am Coll Cardiol 2011;57:1535–1545
3. Di Angelantonio E, Sarwar N, Perry P, et al.; Emerging Risk Factors Collaboration. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009;302:1993–2000
4. von Eckardstein A, Sibler RA. Possible contributions of lipoproteins and cholesterol to the pathogenesis of diabetes mellitus type 2. Curr Opin Lipidol 2011;22:26–32
5. Fioretto P, Dodson PM, Ziegler D, Rosenson RS. Residual microvascular risk in diabetes: unmet needs and future directions. Nat Rev Endocrinol 2010;6:19–25
6. von Eckardstein A. Implications of torcetrapib failure for the future of HDL therapy: is HDL-cholesterol the right target? Expert Rev Cardiovasc Ther 2010;8:345–358
7. Fisher EA, Feig JE, Hewing B, Hazen SL, Smith JD. High-density lipoprotein function, dysfunction, and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2012;32:2813–2820
8. Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. Circ Res 2012;111:1079–1090
9. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. JAMA 2003;290:2292–2300
10. Tardif JC, Grégoire J, L'Allier PL, et al.; Effect of rHDL on Atherosclerosis-Safety and Efficacy (ERASE) Investigators. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. JAMA 2007;297:1675–1682
11. Drew BG, Duffy SJ, Formosa MF, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009;119:2103–2111
12. Schwartz GG, Olsson AG, Abt M, et al.; dal-OUTCOMES Investigators. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med 2012;367:2089–2099
13. Barter PJ, Caulfield M, Eriksson M, et al.; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007;357:2109–2122
14. Boden WE, Probstfield JL, Anderson T, et al.; AIM-HIGH Investigators. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 2011;365:2255–2267
15. Ginsberg HN, Elam MB, Lovato LC, et al.; ACCORD Study Group. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med 2010;362:1563–1574
16. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study. Lancet 2012;380:572–580
17. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol 2013;61:427–436
18. Huang R, Silva RA, Jerome WG, et al. Apolipoprotein A-I structural organization in high-density lipoproteins isolated from human plasma. Nat Struct Mol Biol 2011;18:416–422
19. Shah AS, Tan L, Lu Long J, Davidson WS. The proteomic diversity of high density lipoproteins: Our emerging understanding of its importance in lipid transport and beyond. J Lipid Res 2013 Feb 24 [Epub ahead of print]
20. Wiesner P, Leidl K, Boettcher A, Schmitz G, Liebisch G. Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. J Lipid Res 2009;50:574–585
21. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011;13:423–433
22. Marsche G, Saemann MD, Heinemann A, Holzer M. Inflammation alters HDL composition and function: implications for HDL-raising therapies. Pharmacol Ther 2013;137:341–351
23. Gordon SM, Davidson WS, Urbina EM, et al. The effects of type 2 diabetes on lipoprotein composition and arterial stiffness in male youth. Diabetes 2013;62:2958–2967
24. Kothapalli D, Liu SL, Bae YH, et al. Cardiovascular protection by ApoE and ApoE-HDL linked to suppression of ECM gene expression and arterial stiffening. Cell Rep 2012;2:1259–1271
25. Christoffersen C, Obinata H, Kumaraswamy SB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci USA 2011;108:9613–9618
26. Besler C, Heinrich K, Rohrer L, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest 2011;121:2693–2708
27. Davidson WS, Silva RA, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function. Arterioscler Thromb Vasc Biol 2009;29:870–876